Resveratrol – Compilation of Evidence

Contents:

Intoduction

Resveratrol, a naturally occurring hydroxystilbene identified in over 70 plant species including nuts, grapes, pine trees, certain vines and red wine, is thought to play a role in the prevention of heart disease. Attention was first drawn to resveratrol in 1992 when it was mentioned as a constituent of red wine .Humans have been consuming wine for approximately 7,000 years. Resveratrol, and other polyphenols in wine, are thought to account in part for the so-called French Paradox, the finding that the rate of coronary heart disease mortality in France is lower than observed in other industrialized countries with a similar risk factor profile.

In vitro and animal studies are set forth in this paper showing resveratrol to exhibit antioxidant, anticancer, antiproliferative, antifungal, antiviral, and antibacterial and beneficial in variety of other conditions, including amyotrophic lateral sclerosis, diabetic polyneuropathy, Alzheimer's disease, Parkinson's disease, chronic obstructive pulmonary disease (COPD), and acute pancreatitis .

The human equivalent dose for a 160-pound adult would be about 1575 milligrams of resveratrol to produce the health benefits noted in the Harvard mouse study. The study published last year in the journal Nature, stated that a lower-dose (~364 milligrams for a 160-pound adult) produced similar benefits.

Furthermore, the mice were engorged with fat, 60% of their daily calorie intake. If we take an example of Americans who once consumed about 45% of daily calories from fat (1965), which has dropped to about 34% (2002). we arrive at a lower amount of resveratrol, possbily half as much (~180 mg) for the equivalent effective dose.

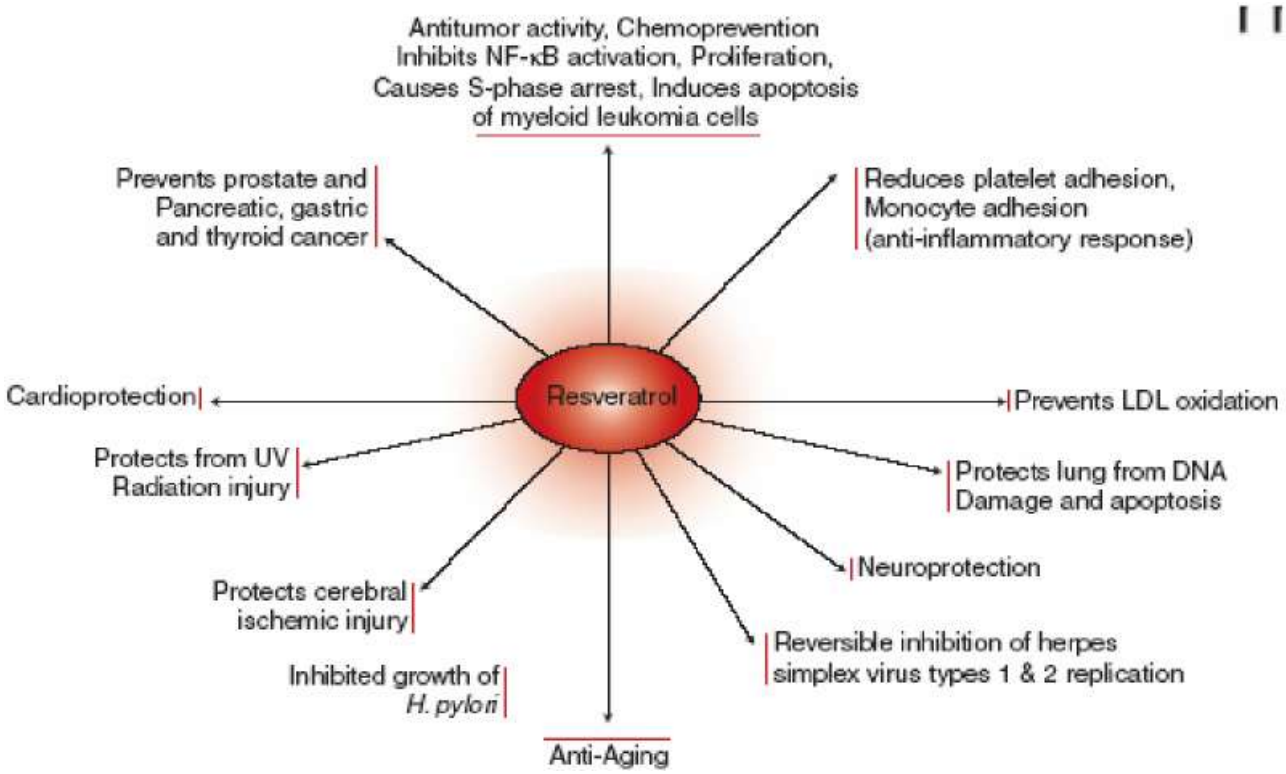
Pharmacokinetic studies revealed that the target organs of resveratrol are the liver and kidneys, where it is concentrated after absorption and is mainly converted to a sulfated form and a glucuronide conjugate Tissue concentrations also show bioavailability in cardiac tissue. In humans, resveratrol is metabolized into two resveratrol-3-O- and 4'-O-glucuronides. : In the human liver, CYP1A2 likely plays a major role in the metabolism of *trans*-resveratrol into piceatannol and tetrahydroxystilbene M1 and also is metabolized by CYP1B1, to form the antileukaemic agent, piceatannol. Resveratrol is excreted in urine as sulfate and glucuronic acid conjugates of the phenolic groups and hydrogenated derivatives of the aliphatic double bond. More elaborate studies are needed to evaluate the specific effects of Resveratrol in the human body, since much of the basic research on resveratrol has been conducted in cultured cells exposed to unmetabolized resveratrol at concentrations that are often 10-100 times greater than peak concentrations observed in human plasma after oral consumption.

Resveratrol is not known to be toxic or cause adverse effects in humans, but there have been few controlled human clinical trials. In rats, daily oral administration of trans-

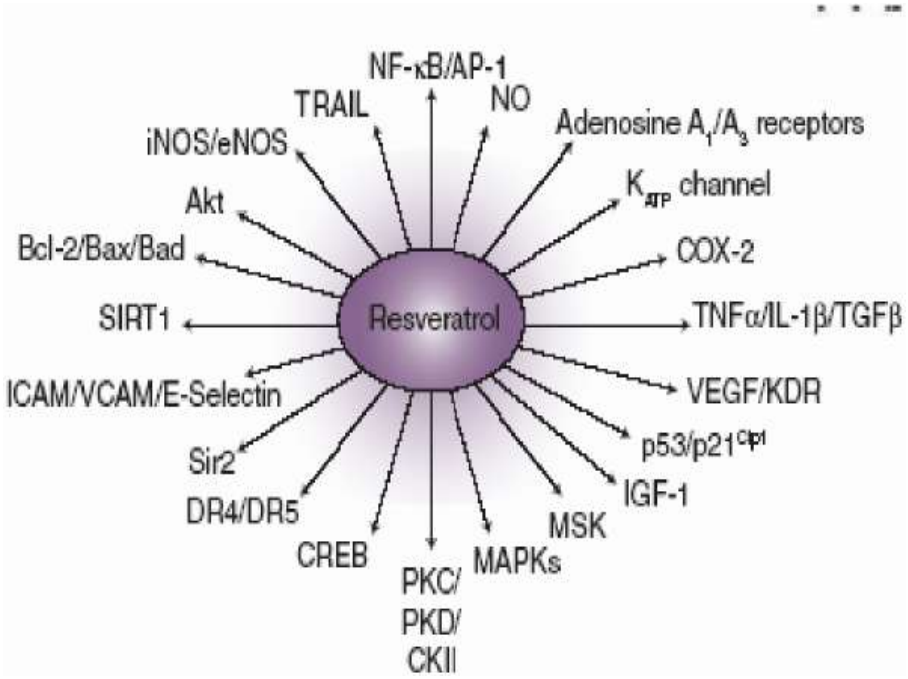
resveratrol at doses up to 300 mg/kg of body weight for 4 weeks resulted in no apparent adverse effects. The safety of resveratrol-containing supplements during pregnancy and lactation has not been established. Since no safe level of alcohol consumption has been established at any stage of pregnancy, pregnant women should avoid consuming wine as a source of resveratrol.

Resveratrol has been found to inhibit human platelet aggregation in vitro Theoretically, high intakes of resveratrol could increase the risk of bleeding when taken with anticoagulant drugs and antiplatelet drugs, non-steroidal anti-inflamatory drugs (NSAIDs).Resveratrol has been reported to inhibit the activity of cytochrome P450 3A4 (CYP3A4) in vitro Although this interaction has not been reported in humans, high intakes of resveratrol could theoretically increase the bioavailability of drugs that undergo extensive first-pass metabolism by CYP3A4.

Health benefits of "Miracle Molecule"- RESVERATROL:



Molecular mechanism:



Resveratrol Safety

The daily oral administration of high doses of trans-resveratrol to rats for 28 days is not harmful.

J Nutr. 2002 Feb;132(2):257-60

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trans-3,5,4'-Trihydroxystilbene (trans-resveratrol) is a phytochemical present in peanuts, grapes and wine with beneficial effects such as protection against cardiovascular disease and cancer prevention. The purpose of this study was to evaluate whether high doses of trans-resveratrol have harmful effects on Sprague-Dawley rats. trans-Resveratrol was administered orally to male rats for 28 d at a dose of 20 mg/(kg x d), 1000 times the amount consumed by a 70-kg person taking 1.4 g of trans-resveratrol/d. Body weight, and food and water consumption did not differ between rats treated with trans-resveratrol and the control group. Hematologic and biochemical variables were not affected by the treatment. Histopathologic examination of the organs obtained at autopsy did not reveal any alterations. These results support the view that repeated consumption of trans-resveratrol at 20 mg/(kg x d) does not adversely affect the variables tested in rats.

Resveratrol-associated renal toxicity.

Toxicol Sci. 2004 Dec;82(2):614-9. Epub 2004 Aug 25

Crowell JA, **Korytko PJ**, **Morrissey RL**, **Booth TD**, **Levine BS**.

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Resveratrol, (3,5,4'-trihydoxystilbene) a compound found in grapes, mulberries, and peanuts, has antimycotic, antiviral, and beneficial cardiovascular and cancer preventive activities. It is being developed for several clinical indications. To evaluate the potential toxicity of resveratrol, rats were administered by gavage 0, 300, 1000, and 3000 mg trans-resveratrol per kilogram body weight per day for 4 weeks. Most of the adverse events occurred in the rats administered 3000 mg per kilogram body weight per day. These included increased clinical signs of toxicity; reduced final body weights and food consumption; elevated BUN, creatinine, alkaline phosphatase, alanine aminotransferase, total bilirubin, and albumin; reduced hemoglobin, hematocrit, and red cell counts; and

increased white cell counts. Increases in kidney weights and clinically significant renal lesions, including an increased incidence and severity of nephropathy, were observed. Diffuse epithelial hyperplasia in the bladder was considered, equivocal and of limited biological significance. No histological effects on the liver were observed, despite the clinical chemistry changes and increased liver weights in the females. Effects seen in the group administered 1000 mg resveratrol per kilogram body weight per day included reduced body weight gain (females only) and elevated white blood cell count (males only). Plasma resveratrol concentrations in blood collected 1 h after dose administration during week 4 were dose related but were relatively low given the high dosage levels; conjugates were not measured. Under the conditions of this study, the no observed adverse effect level was 300 mg resveratrol per kilogram body weight per day in rats.

Resveratrol
and
Breast cancer

Resveratrol modulates roscovitine-mediated cell cycle arrest of human MCF-7 breast cancer cells.

Food Chem Toxicol. 2007 Sep 11

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Human MCF-7 breast cancer cells are relatively resistant to anti-cancer drugs. Recently, we reported that roscovitine (ROSC), a selective cyclin-dependent kinase (CDK) inhibitor, arrested human MCF-7 breast cancer cells in G(2) phase of the cell cycle and concomitantly induced apoptosis. Moreover, we observed that the effect of the CDK inhibitor was dependent on the content of the culture medium. The cell cycle inhibiting action of ROSC was markedly diminished in human MCF-7 cells cultivated in medium supplemented with phenol red. These observations indicated that the therapeutic effects of ROSC can be affected by the components of the tissue medium. Recently, a number of epidemiological and experimental studies indicated that polyphenols (e.g. resveratrol, epicatechins etc.), abundant micronutrients in food, are anti-oxidant agents and could have strong anti-mitotic as well as pro-apoptotic activities. In the present contribution we raised the question whether the ROSC-mediated cell cycle arrest could be additionally modulated by compounds of natural origin, especially by polyphenols. Considering the potential benefits of the dietary components during the post-chemotherapy period, we focused our attention on the effects of resveratrol administration after treatment with ROSC. We analyzed whether the combined treatment with resveratrol would exert any additional effect on the cell cycle status of ROSC-treated human cancer cells. Resveratrol exhibited low direct cytotoxicity. The combined treatment with ROSC enhanced the ROSC-mediated inhibition of cell proliferation and cell cycle arrest. These results indicate that targeted combination of anti-cancer drugs with distinct naturally occurring compounds could increase the efficacy of the therapy and concomitantly reduce the undesired side effects exerted by cytostatic drugs.

Mitochondria, Calcium, and Calpain are Key Mediators of Resveratrol-Induced Apoptosis in Breast Cancer.

Mol Pharmacol. 2007 Sep 11

Sareen D, **Darjatmoko SR**, **Albert DM**, **Polans AS**.

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Resveratrol (RES), a natural plant polyphenol, has gained interest as a non-toxic chemopreventive agent capable of inducing tumor cell death in a variety of cancer types. However, the early molecular mechanisms of RES-induced apoptosis are not well defined. Using the human breast cancer cell lines MDA-MB-231 and MCF-7, we demonstrate that RES is anti-proliferative and induces apoptosis in a concentration- and time-dependent manner. Preceding apoptosis, RES instigates a rapid dissipation of mitochondrial membrane potential (DeltaPsim) by directly targeting mitochondria. This is followed by release of cytochrome c and Smac/DIABLO into the cytoplasm and substantial increase in the activities of caspases-9 and -3 in MDA-MB-231 cells. Additionally, live cell microscopy demonstrates that RES causes an early biphasic increase in the concentration of free intracellular calcium ([Ca(2+)]i), likely resulting from depletion of the endoplasmic reticulum (ER) stores in breast cancer cells. In caspase-3 deficient MCF-7 cells apoptosis is mediated by the Ca(2+)- activated protease, calpain, leading to the degradation of plasma membrane Ca(2+)-ATPase isoform 1 (PMCA1) and fodrin; the degradation is attenuated by buffering [Ca(2+)]i and blocked by calpain inhibitors. Mitochondrial permeability transition pore antagonists also blocked calpain activation. In vivo mouse xenograft studies demonstrate that RES treatment inhibits breast cancer growth with no systemic toxicities. Collectively, these results suggest a critical role for mitochondria not only in the intrinsic apoptotic pathway but also in the Ca(2+) and calpain-dependent cell death initiated by RES. Thus, RES may prove useful as a non-toxic alternative for breast cancer treatment.

Resveratrol inhibits heregulin-beta1-mediated matrix metalloproteinase-9 expression and cell invasion in human breast cancer cells.

Nutr Biochem. 2007 Jul 23;

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The growth factor heregulin-beta1 (HRG-beta1), which is expressed in breast cancer, activates the HER-2 signaling pathway through induction of heterodimeric complexes of HER-2 with HER-3 or HER-4. It has been shown in many studies that HRG-beta1 induces the tumorigenicity and metastasis of breast cancer cells. Matrix metalloproteinase (MMP) 9 is a key enzyme in the degradation of extracellular matrices, and its expression may be dysregulated in breast cancer invasion and metastasis. Resveratrol, a major component in grape, exhibited potential anticarcinogenic activities in both in vitro and in vivo studies. However, the inhibitory effect of resveratrol on HER-2-mediated expression of MMP-9 has not been demonstrated yet. In the present study, we investigated the anti-invasive mechanism of resveratrol in human breast cancer cells. Human breast cancer

MCF-7 cells were exposed to resveratrol (2, 5 and 10 muM). The expression activity of MMP-9 was measured by zymogram analysis. Phosphorylated levels of HER-2 and mitogen-activated protein kinase (MAPK)/ERK were measured by Western blot analysis. Total actin was used as internal control for protein expression. HRG-beta1 induced the phosphorylation of HER-2/neu receptor and MMP-9 expression in human breast cancer MCF-7 cells. Resveratrol significantly inhibited HRG-beta1-mediated MMP-9 expression in human breast cancer cells. MEK inhibitor induced a marked reduction in MMP-9 expression, and it suggested that ERK1/2 cascade could play an important role in HRG-beta1-mediated MMP-9 expression. Furthermore, resveratrol significantly suppressed HRG-beta1-mediated phosphorylation of ERK1/2 and invasion of breast cancer cells. However, resveratrol had negligible effects on either HRG-beta1-mediated phosphorylation of HER-2 receptor or expression of the tissue inhibitor of MMP, tissue inhibitor metalloproteinase protein 1. Taken together, our results suggest that resveratrol inhibited MMP-9 expression in human breast cancer cells. The inhibitory effects of resveratrol on MMP-9 expression and invasion of breast cancer cells are, in part, associated with the down-regulation of the MAPK/ERK signaling pathway.

Resveratrol-induced growth inhibition in MDA-MB-231 breast cancer cells is associated with mitogen-activated protein kinase signaling and protein translation.

Eur J Cancer Prev. 2007 Aug;16(4):334-41

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Resveratrol (3,4',5-trans-trihydroxystilbene) is a natural compound found in grapes and several medicinal plants and has been shown to have anticancer effects on various human cancer cells. The aim of this study was to further investigate the molecular mechanism of this anticancer effect. Resveratrol effect on cell growth, morphology and gene expression was investigated in estrogen receptor-negative MDA-MB-231 human breast cancer cell line. We show here that resveratrol-induced growth inhibition in the estrogen receptor negative MDA-MB-231 breast cancer cells is due to the induction of apoptosis as demonstrated by morphological, nuclear staining and PARP cleavage analysis. Resveratrol-induced growth inhibition was associated with transient activation of p44/42 mitogen-activated protein kinase (MAPK) (Thr202/Tyr204). Most importantly, resveratrol inhibited both the phosphorylation at Ser240/244 and the expression of the pS6 ribosomal protein. This protein is known to play an important role in the translation of mRNAs that have oligopyrimidine tracts in their 5' untranslated regions. Interestingly, only MAPK inhibitor was able to block resveratrol-induced growth inhibition suggesting that effects of resveratrol on cell growth are dependent on MAPK signaling. The data

demonstrated that resveratrol-induced apoptosis is associated with MAPK signaling and with the inhibition of proteins that are involved in protein translation. This is the first data linking resveratrol with downregulation of protein translation via p44/42 MAPK and S6 ribosomal protein. We propose to use these proteins as predictive biomarkers to evaluate the treatment efficacy of resveratrol in estrogen receptor-negative human breast cancer.

Differential expression of genes induced by resveratrol in human breast cancer cell lines.

Nutr Cancer. 2006;56(2):193-203.

Le Corre L, **Chalabi N**, **Delort L**, **Bignon YJ**, **Bernard-Gallon DJ**.

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The phytoalexin, trans-resveratrol (RES), is a polyphenolic compound found in plants and fruits that seems to have a wide spectrum of biological activities. It has been found to possess cancer chemopreventive effects by inhibiting diverse cellular events associated with tumor initiation, promotion, and progression. RES is also a phytoestrogen, which binds to and activates estrogen receptors (ERs) that regulate the transcription of estrogen-responsive target genes. We used two human breast tumor cell lines (MCF7 and MBA-MB-231) and one fibrocystic breast cell line (MCF10a) to examine whether RES altered mRNA expression of genes that are involved in biological pathway frequently altered during carcinogenesis. Two GEarray systems were used to screen the differentially expressed genes between RES-treated cells and control cells. The differentially expressed genes were analyzed further by quantitative reverse transcriptase polymerase chain reaction. Here, we demonstrate that RES regulates mRNA expression of several genes involved in cell cycle control, apoptosis, metastasis, cell-cell adhesion, and ER signaling pathway. This effect of RES on the gene expression appears in correlation with chemoprevention activities of RES described previously. RES is also found to be more active in ER+ than ER- cells.

Resveratrol-induced cell inhibition of growth and apoptosis in MCF7 human breast cancer cells are associated with modulation of phosphorylated Akt and caspase-9.

Appl Biochem Biotechnol. 2006 Dec;135(3):181-92.

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Resveratrol (trans-3,4N,-5-trihydroxystilbene), a phytoalexin present in grapes and red wine, is emerging as a natural compound with potential anticancer properties. Here we show that resveratrol affects the growth of human breast cancer cell lines MCF7, MDA-MB-231, SK-BR-3, and Bcap-37 in a dose-dependent manner and that MCF7 is the most sensitive among the four cell lines. MCF7 cells treated with resveratrol showed typical characteristics of apoptosis including the poly (ADP-ribose) polymerase cleavage, TdT-mediated dUTP nick end labeling-positive staining, and morphologic changes. Phosphorylation of the oncogene product Akt was significantly reduced followed by decreased phosphorylation and increased processing of pro-caspase-9 on resveratrol treatment. These results indicate that resveratrol seems to exert its growth-inhibitory/ apoptotic effect on the breast cancer cell line MCF7 via the Akt-caspase-9 pathway.

Genistein and resveratrol: mammary cancer chemoprevention and mechanisms of action in the rat.

Expert Rev Anticancer Ther. 2006 Dec;6(12):1699-706.

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The environment, including diet, plays a critical role in a woman's subsequent risk of breast cancer. Two dietary polyphenols that have received attention from the health and research communities for their ability to protect against breast cancer are: genistein, a component of soy; and resveratrol, a phytoalexin found in red grapes and red wine. We and others have shown that both genistein and resveratrol can protect against mammary cancer in rodents. The timing of exposure to genistein appears critical for its mammary protective effects. It has been reported that genistein early in life causes enhanced mammary gland differentiation, alterations in cell proliferation and apoptosis, and upregulation of tumor-suppressor genes. With resveratrol in the diet, changes in cell proliferation and apoptosis in terminal ductal structures of the mammary gland might help to explain its protective effects. We conclude that genistein and resveratrol can protect against breast cancer by regulating important mammary growth and differentiation pathways.

Studies on active substance of anticancer effect in Polygonum cuspidatum]

Zhong Yao Cai. 2006 Jul;29(7):689-91.

[Article in Chinese]

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OBJECTIVE: To supply the scientific basis of research and development of the medicinal value of Polygonum cuspidatum. METHODS: One composition was isolated from the roots of Polygonum cuspidatum by cytotoxicity based fractionation and identified by HPLC-MS, UV scanning and IR. The inhibition and morphology of L-02, Hep G2, SHZ-888, MCF-7, MCF-7/ADM cells growth caused by this composition was determined by MTT assay and HE dyeing. RESULTS: This composition was identified as trans-and cis-resveratrol. It could specifically inhibit proliferation of many cancer cells but not human normal liver cell. We investigated the cytotoxicity of resveratrol to adriamycin-resistant MCF-7 cell in virtro. CONCLUSION: Resveratrol is a new anticancer composition which is less toxicity and higher efficiency in Polygonum cuspidatum.

Resveratrol and estradiol exert disparate effects on cell migration, cell surface actin structures, and focal adhesion assembly in MDA-MB-231 human breast cancer cells.

Neoplasia. 2005 Feb;7(2):128-40

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Resveratrol, a grape polyphenol, is thought to be a cancer preventive, yet its effects on metastatic breast cancer are relatively unknown. Since cancer cell invasion is dependent on cell migration, the chemotactic response of MDA-MB-231 metastatic human breast cancer cells to resveratrol, estradiol (E2), or epidermal growth factor (EGF) was investigated. Resveratrol decreased while E2 and EGF increased directed cell migration. Resveratrol may inhibit cell migration by altering the cytoskeleton. Resveratrol induced a rapid global array of filopodia and decreased focal adhesions and focal adhesion kinase (FAK) activity. E2 or EGF treatment did not affect filopodia extension but increased lamellipodia and associated focal adhesions that are integral for cell migration. Combined resveratrol and E2 treatment resulted in a filopodia and focal adhesion response similar to resveratrol alone. Combined resveratrol and EGF resulted in a lamellipodia and focal adhesion response similar to EGF alone. E2 and to a lesser extent resveratrol increased EGFR activity. The cytoskeletal changes and EGFR activity in response to E2 were blocked by EGFR1 inhibitor indicating that E2 may increase cell migration via crosstalk with EGFR signaling. These data suggest a promotional role for E2 in breast cancer cell migration but an antiestrogenic, preventative role for resveratrol.

Resveratrol-induced cyclooxygenase-2 facilitates p53-dependent apoptosis in human breast cancer cells.

Mol Cancer Ther. 2006 Aug;5(8):2034-42

Tang HY, **Shih A**, **Cao HJ**, **Davis FB**, **Davis PJ**, **Lin HY**.

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Cyclooxygenase-2 (COX-2) is antiapoptotic and is implicated in tumorigenesis. Recent reports, however, have also ascribed a proapoptotic action to inducible COX-2. We show here for the first time that a stilbene, resveratrol, induces nuclear accumulation of COX-2 protein in human breast cancer MCF-7 and MDA-MB-231 cell cultures. The induction of COX-2 accumulation by resveratrol is mitogen-activated protein kinase (MAPK; extracellular signal-regulated kinase 1/2)- and activator protein 1- dependent. Nuclear COX-2 in resveratrol-treated cells colocalizes with Ser(15)-phosphorylated p53 and with p300, a coactivator for p53-dependent gene expression. The interaction of COX-2, p53, and p300, as well as resveratrol-induced apoptosis, was inhibited by a MAPK activation inhibitor, PD98059. A specific inhibitor of COX-2, NS398, and small interfering RNA knockdown of COX-2 were associated with reduced p53 phosphorylation and consequent decrease in p53-dependent apoptosis in resveratrol-treated cells. We conclude that nuclear accumulation of COX-2 can be induced by resveratrol and that the COX has a novel intranuclear colocalization with Ser(15)-phosphorylated p53 and p300, which facilitates apoptosis in resveratrol-treated breast cancer cells.

Integrin alphaVbeta3 contains a receptor site for resveratrol.

FASEB J. 2006 Aug;20(10):1742-4. Epub 2006 Jun 21.

Lin HY, **Lansing L**, **Merillon JM**, **Davis FB**, **Tang HY**, **Shih A**, **Vitrac X**, **Krisa S**, **Keating T**, **Cao HJ**, **Bergh J**, **Quackenbush S**, **Davis PJ**.

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Resveratrol is a naturally occurring polyphenol, which causes apoptosis in cultured cancer cells. We describe a cell surface resveratrol receptor on the extracellular domain of hetero-dimeric alphaVbeta3 integrin in MCF-7 human breast cancer cells. This receptor is linked to induction by resveratrol of extracellular-regulated kinases 1 and 2 (ERK1/2)- and serine-15-p53-dependent phosphorylation leading to apoptosis. The integrin receptor is near the Arg-Gly-Asp (RGD) recognition site on the integrin; an integrin-binding RGD peptide inhibits induction by resveratrol of ERK1/2- and p53-dependent apoptosis. Antibody (Ab) to integrin alphaVbeta3, but not to alphaVbeta5, inhibits activation by resveratrol of ERK1/2 and p53 and consequent apoptosis in estrogen receptor-alpha (ERalpha) positive MCF-7, and ERalpha-negative MDA-MB231 cells. Resveratrol is displaced from the purified integrin by an RGD, but not RGE, peptide, and by alphaVbeta3 integrin-specific Ab. Resveratrol action is blocked by siRNAbeta3, but not by siRNAalphaV. [14C]-Resveratrol binds to commercially purified integrin alphaVbeta3 and to alphaVbeta3 prepared from MCF-7 cells; binding of [14C]-resveratrol to the beta3, but not to the alphaV monomer, is displaced by unlabeled resveratrol. In conclusion, binding of resveratrol to integrin alphaVbeta3, principally to the beta3 monomer, is essential for transduction of the stilbene signal into p53-dependent apoptosis of breast cancer cells.

Resveratrol, but not EGCG, in the diet suppresses DMBA-induced mammary cancer in rats.

J Carcinog. 2006 May 15;5:15

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Despite the advent of new and aggressive therapeutics, breast cancer remains a leading killer among women; hence there is a need for the prevention of this disease. Several naturally occurring polyphenols have received much attention for their health benefits, including anti-carcinogenic properties. Two of these are resveratrol, a component of red grapes, and epigallocatechin-3-gallate (EGCG), the major catechin found in green tea. In this study, we tested the hypothesis that these two polyphenols protect against chemically-induced mammary cancer by modulating mammary gland architecture, cell proliferation, and apoptosis. Female Sprague-Dawley CD rats were exposed to either resveratrol (1 g/kg AIN-76A diet), EGCG (0.065% in the drinking water), or control diet (AIN-76A) for the entirety of their life starting at birth. At 50 days postpartum, rats were treated with 60 mg dimethylbenz[a]anthracene (DMBA)/kg body weight to induce mammary cancer. Resveratrol, but not EGCG, suppressed mammary carcinogenesis (fewer tumors per rat

and longer tumor latency). Analysis of mammary whole mounts from 50-day-old rats revealed that resveratrol, but not EGCG, treatment resulted in more differentiated lobular structures. Bromodeoxyuridine (BrdU) incorporation studies showed that resveratrol treatment caused a significant reduction in proliferative cells in mammary terminal ductal structures at 50 days postpartum, making them less susceptible to carcinogen insult. The epithelial cells of terminal end buds in the mammary glands of resveratrol-treated rats also showed an increase in apoptotic cells compared to the control or EGCG-treated rats as measured by a DNA fragmentation assay. At the given doses, resveratrol treatment resulted in a serum resveratrol concentration of 2.00 microM, while treatment with EGCG resulted in a serum EGCG concentration of 31.06 nM. 17beta-Estradiol, progesterone, and prolactin concentrations in the serum were not significantly affected by resveratrol or EGCG. Neither polyphenol treatment resulted in toxicity as tested by alterations in body weights, diet and drink consumptions, and day to vaginal opening. We conclude that resveratrol in the diet can reduce susceptibility to mammary cancer, while EGCG in the drinking water at the dose used was not effective.

The red wine polyphenol resveratrol displays bilevel inhibition on aromatase in breast cancer cells.

Toxicol Sci. 2006 Jul;92(1):71-7. Epub 2006 Apr 11.

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Estrogen plays a crucial role in the development of breast cancer, and the inhibition of estrogen synthesis has been an important target for the prevention and treatment of this disease. The rate-limiting reaction of the hormone biosynthesis is catalyzed by cytochrome P450 (CYP) 19 enzyme or aromatase. It has been of genuine interest to uncover an aromatase-inhibitory compound from a dietary source. Resveratrol is a polyphenolic compound that can be isolated from grape peel. Because of its structural resemblance to estrogen, resveratrol's agonistic and antagonistic properties on estrogen receptor have been examined and demonstrated. In the present study, the effect of resveratrol on the expression and enzyme activity of aromatase was investigated. By assaying on MCF-7 cells stably transfected with CYP19 (MCF-7aro cells), resveratrol inhibited the aromatase activity with an IC(50) value of 25 microM. Kinetic analysis indicated that both competitive and noncompetitive inhibition might be involved. The administration of 10 nmol/l testosterone-a substrate of aromatase-produced a 50% increase in the MCF-7aro cell number. This cell proliferation specifically induced by testosterone was significantly reduced by 10 microM resveratrol. In addition, 50 microM

resveratrol significantly reduced the CYP19-encoding mRNA abundance in SK-BR-3 cells. The transcriptional control of CYP19 gene is tissue specific, and promoter regions I.3 and II have previously been shown to be responsible for CYP19 expression in breast cancer cells. Luciferase reporter gene assays revealed that resveratrol could repress the transcriptional control dictated by the promoter regulation. The present study illustrated that pharmacological dosage of resveratrol inhibited aromatase at both the enzyme and mRNA levels.

Resveratrol down-regulates the growth and telomerase activity of breast cancer cells in vitro.

Int J Oncol. 2006 Mar;28(3):641-8

Lanzilli G, **Fuggetta MP**, **Tricarico M**, **Cottarelli A**, **Serafino A**, **Falchetti R**, **Ravagnan G**, **Turriziani M**, **Adamo R**, **Franzese O**, **Bonmassar E**.

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A number of previous studies investigated the in vitro effects of resveratrol on malignant human breast epithelial cell replication. The aim of the present study was to evaluate the activity of resveratrol on human metastatic breast cancer cells. The study was performed on the MCF-7 tumor cell line. Cell growth, cell cycle perturbation and apoptosis were evaluated by trypan blue dye exclusion assay, flow cytometric analysis and confocal fluorescence microscopy. TRAP assay and Western blot analysis respectively detected levels of telomerase activity and levels of hTERT in intracellular compartments of MCF-7 cells treated with resveratrol. Resveratrol has a direct inhibitory effect on cell proliferation. The results demonstrate that the drug induces apoptosis in MCF-7 cells, in a time- and concentration-related manner. Our results also show that the growth-inhibitory effect of resveratrol on malignant cells is mainly due to its ability to induce S-phase arrest and apoptosis in association with reduced levels of telomerase activity. In particular, TRAP assay and Western blot analysis respectively showed that resveratrol treatment down-regulates the telomerase activity of target cells and the nuclear levels of hTERT, the reverse transcriptase subunit of the telomerase complex. In our experimental model of breast cancer, resveratrol shows direct antiproliferative and pro-apoptotic effects. Studies on telomerase function and intracellular hTERT distribution point out that this agent is endowed with additional suppressive functions on critical tumor biological properties. These results speak in favor of a potential role of resveratrol in chemoprevention/ chemotherapy of breast cancer.

Resveratrol induces apoptosis and inhibits angiogenesis in human breast cancer xenografts in vivo.

Cancer Lett. 2006 Jan 8;231(1):113-22

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Resveratrol, a polyphenol found in grapes and wine, is considered a potential cancer chemopreventive agent. Resveratrol has been shown to induce transcription via both ERalpha and ERbeta. We observed significantly lower tumor growth, decreased angiogenesis, and increased apoptotic index in ERalpha- ERbeta+ MDA-MB-231 tumors in resveratrol-treated nude mice compared with controls. In vitro we found a significant increase in apoptosis in resveratrol-treated MDA-MB-231 cells in addition to significantly reduced extracellular levels of VEGF. This study supports the potential use of resveratrol as a chemotherapeutic agent in breast cancers.

Polyphenol interaction with the T47D human breast cancer cell line.

J Dairy Res. 2005;72 Spec No:44-50

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Experimental and epidemiological studies indicate that antioxidant food polyphenols could have antimitotic activities, interfering with cancer initiation, progression or mortality. Circulating polyphenols are far lower than the nominal value in foods. In the rare studies dealing with polyphenol bioavailability, it was noted that their active concentrations in the blood are <1% of their food concentration. In the present study we investigated the effect of four polyphenols (resveratrol, and the flavonoids quercetin, catechin and epicatechin, major constituents of wine) in the hormone-sensitive human

cancer cell line T47D, at concentrations compatible with their calculated plasma concentrations after ingestion of a moderate quantity of wine (nM or pM). Our results indicate that cell growth was decreased, with cells being arrested at the S phase of the cycle. In addition, we provide evidence of a bimodal modulation of the NO/NOS system, affecting its activity and transcription. We show that modulation of this system is sufficient to explain polyphenol action on this cell line. This result suggests a potential importance of wine ingestion and possibly the consumption of other polyphenol-rich dietary foods and drinks in the control of breast cancer cell growth.

Cyclooxygenase 2 and breast cancer. From biological concepts to clinical trials]

Bull Cancer. 2004 May 1;91 Suppl 2:S99-108

[Article in French]

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Cyclooxygenases (Cox) are prostaglandin synthetase enzymes which play a key role in mammary carcinogenesis. Several connections were demonstrated between Cox and a few oncogenes (v-src, v-Ha-ras, HER-2\neu, Wnt, p53 mutated), alimentary products (PUFAs), transcription factors (c-jun and c-fos), proapoptotic proteins [Bax et Bcl-x(L)] or antiapoptotic (Bcl-2), CYP19 aromatase gene, NFkappaB receptor (RANKL), angiogenesis (via VEGF, TXA2, oxid nitric synthetase, alphaVbeta3 integrin receptor), peroxisome gamma proliferator receptor (PPARgamma) and its ligand PGJ2 and with antitubuline chemotherapy drugs. No correlation of Cox2 expression with hormonal receptors was shown. In epidemiologic studies there is evidence of breast cancer risk reduction for women who take AINS for a lon time. Alimentary factors like resveratrol or insaturated fat acid reduce Cox2 expression in animal and could be investigated in human studies. Clinical trials are planed with the anti Cox2 celecoxib for breast cancer prevention, in adjuvant setting, in metastatic situation combined with exemestane or antitubulin drugs or in neoadjuvant therapy.

Resveratrol-induced apoptosis in MCF-7 human breast cancer cells involves a caspase-independent mechanism with downregulation of Bcl-2 and NF-kappaB.

Int J Cancer. 2005 May 20;115(1):74-84

Pozo-Guisado E, **Merino JM**, **Mulero-Navarro S**, **Lorenzo-Benayas MJ**, **Centeno F**, **Alvarez-Barrientos A**, **Fernandez-Salguero PM**.

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Resveratrol (RES), a chemopreventive molecule, inhibits the proliferation of tumor cells of different etiologies. We previously showed that RES alters the cell cycle and induces apoptosis in MCF-7 breast tumor cells by interfering with the estrogen receptor (ERaalpha)-dependent phosphoinositide 3-kinase (PI3K) pathway. Here, we analyzed signaling downstream of PI3K, to understand the mechanisms of RES-induced apoptosis. Apoptotic death by RES in MCF-7 was mediated by Bcl-2 downregulation since overexpression of this protein abolished apoptosis. Decreased Bcl-2 levels were not related to cytochrome c release, activation of caspases 3/8 or poly(ADP-ribose) polymerase proteolysis. However, RES decreased mitochondrial membrane potential and increased reactive oxygen species and nitric oxide production. NF-kappaB, a regulator of Bcl-2 expression, and calpain protease activity, a regulator of NF-kappaB, were both inhibited by RES. The patterns for NF-kappaB and calpain activities followed that of PI3K and were inhibited by LY294002. NF-kappaB inhibition coincided with diminished MMP-9 activity and cell migration. These data suggest that RES-induced apoptosis in MCF-7 could involve an oxidative, caspase-independent mechanism, whereby inhibition of PI3K signaling converges to Bcl-2 through NF-kappaB and calpain protease activity. Therefore, Bcl-2 and NF-kappaB could be considered potential targets for the chemopreventive activity of RES in estrogen-responsive tumor cells

Identification of a p53-dependent pathway in the induction of apoptosis of human breast cancer cells by the natural product, resveratrol.

J Altern Complement Med. 2004 Apr;10(2):235-9

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OBJECTIVE: Resveratrol, a constituent found in grapes and various other plants, has been shown to have chemo-preventive activity against cancer, and specifically demonstrated to induce apoptosis by p53-dependent pathways in murine cells. The goal of this research was to identify the role of p53-dependent or p53-independent pathways in the induction of apoptosis in human breast cancer cells by this natural product. DESIGN: A number of human breast cancer cell lines, as well as a control of a wild-type line (astrocytoma N 1321N1), were investigated for induction of apoptosis by resveratrol using both microscopic evaluation and DNA fragmentation assays. Concurrently, we established the p53 gene status (wild-type or mutant) of each cell line by Western blot using p53-specific antibody. RESULTS: Apoptosis induced by resveratrol was found to occur only in breast cancer cells expressing wild-type p53 but not in mutant p53-expressing cells. CONCLUSIONS: We therefore conclude that the natural product, resveratrol, induces apoptosis in breast cancer cells via p53-dependent pathways.

Effects of resveratrol on the expression of a panel of genes interacting with the BRCA1 oncosuppressor in human breast cell lines.

Clin Chim Acta. 2004 Jun;344(1-2):115-21

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BACKGROUND: trans-Resveratrol, or 3,5,4'trihydroxy-trans-stilbene, is a polyphenolic compound that seems to provide a protective effect against several types of cancer, notably breast cancer. Through its phytoestrogenic properties it regulates the expression of hormone-dependent genes, such as the oncosuppressor BRCA1, in breast cells. This gene is involved in the majority of hereditary breast cancer, as well as sporadic cancers. METHODS: We used three human breast tumor cell lines (HBL100, MCF7 and MBA-MB-231) and one breast cell line (MCF10a) derived from a fibrocystic disease to study in vitro the effect of resveratrol on the transcription of a group of genes whose proteins interact in different pathways with BRCA1. BRCA1, BRCA2, ER alpha, ER beta, p53, p21(waf1/cip1), CBP/P300, RAD51, pS2 and Ki67 mRNA were quantified using real-time quantitative RT-PCR with an ABI 7700 apparatus. RESULTS: Resveratrol modulated the expression of these genes in a pattern dependent on the status of alpha and beta estrogen receptors. These results show that resveratrol regulates gene expression via the estrogen receptor pathway and also an undetermined pathway. CONCLUSION: Thus, resveratrol seems to have an effect on breast tumor cell lines, on a fibrocystic cell line by affecting several factors regulating the function of BRCA1.

Sensitization for tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by the chemopreventive agent resveratrol.

Cancer Res. 2004 Jan 1;64(1):337-46.

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Survivin is a member of the inhibitor of apoptosis proteins that is expressed at high levels in most human cancers and may facilitate evasion from apoptosis and aberrant mitotic progression. Naturally occurring dietary compounds such as resveratrol have gained considerable attention as cancer chemopreventive agents. Here, we discovered a novel function of the chemopreventive agent resveratrol: resveratrol is a potent sensitizer of tumor cells for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis through p53-independent induction of p21 and p21-mediated cell cycle arrest associated with survivin depletion. Concomitant analysis of cell cycle, survivin expression, and apoptosis revealed that resveratrol-induced G(1) arrest was associated with down-regulation of survivin expression and sensitization for TRAIL-induced apoptosis. Accordingly, G(1) arrest using the cell cycle inhibitor mimosine or induced by p21 overexpression reduced survivin expression and sensitized cells for TRAIL treatment. Likewise, resveratrol-mediated cell cycle arrest followed by survivin depletion and sensitization for TRAIL was impaired in p21- deficient cells. Also, down-regulation of survivin using survivin antisense oligonucleotides sensitized cells for TRAIL-induced apoptosis. Importantly, resveratrol sensitized various tumor cell lines, but not normal human fibroblasts, for apoptosis induced by death receptor ligation or anticancer drugs. Thus, this combined sensitizer (resveratrol)/inducer (e.g., TRAIL) strategy may be a novel approach to enhance the efficacy of TRAIL-based therapies in a variety of human cancers.

Resveratrol inhibits cell proliferation and induces apoptosis of human breast carcinoma MCF-7 cells.

Oncol Rep. 2004 Feb;11(2):441-6

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Resveratrol, which is found in grapes and wine, has been reported to have a variety of important pharmacological effects including anti-inflammatory, anti-platelet, and anti-carcinogenetic properties. In this study, using the human breast cancer cell line MCF-7, we have analyzed a possible mechanism by which resveratrol could interfere with cell cycle control and induce cell death. Resveratrol treatment of MCF-7 cells resulted in a dose-dependent inhibition of the cell growth and the cells accumulated at the S phase transition of the cell cycle at low concentrations, but high concentrations do not induce S phase accumulation. The anti-proliferative effects of resveratrol were associated with a marked inhibition of cyclin D and cyclin-dependent kinase (Cdk) 4 proteins, and induction of p53 and Cdk inhibitor p21WAF1/CIP. Growth suppression by resveratrol was also due to apoptosis, as seen by the appearance of a sub-G1 fraction and chromatin condensation. In addition, the apoptotic process involves activation of caspase-9, a decrease of Bcl-2 as well as Bcl-XL levels, and an increase of Bax levels.

Resveratrol induces growth inhibition and apoptosis in metastatic breast cancer cells via de novo ceramide signaling.

FASEB J. 2003 Dec;17(15):2339-41. Epub 2003 Oct 16

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Resveratrol (3,4',5-trans-trihydroxystilbene), a phytoalexin present in grapes and red wine, is emerging as a natural compound with potential anticancer properties. Here we show that resveratrol can induce growth inhibition and apoptosis in MDA-MB-231, a highly invasive and metastatic breast cancer cell line, in concomitance with a dramatic endogenous increase of growth inhibitory/proapoptotic ceramide. We found that accumulation of ceramide derives from both de novo ceramide synthesis and sphingomyelin hydrolysis. More specifically we demonstrated that ceramide accumulation induced by resveratrol can be traced to the activation of serine palmitoyltransferase (SPT), the key enzyme of de novo ceramide biosynthetic pathway, and neutral sphingomyelinase (nSMase), a main enzyme involved in the sphingomyelin/ceramide pathway. However, by using specific inhibitors of SPT, myriocin and L-cycloserine, and nSMase, gluthatione and manumycin, we found that only the SPT

inhibitors could counteract the biological effects induced by resveratrol. Thus, resveratrol seems to exert its growth inhibitory/apoptotic effect on the metastatic breast cancer cell line MDA-MB-231 by activating the de novo ceramide synthesis pathway.

Suppression of 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in rats by resveratrol: role of nuclear factor-kappaB, cyclooxygenase 2, and matrix metalloprotease 9.

Cancer Res. 2002 Sep 1;62(17):4945-54

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We have reported recently that resveratrol (trans-3,4',5-trihydroxystilbene), a polyphenolic phytoalexin found in grapes, fruits, and root extracts of the weed Polygonum cuspidatum, is a potent inhibitor of nuclear factor (NF)-kappaB activation. Because NF-kappaB suppression has been linked with chemoprevention, this prompted us to investigate the chemopreventive potential of resveratrol by testing it against mammary carcinogenesis induced by 7,12-dimethylbenz(a)anthracene (DMBA) in female Sprague Dawley rats. Dietary administration of resveratrol (10 ppm) had no effect on body weight gain and tumor volume but produced striking reductions in the incidence (45%; $P < 0.05$), multiplicity (55%; $P < 0.001$), and extended latency period of tumor development relative to DMBA-treated animals. Histopathological analysis of the tumors revealed that DMBA induced ductal carcinomas and focal microinvasion in situ (7 of 7), whereas treatment with resveratrol suppressed DMBA-induced ductal carcinoma. Immunohistochemistry and Western blot analysis revealed that resveratrol suppressed the DMBA-induced cyclooxygenase-2 and matrix metalloprotease-9 expression in the breast tumor. Gel shift analysis showed suppression of DMBA-induced NF-kappaB activation by resveratrol. Treatment of human breast cancer MCF-7 cells with resveratrol also suppressed the NF-kappaB activation and inhibited proliferation at S-G(2)-M phase. Overall, our results suggest that resveratrol suppresses DMBA-induced mammary carcinogenesis, which correlates with down-regulation of NF-kappaB, cyclooxygenase-2, and matrix metalloprotease-9 expression.

Effect of resveratrol on growth of 4T1 breast cancer cells in vitro and in vivo.

Biochem Biophys Res Commun. 2002 Mar 8;291(4):1001-5.

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In vitro, resveratrol inhibited growth of 4T1 breast cancer cells in a dose- and time-dependent manner. In vivo, however, resveratrol had no effect on time to tumor take, tumor growth, or metastasis when administered intraperitoneally daily (1, 3, or 5 mg/kg) for 23 days starting at the time of tumor inoculation. Resveratrol had no effect on body weight, organ histology, or estrous cycling of the tumor-bearing mice. Resveratrol, therefore, is a potent inhibitor of 4T1 breast cancer cells in vitro; is nontoxic to mice at 1-5 mg/kg; and has no growth-inhibitory effect on 4T1 breast cancer in vivo.

Effect of resveratrol on the expression of autocrine growth modulators in human breast cancer cells.

Antioxid Redox Signal. 2001 Dec;3(6):969-79

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The effect of resveratrol on the growth of human breast cancer cells was examined. Resveratrol inhibited the growth of estrogen receptor-positive MCF-7 cells cultivated in the presence of estradiol in a dose-dependent fashion. At 10(-5) M, resveratrol maximally inhibited the growth stimulatory effect mediated by 10(-9) M estradiol without affecting cell viability. At the molecular level, resveratrol in a dose-dependent fashion antagonized the stimulation by estradiol of an estrogen response element reporter gene construct and of progesterone receptor gene expression in MCF-7 cells. Resveratrol also inhibited the proliferation of the estrogen-receptor negative human breast carcinoma cell line MDA-MB-468. These later data suggest that resveratrol can also inhibit breast cancer cell proliferation by another mechanism besides estrogen receptor antagonism. We show here that resveratrol altered the expression of several autocrine growth modulators and their receptors in MCF-7 cells. Resveratrol at 10(-5) M inhibited the expression of the autocrine growth stimulators transforming growth factor-alpha (TGF-alpha), PC cell-

derived growth factor, and insulin-like growth factor I receptor mRNA. In addition, resveratrol significantly elevated the expression of the growth inhibitor TGF-beta2 mRNA without changes in TGF-beta1 and TGF-beta3 expression. These data suggest that resveratrol inhibits proliferation by altering autocrine growth modulator pathways in breast cancer cells.

Estrogenic and antiestrogenic properties of resveratrol in mammary tumor models.

Cancer Res. 2001 Oct 15;61(20):7456-63

Bhat KP, **Lantvit D**, **Christov K**, **Mehta RG**, **Moon RC**, **Pezzuto JM**.

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Trans-3,4',5-trihydroxystilbene (resveratrol), a phytoalexin present in grapes and grape products such as wine, has been identified as a chemopreventive agent. Recent studies performed with MCF-7 human breast cancer cells have demonstrated superestrogenic effects with resveratrol. In contrast, studies performed using estrogen receptor-transfected cell lines have shown that resveratrol acts as a mixed agonist/antagonist. The major objective of this study was to characterize the estrogen-modulatory effects of resveratrol in a variety of in vitro and in vivo mammary models. Thus, the effect of resveratrol alone and in combination with 17beta-estradiol (E2) was assessed with MCF-7, T47D, LY2, and S30 mammary cancer cell lines. With cells transfected with reporter gene systems, the activation of estrogen response element-luciferase was studied, and using Western blot analysis, the expression of E2-responsive progesterone receptor (PR) and presnelin 2 protein was monitored. Furthermore, the effect of resveratrol on formation of preneoplastic lesions (induced by 7,12-dimethylbenz(a)anthracene) and PR expression (with or without E2) was evaluated with mammary glands of BALB/c mice placed in organ culture. Finally, the effect of p.o. administered resveratrol on N-methyl-N-nitrosourea-induced mammary tumors was studied in female Sprague Dawley rats. As a result, in transient transfection studies with MCF-7 cells, resveratrol showed a weak estrogenic response, but when resveratrol was combined with E2 (1 nM), a clear dose-dependent antagonism was observed. Similar mixed estrogenic/antiestrogenic effects were noted with S30 cells, whereas resveratrol functioned as a pure estrogen antagonist with T47D and LY2 cells. Furthermore, in MCF-7 cells, resveratrol induced PR protein expression, but when resveratrol was combined with E2, expression of PR was suppressed. With T47D cells, resveratrol significantly down-regulated steady-state and E2-induced protein levels of PR. With LY2 and S30 cells, resveratrol down-regulated

presnelin 2 protein expression. Using the mouse mammary organ culture model, resveratrol induced PR when administered alone, but expression was suppressed in the presence of E2 (1 nM). Furthermore, resveratrol inhibited the formation of estrogen-dependent preneoplastic ductal lesions induced by 7,12-dimethylbenz(a)anthracene in these mammary glands (IC50 = 3.2 microM) and reduced N-methyl-N-nitrosourea-induced mammary tumorigenesis when administered to female Sprague Dawley rats by gavage. Therefore, in the absence of E2, resveratrol exerts mixed estrogen agonist/antagonist activities in some mammary cancer cell lines, but in the presence of E2, resveratrol functions as an antiestrogen. In rodent models, carcinogen-induced preneoplastic lesions and mammary tumors are inhibited. These data suggest that resveratrol may have beneficial effects if used as a chemopreventive agent for breast cancer.

Cell cycle effects and control of gene expression by resveratrol in human breast carcinoma cell lines with different metastatic potentials.

Int J Oncol. 1999 Aug;15(2):245-52

Hsieh TC, **Burfeind P**, **Laud K**, **Backer JM**, **Traganos F**, **Darzynkiewicz Z**, **Wu JM**.

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Trans-resveratrol, a polyphenol present in red wines and various human foods, is an antioxidant also with reported chemopreventive properties. However, whether resveratrol may exert different effects in malignant cells with a common anatomical origin yet displaying different invasive characteristics is not known. Since invasiveness and metastasis are considered to be the most insidious and life-threatening aspects for all cancers, we compared the ability of resveratrol to control growth and cell cycle transition in the highly invasive MDA-MB-435 with the minimally invasive MCF-7 breast carcinoma cells. The data revealed that resveratrol exerted a greater inhibitory effect on the MDA-MB-435 cells. A diminution of percentage of cells in G1 phase and a corresponding accumulation of cells in S phase of the cell cycle was observed. We also studied the effect of resveratrol on a panel of MDA-MB-435 cells transfected with nm23-H1 and nm23-H2 genes, which have been suggested to play a role in controlling metastasis in breast cancer cells. These cells are designated as Vbeta, 1beta, 1Tbeta, 2beta, and 2Tbeta, respectively. The control Vbeta consists of MDA-MB-435 cells transfected with bacterial beta-glucuronidase. Cells labeled 1beta and 1Tbeta correspond to those carrying beta-glucuronidase and overexpressed wild-type (His118) or mutant (Tyr118, catalytically inactive) nm23-H1 genes. The 2beta and 2Tbeta refer to cells

transfected with wild-type and mutant nm23-H2 genes. The responses of these cells to resveratrol were assessed by measuring proliferation, cell cycle phase distribution, and changes in expression of several genes. These studies have shown that resveratrol (25 microM, 3 days) reduced growth of all cell types by 60-80%. Overexpression of both wild-type and catalytically inactive nm23-H1 (1beta, 1Tbeta) but not nm23-H2 (2beta, 2Tbeta) reduced the proportion of cells in G1 phase, compared to the Vbeta control cells. Little changes in expression of PCNA, Rb, p53, and bcl-2 were observed in the five cell types treated with resveratrol, compared to untreated cells. Noted exceptions included reduced expression of Rb protein and increased expression of p53 in 2beta and 2Tbeta cells, and increased expression of bcl-2 in 2beta cells, treated with resveratrol. In contrast, resveratrol upregulated expression of cathepsin D by 50-100% in all cell lines except 1beta. These results suggest that the intrinsic metastatic potential of cancer cells may affect their responses to chemopreventive agents such as resveratrol.

Resveratrol, a natural product derived from grape, exhibits antiestrogenic activity and inhibits the growth of human breast cancer cells.

J Cell Physiol. 1999 Jun;179(3):297-304.

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Resveratrol is a natural phytoalexin compound found in grapes and other food products. In this study, the effect of resveratrol on the growth of human breast cancer cells was examined. Results show that resveratrol inhibits the growth of estrogen receptor(ER)-positive MCF-7 cells in a dose-dependent fashion. Detailed studies with MCF-7 cells demonstrate that resveratrol antagonized the growth-promoting effect of 17-beta-estradiol (E2) in a dose-dependent fashion at both the cellular (cell growth) and the molecular (gene activation) levels. At 5 x 10(-6) M, resveratrol abolished the growth-stimulatory effect mediated by concentrations of E2 up to 10(-9) M. The antiestrogenic effect of resveratrol could be observed at a concentration of 10(-6) M and above. The antiestrogenic effect of resveratrol was also demonstrated at the molecular level. Resveratrol in a dose-dependent fashion antagonized the stimulation by E2 of progesterone receptor gene expression in MCF-7 cells. Moreover, expression of transforming growth factor-alpha and insulin-like growth factor I receptor mRNA was inhibited while the expression of transforming growth factor beta2 mRNA was significantly elevated in MCF-7 cells cultivated in the presence of resveratrol (10(-5) M). In summary, our results show that resveratrol, a partial ER agonist itself, acts as an ER antagonist in the presence of estrogen leading to inhibition of human breast cancer cells.

Resveratrol, a natural product derived from grape, exhibits antiestrogenic activity and inhibits the growth of human breast cancer cells.

J Cell Physiol. 1999 Jun;179(3):297-304

Lu R, **Serrero G**.

Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore 21201, USA.

Resveratrol is a natural phytoalexin compound found in grapes and other food products. In this study, the effect of resveratrol on the growth of human breast cancer cells was examined. Results show that resveratrol inhibits the growth of estrogen receptor(ER)-positive MCF-7 cells in a dose-dependent fashion. Detailed studies with MCF-7 cells demonstrate that resveratrol antagonized the growth-promoting effect of 17-beta-estradiol (E2) in a dose-dependent fashion at both the cellular (cell growth) and the molecular (gene activation) levels. At 5 x 10(-6) M, resveratrol abolished the growth-stimulatory effect mediated by concentrations of E2 up to 10(-9) M. The antiestrogenic effect of resveratrol could be observed at a concentration of 10(-6) M and above. The antiestrogenic effect of resveratrol was also demonstrated at the molecular level. Resveratrol in a dose-dependent fashion antagonized the stimulation by E2 of progesterone receptor gene expression in MCF-7 cells. Moreover, expression of

transforming growth factor-alpha and insulin-like growth factor I receptor mRNA was inhibited while the expression of transforming growth factor beta2 mRNA was significantly elevated in MCF-7 cells cultivated in the presence of resveratrol (10(-5) M). In summary, our results show that resveratrol, a partial ER agonist itself, acts as an ER antagonist in the presence of estrogen leading to inhibition of human breast cancer cells.

Antiproliferative effect of synthetic resveratrol on human breast epithelial cells.

Int J Oncol. 1998 Apr;12(4):865-9

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Resveratrol (3,5,4'-trihydroxy-trans-stilbene), a phytoalexin, is a constituent of the human diet that has been shown to inhibit cellular processes associated with tumor initiation, promotion and progression. In this study, we examined the effect of synthetic resveratrol on the proliferative capacity of immortal and neoplastic human breast epithelial cells in culture. MCF-7, an estrogen receptor-positive breast cancer cell line, MCF-10F, an immortal estrogen receptor-negative breast epithelial cell line, and MDA-MB-231, a malignant estrogen receptor-negative breast epithelial cell line, were treated with 5, 10, 20 or 40 microg/ml resveratrol, and their proliferative activities were determined with the WST-1 colorimetric assay after periods of time ranging from 24 to 144 h of treatment. Our results showed that this phytoalexin inhibited the proliferation of human breast epithelial cells in a dose- and time-dependent manner. Treatment of cells with resveratrol reduced the number of viable cells and prevented the exponential growth of the three cell lines examined. These observations indicate that resveratrol has a direct antiproliferative effect on human breast epithelial cells that is independent of the estrogen receptor status of the cells. Thus, this dietary compound is a potential chemopreventive agent for both hormone responsive and non-responsive breast cancers.

Resveratrol and Cancer

Mechanism-based in vitro screening of potential cancer chemopreventive agents.

Mutat Res. 2003 Feb-Mar;523-524:163-72

Gerhäuser C, Klimo K, Heiss E, Neumann I, Gamal-Eldeen A, Knauft J, Liu GY, Sitthimonchai S, Frank N.

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Identification and use of effective cancer chemopreventive agents have become an important issue in public health-related research. For identification of potential cancer chemopreventive constituents we have set up a battery of cell- and enzyme-based in vitro marker systems relevant for prevention of carcinogenesis in vivo. These systems include modulation of drug metabolism (inhibition of Cyp1A activity, induction of NAD(P)H:quinone reductase (QR) activity in Hepa1c1c7 murine hepatoma cell culture), determination of radical scavenging (DPPH scavenging) and antioxidant effects (scavenging of superoxide anion-, hydroxyl- and peroxyl-radicals), anti-inflammatory mechanisms (inhibition of lipopolysaccharide (LPS)-mediated nitric oxide (NO) generation by inducible NO synthase (iNOS) in Raw 264.7 murine macrophages, cyclooxygenase-1 (Cox-1) inhibition), and anti-tumor promoting activities (inhibition of phorbol ester-induced ornithine decarboxylase (ODC) activity in 308 murine keratinocytes). We have tested a series of known chemopreventive substances belonging to several structural classes as reference compounds for the identification of novel chemopreventive agents or mechanisms. These include organosulfur compounds (phenethylisothiocyanate (PEITC), diallylsulfide, diallyldisulfide), terpenes (limonene, perillyl alcohol, oleanolic acid, 18-beta-glycyrrhetinic acid), short-chain fatty acids (sodium butyrate), indoles (indole-3-carbinol), isoflavonoids (quercetin, silymarin, genistein), catechins ((-)-epigallocatechin gallate (EGCG)), simple phenols (ellagic acid, resveratrol, piceatannol, curcumin), pharmaceutical agents (piroxicam, acetylsalicylic acid, tamoxifen), and vitamins/derivatives (ascorbic acid, Trolox). We confirmed known chemopreventive mechanisms of these compounds. Additionally, we could demonstrate the usefulness of our approach by identification of hitherto unknown mechanisms of selected agents. As an example, we detected anti-inflammatory properties of PEITC, based on NF-kappaB-mediated inhibition of NO production. Further, PEITC inhibited phorbol ester-induced superoxide anion radical production in granulocytes, and ODC induction in the 308 cell line. These mechanisms might contribute to the chemopreventive potential of PEITC.

Resveratrol inhibits the growth and induces the apoptosis of both normal and leukemic hematopoietic cells.

Carcinogenesis. 2002 Aug;23(8):1327-33

Ferry-Dumazet H, **Garnier O**, **Mamani-Matsuda M**, **Vercauteren J**, **Belloc F**, **Billiard C**, **Dupouy M**, **Thiolat D**, **Kolb JP**, **Marit G**, **Reiffers J**, **Mossalayi MD**.

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It is often postulated that trans-3,4',5-trihydroxystilbene (resveratrol, RES) exhibits cell growth regulatory and chemopreventive activities. However, mechanisms by which this polyphenol inhibits tumor cell growth, and its therapeutic potential are poorly understood. Using various human leukemia cells, we have first defined the anti-tumoral doses of this compound. RES inhibited the proliferation and induced the apoptosis of all tested lymphoid and myeloid leukemia cells with IC(50) = 5-43 microM. Prior to apoptosis, RES-induced caspase activity in a dose-dependent manner and cell cycle arrest in G(2)/M-phase, correlating with a significant accumulation of cyclins A and B. Leukemia cell death with RES required both caspase-dependent and -independent proteases, as it was significantly inhibited by simultaneous addition of Z-VAD-FMK and leupeptin to these cultures. While RES did not affect non-activated normal lymphocytes, this agent decreased the growth and induced the apoptosis of cycling normal human peripheral blood lymphocytes at lower concentrations (IC(50) <8 microM) than those required for most leukemia cells. RES also induced the apoptosis of early normal human CD34(+) cells and decreased the number of colonies generated by these precursor cells in a dose-dependent manner (IC(50) = 60 microM). Together, the data point to the complexity of RES-mediated signaling pathways and revealed the high anti-proliferative and proapoptotic activities of RES in normal cycling hemopoietic cells.

Resveratrol induces growth inhibition, S-phase arrest, apoptosis, and changes in biomarker expression in several human cancer cell lines.

Clin Cancer Res. 2002 Mar;8(3):893-903

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PURPOSE: We examined the effects of the phytochemical resveratrol in six human cancer cell lines (MCF7, SW480, HCE7, Seg-1, Bic-1, and HL60). EXPERIMENTAL DESIGN AND RESULTS: Resveratrol induced marked growth inhibition in five of these cell lines, with IC(50) values of approximately 70-150 microM. However, only partial growth inhibition was seen in Bic-1 cells. After treatment with 300 microM resveratrol for 24 h, most of the cell lines were arrested in the S phase of the cell cycle. In addition, induction of apoptosis was demonstrated by the appearance of a sub-G(1) peak and confirmed using an annexin V-based assay. Cyclin B1 expression levels were decreased in all cell lines after 48 h of treatment. In SW480 cells, cyclin A, cyclin B1, and beta-catenin expression levels were decreased within 24 h. There was a decrease in cyclin D1 expression after only 2 h of treatment, and this persisted for up to 48 h. This decrease was partially blocked by concurrent treatment with the proteasome inhibitor calpain inhibitor I. Using a luciferase-based reporter assay, resveratrol did not inhibit cyclin D1 promoter activity in SW480 cells. Furthermore, using a reverse transcription-PCR-based assay, only a higher dose of resveratrol (300 microM) appeared to decrease cyclin D1 mRNA. Seg-1 cells expressed basal levels of cyclooxygenase-2 (cox-2), which was further induced by resveratrol. Neither basal levels nor induction of cox-2 was detectable in the remaining cell lines. Thus, cox-2 does not appear to be a critical target of this compound. CONCLUSIONS: These studies provide support for the use of resveratrol in chemoprevention and cancer therapy trials. Cyclin D1, cyclin B1, beta-catenin, and apoptotic index could be useful biomarkers to evaluate treatment efficacy.

Dose-dependent effect of resveratrol on proliferation and apoptosis in endothelial and tumor cell cultures.

Exp Mol Med. 2000 Jun 30;32(2):88-92

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Experimental data suggest that Resveratrol, a compound found in grapes and other fruits may influence cell proliferation and apoptosis. The aim of our experiments was to study the effect of Resveratrol on tumor cell cultures and an endothelial cell culture in order to examine the effect of various doses of this compound on active cell death and cell proliferation. Human tumor (HT-29, SW-620, HT-1080) and endothelial (HUV-EC-C) cells were treated with various doses of (0.1 to 100.0 microg/ml) Resveratrol in vitro. Cell number, apoptotic and mitotic index was measured 24, 48 and 72 h after treatment. Low doses (0.1-1.0 microg/ml) of Resveratrol enhance cell proliferation, higher doses (10.0-100.0 microg/ml) induce apoptosis and decrease mitotic activity, which is reflected in changes of cell number. Resveratrol influences dose dependently the proliferative and

apoptotic activity of human tumor and endothelial cells. The possible role of formaldehyde in the mechanism of action of Resveratrol is discussed.

Resveratrol-induced G2 arrest through the inhibition of CDK7 and p34CDC2 kinases in colon carcinoma HT29 cells.

Biochem Pharmacol. 2003 Apr 1;65(7):1053-60.

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Resveratrol (3,5,4'-trihydroxystilbene), a phytoalexin found in grapes and other food products, has been shown to have cancer chemopreventive activity. However, the mechanism of the anti-carcinogenic activity is not well understood. Here, we offer a possible explanation of its anti-tumor effect. Based on flow cytometric analysis, resveratrol inhibited the proliferation of HT29 colon cancer cells and resulted in their accumulation in the G(2) phase of the cell cycle. Western blot analysis and kinase assays demonstrated that the perturbation of G(2) phase progression by resveratrol was accompanied by the inactivation of p34(CDC2) protein kinase, and an increase in the tyrosine phosphorylated (inactive) form of p34(CDC2). Kinase assays revealed that the reduction of p34(CDC2) activity by resveratrol was mediated through the inhibition of CDK7 kinase activity, while CDC25A phosphatase activity was not affected. In addition, resveratrol-treated cells were shown to have a low level of CDK7 kinase-Thr(161)-phosphorylated p34(CDC2). These results demonstrated that resveratrol induced cell cycle arrest at the G(2) phase through the inhibition of CDK7 kinase activity, suggesting that its anti-tumor activity might occur through the disruption of cell division at the G(2)/M phase.

Resveratrol-induced apoptosis is associated with Fas redistribution in the rafts and the formation of a death-inducing signaling complex in colon cancer cells.

J Biol Chem. 2003 Oct 17;278(42):41482-90. Epub 2003 Aug 5.

Delmas D, **Rébé C**, **Lacour S**, **Filomenko R**, **Athias A**, **Gambert P**, **Cherkaoui-Malki M**, **Jannin B**, **Dubrez-Daloz L**, **Latruffe N**, **Solary E**.

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Resveratrol, a polyphenol found in grape skin and various other food products, may function as a cancer chemopreventive agent for colon and other malignant tumors and possesses a chemotherapeutic potential through its ability to trigger apoptosis in tumor cells. The present study analyses the molecular mechanisms of resveratrol-induced apoptosis in colon cancer cells, with special attention to the role of the death receptor Fas in this pathway. We show that, in the 10-100 microm range of concentrations, resveratrol activates various caspases and triggers apoptosis in SW480 human colon cancer cells. Caspase activation is associated with accumulation of the pro-apoptotic proteins Bax and Bak that undergo conformational changes and relocalization to the mitochondria. Resveratrol does not modulate the expression of Fas and Fas-ligand (FasL) at the surface of cancer cells, and inhibition of the Fas/FasL interaction does not influence the apoptotic response to the molecule. Resveratrol induces the clustering of Fas and its redistribution in cholesterol and sphingolipid-rich fractions of SW480 cells, together with FADD and procaspase-8. This redistribution is associated with the formation of a death-inducing signaling complex (DISC). Transient transfection of either a dominant-negative mutant of FADD, E8, or MC159 viral proteins that interfere with the DISC function, decreases the apoptotic response of SW480 cells to resveratrol and partially prevents resveratrol-induced Bax and Bak conformational changes. Altogether, these results indicate that the ability of resveratrol to induce the redistribution of Fas receptor in membrane rafts may contribute to the molecule's ability to trigger apoptosis in colon cancer cells.

Involvement of HSP70 in resveratrol-induced apoptosis of human prostate cancer.

Anticancer Res. 2003 Nov-Dec;23(6C):4921-6

Cardile V, **Scifo C**, **Russo A**, **Falsaperla M**, **Morgia G**, **Motta M**, **Renis M**, **Imbriani E**, **Silvestre G**.

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BACKGROUND: 70 KDa heat shock proteins (HSPs70), either as a constitutive or inducible form, are expressed at very high levels in malignant human tumors of various origin. In different cell types, they are known to play an antiapoptotic role. Resveratrol (3,5,4'-trihydroxy-trans-stilbene), a polyphenol present in red wine, grapes and other dietary and medicinal plants, has been shown to be active in inhibiting multistage carcinogenesis, inducing apoptotic cell death. MATERIALS AND METHODS: With the present study, a possible relationship between HSP70 expression and cell death elicited

by resveratrol in DU-145 cells, which mimic the late hormone-refractory stages of prostate carcinoma, was investigated. To this end, we treated DU-145 with different concentrations. (50, 100 and 200 microM) of resveratrol and cell viability, by tetrazolium salts assay (MTT) and membrane breakdown, by lactic dehydrogenase (LDH) release, were measured. The possible induction of oxidative stress was evidenced both by performing a fluorescent analysis of intracellular reactive oxygen species (ROS) production, or evaluating the amount of nitrite/nitrate (NO) in culture medium. In addition, the expression of HSP70 level, evaluated by immunoblotting, was examined and compared with caspase-3 activity (fluorimetrically measured) and DNA damage, determined by Single Cell Gel Electrophoresis or COMET assay. RESULTS: Our data clearly indicate that the addition of resveratrol to DU-145 reduces cell viability and increases membrane breakdown, in a dose-dependent way, without interfering with ROS production or NO synthesis, unless 200 microM resveratrol was added. Furthermore, at low concentration (50-100 microM) resveratrol is able to raise HSP70 levels but, at high concentration (200 microM), the measured levels of protective HSP70 were unmodified with respect to that of the control values. CONCLUSION: Our results confirm the ability of resveratrol to suppress the proliferation of human prostate cancer cells with a typical apoptotic feature, interfering with the expression of HSPs70.

Involvement of p21WAF1/CIP1, pRB, Bax and NF-kappaB in induction of growth arrest and apoptosis by resveratrol in human lung carcinoma A549 cells.

Int J Oncol. 2003 Oct;23(4):1143-9

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Resveratrol, a polyphenolic phytoalexin found in grapes, may have the potential for prevention and therapy for human cancer. We report here that resveratrol inhibits the growth of human lung carcinoma A549 cells and provides molecular understanding of this effect. Resveratrol treatment of A549 cells resulted in a concentration-dependent induction of S phase arrest in cell cycle progression. This anti-proliferative effect of resveratrol was associated with a marked inhibition of the phosphorylation of the retinoblastoma protein (pRB) and concomitant induction of cyclin-dependent kinase (Cdk) inhibitor p21WAF1/CIP, which appears to be transcriptionally upregulated and is p53- dependent. In addition, resveratrol treatment resulted in induction of apoptosis as determined by fluorescence microscopy and flow cytometric analysis. These effects were found to correlate with an activation of caspase-3 and a shift in Bax/Bcl-xL ratio more

towards apoptosis. Resveratrol treatment also inhibited the transcriptional activity of nuclear transcription factor kappaB (NF-kappaB). Taken together, these findings suggest that resveratrol has strong potential for development as an agent for prevention against human lung cancer.

Phytoestrogens in common herbs regulate prostate cancer cell growth in vitro.

Nutr Cancer. 2004;49(2):200-8

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Prostate cancer is an important public health problem in the United States. Seven phytoestrogens found in common herbal products were screened for estrogen receptor binding and growth inhibition of androgen-insensitive (PC-3) and androgen-sensitive (LNCaP) human prostate tumor cells. In a competitive 3H-estradiol ligand binding assay using mouse uterine cytosol, 2.5 M quercetin, baicalein, genistein, epigallocatechin gallate (EGCG), and curcumin displaced > 85% of estradiol binding, whereas apigenin and resveratrol displaced > 40%. From growth inhibition studies in LNCaP cells, apigenin and curcumin were the most potent inhibitors of cell growth, and EGCG and baicalein were the least potent. In PC-3 cells, curcumin was the most potent inhibitor of cell growth, and EGCG was the least potent. In both cell lines, significant arrest of the cell cycle in S phase was induced by resveratrol and EGCG and in G2M phase by quercetin, baicalein, apigenin, genistein, and curcumin. Induction of apoptosis was induced by all of the 7 compounds in the 2 cell lines as shown by TUNEL and DNA fragmentation assays. Androgen responsiveness of the cell lines did not correlate with cellular response to the phytoestrogens. In conclusion, these 7 phytoestrogens, through different mechanisms, are effective inhibitors of prostate tumor cell growth.

Combined effects of resveratrol and paclitaxel on lung cancer cells.

Anticancer Res. 2003 Sep-Oct;23(5A):4039-46.

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Resveratrol (3,4',5-trihydroxystilbene) is a phytoalexin found in grapes and other food products that can prevent cancer. We studied the in vitro biological activity of this compound by examining its effect on proliferation and inducing apoptosis in three lung cancer cell lines (A549, EBC-1, Lu65). Resveratrol inhibited the growth of A549, EBC-1 and Lu65 lung cancer cells by 50% (ED50) at concentrations between 5-10 microM. We also examined the combined effects in these cells of resveratrol and paclitaxel, an essential chemotherapeutic agent against lung cancer. Although simultaneous exposure to resveratrol plus paclitaxel did not result in significant synergy, resveratrol (10 microM, 3 days) significantly enhanced the subsequent antiproliferative effect of paclitaxel. In addition, resveratrol as well as paclitaxel induced apoptosis in EBC-1 and Lu65 cells, as measured by TUNEL and caspase assays, as well as flow cytometry. Resveratrol (10 microM, 3 days) similarly enhanced the subsequent apoptotic effects of paclitaxel. We examined the effects of resveratrol and paclitaxel on levels of p21waf1, p27kip1, E-cadherin, EGFR and Bcl-2 in EBC-1 cells. Resveratrol (10 microM, 3 days) prior to paclitaxel induced p21waf1 expression approximately 4-fold. These results suggest that resveratrol may be a promising alternative therapy for lung cancer and that lung cancer cells exposed to resveratrol have a lowered threshold for killing by paclitaxel.

Inhibitory effect of resveratrol on the proliferation of human and rat hepatic derived cell lines.

FASEB J. 2003 Dec;17(15):2339-41. Epub 2003 Oct 16

Delmas D, **Jannin B**, **Cherkaoui Malki M**, **Latruffe N**.

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Resveratrol is a polyphenolic compound especially produced by grapevine and consequently found in wine. Based on epidemiological studies resveratrol may act as a cancer chemopreventive compound. The ability of resveratrol to inhibit cell proliferation was studied in rat hepatoma Fao cell line and human hepatoblastoma HepG2 cell line. The results show that resveratrol strongly inhibits cell proliferation at the micromolar range in a time- and dose-dependent manner. Concentrations higher than 50 microM become toxic. Fao cells are more sensitive than HepG2 cells. Interestingly, the presence of ethanol lowers the threshold of resveratrol effect. Resveratrol appears to prevent or to delay the entry to mitosis since no inhibition of [3H]thymidine incorporation is observed, while there is an increase of cell number in S and G2/M phases. In conclusion, resveratrol

shows a strong inhibition of hepatic cell proliferation where alcohol may act as an enhancing agent.

Resveratrol blocks interleukin-1beta-induced activation of the nuclear transcription factor NF-kappaB, inhibits proliferation, causes S-phase arrest, and induces apoptosis of acute myeloid leukemia cells.

Blood. 2003 Aug 1;102(3):987-95. Epub 2003 Apr 10

Estrov Z, **Shishodia S**, **Faderl S**, **Harris D**, **Van Q**, **Kantarjian HM**, **Talpaz M**, **Aggarwal BB**.

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Resveratrol, an edible polyphenolic stilbene, has been reported to possess substantial antileukemic activities in different leukemia cell lines. We investigated whether resveratrol is active against fresh acute myeloid leukemia (AML) cells and its mechanism of action. Because interleukin 1beta(IL-1beta) plays a key role in proliferation of AML cells, we first tested the effect of resveratrol on the AML cell lines OCIM2 and OCI/AML3, both of which produce IL-1beta and proliferate in response to it. Resveratrol inhibited proliferation of both cell lines in a dose-dependent fashion (5-75 microM) by arresting the cells at S phase, thus preventing their progression through the cell cycle; IL-1beta partially reversed this inhibitory effect. Resveratrol significantly reduced production of IL-1beta in OCIM2 cells. It also suppressed the IL-1beta-induced activation of transcription factor nuclear factor kappaB (NF-kappaB), which modulates an array of signals controlling cellular survival, proliferation, and cytokine production. Indeed, incubation of OCIM2 cells with resveratrol resulted in apoptotic cell death. Because caspase inhibitors Ac-DEVD-CHO or z-DEVD-FMK partially reversed the antiproliferative effect of resveratrol, we tested its effect on the caspase pathway and found that resveratrol induced the activation of the cysteine protease caspase 3 and subsequent cleavage of the DNA repair enzyme poly (adenosine diphosphate [ADP]-ribose) polymerase. Finally, resveratrol suppressed colony-forming cell proliferation of fresh AML marrow cells from 5 patients with newly diagnosed AML in a dose-dependent fashion. Taken together, our data showing that resveratrol is an effective in vitro inhibitor of AML cells suggest that this compound may have a role in future therapies for AML.

Inhibitory effect of resveratrol on the proliferation of human and rat hepatic derived cell lines.

Oncol Rep. 2000 Jul-Aug;7(4):847-52

Delmas D, **Jannin B**, **Cherkaoui Malki M**, **Latruffe N**.

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Resveratrol is a polyphenolic compound especially produced by grapevine and consequently found in wine. Based on epidemiological studies resveratrol may act as a cancer chemopreventive compound. The ability of resveratrol to inhibit cell proliferation was studied in rat hepatoma Fao cell line and human hepatoblastoma HepG2 cell line. The results show that resveratrol strongly inhibits cell proliferation at the micromolar range in a time- and dose-dependent manner. Concentrations higher than 50 microM become toxic. Fao cells are more sensitive than HepG2 cells. Interestingly, the presence of ethanol lowers the threshold of resveratrol effect. Resveratrol appears to prevent or to delay the entry to mitosis since no inhibition of [3H]thymidine incorporation is observed, while there is an increase of cell number in S and G2/M phases. In conclusion, resveratrol shows a strong inhibition of hepatic cell proliferation where alcohol may act as an enhancing agent.

Anti-hepatoma activity of resveratrol in vitro.

World J Gastroenterol. 2002 Feb;8(1):79-81

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AIM: To study the anti-tumor effect of resveratrol alone and the synergistic effects of resveratrol with 5-FU on the growth of H22 cells line in vitro. METHODS: The number of cells was measured by MTT method the morphological changes of H22 cells were investigated under microscopy and electron microscopy examination. RESULTS: Resveratrol inhibited the growth of hepatoma cells line H22 in a dose- and time-dependent manner, IC50 of the resveratrol on H22 cells was 6.57mg x L(-1),The synergistic anti-tumor effects of resveratrol with 5-FU increased to a greater extent than for H22 cells treated with 5-FU alone (70.2% vs 28.4%) $P<0.05$ .Under microscope and electron microscope, characteristics of apoptosis such as typical apoptotic bodies were commonly found in tumor cells in the drug-treated groups. CONCLUSION: Resveratrol can suppresses the growth of H22 cells in vitro,its anti-tumor activity may occur through the induction of apoptosis.

Resveratrol-induced modification of polyamine metabolism is accompanied by induction of c-Fos.

Carcinogenesis. 2003 Mar;24(3):469-74

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The objective of the current study was to investigate the effect of resveratrol, a naturally occurring polyphenol with cancer chemopreventive properties, on polyamine metabolism in the human colonic adenocarcinoma cell line Caco-2. We demonstrated that inhibition of ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine biosynthesis, was due to attenuated ODC protein and mRNA levels (50-200 microM). The naturally occurring resveratrol analog piceatannol (100 microM) also diminished ODC activity, protein and mRNA levels, whereas the green tea polyphenol (-)-epigallocatechin gallate (EGCG; 100 microM) exerted only weak effects on ODC. The transcription factor c-Myc, a positive regulator of the odc gene was attenuated by resveratrol treatment and to a lesser extent by piceatannol and EGCG. S-Adenosylmethionine decarboxylase, an enzyme that synthesizes higher polyamines, was concomitantly inhibited by resveratrol and piceatannol treatment, whereas EGCG did not affect its activity. In addition resveratrol, piceatannol and EGCG enhanced spermidine/spermine N(1)-acetyltransferase activity, an enzyme that degrades polyamines in cooperation with polyamine oxidase. Intracellular levels of spermine and spermidine were not affected, whereas putrescine and N(8)-acetylspermidine concentrations increased after incubation with resveratrol. These events were paralleled by an increase of the activator protein-1 constituents c-Fos and c-Jun. Whereas DNA-binding activity of c-Jun remained unchanged, DNA-binding activity of c-Fos was significantly enhanced by resveratrol and piceatannol, but inhibited by EGCG. The data suggest that growth arrest by resveratrol is accompanied by inhibition of polyamine synthesis and increased polyamine catabolism. C-Fos seems to play a role in this context. Effects of piceatannol on polyamine synthesis were similar, but not as potent as those exerted by resveratrol.

Increased radiation sensitivity of an eosinophilic cell line following treatment with epigallocatechin-gallate, resveratrol and curcuma.

Int J Mol Med. 2005 Feb;15(2):337-52

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Ionizing radiation is widely used in radiotherapy, in order to promote an apoptotic response in cancerous cells. Since the need to find new substances that would enhance the radiation-induced apoptosis in cancerous cells is great, we studied the effect of epigallocatechin-gallate (EGCG, a tea component), resveratrol (a wine component) and curcuma on cell proliferation and radiation-induced apoptosis in the human leukaemic cell line, EOL-1, derived from a patient with eosinophilic leukaemia. Cells were X-irradiated with 0, 2, 4, 6 or 8 Gy and cultured in the presence of EGCG, resveratrol or curcuma (concentrations ranging from 0 to 200 microM) for 1, 2 or 3 days of culture. Cell proliferation was measured using trypan blue exclusion. Apoptosis was evaluated using light microscopy (morphology study after May-Grunwald Giemsa staining) and flow cytometry (annexin-V staining). Irradiation alone induced a dose-related reduction in cell proliferation and the appearance of polyploid cells in EOL-1 cells. Additionally, EOL-1 cells underwent a dose-related increase of apoptosis which, from the second day on, was accompanied by a dose-related increase of necrosis. When cells were exposed to EGCG, resveratrol or curcuma alone, a decrease in cell proliferation was observed, beginning from 25 microM EGCG and 50 microM resveratrol and curcuma, while an increase in the percentage of apoptotic cells was noted from 50 microM EGCG, 100 microM resveratrol and curcuma in EOL-1 cells, after only one day of culture. Simultaneous exposure to X-irradiation and, EGCG, resveratrol or curcuma resulted in a synergistic decrease of cell proliferation as well as in a synergistic increase of apoptosis and necrosis. These results suggest that, depending on the concentration, EGCG, resveratrol and curcuma enhance radiation-induced apoptosis in the leukaemic cell line, EOL-1 (EGCG >resveratrol >curcuma). In order to further characterise the radiation-induced apoptosis of this leukaemic cell line, other flow cytometrical analyses are in progress.

Anti-proliferative effect of resveratrol, a natural component of grapes and wine, on human colonic cancer cells.

Cancer Lett. 2000 Sep 29;158(1):85-91

Schneider Y, **Vincent F**, **Duranton B**, **Badolo L**, **Gossé F**, **Bergmann C**, **Seiler N**, **Raul F**.

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Resveratrol, a natural polyphenolic phytoalexine present in grapes and wines, has been reported to exert a variety of important pharmacological effects. We investigated the effects of resveratrol on the growth and polyamine metabolism of CaCo-2 human colon

cancer cells. Treatment of the CaCo-2 cells with 25 microM resveratrol caused a 70% growth inhibition. The cells accumulated at the S/G2 phase transition of the cell cycle. No signs of cytotoxicity or apoptosis were detected. Resveratrol caused a significant decrease of ornithine decarboxylase (ODC) activity, a key enzyme of polyamine biosynthesis, which is enhanced in cancer growth. ODC inhibition resulted in the reduction of the intracellular putrescine content, indicating that polyamines might represent one of several targets involved in the anti-proliferative effects of resveratrol.

Suppression of ultraviolet B exposure-mediated activation of NF-kappaB in normal human keratinocytes by resveratrol.

Neoplasia. 2003 Jan-Feb;5(1):74-82

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Chemoprevention by naturally occurring agents is a newer dimension in the management of neoplasia, including skin cancer. Solar ultraviolet (UV) radiation is the major cause of skin cancer. We recently demonstrated that resveratrol (3,5,4'-trihydroxystilbene), a polyphenolic antioxidant found in grapes and red wine, imparts protection from UVB-mediated cutaneous damages in SKH-1 hairless mice. The mechanism of action of resveratrol is not clearly understood. Here, we investigated the involvement of nuclear factor kappa B (NF-kappaB), which is known to play a critical role in skin biology and the development of skin cancer, as the mechanism of chemoprevention of UV damage by resveratrol. In the normal human epidermal keratinocytes, resveratrol blocked UVB-mediated (40 mJ/cm(2)) activation of NF-kappaB in a dose-dependent (5, 10, and 25 micro M resveratrol for 24 hours) as well as time-dependent (5 micro M resveratrol for 12, 24, and 48 hours) fashion. Resveratrol treatment of keratinocytes also inhibited UVB-mediated 1) phosphorylation and degradation of IkappaBalpha, and 2) activation of IKKalpha. We suggest that NF-kappaB pathway plays a critical role in the chemopreventive effects of resveratrol against the adverse effects of UV radiation including photocarcinogenesis.

Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-kappa B, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation.

J Immunol. 2000 Jun 15;164(12):6509-19.

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Resveratrol (trans-3,4',5-trihydroxystilbene), a polyphenolic phytoalexin found in grapes, fruits, and root extracts of the weed Polygonum cuspidatum, exhibits anti-inflammatory, cell growth-modulatory, and anticarcinogenic effects. How this chemical produces these effects is not known, but it may work by suppressing NF-kappaB, a nuclear transcription factor that regulates the expression of various genes involved in inflammation, cytoprotection, and carcinogenesis. In this study, we investigated the effect of resveratrol on NF-kappaB activation induced by various inflammatory agents. Resveratrol blocked TNF-induced activation of NF-kappaB in a dose- and time-dependent manner. Resveratrol also suppressed TNF-induced phosphorylation and nuclear translocation of the p65 subunit of NF-kappaB, and NF-kappaB-dependent reporter gene transcription. Suppression of TNF-induced NF-kappaB activation by resveratrol was not restricted to myeloid cells (U-937); it was also observed in lymphoid (Jurkat) and epithelial (HeLa and H4) cells. Resveratrol also blocked NF-kappaB activation induced by PMA, LPS, H2O2, okadaic acid, and ceramide. The suppression of NF-kappaB coincided with suppression of AP-1. Resveratrol also inhibited the TNF-induced activation of mitogen-activated protein kinase kinase and c-Jun N-terminal kinase and abrogated TNF-induced cytotoxicity and caspase activation. Both reactive oxygen intermediate generation and lipid peroxidation induced by TNF were suppressed by resveratrol. Resveratrol's anticarcinogenic, anti-inflammatory, and growth-modulatory effects may thus be partially ascribed to the inhibition of activation of NF-kappaB and AP-1 and the associated kinases.

Lung carcinogenesis: resveratrol modulates the expression of genes involved in the metabolism of PAH in human bronchial epithelial cells.

Int J Cancer. 2001 Apr 1;92(1):18-25.

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Studies suggest that resveratrol (trans-3,4',5-trihydroxystilbene), which is a diphenolic antioxidant found in plants and foods, has cancer chemopreventive and chemotherapeutic

potential. A lower risk of lung cancer among consumers of wine compared with consumers of other beverages has been observed, which may be partly attributed to the high content of resveratrol particularly in red wine. We have studied the effect of resveratrol on the expression of genes involved in the metabolism of polycyclic aromatic hydrocarbons in the human bronchial epithelial cell line BEP2D. Expression of the cytochrome P450 1A1 (CYP1A1) and 1B1 (CYP1B1), microsomal epoxide hydrolase (mEH), and glutathione S-transferase P1 (GSTP1) genes was measured by quantitative reverse transcriptase-polymerase chain reaction. The cells were treated either with benzo [a]pyrene or 2,3,7,8-tetrachlorodibenzo-p-dioxin in the presence or absence of resveratrol. Resveratrol inhibited both the constitutive and the induced expression of CYP1A1 and CYP1B1 in a dose-dependent manner. In contrast, the expression of the mEH gene was increased in response to resveratrol and no change in the expression of GSTP1 was found. The altered gene expression in response to resveratrol was reflected in a reduced overall level of benzo[a]pyrene metabolism. These data indicate that resveratrol may exert lung cancer chemopreventive activity through altering the expression of genes involved in the metabolism of polycyclic aromatic hydrocarbons, resulting in altered formation of carcinogenic benzo[a]pyrene metabolites in human bronchial epithelial cells

Resveratrol promotes differentiation and induces Fas-independent apoptosis of human medulloblastoma cells.

Neurosci Lett. 2003 Nov 13;351(2):83-6

Wang Q, **Li H**, **Wang XW**, **Wu DC**, **Chen XY**, **Liu J**.

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Resveratrol has tumor-suppressive activities in some in vitro and in vivo experimental systems but its effect on medulloblastoma cells is still unknown. In this study, resveratrol was used to treat four human medulloblastoma cell lines (Med-3, UW228-1, -2 and -3) and its effects on cell growth, differentiation and death were examined by multiple approaches. Expression of Fas, FasL and caspase-3 in the cells without and with resveratrol treatments was examined by immunocytochemical staining and mRNA in situ hybridization and the influence of anti-Fas antibody (200 ng/ml) in cell growth and survival was determined as well. The results demonstrated that resveratrol could suppress growth, promote differentiation and commit its target cells to apoptosis in time- and dose-related fashions. Fas was constitutively expressed but FasL was undetectable in the four lines in spite of resveratrol treatment. Anti-Fas antibody (200 ng/ml) neither inhibited growth nor induced apoptosis of those cell lines. Up-regulated caspase-3 was found in resveratrol-treated populations and appearance of its cleaved form was closely associated with the apoptotic event. These findings suggest for the first time that resveratrol is an

effective anti-medulloblastoma agent that kills medulloblastoma cells through a Fas-independent pathway.

Ultrasensitive assay for three polyphenols (catechin, quercetin and resveratrol) and their conjugates in biological fluids utilizing gas chromatography with mass selective detection.

J Chromatogr B Biomed Sci Appl. 2001 Jun 5;757(1):161-72.

Soleas GJ, **Yan J**, **Goldberg DM**.

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The concentrations of three polyphenols ((+)-catechin, quercetin and trans-resveratrol) in blood serum, plasma and urine, as well as whole blood, have been measured after their oral and intragastric administration, respectively, to humans and rats. The method developed for this purpose utilized ethyl acetate extraction of 100 microl samples and their derivatization with bis(trimethylsilyl)trifluoroacetamide (BSTFA) followed by gas-chromatographic analysis on a DB-5 column followed by mass selective detection employing two target ions and one qualifier ion for each compound. Total run time was 17 min with excellent resolution and linearity. The limits of detection (LOD) and quantitation (LOQ) were an order of magnitude less than for any previously published method, being 0.01 microg/l and 0.1 microg/l, respectively, for all compounds. Recovery at 1 microg/l and 10 microg/l was >80% in all instances but one, and was >90% in 50%. Imprecision was acceptable at 0.25 and 1.0 microg/l, concentrations below the LOQ of previous methods. Aglycones released from conjugates after hydrolysis were easily measurable. Optimal conditions for hydrolysis were established. After oral administration of the three polyphenols to humans, their conjugates vastly exceeded the concentrations of the aglycones in both plasma and urine. Concentrations peaked within 0.5-1.0 h in plasma and within 8 h in urine. During the first 24 h, 5.1% of the (+)-catechin and 24.6% of the trans-resveratrol given were recovered in the urine (free plus conjugated). This method can be proposed as the method of choice to assay these polyphenols and their conjugates in biological fluids.

Resveratrol, a remarkable inhibitor of ribonucleotide reductase.

FEBS Lett. 1998 Jan 16;421(3):277-9

Fontecave M, **Lepoivre M**, **Elleingand E**, **Gerez C**, **Guittet O**.

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Resveratrol, a natural phytoalexin found in grapes, is well known for its presumed role in the prevention of heart disease, associated with red wine consumption. We show here that it is a remarkable inhibitor of ribonucleotide reductase and DNA synthesis in mammalian cells, which might have further applications as an antiproliferative or a cancer chemopreventive agent in humans.

Inhibitory activity of stilbenes from medicinal plants on the expression of cell adhesion molecules on THP1 cells.

Planta Med. 2000 Oct;66(7):641-4.

Ahn KS, **Kim JH**, **Oh SR**, **Ryu SY**, **Lee HK**.

The inhibitory activity of stilbenes isolated from medicinal plants on cell adhesion molecules on the surface of THP-1 human monocytic cell lines was investigated. Among ten stilbenes tested, four stilbenes displayed a significant inhibitory activity on the expression of both intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). A cell-to-cell adhesion assay showed that 3,5-dihydroxy-4'-methoxystilbene and 2,3,4',5-tetrahydroxystilbene-2-O-beta-D-glucopyranoside as well as resveratrol blocked significantly TNF-alpha-inducing cell-cell adhesion between human umbilical vein endothelial cells (HUVEC) and THP-1 cells.

Effect of resveratrol and some other natural compounds on tyrosine kinase activity and on cytolysis.

Drugs Exp Clin Res. 1999;25(2-3):79-85.

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Resveratrol is a phytoalexin with several biological and pharmacological activities including the "French paradox". We investigated the effect of resveratrol on cytolytic activity by oxygen reactive species and on soluble and particulate tyrosine kinases from

human placenta and human prostatic adenoma. These effects were compared with those of piceatannol, quercetin, catechin and epicatechin. Fifty percent of erythrocyte lysis due to H2O2-lactoperoxidase-KI incubation, in which I3-, OI- and oxygen singlet are produced, was obtained after 22 +/- 7 (SD) min in the absence of the tested compounds. The 50% lysis was obtained after 66 +/- 15, 129 +/- 35, 196 +/- 21, 240 +/- 63 and 420 +/- 80 min with 40 microM piceatannol, quercetin, resveratrol, epicatechin and catechin respectively. Protection was concentration dependent. The assay of tyrosine kinase activity was performed using two different substrates as follows: substrate A corresponded to the sequence 1-17 of gastrin, and substrate B to sequence 6-20 of cell division kinase p34cdc2. In all experiments, initial velocity was measured. When assayed with both substrates, tyrosine kinase activities from particulate and cytosolic fractions of placenta were more inhibited by piceatannol and quercetin. Resveratrol significantly inhibited the particulate fraction and the cytosolic fraction respectively when substrates A and B were employed: Catechin acted as an inhibitor with substrate A and particulate fraction while in the other experimental conditions it acted as an activator. Resveratrol inhibited the tyrosine kinase of particulate and cytosolic fractions of prostatic adenoma assayed with substrate A and B.

Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies.

Anticancer Res. 2004 Sep-Oct;24(5A):2783-840.

Aggarwal BB, **Bhardwaj A**, **Aggarwal RS**, **Seeram NP**, **Shishodia S**, **Takada Y**.

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Resveratrol, trans-3,5,4'-trihydroxystilbene, was first isolated in 1940 as a constituent of the roots of white hellebore (Veratrum grandiflorum O. Loes), but has since been found in various plants, including grapes, berries and peanuts. Besides cardioprotective effects, resveratrol exhibits anticancer properties, as suggested by its ability to suppress proliferation of a wide variety of tumor cells, including lymphoid and myeloid cancers; multiple myeloma; cancers of the breast, prostate, stomach, colon, pancreas, and thyroid; melanoma; head and neck squamous cell carcinoma; ovarian carcinoma; and cervical carcinoma. The growth-inhibitory effects of resveratrol are mediated through cell-cycle arrest; upregulation of p21Cip1/WAF1, p53 and Bax; down-regulation of survivin, cyclin D1, cyclin E, Bcl-2, Bcl-xL and clAPs; and activation of caspases. Resveratrol has been shown to suppress the activation of several transcription factors, including NF-kappaB, AP-1 and Egr-1; to inhibit protein kinases including IkappaBalpha kinase, JNK, MAPK, Akt, PKC, PKD and casein kinase II; and to down-regulate products of genes such as COX-2, 5-LOX, VEGF, IL-1, IL-6, IL-8, AR and PSA. These activities account for the

suppression of angiogenesis by this stilbene. Resveratrol also has been shown to potentiate the apoptotic effects of cytokines (e.g., TRAIL), chemotherapeutic agents and gamma-radiation. Phamacokinetic studies revealed that the target organs of resveratrol are liver and kidney, where it is concentrated after absorption and is mainly converted to a sulfated form and a glucuronide conjugate. In vivo, resveratrol blocks the multistep process of carcinogenesis at various stages: it blocks carcinogen activation by inhibiting aryl hydrocarbon-induced CYP1A1 expression and activity, and suppresses tumor initiation, promotion and progression. Besides chemopreventive effects, resveratrol appears to exhibit therapeutic effects against cancer. Limited data in humans have revealed that resveratrol is pharmacologically quite safe. Currently, structural analogues of resveratrol with improved bioavailability are being pursued as potential therapeutic agents for cancer.

Resveratrol causes WAF-1/p21-mediated G(1)-phase arrest of cell cycle and induction of apoptosis in human epidermoid carcinoma A431 cells.

Clin Cancer Res. 2001 May;7(5):1466-73.

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Resveratrol (trans-3,4',5,-trihydroxystilbene), a phytoalexin found in grapes, nuts, fruits, and red wine, is a potent antioxidant with cancer-preventive properties. The mechanism by which resveratrol imparts cancer chemopreventive effects is not clearly defined. Here, we demonstrate that resveratrol, via modulations in cyclin-dependent kinase (cdk) inhibitor-cyclin-cdk machinery, results in a G(1)-phase arrest of the cell cycle followed by apoptosis of human epidermoid carcinoma (A431) cells. Resveratrol treatment (1-50 microM for 24 h) of A431 cells resulted in a dose-dependent (a) inhibition of cell growth as shown by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, (b) G (1)-phase arrest of the cell cycle as shown by DNA cell cycle analysis, and (c) induction of apoptosis as assessed by ELISA. The immunoblot analysis revealed that resveratrol treatment causes a dose- and time-dependent (a) induction of WAF1/p21; (b) decrease in the protein expressions of cyclin D1, cyclin D2, and cyclin E; and (c) decrease in the protein expressions of cdk2, cdk4, and cdk6. Resveratrol treatment was also found to result in a dose- and time-dependent decrease in kinase activities associated with all of the cdks examined. Taken together, our study suggests that resveratrol treatment of the cells causes an induction of WAF1/p21 that inhibits cyclin D1/D2-cdk6, cyclin D1/D2-cdk4, and cyclin E-cdk2 complexes, thereby imposing an artificial checkpoint at the G (1)-->S transition of the cell cycle. This series of events results in a G(1)-phase arrest of the cell cycle, which is an irreversible process that ultimately results in the apoptotic

death of cancer cells. To our knowledge, this is the first systematic study showing the involvement of each component of cdk inhibitor-cyclin-cdk machinery during cell cycle arrest and apoptosis of cancer cells by resveratrol.

Sensitization for tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by the chemopreventive agent resveratrol.

Cancer Res. 2004 Jan 1;64(1):337-46

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Survivin is a member of the inhibitor of apoptosis proteins that is expressed at high levels in most human cancers and may facilitate evasion from apoptosis and aberrant mitotic progression. Naturally occurring dietary compounds such as resveratrol have gained considerable attention as cancer chemopreventive agents. Here, we discovered a novel function of the chemopreventive agent resveratrol: resveratrol is a potent sensitizer of tumor cells for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis through p53-independent induction of p21 and p21-mediated cell cycle arrest associated with survivin depletion. Concomitant analysis of cell cycle, survivin expression, and apoptosis revealed that resveratrol-induced G(1) arrest was associated with down-regulation of survivin expression and sensitization for TRAIL-induced apoptosis. Accordingly, G(1) arrest using the cell cycle inhibitor mimosine or induced by p21 overexpression reduced survivin expression and sensitized cells for TRAIL treatment. Likewise, resveratrol-mediated cell cycle arrest followed by survivin depletion and sensitization for TRAIL was impaired in p21- deficient cells. Also, down-regulation of survivin using survivin antisense oligonucleotides sensitized cells for TRAIL-induced apoptosis. Importantly, resveratrol sensitized various tumor cell lines, but not normal human fibroblasts, for apoptosis induced by death receptor ligation or anticancer drugs. Thus, this combined sensitizer (resveratrol)/inducer (e.g., TRAIL) strategy may be a novel approach to enhance the efficacy of TRAIL-based therapies in a variety of human cancers.

Grape polyphenol resveratrol and the related molecule 4-hydroxystilbene induce growth inhibition, apoptosis, S-phase arrest, and upregulation of cyclins A, E, and B1 in human SK-Mel-28 melanoma cells.

J Agric Food Chem. 2003 Jul 30;51(16):4576-84

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The effect of the naturally occurring polyphenol resveratrol (3,5,4'-trihydroxy-trans-stilbene; RES) on growth, cell cycle, and cyclins A, E, and B1 expression was investigated in the human SK-Mel-28 melanoma cell line. In addition, the structurally related compounds 4-hydroxy-trans-stilbene (4HST), piceatannol (3,5,3',4'-tetrahydroxy-trans-stilbene (PICE), and 4-trans-stilbenemethanol (4STMe) were also assayed in order to investigate the requirements of stilbenes to exert activity against melanoma cells. Both RES and 4HST inhibited cell growth in a dose- and time-dependent manner and upregulated the expression of cyclins A, E, and B1 with subsequent irreversible arrest of melanoma cells in the S-phase, concomitant with a decrease in G0/G1 and G2/M phases. In addition, potent apoptosis-mediated cell death was detected with the annexin V assay whereas no apoptosis was observed by flow cytometry, which encourages the assay of different methodologies to evaluate the effect of polyphenols on cell lines. The effect of PICE was not evaluated because of its instability in the reaction medium. No effect on cell cycle and cyclins expression was observed when 4STMe was assayed, which supported the critical requirement of the 4'-hydroxystyryl moiety to exert the above effects. In addition, this structural requirement also influenced the cellular uptake of stilbenes. The presence of two extra hydroxyl groups in RES increased its cytotoxicity whereas it diminished its efficiency to inhibit cell growth, upregulate cyclins expression, and arrest cell cycle in the S-phase with respect to 4HST. The present study suggests that the antimelanoma properties of dietary stilbenes, such as grape RES, cannot be ruled out, taking into account previous studies concerning the relationship between plasma and tissue concentrations and pharmacological activity of RES in animal models.

Role of Bax in resveratrol-induced apoptosis of colorectal carcinoma cells.

BMC Cancer. 2002 Oct 17;2:27

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BACKGROUND: The natural plant polyphenol resveratrol present in some foods including grapes, wine, and peanuts, has been implicated in the inhibition, delay, and reversion of cellular events associated with heart diseases and tumorigenesis. Recent

work has suggested that the cancer chemoprotective effect of the compound is primarily linked to its ability to induce cell division cycle arrest and apoptosis, the latter possibly through the activation of pro-apoptotic proteins such as Bax. METHODS: The expression, subcellular localization, and importance of Bax for resveratrol-provoked apoptosis were assessed in human HCT116 colon carcinoma cells and derivatives with both bax alleles inactivated. RESULTS: Low to moderate concentrations of resveratrol induced co-localization of cellular Bax protein with mitochondria, collapse of the mitochondrial membrane potential, activation of caspases 3 and 9, and finally, apoptosis. In the absence of Bax, membrane potential collapse was delayed, and apoptosis was reduced but not absent. Resveratrol inhibited the formation of colonies by both HCT116 and HCT116 bax -/- cells. CONCLUSION: Resveratrol at physiological doses can induce a Bax-mediated and a Bax-independent mitochondrial apoptosis. Both can limit the ability of the cells to form colonies.

Resveratrol induces extensive apoptosis by depolarizing mitochondrial membranes and activating caspase-9 in acute lymphoblastic leukemia cells.

Cancer Res. 2001 Jun 15;61(12):4731-9

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Resveratrol, a plant antibiotic, has been found to have anticancer activity and was recently reported to induce apoptosis in the myeloid leukemia line HL60 by the CD95-CD95 ligand pathway. However, many acute lymphoblastic leukemias (ALLs), particularly of B-lineage, are resistant to CD95-mediated apoptosis. Using leukemia lines derived from patients with pro-B t(4;11), pre-B, and T-cell ALL, we show in this report that resveratrol induces extensive apoptotic cell death not only in CD95-sensitive leukemia lines, but also in B-lineage leukemic cells that are resistant to CD95-signaling. Multiple dose treatments of the leukemic cells with 50 microM resveratrol resulted in >/=80% cell death with no statistically significant cytotoxicity against normal peripheral blood mononuclear cells under identical conditions. Resveratrol treatment did not increase CD95 expression or trigger sensitivity to CD95-mediated apoptosis in the ALL lines. Inhibition of CD95-signaling with a CD95-specific antagonistic antibody indicated that CD95-CD95 ligand interactions were not involved in initiating resveratrol-induced apoptosis. However, in each ALL line, resveratrol induced progressive loss of mitochondrial membrane potential as measured by the dual emission pattern of the mitochondria-selective dye JC-1. The broad spectrum caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone failed to block the depolarization of mitochondrial membranes induced by resveratrol, further indicating that resveratrol

action was independent of upstream caspase-8 activation via receptor ligation. However, increases in caspase-9 activity ranged from 4- to 9-fold in the eight cell lines after treatment with resveratrol. Taken together, these results point to a general mechanism of apoptosis induction by resveratrol in ALL cells that involves a mitochondria/caspase-9-specific pathway for the activation of the caspase cascade and is independent of CD95-signaling.

Resveratrol--from the bottle to the bedside?

Leuk Lymphoma. 2001 Feb;40(5-6):491-8

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Resveratrol, a naturally occurring plant antibiotic has been the focus of a number of studies investigating its biological attributes, which include anti-oxidant activity, anti-platelet aggregation effect, anti-atherogenic property, estrogen-like growth promoting effect, growth inhibiting activity, immunomodulation, and chemoprevention. More recently, since the first report on the apoptosis inducing activity of resveratrol in human cancer cells, the interest in this molecule as a potential chemotherapy agent has significantly intensified. Not only has its role as an anti-cancer agent been corroborated, but the precise mechanism(s) of the anti-cancer activity of resveratrol is/are being elucidated. Our group has been active in studying the cross talk between the caspase family of proteases and mitochondria, in drug-induced apoptosis. In this regard, we have shown that the cancer preventive activity of resveratrol could be attributed to its ability to trigger apoptosis in human leukemia and breast carcinoma cells. The cytotoxicity of resveratrol is restricted against these transformed cell types due to its ability to selectively upregulate CD95-CD95L interaction on the tumor cell surface, unlike normal peripheral blood cells. Despite the involvement of the CD95 signaling pathway, apoptosis induced by resveratrol is not accompanied by robust caspase 8 activation, but involves mitochondrial release of cytochrome C and downstream activation of caspases 9 and 3. We also extrapolate these in vitro findings in a murine model of carcinogensis, and demonstrate in vivo induction of apoptosis in mouse skin papillomas. These findings highlight the chemotherapeutic potential of this polyphenolic compound.

Resveratrol inhibits drug-induced apoptosis in human leukemia cells by creating an intracellular milieu nonpermissive for death execution.

Cancer Res. 2004 Feb 15;64(4):1452-9

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Efficient apoptotic signaling is a function of a permissive intracellular milieu created by a decrease in the ratio of superoxide to hydrogen peroxide and cytosolic acidification. Resveratrol (RSV) triggers apoptosis in some systems and inhibits the death signal in others. In this regard, the inhibitory effect on hydrogen peroxide-induced apoptosis is attributed to its antioxidant property. We provide evidence that exposure of human leukemia cells to low concentrations of RSV (4-8 micro M) inhibits caspase activation, DNA fragmentation, and translocation of cytochrome c induced by hydrogen peroxide or anticancer drugs C2, vincristine, and daunorubicin. Interestingly, at these concentrations, RSV induces an increase in intracellular superoxide and inhibits drug-induced acidification. Blocking the activation of NADPH oxidase complex neutralized RSV-induced inhibition of apoptosis. Furthermore, our results implicate intracellular hydrogen peroxide as a common effector mechanism in drug-induced apoptosis that is inhibited by preincubation with RSV. Interestingly, decreasing intracellular superoxide with the NADPH oxidase inhibitor diphenyliodonium reversed the inhibitory effect of RSV on drug-induced hydrogen peroxide production. These data show that low concentrations of RSV inhibit death signaling in human leukemia cells via NADPH oxidase-dependent elevation of intracellular superoxide that blocks mitochondrial hydrogen peroxide production, thereby resulting in an intracellular environment nonconducive for death execution.

Chemopreventive properties of trans-resveratrol are associated with inhibition of activation of the IkappaB kinase.

Cancer Res. 2000 Jul 1;60(13):3477-83.

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trans-Resveratrol (Res), a phytoalexin found at high levels in grapes and in grape products such as red wine, has been shown to have anti-inflammatory and antioncogenic properties. Because the transcription factor nuclear factor kappaB (NF-kappaB) is involved in inflammatory diseases and oncogenesis, we tested whether Res could

modulate NF-kappaB activity. Res was shown to be a potent inhibitor of both NF-kappaB activation and NF-kappaB-dependent gene expression through its ability to inhibit IkappaB kinase activity, the key regulator in NF-kappaB activation, likely by inhibiting an upstream signaling component. In addition, Res blocked the expression of mRNA-encoding monocyte chemoattractant protein-1, a NF-kappaB-regulated gene. Relative to cancer chemopreventive properties, Res induced apoptosis in fibroblasts after the induced expression of oncogenic H-Ras. Thus, Res is likely to function by inhibiting inflammatory and oncogenic diseases, at least in part, through the inhibition of NF-kappaB activation by blocking IkappaB kinase activity. These data may also explain aspects of the so-called "French paradox" that is associated with reduced mortality from coronary heart disease and certain cancers and provide a molecular rationale for the role of a potent chemopreventive compound in blocking the initiation of inflammation and oncogenesis.

Resveratrol is a potent inducer of apoptosis in human melanoma cells.

Cancer Lett. 2003 Feb 20;190(2):157-63

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Resveratrol is a plant polyphenol found in grapes and red wine. It has been found to have beneficial effects on the cardiovascular system. Resveratrol also inhibits the growth of various tumor cell lines in vitro and inhibits carcinogenesis in vivo. In this study we examined the effect of resveratrol on growth of two human melanoma cell lines. We found that this plant polyphenol inhibited growth and induced apoptosis in both cell lines, with the amelanotic cell line A375 being more sensitive. The potential involvement of different MAP kinases in the action of resveratrol was also examined. Although resveratrol did not alter the phosphorylation of p38 or JNK MAP kinases in either cell line, it induced phosphorylation of ERK1/2 in A375, but not in SK-mel28 cells. These results suggest that in vivo studies of the effect of resveratrol on melanoma are warranted and that this plant polyphenol might have effectiveness as either a therapeutic or chemopreventive agent against melanoma.

The egr-1 gene is induced by DNA-damaging agents and non-genotoxic drugs in both normal and neoplastic human cells.

Life Sci. 2003 May 16;72(26):2975-92.

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The human egr-1 gene encodes a zinc finger transcription factor induced by endogenous and exogenous stimuli such as growth factors, cytokines, and mitogens. Egr-1 regulates other genes involved in growth and differentiation. The present study investigated the influence of genotoxic agents, such as chemotherapy drugs and other DNA damaging agents, on egr-1 expression in normal and neoplastic cells. A transcriptional fusion between the human egr-1 promoter and the enhanced green fluorescent protein (EGFP) gene was used for direct visualization of intracellular Egr-1 regulation. The transcriptional activity of the egr-1 promoter in this reporter system faithfully reflects intrinsic egr-1 expression and induction, as demonstrated by FACS analysis of fluorescence and by RT-PCR for egr-1. EGFP was expressed under the control of the egr-1 promoter in stably transfected immortalized cell lines, such as HEK293, T98G, LNZ308, and 9L, which were then treated with genotoxic agents.A multitude of DNA damaging agents and therapeutic drugs caused significant upregulation of egr-1 transcription. Furthermore, cytotoxic compounds without a direct DNA damaging effect, such as resveratrol and vincristine, which interfere with DNA replication and cell division, were also able to activate egr-1 transcription. This suggests that cell cycle arrest rather than DNA damage seems to be the condition triggering egr-1 transcription. Moreover, treatment with the MAP kinase (MAPK) inhibitor SB203580, which specifically blocks the stress inducible p38/SAPK2 pathway, did not alter egr-1 induction. On the other hand, treatment with the inhibitor PD98059, which specifically blocks the MAPK/ERK pathway, partially suppressed the induction effect. In addition, the egr-1 induction effect caused by genotoxic stress was found to be at least in part independent from the cellular p53 status, as it was observed in p53-deficient as well as in wild type p53 cell lines.These results suggest that induction of egr-1, a gene to which until now no relation to DNA repair has been assigned, may belong to the fundamental cellular responses elicited by genotoxic and mitotic stress in normal as well as in neoplastic cells, and that enhanced levels of Egr-1 protein may be needed to regulate genes involved in DNA repair, cell survival, and apoptosis.

Resveratrol antagonizes EGFR-dependent Erk1/2 activation in human androgen-independent prostate cancer cells with associated isozyme-selective PKC alpha inhibition.

Invest New Drugs. 2004 Apr;22(2):107-17

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The development of androgen-independent prostate cancer (AI PrCa) involves constitutive Erk1/2 activation sustained by the epidermal growth factor/transforming growth factor-alpha/EGF receptor (EGF/TGFalpha/EGFR) axis and other trophic signaling mechanisms in neoplastic human prostate epithelial cells in vivo. In this report, we show that growth-inhibitory concentrations of the dietary phytochemical resveratrol suppress EGFR-dependent Erk1/2 activation pathways stimulated by EGF and phorbol ester (12- O -tetradecanoyl phorbol 13-acetate, TPA) in human AI PrCa PC-3 cells in vitro. Because protein kinase C (PKC) is the major cellular receptor for phorbol esters and taking into consideration that resveratrol is PKC-inhibitory, we investigated resveratrol effects on cellular PKC isozymes associated with the suppression of TPA-induced Erk1/2 activation. The PKC isozyme composition of PC-3 cells was defined by Western analysis of the cell lysate with a comprehensive set of isozyme-selective PKC Ab's. PC-3 cells expressed PKCalpha, epsilon, zeta, iota, and PKD (PKCmicro), as did another human AI PrCa cell line of distinct genetic origin, DU145. The effects of resveratrol on TPA-induced PKC isozyme activation were defined by monitoring PKC isozyme translocation and autophosphorylation. Under conditions where resveratrol suppressed TPA-induced Erk1/2 activation, the phytochemical produced isozyme-selective interference with TPA-induced translocation of cytosolic PKCalpha to the membrane/cytoskeleton and selectively diminished the amount of autophosphorylated PKCalpha in the membrane/cytoskeleton of the TPA-treated cells. These results demonstrate that resveratrol abrogation of a PKC-mediated Erk1/2 activation response in PC-3 cells correlates with isozyme-selective PKCalpha inhibition. The results provide evidence that resveratrol may have value as an adjuvant cancer therapeutic in advanced prostate cancer.

Resveratrol-induced inactivation of human gastric adenocarcinoma cells through a protein kinase C-mediated mechanism.

Biochem Pharmacol. 2001 Nov 15;62(10):1423-32.

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Resveratrol, a polyphenolic phytochemical present in berries, grapes, and wine, has emerged as a promising chemopreventive candidate. Because there is scant information

regarding natural agents that prevent, suppress, or reverse gastric carcinogenesis, the aim of the present study was to determine the chemopreventive potential of resveratrol against gastric cancer by investigating cellular and molecular events associated with resveratrol treatment of human gastric adenocarcinoma cells. We determined the action of resveratrol on cellular function and cellular integrity by measuring DNA synthesis, cellular proliferation, cell cycle distribution, cytolysis, apoptosis, and phosphotransferase activities of two key signaling enzymes, protein kinase C (PKC) and mitogen-activated protein kinases (ERK1/ERK2), in human gastric adenocarcinoma KATO-III and RF-1 cells. Resveratrol inhibited [3H]thymidine incorporation into cellular DNA of normally proliferating KATO-III cells and of RF-1 cells whose proliferation was stimulated with carcinogenic nitrosamines. Treatment with resveratrol arrested KATO-III cells in the G (0)/G(1) phase of the cell cycle and eventually induced apoptotic cell death, but had a minimal effect on cell lysis. Resveratrol treatment had no effect on ERK1/ERK2 activity but significantly inhibited PKC activity of KATO-III cells and of human recombinant PKCalpha. Results indicate that resveratrol has potential as a chemopreventive agent against gastric cancer because it exerts an overall deactivating effect on human gastric adenocarcinoma cells. Resveratrol-induced inhibition of PKC activity and of PKCalpha, without any change in ERK1/ERK2 activity, suggests that resveratrol utilizes a PKC-mediated mechanism to deactivate gastric adenocarcinoma cells.

Flavonoid effects relevant to cancer.

J Nutr. 2002 Nov;132(11 Suppl):3482S-3489S.

Brownson DM, **Azios NG**, **Fuqua BK**, **Dharmawardhane SF**, **Mabry TJ**.

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Flavonoids, such as daidzein and genistein, present in dietary plants like soybean, have unique chemical properties with biological activity relevant to cancer. Many flavonoids and polyphenols, including resveratrol in red wine and epigallocatechin gallate in green tea, are known antioxidants. Some of these compounds have estrogenic (and antiestrogenic) activity and are commonly referred to as phytoestrogens. A yeast-based estrogen receptor (ER) reporter assay has been used to measure the ability of flavonoids to bind to ER and activate estrogen responsive genes. Recently, estrogenic compounds were also shown to trigger rapid, nongenomic effects. The molecular mechanisms, however, have not been completely detailed and little information exists regarding their relevance to cancer progression. As a preliminary step toward elucidating rapid phytoestrogen action on breast cancer cells, we investigated the effect of 17-beta estradiol (E2), genistein, daidzein and resveratrol on the activation status of signaling proteins that regulate cell survival and invasion, the cell properties underlying breast cancer progression. The effect of these estrogenic compounds on the activation, via

phosphorylation, of Akt/protein kinase B (Akt) and focal adhesion kinase (FAK) were analyzed in ER-positive and -negative human breast cancer cell lines. E2, genistein and daidzein increased whereas resveratrol decreased both Akt and FAK phosphorylation in nonmetastatic ER-positive T47D cells. In metastatic ER-negative MDA-MB-231 cells, all estrogenic compounds tested increased Akt and FAK phosphorylation. The inhibitory action of resveratrol on cell survival and proliferation is ER dependent. Therefore, all estrogenic compounds tested, including resveratrol, may exert supplementary ER-independent nongenomic effects on cell survival and migration in breast cancer cells.

Resveratrol induced serine phosphorylation of p53 causes apoptosis in a mutant p53 prostate cancer cell line.

J Urol. 2002 Aug;168(2):748-55

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PURPOSE: Resveratrol (Calbiochem, La Jolla, California) is a naturally occurring stilbene reported to cause apoptosis in various cultured cancer cells. In the current study the effect of resveratrol was determined in the androgen insensitive DU 145 prostate cancer cell line. Induction of apoptosis and activation of apoptosis related signal transduction pathways were measured. MATERIALS AND METHODS: DU 145 cells were treated with resveratrol and apoptosis was measured by determining nucleosome content. Activation of mitogen activated protein kinase (MAPK) (extracellular signal-regulated kinase 1/2), p53 content and serine-15 phosphorylation of p53 were measured by immunoblot. Electrophoretic mobility shift assay of p53 binding to DNA, and measurement of p21 and glyceraldehyde-3-phosphate dehydrogenase messenger RNA were also done. RESULTS: Resveratrol induced apoptosis in DU 145 cells. The stilbene activated MAPK and caused increased abundance of p53 and serine-15 phosphorylated p53. Resveratrol induced serine-15 phosphorylation of p53 was blocked by PD 98059 (Calbiochem), a MAPK kinase inhibitor, implicating MAPK activation in the phosphorylation of p53. PD 98059 also inhibited resveratrol induced apoptosis. These results suggest that apoptosis induction by resveratrol in DU 145 cells requires serine-15 phosphorylation of p53 by MAPK. Inhibition of MAPK dependent serine-15 phosphorylation resulted in reduced p53 binding to a p53 specific oligonucleotide on electrophoretic mobility shift assay. Pifithrin-alpha (Calbiochem), a p53 inhibitor, blocked resveratrol induced serine-15 phosphorylation of p53 and p53 binding to DNA. Resveratrol caused a p53 stimulated increase in p21 messenger RNA. Transfection of additional wild-type p53 into DU 145 cells induced apoptosis, which was further enhanced by resveratrol treatment. CONCLUSIONS: Resveratrol causes apoptosis in DU

145 prostate cancer cells. This action depends on the activation of MAPK, increase in cellular p53 content, serine-15 phosphorylation of p53 and increased p53 binding to DNA.

Different short- and long-term effects of resveratrol on nuclear factor-kappaB phosphorylation and nuclear appearance in human endothelial cells.

Am J Clin Nutr. 2003 May;77(5):1220-8

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BACKGROUND: Resveratrol (a naturally occurring phytoalexin found in grapes and wine) has cardiovascular protective effects that suggest the antiatherogenic (ie, antiinflammatory) activities of the compound on endothelial cells. OBJECTIVE: The antiinflammatory activity of resveratrol could be mediated by its interference with nuclear factor-kappaB (NF-kappaB)-dependent transcription. Thus, we studied the in vitro influence of physiologic concentrations of resveratrol (<or= 1 micromol/L) on the NF-kappaB signaling pathway after tumor necrosis factor alpha (TNF-alpha) stimulation of endothelial cells. DESIGN: The effects of a 30-min (acute) and an overnight incubation of resveratrol on the nuclear appearance of p50-NF-kappaB and p65-NF-kappaB on serine and tyrosine phosphorylation of the inhibitory subunit kappaB alpha (IkappaBalpha), cytoplasmic concentrations of IkappaBalpha, NF-kappaB phosphorylation or nitrosylation, the reduction of the mitotic inhibitor p21, and the activation of peroxisome proliferator-activated receptor alpha were evaluated. RESULTS: The nuclear appearance of p50-NFkappaB and p65-NFkappaB acutely induced by TNF-alpha was not modified by resveratrol but was increased after overnight incubation with resveratrol alone or in combination with TNF-alpha. Acute treatment with resveratrol did not modify TNF-alpha-induced cytoplasmic IkappaBalpha serine phosphorylation but did increase IkappaBalpha tyrosine phyophorylation. Resveratrol increased the tyrosine phosphorylation (but not nitrosylation) of immunoprecipitated NF-kappaB, did not decrease cellular p21, and did not increase peroxisome proliferator-activated receptor alpha activity. CONCLUSIONS: Acute resveratrol treatment does not inhibit the nuclear appearance of NF-kappaB in human umbilical vein endothelial cells, but overnight treatment does. The increase in tyrosine phosphorylation of IkappaBalpha, p50-NF-kappaB, and p65-NF-kappaB suggests the involvement of such alterations in the modulation of NF-kappaB transcription activity.

Effects of resveratrol on the autophosphorylation of phorbol ester-responsive protein kinases: inhibition of protein kinase D but not protein kinase C isozyme autophosphorylation.

Biochem Pharmacol. 2000 Nov 1;60(9):1355-9.

Stewart JR, **Christman KL**, **O'Brian CA**.

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The natural product resveratrol is a potent antagonist of phorbol ester-mediated tumor promotion and in vitro cellular responses to phorbol-ester tumor promoters, but it is only weakly inhibitory against the phosphorylation of conventional exogenous substrates by phorbol ester-responsive protein kinase C (PKC) isozymes. In this report, we compare the effects of resveratrol against the autophosphorylation reactions of PKC isozymes versus the novel phorbol ester-responsive kinase, protein kinase D (PKD). We found that resveratrol inhibits PKD autophosphorylation in a concentration-dependent manner, but has only negligible effects against the autophosphorylation reactions of representative members of each PKC isozyme subfamily (cPKC-alpha, -beta(1), and -gamma, nPKC-delta and -epsilon, and aPKC-zeta). Resveratrol was comparably effective against PKD autophosphorylation (IC(50) = 52 microM) and PKD phosphorylation of the exogenous substrate syntide-2 (IC(50) = 36 microM). The inhibitory potency of resveratrol against PKD is in line with the potency of resveratrol observed in cellular systems and with its potency against other purified enzymes and binding proteins that are implicated in the cancer chemopreventive activity of the polyphenol. Thus, PKD inhibition may contribute to the cancer chemopreventive action of resveratrol.

Downregulation of the cyclin D1/Cdk4 complex occurs during resveratrol-induced cell cycle arrest in colon cancer cell lines.

J Nutr. 2001 Aug;131(8):2197-203.

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Resveratrol is a naturally occurring polyphenol with cancer chemopreventive properties. The objective of the current study was to investigate the effect of resveratrol on the human colonic adenocarcinoma cell line Caco-2. The compound inhibited cell growth and proliferation of Caco-2 cells in a dose-dependent manner (12.5-200 micromol/L) as assessed by crystal violet assay, [(3)H]thymidine and [(14)C]leucine incorporation. Furthermore, apoptosis was determined by measuring caspase-3 activity, which increased significantly after 24 and 48 h of treatment with 200 micromol/L resveratrol. Perturbed cell cycle progression from the S to G2 phase was observed for concentrations up to 50 micromol/L, whereas higher concentrations led to reversal of the S phase arrest. These effects were specific for resveratrol; they were not observed after incubation with the stilbene analogs stilbenemethanol and rhapontin. Levels of cyclin D1 and cyclin-dependent kinase (cdk) 4 proteins were decreased, as revealed by immunoblotting. In addition, resveratrol enhanced the expression of cyclin E and cyclin A. The protein levels of cdk2, cdk6 and proliferating cell nuclear antigen were unaffected. Similar results were obtained for the colon carcinoma cell line HCT-116, indicating that cell cycle inhibition by resveratrol is independent of cyclooxygenase inhibition. The phosphorylation state of the retinoblastoma protein in Caco-2 cells was shifted from hyperphosphorylated to hypophosphorylated at 200 micromol/L, which may account for reversal of the S phase block at concentrations exceeding 50 micromol/L. These findings suggest that resveratrol exerts chemopreventive effects on colonic cancer cells by inhibition of the cell cycle.

Inhibition of protein kinase CKII activity by resveratrol, a natural compound in red wine and grapes.

Life Sci. 2002 Sep 20;71(18):2145-52.

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Resveratrol is a phytoalexin found in grapes and other foods that has been shown to have anticancer and anti-inflammatory effects. Because protein kinase CKII is involved in cell proliferation and oncogenesis, we examined whether resveratrol could modulate CKII activity. Resveratrol was shown to inhibit the phosphotransferase activity of CKII with IC (50) of about 10 microM. Steady state studies revealed that resveratrol acted as a competitive inhibitor with respect to the substrate ATP. A value of 1.2 microM was obtained for the apparent K(i). Resveratrol also inhibited the catalytic reaction of CKII with GTP as substrate. Furthermore, resveratrol inhibits endogenous CKII activity on protein substrates in HeLa cell lysates. These results suggest that resveratrol is likely to function by inhibiting oncogenic disease, at least in part, through the inhibition of CKII activity.

Involvement of HSP70 in resveratrol-induced apoptosis of human prostate cancer.

Anticancer Res. 2003 Nov-Dec;23(6C):4921-6

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BACKGROUND: 70 KDa heat shock proteins (HSPs70), either as a constitutive or inducible form, are expressed at very high levels in malignant human tumors of various origin. In different cell types, they are known to play an antiapoptotic role. Resveratrol (3,5,4'-trihydroxy-trans-stilbene), a polyphenol present in red wine, grapes and other dietary and medicinal plants, has been shown to be active in inhibiting multistage carcinogenesis, inducing apoptotic cell death. MATERIALS AND METHODS: With the present study, a possible relationship between HSP70 expression and cell death elicited by resveratrol in DU-145 cells, which mimic the late hormone-refractory stages of prostate carcinoma, was investigated. To this end, we treated DU-145 with different concentrations. (50, 100 and 200 microM) of resveratrol and cell viability, by tetrazolium salts assay (MTT) and membrane breakdown, by lactic dehydrogenase (LDH) release, were measured. The possible induction of oxidative stress was evidenced both by performing a fluorescent analysis of intracellular reactive oxygen species (ROS) production, or evaluating the amount of nitrite/nitrate (NO) in culture medium. In addition, the expression of HSP70 level, evaluated by immunoblotting, was examined and compared with caspase-3 activity (fluorimetrically measured) and DNA damage, determined by Single Cell Gel Electrophoresis or COMET assay. RESULTS: Our data clearly indicate that the addition of resveratrol to DU-145 reduces cell viability and increases membrane breakdown, in a dose-dependent way, without interfering with ROS production or NO synthesis, unless 200 microM resveratrol was added. Furthermore, at low concentration (50-100 microM) resveratrol is able to raise HSP70 levels but, at high concentration (200 microM), the measured levels of protective HSP70 were unmodified with respect to that of the control values. CONCLUSION: Our results confirm the ability of resveratrol to suppress the proliferation of human prostate cancer cells with a typical apoptotic feature, interfering with the expression of HSPs70.

Resveratrol induces apoptosis in human esophageal carcinoma cells.

World J Gastroenterol. 2003 Mar;9(3):408-11

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AIM: To investigate the apoptosis in esophageal cancer cells induced by resveratrol, and the relation between this apoptosis and expression of Bcl-2 and Bax. METHODS: In in vitro experiments, MTT assay was used to determine the cell growth inhibitory rate. Transmission electron microscope and TUNEL staining method were used to quantitatively and qualitively detect the apoptosis status of esophageal cancer cell line EC-9706 before and after the resveratrol treatment. Immunohistochemical staining was used to detect the expression of apoptosis-regulated gene Bcl-2 and Bax. RESULTS: Resveratrol inhibited the growth of esophageal cancer cell line EC-9706 in a dose-and time-dependent manner. Resveratrol induced EC-9706 cells to undergo apoptosis with typically apoptotic characteristics, including morphological changes of chromatin condensation, chromatin crescent formation, nucleus fragmentation and apoptotic body formation. TUNEL assay showed that after the treatment of EC-9706 cells with resveratrol (10 mmol/L) for 24 to 96 hours, the AIs were apparently increased with treated time ($P<0.05$). Immunohistochemical staining showed that after the treatment of EC-9706 cells with resveratrol (10 mmol/L) for 24 to 96 hours, the PRs of Bcl-2 proteins were apparently reduced with treated time ($P<0.05$) and the PRs of Bax proteins were apparently increased with treated time ($P<0.05$). CONCLUSION: Resveratrol is able to induce the apoptosis in esophageal cancer. This apoptosis may be mediated by down-regulating the apoptosis-regulated gene Bcl-2 and up-regulating the expression of apoptosis-regulated gene bax.

Pro-oxidant activity of low doses of resveratrol inhibits hydrogen peroxide-induced apoptosis.

Ann N Y Acad Sci. 2003 Dec;1010:365-73.

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We have recently shown that efficient apoptotic signaling is a function of a permissive intracellular milieu created by a decrease in the ratio of superoxide to hydrogen peroxide and cytosolic acidification. Resveratrol, a phytoalexin found in grapes and wines, triggers apoptosis in some systems and inhibits the death signal in others. In this regard, the reported inhibitory effect on hydrogen peroxide-induced apoptosis has been attributed to its antioxidant property. Here, we provide evidence that exposure of human leukemia

cells to low concentrations of resveratrol (4-8 micro M) inhibits caspase activation and DNA fragmentation induced by incubation with hydrogen peroxide or upon triggering apoptosis with a novel compound that kills via intracellular hydrogen peroxide production. At these concentrations, resveratrol elicits pro-oxidant properties as evidenced by an increase in intracellular superoxide concentration. This pro-oxidant effect is further supported by our observations that the drop in intracellular superoxide and cytosolic acidification induced by hydrogen peroxide is completely blocked in cells preincubated with resveratrol. Thus, the inhibitory effect of resveratrol on hydrogen peroxide-induced apoptosis is not due to its antioxidant activity, but contrarily via a pro-oxidant effect that creates an intracellular environment nonconducive for apoptotic execution.

Possible candidates for the compound which is expected to attenuate dioxin toxicity]

Fukuoka Igaku Zasshi. 2005 May;96(5):204-13.

[Article in Japanese]

Ishii Y, **Ishida T**, **Mutoh J**, **Yamada H**, **Oguri K**.

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This review deals with the three candidate compounds which may combat with dioxins' toxicity. Geranylgeranylacetone (GGA), an antiulcer drug, counteracts suppression of body weight gain and lethality produced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in C57BL/6J mice. Similarly, curcumin, a food ingredient anticipates the TCDD's toxicity on body weight gain. Both GGA and curcumin had no effect on the induction of hepatic ethoxyresorufin O-deethylase activity by TCDD. These data suggest that both compounds exhibit a protective effect against some forms of dioxin toxicity by a mechanism without involving inhibition of aryl hydrocarbon receptor activation. Further, the mechanism involved in resveratrol action on dioxin's toxicity was also discussed. Prior to the application of these compounds to Yusho patients, the limitation and possibility of these candidate compounds are seemed to be further studied.

Resveratrol enhances the expression of non-steroidal anti-inflammatory drug-activated gene (NAG-1) by increasing the expression of p53.

Carcinogenesis. 2002 Mar;23(3):425-34.

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Dietary phenolic substances including resveratrol, a stillbene compound, are found in several fruits and vegetables, and these compounds have been reported to have anti-oxidant, anti-inflammatory and antitumorigenic activities. However, the molecular mechanisms underlying the antitumorigenic or chemopreventive activities of these compounds remain largely unknown. The expression of NAG-1 [non-steroidal anti-inflammatory (NSAID) drug-activated gene-1], a member of the transforming growth factor-beta (TGF-beta) superfamily, has been shown to be associated with pro-apoptotic and antitumorigenic activities. Here, we have demonstrated that resveratrol induces NAG-1 expression and apoptosis in a concentration-dependent manner. Resveratrol increases the expression of p53, tumor suppressor protein, prior to NAG-1 induction, indicating that NAG-1 expression by resveratrol is mediated by p53 expression. We also show that the p53 binding sites within the promoter region of NAG-1 play a pivotal role to control NAG-1 expression by resveratrol. Derivatives of resveratrol were examined for NAG-1 induction, and the data suggest that resveratrol-induced NAG-1 and p53 induction is not dependent on its anti-oxidant activity. The data may provide linkage between p53, NAG-1 and resveratrol, and in part, a new clue to the molecular mechanism of the antitumorigenic activity of natural polyphenolic compounds.

Effects of food factors on signal transduction pathways.

Biofactors. 2000;12(1-4):17-28.

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Consumption of plant-derived foods, especially fruits and vegetables, has been linked to decreased risk of cancer. Laboratory studies with animals and cells in culture have shown cancer preventive activity of chemicals isolated from soy, tea, rice and many green, yellow and orange fruits and vegetables. Using cell culture, transgenic mice and knockout mice models to examine the anti-cancer effects of these dietary factors at the molecular level, we found that (11) (-)-epigallocatechin gallate (EGCG), the major active polyphenol in green tea, and theaflavins, the major active components in black tea, inhibit epidermal growth factor (EGF)- or 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced JB6 cell transformation. At the same dose range that inhibited cell transformation, EGCG

and theaflavins inhibited activator protein-1 (AP-1) activation. These compounds also inhibited ultraviolet B (UVB)-induced AP-1 and nuclear factor kappa B (NFkappaB)-dependent transcriptional activation; (2) resveratrol, found at high levels in grapes, inhibited cell transformation through the induction of apoptosis, mediated through JNK and p53-dependent pathways; (3) inositol hexaphosphate (InsP6), an active compound from rice and other grains, inhibited TPA- or EGF-induced transformation and signal transduction through its effects on phosphatidylinositol-3 kinase (PI-3) kinase; (4) phenethyl isothiocyanate (PEITC), which occurs as a conjugate in certain cruciferous vegetables, inhibited cell transformation corresponding with the induction of apoptosis. An elevation of p53 is required for PEITC-induced apoptosis. Our studies indicated that the chemopreventive effect of these food factors may be mediated by their effects on different signal transduction pathways; (5) retinoids (vitamin A and its metabolites) inhibited tumor promoter-induced cell transformation and tumor promotion in transgenic mice through the inhibition of AP-1 action but not through the activation of retinoic acid response element (RARE).

Substrate-specific activation of sirtuins by resveratrol.

J Biol Chem. 2005 Apr 29;280(17):17038-45. Epub 2005 Jan 31.

Kaeberlein M, **McDonagh T**, **Heltweg B**, **Hixon J**, **Westman EA**, **Caldwell SD**, **Napper A**, **Curtis R**, **DiStefano PS**, **Fields S**, **Bedalov A**, **Kennedy BK**.

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Resveratrol, a small molecule found in red wine, is reported to slow aging in simple eukaryotes and has been suggested as a potential calorie restriction mimetic. Resveratrol has also been reported to act as a sirtuin activator, and this property has been proposed to account for its anti-aging effects. We show here that resveratrol is a substrate-specific activator of yeast Sir2 and human SirT1. In particular, we observed that, in vitro, resveratrol enhances binding and deacetylation of peptide substrates that contain Fluor de Lys, a non-physiological fluorescent moiety, but has no effect on binding and deacetylation of acetylated peptides lacking the fluorophore. Consistent with these biochemical data we found that in three different yeast strain backgrounds, resveratrol has no detectable effect on Sir2 activity in vivo, as measured by rDNA recombination, transcriptional silencing near telomeres, and life span. In light of these findings, the mechanism accounting for putative longevity effects of resveratrol should be reexamined.

Wine antioxidant polyphenols inhibit the proliferation of human prostate cancer cell lines.

Nutr Cancer. 2000;37(2):223-33

Kampa M, **Hatzoglou A**, **Notas G**, **Damianaki A**, **Bakogeorgou E**, **Gemetzi C**, **Kouroumalis E**, **Martin PM**, **Castanas E**.

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The effect of different wine antioxidant polyphenols (catechin, epicatechin, quercetin, and resveratrol) on the growth of three prostate cancer cell lines (LNCaP, PC3, and DU145) was investigated. A dose- and time-dependent inhibition of cell growth by polyphenols was found at nanomolar concentrations. The proliferation of LNCaP and PC3 cells was preferentially inhibited by flavonoids (catechin, epicatechin, and quercetin), whereas resveratrol was the most potent inhibitor of DU145 cell growth. Possible mechanisms of action were investigated: 1) The competition of polyphenols for androgen binding in LNCaP cells revealed significant interaction only in the case of high concentrations of quercetin, at least at five orders of magnitude higher than the concentrations needed for cell growth inhibition. All other phenols showed low interactions. 2) Oxygen species production after mitogen stimulation and H2O2 sensitivity of these cell lines did not correlate with the observed antiproliferative effects, ruling out such a mode of action. 3) NO production revealed two different patterns: LNCaP and DU145 cells produced high concentrations of NO, whereas PC3 cells produced low concentrations. Phorbol ester stimulation of cells did not reveal any additional effect in LNCaP and DU145 cells, whereas it enhanced the secretion of NO in PC3 cells. Polyphenols decreased NO secretion. This effect correlates with their antiproliferative action and the inhibition of inducible NO synthase. It is therefore proposed that the antiproliferative effect of polyphenols is mediated through the modulation of NO production. In conclusion, our data show a direct inhibitory effect of low concentrations of antioxidant wine phenols on the proliferation of human prostate cancer cell lines mediated by the production of NO, further suggesting potential beneficial effects of wine and other phenol-containing foods or drinks for the control of prostate cancer cell growth.

Resveratrol- induced apoptosis is mediated by p53-dependent pathway in Hep G2 cells.

Life Sci. 2002 Nov 22;72(1):23-34

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Resveratrol, a phytoalexin found in many plants, has been reported to possess a wide range of pharmacological properties and is one of the promising chemopreventive agents for cancer. Here, we examined the antiproliferation effect of resveratrol in two human liver cancer cell lines, Hep G2 and Hep 3B. Our results showed that resveratrol inhibited cell growth in p53-positive Hep G2 cells only. This anticancer effect was a result of cellular apoptotic death induced by resveratrol via the p53-dependent pathway. Here we demonstrated that the resveratrol-treated cells were arrested in G1 phase and were associated with the increase of p21 expression. In addition, we also illustrated that the resveratrol-treated cells had enhanced Bax expression but they were not involved in Fas/APO-1 apoptotic signal pathway. In contrast, the p53-negative Hep 3B cells treated with resveratrol did not show the antiproliferation effect neither did they show significant changes in p21 nor Fas/APO-1 levels. In summary, our study demonstrated that the resveratrol effectively inhibited cell growth and induced programmed cell death in Hepatoma cells on a molecular basis. Furthermore, these results implied that resveratrol might also be a new potent chemopreventive drug candidate for liver cancer as it played an important role to trigger p53-mediated molecules involved in the mechanism of p53-dependent apoptotic signal pathway.

Anticancer activity of resveratrol on implanted human primary gastric carcinoma cells in nude mice.

World J Gastroenterol. 2005 Jan 14;11(2):280-4

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AIM: To investigate the apoptosis of implanted primary gastric cancer cells in nude mice induced by resveratrol and the relation between this apoptosis and expression of bcl-2 and bax. METHODS: A transplanted tumor model was established by injecting human primary gastric cancer cells into subcutaneous tissue of nude mice. Resveratrol (500 mg/kg, 1,000 mg/kg and 1,500 mg/kg) was directly injected beside tumor body 6 times at an interval of 2 d. Then changes of tumor volume were measured continuously and tumor inhibition rate of each group was calculated. We observed the morphologic alterations by electron microscope, measured the apoptotic rate by TUNEL staining method, detected the expression of apoptosis-regulated genes bcl-2 and bax by immunohistochemical staining and PT-PCR. RESULTS: Resveratrol could significantly inhibit carcinoma growth when it was injected near the carcinoma. An inhibitory effect was observed in all therapeutic groups and the inhibition rate of resveratrol at the dose of 500 mg/kg, 1,000 mg/kg and 1,500 mg/kg was 10.58%, 29.68% and 39.14%, respectively. Resveratrol induced implanted tumor cells to undergo apoptosis with apoptotic characteristics, including morphological changes of chromatin condensation, chromatin crescent

formation, nucleus fragmentation. The inhibition rate of 0.2 mL of normal saline solution, 1,500 mg/kg DMSO, 500 mg/kg resveratrol, 1 000 mg/kg resveratrol, and 1 500 mg/kg resveratrol was 13.68+/-0.37%, 13.8+/-0.43%, 48.7+/-1.07%, 56.44+/-1.39% and 67 +/-0.96%, respectively. The positive rate of bcl-2 protein of each group was 29.48 +/-0.51%, 27.56+/-1.40%, 11.86+/-0.97%, 5.7+/-0.84% and 3.92+/-0.85%, respectively by immunohistochemical staining. The positive rate of bax protein of each group was 19.34+/-0.35%, 20.88+/-0.91%, 40.02+/-1.20%, 45.72+/-0.88% and 52.3+/-1.54%, respectively by immunohistochemical staining. The density of bcl-2 mRNA in 0.2 mL normal saline solution, 1,500 mg/kg DMSO, 500 mg/kg resveratrol, 1,000 mg/kg resveratrol, and 1,500 mg/kg resveratrol decreased progressively and the density of bax mRNA in 0.2 mL normal saline solution, 1,500 mg/kg DMSO, 500 mg/kg resveratrol, 1,000 mg/kg resveratrol, and 1,500 mg/kg increased progressively with elongation of time by RT-PCR. CONCLUSION: Resveratrol is able to induce apoptosis of transplanted tumor cells. This apoptosis may be mediated by down-regulating apoptosis-regulated gene bcl-2 and up-regulating the expression of apoptosis-regulated gene bax.

Differential effects on growth, cell cycle arrest, and induction of apoptosis by resveratrol in human prostate cancer cell lines.

Exp Cell Res. 1999 May 25;249(1):109-15

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Epidemiologic studies have suggested that nutrition plays an important role in carcinogenesis and that 30% of cancer morbidity and mortality can potentially be prevented with proper adjustment of diets. Resveratrol, a polyphenol present in red wines and a variety of human foods, has recently been reported to exhibit chemopreventive properties when tested in a mouse skin cancer model system. In this study, we investigated the effects of resveratrol on growth, induction of apoptosis, and modulation of prostate-specific gene expression using cultured prostate cancer cells that mimic the initial (hormone-sensitive) and advanced (hormone-refractory) stages of prostate carcinoma. Androgen-responsive LNCaP and androgen-nonresponsive DU-145, PC-3, and JCA-1 human prostate cancer cells were cultured with different concentrations of resveratrol (2. 5 x 10(-5)-10(-7) M). Cell growth, cell cycle distribution, and apoptosis were determined. Addition of 2.5 x 10(-5) M resveratrol led to a substantial decrease in growth of LNCaP and in PC-3 and DU-145 cells, but only had a modest inhibitory effect on proliferation of JCA-1 cells. Flow cytometric analysis showed resveratrol to partially disrupt G1/S transition in all three androgen-nonresponsive cell lines, but had no effect in the androgen-responsive LNCaP cells. In difference to the androgen-nonresponsive

prostate cancer cells however, resveratrol causes a significant percentage of LNCaP cells to undergo apoptosis and significantly lowers both intracellular and secreted prostate-specific antigen (PSA) levels without affecting the expression of the androgen receptor (AR). These results suggest that resveratrol negatively modulates prostate cancer cell growth, by affecting mitogenesis as well as inducing apoptosis, in a prostate cell-type-specific manner. Resveratrol also regulates PSA gene expression by an AR-independent mechanism.

Grape-derived chemopreventive agent resveratrol decreases prostate-specific antigen (PSA) expression in LNCaP cells by an androgen receptor (AR)-independent mechanism.

Anticancer Res. 2000 Jan-Feb;20(1A):225-8.

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Trans-resveratrol, a polyphenol present in red wines and various human foods, inhibited the proliferation of LNCaP cells and the expression of a prostate specific gene, PSA. A 4-day treatment with resveratrol reduced the levels of intracellular and secreted PSA by approximately 80%, as compared to controls. To test whether this decrease was coordinated with changes in AR expression, levels of AR were assayed by Western blot analysis, using the cognate antibody, or by binding with the radioactive ligand methyltrienolone [3H]R1881. With either assay, little or no change in AR expression could be detected between control and resveratrol-treated cells. Thus, it would appear that the prostate tumor marker PSA is down regulated by resveratrol, by a mechanism independent of changes in AR.

Resveratrol inhibits the expression and function of the androgen receptor in LNCaP prostate cancer cells.

Cancer Res. 1999 Dec 1;59(23):5892-5

Mitchell SH, **Zhu W**, **Young CY**.

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Androgens via their receptor (AR) may play a role in prostate cancer etiology. This study focuses on the inhibitory effects of resveratrol on androgen action in the LNCaP prostate cancer cell line. We found that resveratrol represses different classes of androgen up-regulated genes at the protein or mRNA level including prostate-specific antigen, human glandular kallikrein-2, AR-specific coactivator ARA70, and the cyclin-dependent kinase inhibitor p21. This inhibition is likely attributable to a reduction in AR contents at the transcription level, inhibiting androgen-stimulated cell growth and gene expression. This study suggests that resveratrol may be a useful chemopreventive/chemotherapeutic agent for prostate cancer.

Resveratrol, a natural phenolic compound, inhibits cell proliferation and prevents oxidative DNA damage.

Mutat Res. 2001 Sep 20;496(1-2):171-80

Sgambato A, **Ardito R**, **Faraglia B**, **Boninsegna A**, **Wolf FI**, **Cittadini A**.

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Resveratrol (3,4',5-trihydroxystilbene) is a naturally occurring phenolic compound which is present at high levels in wine and has been recently proposed as a potential cancer chemopreventive and chemoterapeutic agent. In this study, we evaluated the antiproliferative activity of resveratrol on a panel of cell lines of various histogenetic origin, including normal rat fibroblasts and mouse mammary epithelial cells compared to human breast, colon and prostate cancer cells. The concentration of resveratrol inhibiting cell growth by 50% (IC(50)) ranged from about 20 to 100 microM. At such concentration, we were unable to detect a significant increase in the apoptotic index in most of the cell lines analyzed.We also studied the effects of resveratrol on cell cycle distribution. The most striking effect was a reduction in the percentage of cells in the G2/M phase which was most frequently associated with an increase of cells in the S phase of the cell cycle. We also found that resveratrol is able to prevent the increase in reactive oxygen species (ROS) following exposure to oxidative agents (i.e. tobacco-smoke condensate (TAR) and H(2)O(2)). Resveratrol also reduced nuclear DNA fragmentation, as assessed by single cell gel electrophoresis (comet test). Taken together our results suggest that resveratrol can act as an antimutagenic/anticarcinogenic agent by preventing oxidative DNA damage which plays a pivotal role in the carcinogenic activity of many genotoxic agents.

Antioxidant activity

In vitro biological activity of prenylflavanones.

Anticancer Res. 2001 Jan-Feb;21(1A):275-80

Shirataki Y, **Motohashi N**, **Tani S**, **Sakagami H**, **Satoh K**, **Nakashima H**, **Mahapatra SK**, **Ganguly K**, **Dastidar SG**, **Chakrabarty AN**.

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The biological activity of ten prenylflavanones purified from Sophora tomentosa L., and Sophora moorcroftiana Benth. ex Baker (Leguminosae) was investigated. The flavanones with prenyl-, lavandulyl- or geranyl groups on A ring, and two bioactive flavonostilbenes on ring B and stilbene (resveratrol) showed tumor-specific cytotoxic activity, antimicrobial activity, and anti-HIV activity, radical generation, and O2- scavenging activity. There was a positive relationship between radical generation and O2- scavenging activity in these prenylflavanones. These data suggest the medicinal significance of prenylflavanones.

Resveratrol, a polyphenol found in wine, reduces ischemia reperfusion injury in rat kidneys.

J Cardiovasc Pharmacol. 2001 Mar;37(3):262-70

Giovannini L, **Migliori M**, **Longoni BM**, **Das DK**, **Bertelli AA**, **Panichi V**, **Filippi C**, **Bertelli A**.

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Reactive oxygen species have been implicated in the pathophysiology of renal ischemia reperfusion injury. Antioxidants including polyphenolics have been found to protect renal cells from the cellular injury induced by ischemia and reperfusion. Resveratrol, a stilbene polyphenol found in grapes and red wine, has recently been found to protect isolated rat heart from ischemia reperfusion injury. This study was sought to determine if resveratrol could also protect renal cells from ischemic injury. Male Wistar rats were treated with control, resveratrol (0.23 microg/kg), vehicle used to solubilize resveratrol, and resveratrol plus L-NAME (15 mg/kg body wt), a nitric oxide blocker. Our results demonstrated that resveratrol administration reduced the mortality of ischemic rats from 50% to 10% and renal damage was reduced as indicated by histologic examination and serum creatinine level. The short-term administration of resveratrol also inhibited renal

lipid peroxidation induced by ischemia and reperfusion both in cortex and in medulla. Electron paramagnetic resonance detected an increased formation of nitric oxide in the resveratrol-treated kidney that was reduced to the baseline value after treating the rats with L-NAME in addition to resveratrol. The results suggest that resveratrol reduced the renal ischemia reperfusion injury through a nitric oxide-dependent mechanism.

The protective effects of resveratrol against changes in blood platelet thiols induced by platinum compounds.

J Physiol Pharmacol. 2004 Jun;55(2):467-76

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Cisplatin (cis-diamminedichloroplatinum II, cisPt) is especially useful in the treatment of epithelial malignancies, however, the use of cisplatin is accompanied by several toxicities including haematological toxicity. Contrary to cisplatin, selenium-cisplatin conjugate ((NH(3))(2)Pt(SeO(3)); Se-Pt) has only a slight toxicity effect on blood platelet function. In the mechanism of platinum compounds action on platelets thiols are involved. The aim of the present studies was to examine in vitro how trans-resveratrol (trans-3,4',5-trihydroxystilbene) acts on the levels of platelet glutathione (GSH) and other thiol-containing compounds and how, as an antioxidant, protecs blood platelets against the oxidative stress caused by platinum compounds (cisPt and Se-Pt). To analyse the level of thiols in human blood platelets treated with platinum compounds and with resveratrol the classical technique HPLC has been used. Blood platelets isolated by differential centrifugation of human blood were incubated (30 min, 37 degrees C) with cisPt or Se-Pt at dose of 10 microg/ml that inhibits platelet function and with resveratrol (25 microg/ml). The obtained results indicate that platinum compounds caused in platelets a decrease of both, reduced glutathione (GSH) and free thiols of cysteine (CSH) and cysteinylglycine (CGSH). The pool of these compounds in unreduced form was increased. Platinum compounds caused the reduction of platelet protein thiols. Resveratrol (after 30 min action) at the concentration of 25 microg/ml partly reduced the platinum compounds induced decrease of platelet thiols, particularly thiols in acid-soluble fraction.

On-line EPR study of free radicals induced by peroxidase/H(2)O(2) in human low-density lipoprotein.

Biochim Biophys Acta. 2002 Jul 11;1583(2):176-84

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The aim of this study was to use direct electron paramagnetic resonance (EPR) spectroscopy at 37 degrees C and spin trapping techniques to study radical species formed during horseradish peroxidase/H(2)O(2)-initiated low-density lipoprotein (LDL) oxidation. Using direct EPR, we obtained evidence for the formation not only of the alpha-tocopheroxyl radical but also of a protein radical(s), assigned to a tyrosyl radical(s) of apolipoprotein B-100 (apo B-100). Spin trapping with 2-methyl-2-nitrosopropane revealed (i) the formation of a mobile adduct with beta-hydrogen coupling assigned to a lipid radical and (ii) a partially immobilised adduct detected in LDL as well as in apo B-100, assigned after proteolytic digestion to the trapping of a radical centred on a tertiary carbon atom of an aromatic residue, probably tyrosine. Our results support the hypothesis that radicals are initiators of the oxidative process, and show that their formation is an early event in peroxidase-mediated oxidation. We also tested the effects of resveratrol (RSV), a polyphenolic antioxidant present in red wine. Our data indicate that 1-10 microM RSV is able to accelerate alpha-tocopherol consumption, conjugated dienes formation and the decay kinetics of LDL-centred radicals. Since phenols are substrates for peroxidases, this result may be ascribed to a RSV-mediated catalysis of peroxidase activity.

[The Mediterranean diet--healthy but and still delicious]

Ther Umsch. 2000 Mar;57(3):167-72

[Article in German]

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Mediterranean diets are characterized by a high portion of monounsaturated fatty acids (in particular olive oil) and a low intake of saturated fatty acids (from animal origin apart from fish). Moreover, high intakes of fruits, vegetables, cereals (in form of bread), and moderate (regular) wine consumption are typical for the mediterranean diet. Contrary to the current opinion, extra virgin olive oil has no advantage compared to ordinary olive oil. Canola oil is an alternative to olive oil because of its similar fatty acid composition. Based on the new medical literature, mediterranean diet has strong secondary preventive effects after myocardial infarction resulting in a decrease of total mortality, cardiac death and non fatal reinfarction. The high portion of monounsaturated fatty acids in olive oil

cause a decrease in LDL cholesterol and an increase in HDL cholesterol. In addition, antioxidative vitamins such as vitamin E, C and beta-carotene and phenolic substances such as the flavonoids, e.g. quercetin and resveratrol, decrease the oxidation of LDL cholesterol and thus atherogenicity. Mediterranean diet has at least no negative effects in the initiation and promotion of cancer, however, its potential positive effects are so far less well known.

Comparison of the antioxidant effects of equine estrogens, red wine components, vitamin E, and probucol on low-density lipoprotein oxidation in postmenopausal women.

Menopause. 2001 Nov-Dec;8(6):408-19

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OBJECTIVE: Oxidized low-density lipoprotein (LDL) seems to play an important role in the etiology of atherosclerosis. To further study this, we performed two studies: (1) we determined the ability of 10 estrogen components of the drug, conjugated equine estrogen (CEE), trans-resveratrol (t-resveratrol) and quercetin (red wine components), trolox (vitamin E analog), and probucol (a serum cholesterol-lowering drug) to delay or prevent the oxidation of plasma LDL isolated from untreated postmenopausal women, and (2) we assessed the effect of long-term (>1 year) estrogen replacement therapy and hormone replacement therapy on LDL oxidation by ex vivo methods. DESIGN: For the in vivo study, three groups of postmenopausal women were selected based on whether they were on long-term CEE therapy (group A: 0.625 mg CEE; n = 21), on combination CEE plus progestogen therapy (group B: 0.625 mg CEE + 5.0 mg medroxyprogesterone acetate, 10 days; n = 20), or not on any hormone therapy (group C; n = 37). For the in vitro study, only LDL samples obtained from group C were used. The kinetics of LDL oxidation were measured by continuously monitoring the formation of conjugated dienes followed by determination of the lag time. RESULTS: All compounds tested protected the LDL from oxidative damage. The relative antioxidant potency of estrogen components was generally greater than that of the other compounds. The minimum dose (nmoles) required to double the lag time from the control lag time of 57 +/- 2 min was 0.47 for 17beta-dihydroequilenin, 17alpha-dihydroequilenin, Delta 8 -estrone; 0.6 to 0.7 for Delta 8 -17beta-estradiol, equilenin, and quercetin; 0.9 for 17beta-dihydroequilin and 17alpha-dihydroequilin; 1.3 for equilin, estrone, 17beta-estradiol, 17alpha-estradiol; 1.4 for trolox; 1.9 for probucol; and 3.0 for t-resveratrol. The data from the in vivo study indicate that after long-term estrogen replacement therapy (group A) and hormone replacement therapy (group B), the LDL was significantly ($p < 0.01$) protected (higher lag time) against oxidation compared with the control (group C). There was no difference between groups

A and B. CONCLUSIONS: The oxidation of LDL isolated from postmenopausal women is inhibited differentially by various estrogens and other antioxidants. The unique ring B unsaturated estrogen components of CEE were the most potent, and t-resveratrol, the red wine component, was the least potent. Long-term CEE or CEE + medroxyprogesterone acetate administration to postmenopausal women protects the LDL against oxidation to the same extent. These combined data support the hypothesis that some of the cardioprotective benefits associated with CEE therapy and perhaps red wine consumption may be due to the ability of their components to protect LDL against oxidative modifications.

Class A scavenger receptor up-regulation in smooth muscle cells by oxidized low density lipoprotein. Enhancement by calcium flux and concurrent cyclooxygenase-2 up-regulation.

J Biol Chem. 2000 Jun 9;275(23):17661-70.

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Oxidative stress caused by phorbol esters or reactive oxygen up-regulates the class A scavenger receptor (SR-A) in human smooth muscle cells (SMC), which normally do not express this receptor. The increase in SR-A expression correlates with activation of the redox-sensitive transcription factors activating protein-1 c-Jun and CCAAT enhancer-binding protein beta. Here we show that coincubation of SMC with macrophages or oxidized low density lipoproteins (LDL) from macrophage-conditioned medium activates these same regulatory pathways and stimulates SR-A expression. The increased SR-A gene transcription induced by cell-oxidized LDL up-regulated SR-A mRNA and increased by 30-fold the uptake of acetyl LDL, a ligand for the SR-A. Copper-oxidized LDL also increased SR-A receptor expression. Oxidized LDL with a lipid peroxide level of 80-100 nmol/mg of LDL protein and an electrophoretic mobility approximately 1.5 times that of native LDL exhibited the greatest bioactivity. Inhibition of calcium flux suppressed SR-A induction by oxidized LDL. Conversely, calcium ionophore greatly enhanced SR-A up-regulation by oxidized LDL or other treatments that promote intracellular oxidative stress. This enhancement was dependent upon concurrent up-regulation of SMC cyclooxygenase-2 expression and activity and was blocked by the cyclooxygenase-2 inhibitors NS-398 and Resveratrol. In THP-1 cells, oxidized LDL induced monocyte-to-macrophage differentiation and increased SR-A expression. These findings support a role for mildly oxidized LDL in the redox regulation of macrophage differentiation and SR-A expression and suggest that increased vascular oxidative stress may contribute to the formation of both SMC and macrophage foam cells.

Resveratrol inhibits copper ion-induced and azo compound-initiated oxidative modification of human low density lipoprotein.

Biochem Mol Biol Int. 1999 Jun;47(6):1089-96.

Zou JG, **Huang YZ**, **Chen Q**, **Wei EH**, **Hsieh TC**, **Wu JM**.

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To investigate whether resveratrol, a polyphenolic compound in red wine, affects the oxidation of human low density lipoprotein (LDL), LDL purified from normolipidemic subjects was subjected to Cu(2+)-induce and azo compound-initiated oxidative modification, with and without the addition of varying concentrations of resveratrol. Modification of LDL was assessed by the formation of thiobarbituric acid reactive substances (TBARS) and changes in the relative electrophoretic mobility (REM) of LDL on agarose gels. Resveratrol (50 microM) reduced TBARS and REM of LDL during Cu(2+)-induced oxidation by 70.5% and 42.3%, respectively ($p < 0.01$), and prolonged the lag phase associated with the oxidative modification of LDL by copper ion or azo compound. These in vitro results suggest that resveratrol may afford protection of LDL against oxidative damage resulting from exposure to various environmental challenges, possibly by acting as a free radical scavenger.

Effects of resveratrol on oxidative modification of human low density lipoprotein.

Chin Med J (Engl). 2000 Feb;113(2):99-102.

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OBJECTIVE: To determine the antioxidative effects of resveratrol (RES), a polyphenolic compound in red wine, on the oxidation of human low density lipoprotein (LDL) using two different oxidation systems. METHODS: Oxidation of LDL was induced by adding either Cu2+ or an azo compound. The extent of LDL modification was assessed by measuring the formation of thiobarbituric acid reactive substances (TBARS), the relative electrophoretic mobilities (REM), and the amount of oxidized LDL degradation by macrophages. RESULTS: During Cu(2+)-induced oxidation, RES reduced TBARS

formation in LDL by 70.5%, REM of LDL by 42.3% and the amount of macrophage degradation by 65.7%, respectively. The lag phase of LDL oxidation was also delayed by adding RES both in the copper ion and azo compound-induced oxidation systems. CONCLUSION: RES can protect LDL against both Cu(2+)-induced and azo compound-initiated oxidative modification in vitro, which might be due to its free radical scavenging capacity.

Effects of trans-resveratrol on copper-dependent hydroxyl-radical formation and DNA damage: evidence for hydroxyl-radical scavenging and a novel, glutathione-sparing mechanism of action.

Arch Biochem Biophys. 2000 Sep 15;381(2):253-63

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Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a natural product occurring in grapes and various other plants with medicinal properties. The phenolic antioxidant has been identified as a potential cancer chemopreventative agent and its presence in red wine has been suggested to be linked to the low incidence of heart disease in some regions of France. Recently, however, resveratrol was reported to promote DNA fragmentation in the presence of copper ions (K. Fukuhara and N. Miyata, 1998, Bioorg. Med. Chem. Lett. 8, 3187-3192), prompting us to investigate this phenomenon in mechanistic detail. By acting as a reducing agent, resveratrol was found to promote hydroxyl-radical (*OH) formation by DNA-bound Cu(H) ions. However, in the presence of either ascorbic acid or glutathione (i.e., under more physiological conditions), the phenolic lost this property and behaved as an antioxidant. In the ascorbate system, resveratrol had no effect on the rate of *OH formation, but protected DNA from damage by acting as a radical-scavenging antioxidant. In contrast, in the glutathione system, resveratrol inhibited *OH formation via a novel mechanism involving the inhibition of glutathione disulfide formation. We have concluded, therefore, that the DNA-damaging properties of resveratrol, identified recently by Fukuhara and Miyata, will be of no significance under physiological conditions. To the contrary, we have demonstrated that the phenolic behaves as a powerful antioxidant, both via classical, hydroxyl-radical scavenging and via a novel, glutathione-sparing mechanism.

Protection against damaged DNA in the single cell by polyphenols.

Pharmazie. 2002 Dec;57(12):852-4

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The protective properties of seven polyphenols against hydrogen peroxide induced DNA damage in human peripheral blood lymphocytes (PBL) were studied using single cell micro-gel electrophoresis. Hydrogen peroxide causes a concentration-dependent increase in single cell DNA strand breakage in human PBL. Quercetin and 7,8-dihydroxy-4-methyl coumarin exhibited the strongest protection, significantly inhibiting 50 microM H2O2-induced DNA damage at a range of concentrations of 3.1-25 microM. Curcumin, resveratrol and vanillin protected against DNA damage induced by 50 microM H2O2 at a range of concentrations of 6.25-25 microM, but rutin and 7-hydroxy-4-methyl coumarin failed to provide any protection even at concentrations up to 50 microM. Quercetin, 7,8-dihydroxy-4-methyl coumarin, curcumin, resveratrol and vanillin are therefore effective in protection of human single cell DNA from oxidative attack.

Effect of resveratrol on some activities of isolated and in whole blood human neutrophils.

Physiol Res. 2003;52(5):555-62

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Resveratrol, which is a polyphenol present in red wines and vegetables included in human diets, exerts many biological effects. The aim of the present study was to investigate its effect on some activities of polymorphonuclear leukocytes, particularly the generation of superoxide anion ((O2)(-)) in whole blood, hypochlorous acid (HOCl) and nitric oxide (NO) production by isolated cells, and chemotaxis. Resveratrol showed significant dose-dependent inhibitory effect on all these activities. In particular, it inhibited O2(-) generation in stimulated but not in resting neutrophils, decreased HOCl much more than O2(-) production indicating an effect on myeloperoxidase secretion since HOCl production is directly and proportionally dependent on O2(-) generation and reduced cell motility. The small dose of resveratrol (4.38 nM) used is attainable with a diet including red wine and vegetables confirming its protective role against some pathological processes such as inflammation, coronary heart disease, and cancer.

Resveratrol inhibits TCDD-induced expression of CYP1A1 and CYP1B1 and catechol estrogen-mediated oxidative DNA damage in cultured human mammary epithelial cells.

Carcinogenesis. 2004 Oct;25(10):2005-13. Epub 2004 May 13

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Resveratrol (3,5,4'-trihydroxystilbene), a naturally occurring phytoalexin present in grapes and other foods, has been reported to possess chemopreventive effects as revealed by its striking inhibition of diverse cellular events associated with tumor initiation, promotion and progression. In our present study, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), when treated with the cultured human mammary epithelial (MCF-10A) cells, induced the expression of cytochrome P450 1A1 (CYP1A1) and 1B1 (CYP1B1) that are responsible for the oxidation of 17beta-estradiol to produce catechol estrogens. Resveratrol strongly inhibited the TCDD-induced aryl hydrocarbon receptor (AhR) DNA binding activity, the expression of CYP1A1 and CYP1B1 and their catalytic activities in MCF-10A cells. It also reduced the formation of 2-hydroxyestradiol and 4-hydroxyestradiol from 17beta-estradiol by recombinant human CYP1A1 and CYP1B1, respectively. Furthermore, resveratrol significantly attenuated the intracellular reactive oxygen species (ROS) formation and oxidative DNA damage as well as the cytotoxicity induced by the catechol estrogens. Our data suggest that CYP1A1- and CYP1B1-catalyzed catechol estrogen formation might play a key role in TCDD-induced oxidative damage, and resveratrol can act as a potential chemopreventive against dioxin-induced human mammary carcinogenesis by blocking the metabolic formation of the catechol estrogens and scavenging the ROS generated during their redox cycling.

Inhibitory effects of resveratrol analogs on unopsonized zymosan-induced oxygen radical production.

Biochem Pharmacol. 1999 Mar 15;57(6):705-12

Jang DS, **Kang BS**, **Ryu SY**, **Chang IM**, **Min KR**, **Kim Y**.

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Resveratrol, a natural hydroxystilbene, has been reported to have anti-inflammatory and anticarcinogenic activities. Inhibitory effects of resveratrol and its analogs on reactive oxygen species (ROS) production in unopsonized zymosan-stimulated murine macrophage Raw264.7 cells, human monocytes, and neutrophils were analyzed to investigate if the anti-inflammatory and anticarcinogenic activities of resveratrol are related to the inhibition of ROS production. Resveratrol was a potent inhibitor of ROS production in both unopsonized zymosan-stimulated Raw264.7 cells and human monocytes and neutrophils. Resveratrol exhibited 50% inhibition values (IC50) of 17 microM in activated Raw264.7 cells, 18 microM in human monocytes, and 23 microM in human neutrophils. 3,5-Dihydroxy-4'-methoxystilbene or 3,4'-dimethoxy-5-hydroxystilbene exhibited IC50 values of 63 or 73 microM in Raw264.7 cells, 51 or >100 microM in human monocytes, and 10 or 37 microM in human neutrophils, respectively. Trimethylresveratrol, piceid, and 3,5-dihydroxy-4'-methoxystilbene-3-O-beta-D-glucoside were weak inhibitors of ROS production. Thus, resveratrol was identified as a potent inhibitor of ROS production, which might be one biochemical mechanism related to its anti-inflammatory and anticarcinogenic activities. The number and position of hydroxy substituents in resveratrol analogs seem to play an important role in the inhibitory potency of ROS production.

Chemoprevention of cancer and cardiovascular disease by resveratrol.

Proc Natl Sci Counc Repub China B. 1999 Jul;23(3):99-106

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Resveratrol (trans-3,4',5-trihydroxystibene) is a phytopolyphenol isolated from the seeds and skins of grapes. Recent studies indicate that resveratrol can block the process of multistep carcinogenesis, namely, tumor initiation, promotion and progression. Resveratrol can also reduce the risk of cardiovascular disease in man. The molecular mechanisms of resveratrol in chemoprevention of cancer and cardiovascular disease are interesting and under intensive investigation. Resveratrol was found to strongly inhibit nitric oxide (NO) generation in activated macrophages, as measured by the amount of nitrite released into the culture medium, and resveratrol strongly reduced the amount of cytosolic inducible nitric oxide synthase (iNOS) protein. The activation of nuclear factor kappa B (NF kappa B) induced by lipopolysaccharide (LPS) was inhibited by resveratrol. The phosphorylation and degradation of nuclear factor inhibitor kappa B alpha (I kappa B alpha) were inhibited by resveratrol simultaneously. Reactive oxygen species (ROS) are regarded as having carcinogenic potential and have been associated with tumor promotion. Resveratrol may act as a reactive oxygen species scavenger to suppress tumor

development. In addition, resveratrol may block multistep carcinogenesis through mitotic signal transduction blockade. Reactive oxygen species are pivotal factors in the genesis of heart disease. Meanwhile, efficient endogenous antioxidants, including superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase, are present in tissues. A fine balance between reactive oxygen species and endogenous antioxidants is believed to exist. Any disturbance of this balance in favor of reactive oxygen species causes an increase in oxidative stress and initiates subcellular changes, leading to cardiomyopathy and heart failure. The experimental results indicate that exogenous antioxidant resveratrol is of value in chemopreventing the development of heart disease. It is urgent that more efforts be made to investigate newer therapies employing antioxidants for the chemoprevention of cardiovascular disease and cancer.

Inhibition of cyclic strain-induced endothelin-1 gene expression by resveratrol.

Hypertension. 2003 Dec;42(6):1198-205. Epub 2003 Nov 17

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Resveratrol is a phytoestrogen naturally found in grapes and is among the major constituents of wine thought to have a cardioprotective effect. Endothelin-1 (ET-1) is a potent vasopressor synthesized by endothelial cells both in culture and in vivo. The aims of this study were to test the hypothesis that resveratrol may alter strain-induced ET-1 gene expression and to identify the putative underlying signaling pathways in endothelial cells. We show that resveratrol indeed potently inhibits strain-induced ET-1 secretion, ET-1 mRNA level, and ET-1 promoter activity. Resveratrol also inhibits strain-increased NADPH oxidase activity, reactive oxygen species formation, and extracellular signal-regulated kinases1/2 (ERK1/2) phosphorylation. Furthermore, pretreating cells with resveratrol or antioxidant N-acetyl-cysteine decreases strain-increased or hydrogen peroxide-increased ET-1 secretion, ET-1 promoter activity, and ET-1 mRNA and ERK1/2 phosphorylation. Using both the electrophoretic mobility shift assay and a reporter gene assay, resveratrol and N-acetyl-cysteine also attenuated the strain-stimulated activator protein-1 binding activity and activator protein-1 reporter activity. In summary, we demonstrate for the first time that resveratrol inhibits strain-induced ET-1 gene expression, partially by interfering with the ERK1/2 pathway through attenuation of reactive oxygen species formation. Thus, this study provides important new insights in the molecular pathways that may contribute to the proposed beneficial effects of resveratrol in the cardiovascular system.

Inhibition of the respiratory burst by resveratrol in human monocytes: correlation with inhibition of PI3K signaling.

Free Radic Biol Med. 2005 Jul 1;39(1):118-32. Epub 2005 Apr 1

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trans-Resveratrol (t-RVT) has been shown to have a wide range of anti-inflammatory properties, some of which have been suggested to contribute to the molecular explanation of the French Paradox, a possible reason for the low incidence of heart disease in France. The ability of t-RVT to inhibit the production of reactive oxygen species (ROS) from monocytes (differentiated U937) was investigated using isoluminol, luminol, lucigenin, and 2',7'-dichlorofluorescein (DCF). t-RVT (0.1-50 microM) was found to significantly inhibit cellular ROS production stimulated by f-Met-Leu-Phe (fMLP), 12-phorbol 13-myristate, and arachidonic acid after a 1-h preincubation. The efficacy of t-RVT could be increased if it was added directly into the assay. NADPH-dependent superoxide production was measured in cell homogenates and t-RVT (10-50 microM) was found to have no effect on this activity. The majority of these redox probes require a peroxidase to be oxidized; therefore, the inhibitory effect of t-RVT on ROS measured by these probes is complicated by its ability to be oxidized by peroxidase enzymes and thus compete with the probe. t-RVT, known to be oxidized by the horseradish peroxidase (HRP)/H(2)O(2) system, was found to inhibit the HRP-dependent oxidation of the fluorescent probe DCF and the chemiluminescent probe isoluminol. However, using a redox probe that did not require oxidation by a peroxidase (lucigenin), significant inhibition was still observed. Moreover, the inhibitory effects of t-RVT on fMLP-induced ROS production correlated with significant inhibitory effects on fMLP-induced phosphatidylinositol 3-kinase (PI3K) activity at 50 microM and Akt phosphorylation (10-50 microM). Other known inhibitors of both PI3K and Akt were also found to inhibit this response. Therefore, inhibition of signaling through the PI3K to NADPH oxidase by t-RVT might represent an important anti-inflammatory mechanism.

A reappraisal of the potential chemopreventive and chemotherapeutic properties of resveratrol.

Carcinogenesis. 2001 Aug;22(8):1111-7

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Resveratrol, a phytoalexin found in grapes and wines, has been reported to exhibit a wide range of pharmacological properties and is believed to play a role in the prevention of human cardiovascular disease (the so-called 'French paradox'). This molecule may also play a major role in both cancer prevention and therapy. In this review article we summarize the recent advances that have provided new insights into the molecular mechanisms underlying the promising properties of resveratrol. These include cyclooxygenase, nitric oxide synthase and cytochrome P450 inhibition, as well as cell cycle effects, apoptosis modulation and hormonal activity.

Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase.

Circulation. 2002 Sep 24;106(13):1652-8.

Wallerath T, **Deckert G**, **Ternes T**, **Anderson H**, **Li H**, **Witte K**, **Förstermann U**.

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BACKGROUND: Estrogens can upregulate endothelial nitric oxide synthase (eNOS) in human endothelial cells by increasing eNOS promoter activity and enhancing the binding activity of the transcription factor Sp1. Resveratrol, a polyphenolic phytoalexin found in grapes and wine, has been reported to act as an agonist at the estrogen receptor. Therefore, we tested the effect of this putative phytoestrogen on eNOS expression in human endothelial cells. METHODS AND RESULTS: Incubation of human umbilical vein endothelial cells (HUVEC) and HUVEC-derived EA.hy 926 cells with resveratrol for 24 to 72 hours upregulated eNOS mRNA expression in a time- and concentration-dependent manner (up to 2.8-fold). eNOS protein expression and eNOS-derived NO production were also increased after long-term incubation with resveratrol. Resveratrol increased the activity of the eNOS promoter (3.5-kb fragment) in a concentration-dependent fashion, with the essential trans-stimulated sequence being located in the proximal 263 bp of the promoter sequence. In addition, eNOS mRNA was stabilized by resveratrol. The effect of resveratrol on eNOS expression was not modified by the estrogen receptor antagonists ICI 182780 and RU 58668. In electrophoretic mobility shift assays, nuclear extracts from resveratrol-incubated EA.hy 926 cells showed no enhanced binding activity of the eNOS promoter-relevant transcription factors Sp1, GATA, PEA3, YY1, or Elf-1. In addition to its long-term effects on eNOS expression, resveratrol also enhanced the production of bioactive NO in the short-term (after a 2-minute incubation).

CONCLUSIONS: In concert with other effects, the stimulation of eNOS expression and activity may contribute to the cardiovascular protective effects attributed to resveratrol.

Divergent effects of resveratrol, a polyphenolic phytostilbene, on free radical levels and type of cell death induced by the histone deacetylase inhibitors butyrate and trichostatin A.

J Steroid Biochem Mol Biol. 2005 Feb;94(1-3):39-47. Epub 2005 Feb 1

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We investigated the effects of the polyphenolic phytostilbene resveratrol on the steady-state free radical (FR) concentration and mode of cell death induced by the histone deacetylase inhibitors butyrate and trichostatin A. (i) There was no correlation between cell death induction by butyrate or trichostatin A (TSA) and FR levels. (ii) Treatment with resveratrol or N-acetyl-l-cystein (NAC) of cells, in which the FR concentration was high, resulted in an almost complete reduction of FR levels. (iii) When, however, the cellular FR concentration was marginal, resveratrol caused a minor, and NAC a marked increase of FRs as well as of the extent of cell death. Thus, resveratrol and NAC acted as antioxidants only when the cellular FR levels were high, and acted as pro-oxidants when facing a low FR concentration. (iv) Since resveratrol and the antioxidant NAC exhibited analogous effects, it is concluded that the observed actions of resveratrol are due to polyphenolic redox reactions and not related to the stilbene moiety of the molecule. (v) The results indicate that the redox status of a given cell type plays an important role in determining whether resveratrol and other antioxidants promote cell death or protect cells from it.

Putative mechanism for anticancer and apoptosis-inducing properties of plant-derived polyphenolic compounds.

IUBMB Life. 2000 Sep;50(3):167-71.

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Several plant-derived polyphenolic compounds are considered to possess anticancer and apoptosis-inducing properties in cancer cells. Such compounds are recognized as naturally occurring antioxidants but also exhibit prooxidant properties under appropriate conditions. Evidence in the literature suggests that the antioxidant properties of polyphenolics such as gallotannins, curcumin, and resveratrol may not fully account for their chemopreventive effects. We propose a mechanism for the cytotoxic action of these compounds against cancer cells that involves mobilization of endogenous copper and the consequent prooxidant action.

Immune activity

Effects of resveratrol on human immune cell function.

Life Sci. 2001 Nov 21;70(1):81-96

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Resveratrol (3,5,4'-trihydroxystilbene), a polyphenol found in grapes and grape products such as red wine, has been reported to exhibit a wide range of biological and pharmacological activities both in vitro and in vivo. Because many of the biological activities of resveratrol, like the inhibition of cyclooxygenase, induction of CD95 signaling-dependent apoptosis, effects on cell division cycle and modulation of NF-kB activation, suggest a possible effect on the immune system, we evaluated the in vitro effects of resveratrol in three immune response models: i) development of cytokine-producing CD4+ and CD8+ T cells induced by stimulation of peripheral blood mononuclear cells (PBMC) with anti-CD3/anti-CD28; ii) specific antigen-induced generation of cytotoxic T lymphocytes; iii) natural killer (NK) activity of PBMC. The results showed that in vitro exposure to resveratrol produces a biphasic effect on the anti-CD3/anti-CD28-induced development of both IFN-gamma- IL2- and IL4-producing CD8 + and CD4+ T cells, with stimulation at low resveratrol concentrations and suppression at high concentrations. Similarly, the compound was found to induce a significant enhancement at low concentrations and suppression at high concentrations of both CTL and NK cell cytotoxic activity. On the whole, the results of the study indicate that resveratrol modulates several human immune cell functions and suggest that this activity may be related to its effects on cytokine production by both CD4+ and CD8+ T cells.

Effects of resveratrol on lymphocyte proliferation and cytokine release.

Ann Clin Lab Sci. 2003 Spring;33(2):226-31.

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Resveratrol, synthesized in dietary plants and contained in wine, has been reported to play a beneficial role in certain cardiovascular regulatory mechanisms and to inhibit

carcinogenesis by activating immune and inflammatory responses and apoptosis. The object of this study was to elucidate the "in vitro" effects of different concentrations of resveratrol (10(-4), 10(-5), and 10(-7) M) on human peripheral blood mononuclear cell (PBMC) proliferation and cytokine release. Spontaneous PBMC proliferation was unaffected by resveratrol, while the compound at 10(-4) M inhibited (69%) the PHA-stimulated PBMC proliferation. The proliferation stimulation index (ie, the ratio of PHA-stimulated PBMC proliferation/spontaneous PBMC proliferation) of cultures containing 10(-4) M resveratrol was very low in relation to the control, while the proliferation stimulation index values at 10(-5) and 10(-7) M were similar and slightly higher (without statistical significance), respectively. At 10(-4) M, resveratrol strongly inhibited PHA-stimulated IFN-gamma and TNF-alpha release from PBMC, but it did not cause inhibition at 10(-5) or 10(-7) M. The concomitant immune effects of resveratrol on PBMC proliferation and release of IFN-gamma and TNF-alpha may be explained by an inhibitory effect on transcription factor NF-kappaB. This study suggests that resveratrol, which is typically present in red wine at about 10(-5) M, is unlikely to cause inhibitory immune effects. However, a stimulatory effect of low concentrations of resveratrol on the immune system cannot be excluded.

Anti-inflammatory activity

Rational design of potent human transthyretin amyloid disease inhibitors.

Nat Struct Biol. 2000 Apr;7(4):312-21.

Klabunde T, **Petrassi HM**, **Oza VB**, **Raman P**, **Kelly JW**, **Sacchettini JC**.

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The human amyloid disorders, familial amyloid polyneuropathy, familial amyloid cardiomyopathy and senile systemic amyloidosis, are caused by insoluble transthyretin (TTR) fibrils, which deposit in the peripheral nerves and heart tissue. Several nonsteroidal anti-inflammatory drugs and structurally similar compounds have been found to strongly inhibit the formation of TTR amyloid fibrils in vitro. These include flufenamic acid, diclofenac, flurbiprofen, and resveratrol. Crystal structures of the protein-drug complexes have been determined to allow detailed analyses of the protein-drug interactions that stabilize the native tetrameric conformation of TTR and inhibit the formation of amyloidogenic TTR. Using a structure-based drug design approach ortho-trifluormethylphenyl anthranilic acid and N-(meta-trifluoromethylphenyl) phenoxazine 4, 6-dicarboxylic acid have been discovered to be very potent and specific TTR fibril formation inhibitors. This research provides a rationale for a chemotherapeutic approach for the treatment of TTR-associated amyloid diseases.

Plant-derived anti-inflammatory compounds affect MIF tautomerase activity.

Int Immunopharmacol. 2005 May;5(5):849-56. Epub 2005 Jan 27

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The cytokine macrophage migration inhibitory factor (MIF) has recently emerged as a crucial factor in the pathogenesis of rheumatoid arthritis (RA). It is debated whether the MIF mediated tautomeric conversion of either phenylpyruvate or of its other phenolic substrates is implicated in the pro-inflammatory action of this cytokine. Traditional herbal remedies have been used for centuries to alleviate inflammatory ailments of many kinds

including arthritis. Several of their active ingredients identified are mono- or poly-phenol derivatives. In the present study the effect of some anti-inflammatory plant phenols on MIF mediated tautomerism of phenylpyruvate was investigated. Curcumin and caffeic acid were found to be the most potent inhibitors, exhibiting IC(50) values in the submicromolar range in the ketonase assay. Resveratrol and umbelliferon were almost as potent inhibitors as the antipyretic-analgetic drug acetaminophen. Our results reveal MIF as a possible target for the herbal anti-rheumatic agents.

Resveratrol: a medical drug for acute pancreatitis.

World J Gastroenterol. 2005 Jun 7;11(21):3171-4

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Accumulating evidence demonstrates that resveratrol, a natural polyphenolic compound extracted from plants, inhibit inflammation when administered. It has direct effects on suppression of platelet coagulation and cytokines production in many experimental models. Because microcirculation occlusion and cytokines over-production is involved in many diseases such as acute pancreatitis (AP), the discovery of resveratrol as platelet and cytokines inhibitors has shed light on the treatment of AP, which still has significant mortality and morbidity. It is anticipated that this natural polyphenol could serve as a therapeutic compound in managing AP through different pathways.

Effect of trans-resveratrol, a natural polyphenolic compound, on human polymorphonuclear leukocyte function.

Br J Pharmacol. 1998 Apr;123(8):1691-9.

Rotondo S, **Rajtar G**, **Manarini S**, **Celardo A**, **Rotillo D**, **de Gaetano G**, **Evangelista V**, **Cerletti C**.

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1. Polymorphonuclear leukocytes (PMN) may contribute to the pathogenesis of acute coronary heart disease (CHD). 2. Epidemiological and laboratory evidence suggests that red wine, by virtue of its polyphenolic constituents, may be more effective than other alcoholic beverages in reducing the risk of CHD mortality. 3 The aim of the present study was to investigate the effects of trans-resveratrol (3,4',5-trihydroxy-trans-stilbene), a polyphenol present in most red wines, on functional and biochemical responses of PMN, upon in vitro activation. 4. trans-Resveratrol exerted a strong inhibitory effect on reactive oxygen species produced by PMN stimulated with 1 microM formyl methionyl leucyl phenylalamine (fMLP) (IC50 1.3+/-0.13 microM, mean+/-s.e.mean), as evaluated by luminol-amplified chemiluminescence. 5. trans-Resveratrol prevented the release of elastase and beta-glucuronidase by PMN stimulated with the receptor agonists fMLP (1 microM, IC50 18.4+/-1.8 and 31+/-1.8 microM), and C5a (0.1 microM, IC50 41.6+/-3.5 and 42+/-8.3 microM), and also inhibited elastase and beta-glucuronidase secretion (IC50 37.7+/-7 and 25.4+/-2.2 microM) and production of 5-lipoxygenase metabolites leukotriene B4 (LTB4), 6-trans-LTB4 and 12-trans-epi-LTB4 (IC50 48+/-7 microM) by PMN stimulated with the calcium ionophore A23187 (5 microM). 6. trans-Resveratrol significantly reduced the expression and activation of the beta2 integrin MAC-1 on PMN surface following stimulation, as revealed by FACS analysis of the binding of an anti-MAC-1 monoclonal antibody (MoAb) and of the CBRM1/5 MoAb, recognizing an activation-dependent epitope on MAC-1. Consistently, PMN homotypic aggregation and formation of mixed cell-conjugates between PMN and thrombin-stimulated fixed platelets in a dynamic system were also prevented by transresveratrol. 7. These results, indicating that trans-resveratrol interferes with the release of inflammatory mediators by activated PMN and down-regulates adhesion-dependent thrombogenic PMN functions, may provide some biological plausibility to the protective effect of red wine consumption against CHD.

Effects of stilbenes isolated from medicinal plants on arachidonate metabolism and degranulation in human polymorphonuclear leukocytes.

J Ethnopharmacol. 1995 Feb;45(2):131-9

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Studies were made on the effects of stilbene derivatives isolated from medicinal plants on arachidonate metabolism and degranulation in human polymorphonuclear leukocytes (PMN-L). Resveratrol (3,4',5-trihydroxystilbene) isolated from the roots of Reynoutria japonica was found to inhibit the 5-lipoxygenase products 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE), 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid (5,12-diHETE) and leukotriene C4(LTC4); its concentrations for 50% inhibition (IC50) were

8.90 x 10(-6) M, 6.70 x 10(-6) M and 1.37 x 10(-6) M, respectively. The IC50 of 5-HETE, 5,12-diHETE and LTC4 formations of synthetic 3,3',4-trihydroxystilbene were 5.90 x 10(-6) M, 6.30 x 10(-7) M and 8.80 x 10(-7) M, respectively. Moreover, they inhibited the release of lysosomal enzyme such as lysozyme and beta-glucuronidase induced by calcium ionophore A 23187 from human PMN-L at 10(-3)-10(-4) M.

Inhibition of COX isoforms by nutraceuticals.

J Herb Pharmacother. 2004;4(2):11-8.

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Humans have two isoforms of Prostaglandin H Synthase or cyclooxygenase: COX-1 and COX-2. COX-1 is cytoprotective. COX-2 inhibitors reduce inflammation without the risk of ulceration and kidney damage. The ideal nutraceutical would inhibit COX-2 synthesis while preserving COX-1 synthesis. The hypothesis for this research was that COX inhibitors would fall primarily into three categories: COX-2 specific inhibition, non-specific inhibition (COX-1 and COX-2), and minimal inhibition. The human Cayman COX inhibitor screening assay was used to determine the inhibitory concentration 50 (IC50) of COX-1/ COX-2 activity of each nutraceutical. The assay was run, in duplicate, with three concentrations of a suspected inhibitor, a standard curve of eight concentrations, a non-specific binding sample, and a maximum binding sample. The inhibition and concentration of each sample was then put on a multiple regression best-fit line and the IC50 determined. For comparison, ibuprofen, rofecoxib, naproxen, and indomethacin were used. Positive results were seen for ipriflavone, resveratrol, MSV-60, amentoflavone, ruscus extract and notoginseng. Glucosamine, nexrutine, and berberine did not inhibit either isoform.

Resveratrol is a peroxidase-mediated inactivator of COX-1 but not COX-2: a mechanistic approach to the design of COX-1 selective agents.

J Biol Chem. 2004 May 21;279(21):22727-37. Epub 2004 Mar 12

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Resveratrol (3,4',5-trihydroxy-trans-stilbene) is a phytoalexin found in grapes that has anti-inflammatory, cardiovascular protective, and cancer chemopreventive properties. It has been shown to target prostaglandin H(2) synthase (COX)-1 and COX-2, which catalyze the first committed step in the synthesis of prostaglandins via sequential cyclooxygenase and peroxidase reactions. Resveratrol discriminates between both COX isoforms. It is a potent inhibitor of both catalytic activities of COX-1, the desired drug target for the prevention of cardiovascular disease, but only a weak inhibitor of the peroxidase activity of COX-2, the isoform target for nonsteroidal anti-inflammatory drugs. We have investigated the unique inhibitory properties of resveratrol. We find that it is a potent peroxidase-mediated mechanism-based inactivator of COX-1 only (k(inact) = 0.069 +/- 0.004 s(-1), K(i(inact)) = 1.52 +/- 0.15 microm), with a calculated partition ratio of 22. Inactivation of COX-1 was time- and concentration-dependent, it had an absolute requirement for a peroxide substrate, and it was accompanied by a concomitant oxidation of resveratrol. Resveratrol-inactivated COX-1 was devoid of both the cyclooxygenase and peroxidase activities, neither of which could be restored upon gel-filtration chromatography. Inactivation of COX-1 by [(3)H]resveratrol was not accompanied by stable covalent modification as evident by both SDS-PAGE and reverse phase-high performance liquid chromatography analysis. Structure activity relationships on methoxy-resveratrol analogs showed that the m-hydroquinone moiety was essential for irreversible inactivation of COX-1. We propose that resveratrol inactivates COX-1 by a "hit-and-run" mechanism, and offers a basis for the design of selective COX-1 inactivators that work through a mechanism-based event at the peroxidase active site.

Effect of resveratrol, a natural polyphenolic compound, on reactive oxygen species and prostaglandin production.

Biochem Pharmacol. 2000 Apr 1;59(7):865-70.

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Resveratrol is a natural molecule with antioxidant action. Moreover, resveratrol is also considered to be a molecule with anti-inflammatory action, an effect attributed to suppression of prostaglandin (PG) biosynthesis. The aim of the present study was to investigate the effects of resveratrol, a polyphenol present in most red wines, on reactive oxygen species formation as well as on arachidonic acid (AA) release, cyclooxygenase expression, and PG synthesis in murine resident peritoneal macrophages. Results show that resveratrol exerted a strong inhibitory effect on superoxide radical (O2-) and hydrogen peroxide (H2O2) produced by macrophages stimulated by lipopolysaccharides (LPS) or phorbol esters (PMA). Resveratrol also significantly decreased [3H]AA release induced by LPS and PMA or by exposure to O2- or H2O2. Resveratrol treatment caused a significant impairment of cyclooxygenase-2 (COX-2) induction stimulated by LPS and

PMA or by O2- or H2O2 exposure. These effects of resveratrol on [3H]AA release and COX-2 overexpression were correlated with a marked reduction of PG synthesis. Our results indicate that the antioxidant action of resveratrol affects AA mobilization and COX-2 induction.

Suppression of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells by chemopreventive agents with a resorcin-type structure.

Carcinogenesis. 2000 May;21(5):959-63

Mutoh M, **Takahashi M**, **Fukuda K**, **Matsushima-Hibiya Y**, **Mutoh H**, **Sugimura T**, **Wakabayashi K**.

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Cyclooxygenase-2 (COX-2) is abundantly expressed in colon cancer cells. It has been reported that inhibition of COX-2 enzyme activity is shown to prevent colon carcinogenesis. Thus, suppression of COX-2 expression may also be an effective chemopreventive strategy. In the present study, we constructed a beta-galactosidase reporter gene system in human colon cancer DLD-1 cells, and measured COX-2 promoter-dependent transcriptional activity in the cells. Interferon gamma suppressed this COX-2 promoter activity, while 12-O-tetradecanoylphorbol-13-acetate and transforming growth factor alpha (TGFalpha) exerted enhancing effects. We then tested the influence of 14 candidate cancer chemopreventive compounds on COX-2 promoter activity. Chemopreventive agents such as quercetin, kaempferol, genistein, resveratrol and resorcinol, all having a common resorcin moiety, were found to effectively suppress the COX-2 promoter activity with and without TGFalpha-stimulation in DLD-1 cells. Since all these compounds have a resorcin moiety as a common structure, a resorcin-type structure may play an active role in the inhibition of COX-2 expression in colon cancer cells.

Resveratrol inhibits cyclooxygenase-2 transcription in human mammary epithelial cells.

Ann N Y Acad Sci. 1999;889:214-23

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A large body of evidence suggests that inhibiting cyclooxygenase-2 (COX-2), the inducible form of COX, will be an important strategy for preventing cancer. In this study, we investigated whether resveratrol, a chemopreventive agent found in grapes, could suppress phorbol ester (PMA)-mediated induction of COX-2 in human mammary and oral epithelial cells. Treatment of cells with PMA induced COX-2 mRNA, COX-2 protein, and prostaglandin synthesis. These effects were inhibited by resveratrol. Nuclear runoffs revealed increased rates of COX-2 transcription after treatment with PMA, an effect that was inhibited by resveratrol. Resveratrol inhibited PMA-mediated activation of protein kinase C and the induction of COX-2 promoter activity by c-Jun. Phorbol ester-mediated induction of AP-1 activity was blocked by resveratrol. These data are likely to be important for understanding the anticancer and anti-inflammatory properties of resveratrol.

Resveratrol inhibits matrix metalloproteinase-9 transcription in U937 cells.

Acta Pharmacol Sin. 2003 Nov;24(11):1167-71

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AIM: To examine the inhibitory effect of resveratrol on matrix metalloroteinase-9 (MMP-9) and explore its mechanism. METHODS: MMP-9 activity was analyzed by gelatin zymography; MMP-9 protein was detected by Western blot; MMP-9 mRNA expression was investigated by RT-PCR. Activation of activator protein -1 (AP-1) was measured by electrophoretic mobility shift assay (EMSA). RESULTS: MMP-9 activity in U937 cells increased significantly after exposed to PMA at 10 nmol/L for 24 h without FCS ($P<0.01$). Resveratrol at 1 and 10 micromol/L showed significant inhibition on MMP-9 activity ($P<0.05$ and $P<0.01$, respectively). Western blot and RT-PCR experiments displayed that MMP-9 protein ($P<0.01$) and mRNA expression ($P<0.01$) increased significantly in PMA-treated U937 cells. Resveratrol at 1 and 10 micromol/L showed inhibitory effects on MMP-9 protein production and MMP-9 mRNA expression ($P<0.05$). The activation of AP-1 induced by PMA was also extensively inhibited by resveratrol at 0.1, 1, and 10 micromol/L. CONCLUSION: The inhibitory effect of resveratrol on MMP-9 activity may be partly through suppression of activation of nuclear transcription factor AP-1, and inhibition of MMP-9 mRNA expression and MMP-9 protein production.

[Inhibition of dexamethasone, indomethacin and resveratrol on matrix metalloproteinase-9 and the mechanism of inhibition]

Yao Xue Xue Bao. 2003 Jul;38(7):501-4.

[Article in Chinese]

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AIM: To investigate the expression of matrix metalloproteinase-9 (MMP-9) in mouse ears induced with croton oil and the inhibitory effect of dexamethasone, indomethacin and resveratrol on MMP-9 expression, and further explore the relationship between anti-inflammation and MMP-9 inhibition of these three medicines. METHODS: Immuno-histochemistry was used to detect the expression of MMP-9 in mouse ears. Expression of MMP-9 in U937 cells was analyzed by gelatin zymography. RESULTS: Mouse ear edema induced with croton oil was inhibited significantly by dexamethasone and indomethacin at the dose of 10 mg.kg-1 and resveratrol at 50 mg.kg-1 administered subcutaneously. The inhibitory rate was 76.2% ($P < 0.001$), 56.7% ($P < 0.001$) and 36.9% ($P < 0.001$) respectively. The MMP-9 expression increased in mouse ears induced with croton oil and inhibited by dexamethasone, indomethacin and resveratrol at above doses. Gelatin zymography results showed that MMP-9 expression in U937 cells increased significantly after exposed to PMA at 1 x 10(-8) mol.L-1 ($P < 0.001$); MMP-9 expression induced with phorbol myristate acetate(PMA) was inhibited by dexamethasone at 1 x 10 (-9), 1 x 10(-7) and 1 x 10(-5) mol.L-1, indomethacin at 1 x 10(-6) and 1 x 10(-5) mol.L-1 and resveratrol at 1 x 10(-6) and 1 x 10(-5) mol.L-1. CONCLUSION: The inhibition of MMP-9 expression may be one of the anti-inflammatory mechanisms of dexamethasone, indomethacin and resveratrol.

Antiplatelet activity

Antiplatelet activity of synthetic and natural resveratrol in red wine.

Int J Tissue React. 1995;17(1):1-3

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The antiaggregating effect of the phytoalexin resveratrol (3,4,5-trihydroxystilbene), alone or associated with red wine, and polyphenol have been evaluated in vitro at different concentrations on platelet-rich plasma from healthy volunteers. Resveratrol at the concentration of 3.56 micrograms/l was able to lower platelet aggregation by 50.3% +/- 1.83. Red wine containing 1.2 mg/l of natural trans-resveratrol and 3.6 milligrams of polyphenols diluted 1000-fold (final resveratrol concentration: 1.2 micrograms/l) inhibited platelet aggregation by 41.9% +/- 2.11. By adding resveratrol to the wine up to a concentration of 1.2 micrograms/l, inhibition was raised to 78.5% +/- 4.70. These results suggest that the antiaggregating activity of resveratrol is related to its concentration in wine.

The red wine phenolics trans-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: implications for protection against coronary heart disease.

Clin Chim Acta. 1995 Mar 31;235(2):207-19

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A number of lines of evidence suggest that red wine may be more effective than other alcoholic beverages in decreasing the risk of coronary heart disease (CHD) mortality. This protection over and above that due to ethanol itself may be explained by phenolic components with which red wines are richly endowed. We have studied the effects of the trihydroxy stilbene trans-resveratrol on human platelet aggregation and on the synthesis of three eicosanoids from arachidonate by platelets, i.e. thromboxane B2 (TxB2), hydroxyheptadecatrienoate (HHT) and 12-hydroxyeicosatetraenoate (12-HETE). These effects were compared with the actions of other wine phenolics (quercetin, catechin and

epicatechin) and antioxidants (alpha-tocopherol, hydroquinone and butylated hydroxytoluene). trans-Resveratrol and quercetin demonstrated a dose-dependent inhibition of both thrombin-induced and ADP-induced platelet aggregation, whereas ethanol inhibited only thrombin-induced aggregation. The other compounds tested were inactive. trans-Resveratrol also inhibited the synthesis of TxB2, HHT, and to a lesser extent 12-HETE, from arachidonate in a dose-dependent manner. Quercetin inhibited only 12-HETE synthesis, and hydroquinone caused slight inhibition of TxB2 synthesis, the remaining compounds being ineffective. De-alcoholized red wines inhibited platelet aggregation; their ability to inhibit the synthesis of TxB2 but not that of 12-HETE from labelled arachidonate by washed human platelets was proportional to their trans-resveratrol concentration. These results are consistent with the notion that trans-resveratrol may contribute to the presumed protective role of red wine against atherosclerosis and CHD.

Antiplatelet activity of cis-resveratrol.

Drugs Exp Clin Res. 1996;22(2):61-3

Bertelli AA, **Giovannini L**, **Bernini W**, **Migliori M**, **Fregoni M**, **Bavaresco L**, **Bertelli A**.

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The anti-aggregating effect of cis-resveratrol (cis-3,4,5- trihydroxystilbene) has been evaluated in vitro in different concentrations on platelet-rich plasma from health volunteers. Cis-resveratrol at the concentration of 1 x 10(-5) and 1 x 10(-6) M was able to decrease collagen-induced platelet aggregation by 43.5 +/-11.4% and 26.8 +/- 14.6%, while trans-resveratrol at the same concentration showed a slightly lower activity. In view of the behaviour of these two isomers in biological fluids, the evaluation of resveratrol activity in animals and humans take into account the total amount of the two isomers.

trans-Resveratrol inhibits calcium influx in thrombin-stimulated human platelets.

Br J Pharmacol. 1999 Sep;128(1):149-57

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1. The phytoestrogenic compound trans-resveratrol (trans-3,5, 4'-trihydroxystilbene) is found in appreciable quantities in grape skins and wine. It has been shown that both products rich in trans-resveratrol and pure trans-resveratrol inhibit platelet aggregation both in vivo and in vitro. However the mechanism of this action still remains unknown. 2. An essential component of the aggregation process in platelets is an increase in intracellular free Ca2+ ([Ca2+]i). Ca2+ must enter the cell from the external media through specific and tightly regulated Ca2+ channels in the plasma membrane. The objective of this study was to characterize what effect trans-resveratrol had on the Ca2+ channels in thrombin stimulated platelets. 3. In this study we showed that trans-resveratrol immediately inhibited Ca2+ influx in thrombin-stimulated platelets with an IC50 of 0.5 microM. trans-Resveratrol at 0.1, 1.0 and 10.0 microM produced 20+/-6, 37 +/-6 and 57+/-4% inhibition respectively of the effect of thrombin (0.01 u ml(-1)) to increase [Ca2+]i. 4. trans-Resveratrol also inhibited spontaneous Ba2+ entry into Fura-2 loaded platelets, with 0.1, 1.0 and 10.0 microM trans-resveratrol producing 10+/-5, 30 +/-5 and 50+/-7% inhibition respectively. This indicated that trans-resveratrol directly inhibited Ca2+ channel activity in the platelets in the absence of agonist stimulation. 5. trans-Resveratrol also inhibited thapsigargin-mediated Ca2+ influx into platelets. This suggests that the store-operated Ca2+ channels are one of the possible targets of trans-resveratrol. These channels rely on the emptying of the internal Ca2+ stores to initiate influx of Ca2+ into the cell. 6. The phytoestrogens genistein, daidzein, apigenin and genistein-glucoside (genistin) produced inhibitory effects against thrombin similar to those seen with trans-resveratrol. 7. We conclude that trans-resveratrol is an inhibitor of store-operated Ca2+ channels in human platelets. This accounts for the ability of trans-resveratrol to inhibit platelet aggregation induced by thrombin.

Investigation into the rapid effects of 17 beta-estradiol and neuroactive steroids upon beta-amyloid(25-35)-induced activation of phosphoinositide-specific phospholipase C in human platelets.

Methods Find Exp Clin Pharmacol. 2000 Oct;22(8):615-20

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In the present study, the rapid effects of five steroids (17 beta-estradiol, progesterone, allopregnanolone, 3 alpha-hydroxy-5 alpha-pregnan-20-one and 3 alpha-hydroxy-5 beta-pregnan-20-one) and the plant steroid trans-resveratrol upon the calcium response to beta-amyloid(25-35) peptide (A beta(25-35)) in human platelets was measured. A beta(25-35) produced a robust increase in intracellular calcium due to a direct activation of phosphoinositide-specific phospholipase C. None of the steroids significantly affected the

response to A beta(25-35). In contrast, trans-resveratrol appeared to increase the response to A beta(25-35) at a concentration that decreased the response to thrombin, although the possibility that these changes are artifactual could not be ruled out. It is concluded that although steroids affect human platelet Ca2+ homeostasis, this is not a rapid event, unless very high concentrations are used.

Resveratrol decreases early signaling events in washed platelets but has little effect on platelet in whole blood.

Blood Cells Mol Dis. 2000 Apr;26(2):144-50.

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Resveratrol, a polyphenolic compound found in red wines, is believed to be a contributor in decreasing the incidence of coronary heart disease. Although its primary target is unknown, it blocks aggregation of washed platelets by an ill-defined mechanism. We show that resveratrol, at 10-50 microM, blocked aggregation induced by collagen (5 microg/ml), thrombin (0.2 units/ml), and ADP (10 microM). This affect was not overcome by adding exogenous human fibrinogen to the assay, suggesting that an early (wave I) signaling step in the alpha(IIb)beta(3) activation cascade was impaired. To explore this possibility we examined the effect of resveratrol on activation of MAP kinases. In the platelet, MAP kinases become activated as a consequence of agonist binding and not of aggregation, which itself induces signaling events. In fact, we find that collagen-induced activation of MAP kinases is superinduced in the presence of RGDS, an aggregation-blocking peptide. Resveratrol, at concentrations of 10 microM and greater, inhibited MAP kinase activation induced by collagen (in the absence and presence of RGDS peptide), thrombin, and ADP. These data indicate that resveratrol blocks receptor-mediated signaling events in washed platelets. In comparison, resveratrol has poor antiplatelet activity in whole blood. Under these conditions aggregation was not affected by 50-100 microM resveratrol. Concentrations of 200 microM resveratrol were needed to cause a 30-60% decrease in platelet aggregation in whole blood. Together these studies suggest that resveratrol is a potent inhibitor of platelet signaling responses, but its antiplatelet activity is weakened or masked in circulation. Thus, although resveratrol may function as a protective agent of coronary heart disease, its affects are not solely attributed to its effects on platelets in circulation.

Effect of resveratrol, a natural polyphenolic compound, on platelet activation induced by endotoxin or thrombin.

Thromb Res. 2002 Aug 15;107(3-4):141-5

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Resveratrol (3, 4', 5-trihydroxystilbene), a natural polyphenol, is found in some plants that are used in human nutrition. Grapes are a major source for resveratrol, and a significant amount can also be found in red wine. Several experimental studies have demonstrated biological properties of resveratrol, especially its anti-inflammatory, antioxidant, anti-platelet and antitumor effects. In the present study, we investigated the first step of platelet activation-platelet adhesion stimulated by lipopolysaccharide (LPS) from Proteus mirabilis (weak stimulator) and thrombin (strong activator) in the presence of resveratrol. Our studies show that endotoxin (0.3 microg/10(8) platelets), like thrombin (0.2 U/10(8) platelets), induced the adhesion of platelets (expressed as absorbance of cell attached proteins) to collagen and fibrinogen. Preincubation of washed platelets with resveratrol at physiological plasma concentrations (25-100 microg/ml, 30 min, 37 degrees C) had an inhibitory effect on adhesion of platelets to collagen after activation by LPS alone or LPS with thrombin. The strongest effect on this process was caused by resveratrol at the concentration of 100 microg/ml. Pretreatment of platelets with resveratrol (25-100 microg/ml, 30 min, 37 degrees C) had also inhibitory effects on adhesion of platelets to fibrinogen after stimulation of these cells by LPS alone or by LPS with thrombin at the same concentration. In conclusion, we suggest that resveratrol present in human diet may be an important compound responsible for the reduction of platelet adhesion and changed reactivity of blood platelets in inflammatory process

Resveratrol, a polyphenolic compound found in wine, inhibits tissue factor expression in vascular cells : A possible mechanism for the cardiovascular benefits associated with moderate consumption of wine.

Arterioscler Thromb Vasc Biol. 1999 Feb;19(2):419-26

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A number of studies suggest that moderate consumption of red wine may be more effective than other alcoholic beverages in decreasing the risk of coronary heart disease

mortality. The phytochemical resveratrol found in wine, derived from grapes, has been thought to be responsible for cardiovascular benefits associated with wine consumption because it was shown to have antioxidant and antiplatelet activities. In the present investigation, we examined the effect of resveratrol on induction of tissue factor (TF) expression in vascular cells that were exposed to pathophysiological stimuli. The data presented herein show that resveratrol, in a dose-dependent manner, inhibited the expression of TF in endothelial cells stimulated with a variety of agonists, including interleukin-1beta (IL-1beta), tumor necrosis factor-alpha (TNFalpha) and lipopolysaccharide (LPS). A similar inhibition of TF induction was also seen in LPS stimulated monocytes that were pretreated with resveratrol before their stimulation with LPS. In addition, resveratrol was shown to inhibit the LPS-induced expression of TNFalpha mRNA in endothelial cells and of TNFalpha and IL-1beta mRNA in monocytes. Nuclear run-on analysis in endothelial cells showed that resveratrol inhibited TF expression at the level of transcription. However, resveratrol did not significantly alter the binding of the transcription factors c-Fos/c-Jun and c-Rel/p65, the transcription factors required for the induction of TF promoter in both endothelial cells and monocytes. Similarly, resveratrol had no significant effect on the binding of NF-kappaB in endothelial cells stimulated with IL-1beta, TNFalpha, and LPS. Overall, our data show that resveratrol could effectively suppress the aberrant expression of TF and cytokines in vascular cells, but it requires further investigation to understand how resveratrol exerts its inhibitory effect.

Resveratrol: a molecule whose time has come? And gone?

Clin Biochem. 1997 Mar;30(2):91-113

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OBJECTIVES: Resveratrol (3,5,4'-trihydroxystilbene) is the parent compound of a family of molecules, including glucosides and polymers, existing in cis and trans configurations in a narrow range of spermatophytes of which vines, peanuts and pines are the prime representatives. Its synthesis from p-coumaroyl CoA and malonyl CoA is induced by stress, injury, infection or UV-irradiation, and it is classified as a phytoalexin anti-fungicide conferring disease resistance in the plant kingdom. RESULTS: In vitro, ex vivo and animal experiments have shown that it possesses many biological attributes that favour protection against atherosclerosis, including antioxidant activity, modulation of hepatic apolipoprotein and lipid synthesis, inhibition of platelet aggregation as well as the production of pro-atherogenic eicosanoids by human platelets and neutrophils. Red wine represents its main source in the human diet, and it has been proposed as a major constituent of the polyphenol fraction to which the health benefits of red wine

consumption have been attributed. CONCLUSIONS: The past several years have witnessed intense research devoted to its measurement in wine and the factors likely to promote its enrichment in this beverage. Up to the present, conclusive evidence for its absorption by human subjectsin biologically significant amounts is lacking, and it is questionable (but not yetexcluded) that its powerful and beneficial in vitro activities are reproduced as a consequence of sustained moderate red wine consumption.

Antioxidants with carcinostatic activity (resveratrol, vitamin E and selenium) in modulation of blood platelet adhesion.

Drugs Exp Clin Res. 1998;24(3):133-8

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Compounds with potential antiplatelet activity can be used in the therapy of cardiovascular disorders. We investigated the effects of three different antioxidants with carcinostatic property: trans-resveratrol, Trolox a water-soluble analog of vitamin E, and inorganic selenocompounds (sodium selenite and selenate) on blood platelet adhesion to fibrinogen (Fg). Adhesion, the initial step of platelet activation, was estimated by the colorimetric method with BCA (bicinchoninic acid) solution in 96-well Fg-coated microtiter dishes. It was shown that resveratrol significantly inhibited adhesion of both thrombin- and ADP-activated platelets to Fg. After incubation of platelets for 30 min. at 37 degrees C with resveratrol at the concentration of 100 microg/ml above 40% inhibition of adhesion was achieved. The inhibition of platelet adhesion of Fg caused by Trolox was lower than by resveratrol and at higher concentration (1 mM) reached maximum 12%. We also demonstrated that neither sodium selenite nor selenate significantly altered platelet adhesion to Fg. We conclude that changed adhesion of blood platelets to Fg in the presence of resveratrol and Trolox, but not selenium may be the result of different antioxidative activities of tested compounds.

Plasma and tissue resveratrol concentrations and pharmacological activity.

Drugs Exp Clin Res. 1998;24(3):133-8

Bertelli A, **Bertelli AA**, **Gozzini A**, **Giovannini L**.

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In this study a comparison was made between results obtained from resveratrol dosages which have been shown to be pharmacologically active, in vitro and in vivo, and the results of plasma and tissue concentrations obtained after a single administration or after prolonged administration of red wine with a known resveratrol content. The dosages used by different investigators in the tests are very different and, in general, rather high in relation to the concentrations which are found in wine or grapes. The results of our tests on platelet aggregation confirm that even with modest dosages of resveratrol, a pharmacological effect can be observed, and that these dosages can be compatible with the resveratrol concentrations obtained after oral administration. The data obtained from these tests on animals can lead to the conclusion that even an average drinker of wine can, particularly in the long term, absorb a sufficient quantity of resveratrol to explain the beneficial effect of red wine on health, which has been observed in epidemiological studies carried out in populations whose daily diet includes the drinking of wine.

Dietary phytoestrogens and their synthetic structural analogues as calcium channel blockers in human platelets.

J Cardiovasc Pharmacol. 2002 Sep;40(3):399-410

Dobrydneva Y, **Williams RL**, **Morris GZ**, **Blackmore PF**.

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Phytoestrogens have been shown to inhibit platelet activation by blocking platelet calcium channels. This study examined the effect of several synthetic derivatives of trans-resveratrol, genistein, and daidzein on platelet free intracellular calcium ([Ca2+]i) elevation in thrombin-activated platelets and the possible mechanisms of this inhibitory effect. Studies were conducted on fresh human platelets from healthy volunteers. The fluorescent dye fura-2 was used to monitor [Ca2+]i in platelets. At 10 microM-resveratrol, triacetyl-trans-resveratrol, and trimethoxy-trans-resveratrol produced, respectively, 57 +/- 4%, 40 +/- 4%, and 21 +/- 1% inhibition; genistein, acetylgenistein, and dihydrogenistein produced 51 +/- 10%, 26 +/- 7%, and 16 +/- 2% inhibition, respectively; daidzein and diacetyldaidzein produced 56 +/- 5% and 45 +/- 10% inhibition of thrombin-induced [Ca2+]i elevation. The inhibitory effect was immediate and appeared to directly affect the calcium influx channels. Phytoestrogen action on [Ca2+]i did not cause alteration in nitric oxide signaling. Tyrosine phosphorylation was not involved in the inhibition of [Ca2+]i elevation by phytoestrogens, because the percent inhibition produced by the tyrosine kinase inhibitor genistein and its inactive analogue

daidzein on thrombin-induced and thapsigargin-induced [Ca2+]i elevation was not significantly different for either compound at any concentration tested. Structure-activity relationship studies on this limited set of compounds reveal the requirements for the stilbene pharmacophore for the calcium-blocking activity.

Wines and grape juices as modulators of platelet aggregation in healthy human subjects.

Clin Chim Acta. 1996 Mar 15;246(1-2):163-82

Pace-Asciak CR, **Rounova O**, **Hahn SE**, **Diamandis EP**, **Goldberg DM**.

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To test the hypothesis that red wine, by virtue of its relatively high concentration of polyphenols, is more protective against atherosclerosis and coronary heart disease (CHD) than white wine, and that grape juice enriched in one of these, trans-resveratrol, may share some of these properties, studies were performed on 24 healthy males aged 26-45 years. Each consumed the following beverages for periods of 4 weeks: red wine, white wine, commercial grape juice and the same grape juice enriched with trans-resveratrol. Apart from the last beverage, 2 weeks abstinence was maintained before commencing the schedule. Blood was taken at the beginning and end of each schedule to determine plasma thromboxane B2 (TxB2) concentration and the IC50 (concentration required for 50% aggregation) for ADP and thrombin-induced platelet aggregation. White wine ($P < 0.05$) but not red wine increased the IC50 for ADP. Both wines increased the IC50 for thrombin ($P < 0.02$ and $P < 0.001$, respectively) and also lowered plasma TxB2 concentrations ($P < 0.01$ and $P < 0.025$, respectively). Neither grape juice altered ADP-induced aggregation or TxB2 concentrations, but the commercial juice lowered the IC50 for thrombin ($P < 0.001$) whereas the resveratrol-enriched juice caused a dramatic increase ($P < 0.001$). In vitro experiments demonstrated that the aggregation of fresh washed human platelets by ADP and thrombin was moderately reduced by both grape juices, strongly by red wine and not at all by white wine. The synthesis of TxB2 by platelets from labelled arachidonate was stimulated by commercial grape juice, slightly enhanced by resveratrol-enriched juice and strongly inhibited by red wine with white wine having little effect. Platelets from subjects consuming the commercial juice had a higher ratio of cyclo-oxygenase to lipoxygenase product formation and those consuming the resveratrol-enriched juice a lower ratio than during the control period. We conclude that trans-resveratrol can be absorbed from grape juice in biologically active quantities and in amounts that are likely to cause reduction in the risk of atherosclerosis. The failure of red wines (which have a 20-fold excess of polyphenols over white wines) to show any advantage suggests that, in vivo, ethanol is the dominant anti-aggregatory component in these beverages which are more potent than grape juices in preventing platelet aggregation in humans.

Role of free radicals in blood platelet activation

[Article in Czech]

Cas Lek Cesk. 2002 Sep 22;141 Suppl:47-9

Suttnar J, **Másová L**, **Scheiner T**, **Sorelová V**, **Dyr JE**.

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BACKGROUND: Free radicals and reactive oxygen containing substances are in addition to negative effects on biological systems important as signal molecules. Influencing their concentration by the action of antioxidants has a basic influence on the course of a number of cellular responses. The function of platelets is modulated in a significant way by the presence of vitamin E and resveratrol. The objective of the submitted paper is to assess the effect of Trolox (a stable analogue of vitamin E) and resveratrol sorbed by platelets on the aggregation response of washed platelets activated by collagen. METHODS AND RESULTS: To investigate the effects of the mentioned antioxidants on platelet aggregation washed platelets were prepared. The concentrations of the two antioxidants sorbed by platelets were assessed by the method of high performance liquid chromatography. From the total Trolox concentration (4200 microM) the platelets sorbed 33.5 nmol/10(9) platelets and from the total concentration of resveratrol (300 microM) the platelets sorbed 6.5 nmol/10(9) platelets. Inhibited aggregation by collagen was 57% for Trolox and 98% for resveratrol. CONCLUSIONS: The antioxidant capacity of both antioxidants is identical. The resveratrol concentration in platelets which led to almost complete inhibition of platelet aggregation by collagen was five times lower than for Trolox which caused a 57% inhibition of aggregation. Thus also other factors participate in the antioxidant activity of resveratrol. One of these factors is very probably the effect on arachidonic acid cycle.

Effect of resveratrol on platelet aggregation in vivo and in vitro.

Chin Med J (Engl). 2002 Mar;115(3):378-80

Wang Z, **Zou J**, **Huang Y**, **Cao K**, **Xu Y**, **Wu JM**.

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OBJECTIVE: Low or moderate consumption of red wine has a greater benefit than the consumption of other beverages in the prevention of atherosclerosis and coronary heart

disease and this is increasingly attributed to the polyphenol compounds in red wine, such as resveratrol. In the present study, we investigated the effect of resveratrol on platelet aggregation in vitro and in vivo. METHODS: Platelet aggregation in rabbits and normal subjects was measured using Born's method. RESULTS: Resveratrol, at 10 - 1000 micromol/L, significantly inhibited platelet aggregation in vitro induced by collagen, thrombin, and ADP in healthy subjects. The inhibitory effect was concentration-dependent. Hypercholesterolemia induced by high-cholesterol diet enhanced ADP-induced platelet aggregation. Resveratrol 4 mg x kg(-1) x d(-1) inhibited ADP-induced platelet aggregation in vivo despite no changes in serum lipid levels. CONCLUSIONS: Resveratrol inhibits platelet aggregation both in vitro and in vivo. This may be one of the mechanisms by which resveratrol prevents atherosclerosis.

Resveratrol and Cholesterol

Statin and resveratrol in combination induces cardioprotection against myocardial infarction in hypercholesterolemic rat.

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J Mol Cell Cardiol. 2007 Mar;42(3):508-16. Epub 2006 Dec 26

Hypercholesterolemia (HC) is a common health problem that significantly increases risk of cardiovascular disease. Both statin (S) and resveratrol (R) demonstrated cardioprotection through nitric oxide-dependent mechanism. Therefore, the present study was undertaken to determine whether combination therapy with statin and resveratrol is more cardioprotective than individual treatment groups in ischemic rat heart model. The rats were fed with 2% high cholesterol diet and after 8 weeks of high cholesterol diet the animals were treated with statin (1 mg/kg bw/day) and resveratrol (20 mg/kg bw/day) for 2 weeks. The rats were assigned to: (1) Control (C), (2) HC, (3) HCR, (4) HCS and (5) HCRS. The hearts, subjected to 30-min global ischemia followed by 120-min reperfusion were used as experimental model. The left ventricular functional recovery (+dp/dt(max)) was found to be significantly better in the HCRS (1926+/-43), HCR (1556+/-65) and HCS (1635+/-40) compared to HC group (1127+/-16). The infarct sizes in the HCRS, HCS and HCR groups were 37+/-3.6, 43+/-3.3 and 44+/-4.2 respectively compared to 53 +/-4.6 in HC. The lipid level was found to be decreased in all the treatment groups when compared to HC more significantly in HCS and HCRS groups when compared to HCR. Increased phosphorylation of Akt and eNOS was also observed in all the treatment groups resulting in decreased extent of cardiomyocyte apoptosis but the extent of reduction in apoptosis was more significant in HCRS group compared to all other groups. In vivo rat myocardial infarction (MI) model subjected to 1 week of permanent left descending coronary artery (LAD) occlusion documented increased capillary density in HCR and HCRS treated group when compared to HCS treatment group. We also documented increased beta-catenin translocation and increased VEGF mRNA expression in all treatment groups. Thus, we conclude that the acute as well as chronic protection afforded by combination treatment with statin and resveratrol may be due to pro-angiogenic, anti-hyperlipidemic and anti-apoptotic effects and long-term effects may be caused by increased neo-vascularization of the MI zone leading to less ventricular remodeling.

Dealcoholized red wine containing known amounts of resveratrol suppresses atherosclerosis in hypercholesterolemic rabbits without affecting plasma lipid levels.

Wang Z, **Zou J**, **Cao K**, **Hsieh TC**, **Huang Y**, **Wu JM**.

Int J Mol Med. 2005 Oct;16(4):533-40.

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Moderate consumption of red wine is associated with a reduced risk of coronary heart disease (CHD). This phenomenon is based on data from epidemiological observations known as the French paradox, and has been attributed to CHD-protective phytochemicals, e.g. resveratrol in red wine. Since red wine also contains alcohol, it is conceivable that alcohol interacts with resveratrol to elicit the observed cardioprotective effects. To determine whether resveratrol has alcohol-independent affects, we compared cardioprotective properties of dealcoholized Chinese red wine with alcohol-containing Chinese red wine having comparable amounts of resveratrol, using a hypercholesterolemic rabbit model and resveratrol as a reference. Animals fed a high cholesterol (1.5%) diet were simultaneously given water containing resveratrol (3 mg/kg/day) or red wine (4 ml/kg/day) containing 3.98 mg/l and 3.23 mg/l resveratrol for regular and dealcoholized red wine, respectively, for a 12-week duration. Total, HDL- and LDL-cholesterol and triglyceride levels in the plasma were measured before and after the cholesterol challenge. Atherosclerotic plaques in the thoracic aorta were evaluated using histochemical methods. Vascular and endothelial functions in the femoral artery were also assessed by ultrasonographic image analysis. High cholesterol-fed animals showed a significant increase in plasma levels of total, HDL- and LDL-cholesterol, but not triglycerides, compared to those fed a regular diet. Dietary cholesterol-elicited lipid changes were similarly observed in animals concurrently fed dealcoholized red wine, red wine or resveratrol. In contrast, whereas atherosclerotic lesions were clearly evident in specimens prepared from the thoracic aorta of high cholesterol-fed animals, the size, density, and mean area of atherosclerotic plaques, and thickness of the intima layer were significantly reduced in rabbits given dealcoholized red wine, red wine, or resveratrol. These results were in agreement with data obtained by an ultrasound analysis of endothelial function, which showed a 25% reduction in flow-mediated dilation (FMD) in rabbits fed a high cholesterol diet compared to animals on control diet. This decrease was effectively prevented by the simultaneous exposure to dealcoholized red wine, red wine, or resveratrol. Our study shows that animals given dealcoholized red wine exhibited cardio-active effects comparable to those of animals orally administered resveratrol, and suggests that wine polyphenolics, rather than alcohol present in red wine, suffice in exerting cardioprotective properties. The results also provide support for the notion that

resveratrol and phytochemicals in red wine can suppress atherosclerosis without affecting plasma lipid levels.

Grape polyphenols exert a cardioprotective effect in pre- and postmenopausal women by lowering plasma lipids and reducing oxidative stress.

Zern TL, **Wood RJ**, **Greene C**, **West KL**, **Liu Y**, **Aggarwal D**, **Shachter NS**, **Fernandez ML**.

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J Nutr. 2005 Aug;135(8):1911-7

To evaluate the effects of grape polyphenols on plasma lipids, inflammatory cytokines, and oxidative stress, 24 pre- and 20 postmenopausal women were randomly assigned to consume 36 g of a lyophilized grape powder (LGP) or a placebo for 4 wk. The LGP consisted of 92% carbohydrate and was rich in flavans, anthocyanins, quercetin, myricetin, kaempferol, and resveratrol. After a 3-wk washout period, subjects were assigned to the alternate treatment for an additional 4 wk. The placebo consisted of an equal ratio of fructose and dextrose and was similar in appearance and energy content (554 kJ) to LGP. Plasma triglyceride concentrations were reduced by 15 and 6% in pre- and postmenopausal women, respectively ($P < 0.01$) after LGP supplementation. In addition, plasma LDL cholesterol and apolipoproteins B and E were lower due to LGP treatment ($P < 0.05$). Further, cholesterol ester transfer protein activity was decreased by approximately 15% with intake of LGP ($P < 0.05$). In contrast to these beneficial effects on plasma lipids, LDL oxidation was not modified by LGP treatment. However, whole-body oxidative stress as measured by urinary F(2)-isoprostanes was significantly reduced after LGP supplementation. LGP also decreased the levels of plasma tumor necrosis factor-alpha, which plays a major role in the inflammation process. Through alterations in lipoprotein metabolism, oxidative stress, and inflammatory markers, LGP intake beneficially affected key risk factors for coronary heart disease in both pre- and postmenopausal women.

Effect of trans-resveratrol on the thrombogenicity and atherogenicity in apolipoprotein E-deficient and low-density lipoprotein receptor-deficient mice.

Fukao H, **Ijiri Y**, **Miura M**, **Hashimoto M**, **Yamashita T**, **Fukunaga C**, **Oiwa K**, **Kawai Y**, **Suwa M**, **Yamamoto J**.

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Blood Coagul Fibrinolysis. 2004 Sep;15(6):441-6

Resveratrol is one of the major polyphenolics in red wine that has been shown to exert the preventive effects against cardiovascular diseases. The effect of trans-resveratrol (t-RES) administered as an ingredient of the diet on the atherothrombotic tendency was assessed in genetically hypercholesterolemic mice after laser-induced damage on endothelium. Mice lacking both apolipoprotein E and low-density lipoprotein receptor (apoE-/-/ LDLR-/-) were fed with a high-fat diet with or without t-RES (9.6 and 96 mg/kg diet) for 8 weeks. The atherosclerotic tendency was morphometrically analyzed in their aortae. The thrombotic tendency was determined by inducing thrombus by the irradiation of a helium-neon laser on carotid arteries of these mice with injection of Evans blue. Atherosclerotic area and thrombus size were evaluated by image analyzing in a computer system. Even though the plasma concentrations of lipids (total cholesterol and triacylglycerol) did not change in the control and t-RES groups, a significant decrease (approximately 30%) in the formation of atheroma was observed in the aortae of the t-RES group. The size of laser-induced thrombus that mostly consisted of platelet aggregates was significantly reduced (approximately 25%) in the t-RES group compared with that in the control group. Thus, t-RES orally administrated with a high-fat diet in apoE-/-/LDLR-/- mice significantly suppressed atherosclerosis in their aortae and reduced the laser-induced thrombosis in their carotid arteries.

Inhibitory effects of Polygonum cuspidatum water extract (PCWE) and its component resveratrol [correction of rasveratrol] on acyl-coenzyme A-cholesterol acyltransferase activity for cholesteryl ester synthesis in HepG2 cells.

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Vascul Pharmacol. 2004 Jan;40(6):279-84

The pharmacological effects of Polygonum cuspidatum water extract (PCWE) on lipid biosynthesis were investigated in cultured human hepatocyte HepG2 cells. The addition of PCWE (5 and 20 microg/ml), which had no effect on cell proliferation and cellular protein content, caused a marked decrease in the cellular cholesterol content, particularly, the cholesteryl ester content following 24 h of incubation. The incorporation of (14)C-oleate into the cellular cholesteryl ester fraction was also reduced remarkably during incubation for 6 and 24 h. The effect of PCWE on acyl-coenzyme A-cholesterol acyltransferase (ACAT) activity were studied in vitro to explore the mechanism by which PCWE inhibits cholesterol ester formation. The data confirmed that PCWE, in a dose dependent manner, remarkably inhibits ACAT activity. Among the main active chemicals of P. cuspidatum, resveratrol, a kind of flavonoid, decreased ACAT activity in a dose-dependent manner from the level of 10(-3) M. Theses results strongly suggest that PCWE reduces the cholesteryl ester formation in human hepatocytes by inhibiting ACAT.

Cardiovascular protective effects of resveratrol.

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Cardiovasc Drug Rev. 2004 Fall;22(3):169-88

Resveratrol (3,4',5-trihydroxy-trans-stilbene), a phytoalexin found in grape skins, peanuts, and red wine, has been reported to have a wide range of biological and pharmacological properties. It has been speculated that at low doses (such as consumed in the common diet) resveratrol may have cardioprotective activity. In this article we describe recent in vitro and in vivo studies in animal models. The results of these studies suggest that resveratrol modulates vascular cell function, inhibits LDL oxidation, suppresses platelet aggregation and reduces myocardial damage during ischemia-reperfusion. Although the reported biological data indicate that resveratrol is a highly promising cardiovascular protective agent, more studies are needed to establish its bioavailability and in vivo cardioprotective effects, particularly in humans.

Hypolipidemic action of dietary resveratrol, a phytoalexin in grapes and red wine, in hepatoma-bearing rats.

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Life Sci. 2003 Aug 1;73(11):1393-400.

Resveratrol is an antioxidant present in grapes and their related products. We investigated whether dietary resveratrol could inhibit the proliferation and metastasis of tumors and hyperlipidemia in Donryu rats subcutaneously implanted with an ascites hepatoma cell line of AH109A. By feeding 10 or 50 ppm resveratrol in the diet to hepatoma-bearing rats for 20 days, solid tumor growth and metastasis tended to be suppressed dose-dependently. Resveratrol (50 ppm) significantly suppressed the serum lipid peroxide level, indicating its antioxidative properties or those of its metabolite(s) in vivo. Resveratrol dose-dependently suppressed both the serum triglyceride and very-low-density lipoprotein + low-density lipoprotein (VLDL + LDL)-cholesterol levels. The hypocholesterolemic action of resveratrol is attributed, at least in part, to an increased excretion of neutral sterols and bile acids into feces. These results suggest that dietary resveratrol is hypolipidemic with a tendency for anti-tumor-growth and anti-metastasis effects in hepatoma-bearing rats.

Grape polyphenols decrease plasma triglycerides and cholesterol accumulation in the aorta of ovariectomized guinea pigs.

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J.Nutr. 2003 Jul;133(7):2268-72

Female ovariectomized guinea pigs, a model for menopausal women, were fed either a control diet or a diet containing 10 g/100 g of a lyophilized grape preparation for 12 wk. The macronutrient composition of the grape preparation was: simple carbohydrates, 90 g/100 g; protein, 4 g/100 g; and dietary fiber, 6 g/100 g. Control and grape diets had the same composition except for the percentage of macronutrients provided by the grape preparation. Polyphenols were present in the grape preparation at 0.58 g/100 g and included flavans, anthocyanins, quercetin, myricetin, kaempferol and resveratrol. Dietary cholesterol was 0.33 g/100 g to raise plasma cholesterol concentrations and ensure the

development of atherosclerosis. Plasma LDL cholesterol concentrations did not differ between groups, whereas plasma triglycerides and VLDL cholesterol were 39 and 50% lower, respectively in guinea pigs fed the grape diet compared with controls ($P < 0.05$). Significant modifications in LDL particles included 58 and 30% lower triglycerides and phospholipids, respectively ($P < 0.0001$). Hepatic acyl CoA:cholesteryl acyltransferase activity was 27% lower ($P < 0.05$) in the grape diet-fed group compared with controls. In addition, concentrations of cholesterol in the aorta were 33% lower ($P < 0.05$) in guinea pigs fed the grape diet. These results suggest that grape intake in ovariectomized guinea pigs alters hepatic cholesterol metabolism, which may affect VLDL secretion rates and result in less accumulation of cholesterol in the aorta.

Red wine polyphenolics increase LDL receptor expression and activity and suppress the secretion of ApoB100 from human HepG2 cells.

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J Nutr. 2003 Mar;133(3):700-6

Epidemiologic studies suggest that the consumption of red wine may lower the risk of cardiovascular disease. The cardioprotective effect of red wine has been attributed to the polyphenols present in red wine, particularly resveratrol (a stilbene, with estrogen-like activity), and the flavonoids, catechin, epicatechin, quercetin and phenolic acids such as gallic acid. At present, very little is known about the mechanisms by which red wine phenolic compounds benefit the cardiovascular system. Therefore, the aim of this study was to elucidate whether red wine polyphenolics reduce lipoprotein production and clearance by the liver. Cultured HepG2 cells were incubated in the presence of dealcoholized red wine, alcohol-containing red wine and atorvastatin for 24 h. The apolipoprotien B100 (apoB100) protein (marker of hepatic lipoproteins) was quantified on Western blots with an anti-apoB100 antibody and the enhanced chemiluminescence detection system. Apolipoprotein B100 levels in the cells and that secreted into the media were significantly reduced by 50% in liver cells incubated with alcohol-stripped red wine compared with control cells. This effect of dealcoholized red wine on apoB100 production in HepG2 cells was similar to the effect of atorvastatin. Apo B100 production was significantly attenuated by 30% in cells incubated with alcoholized red wine, suggesting that the alcohol was masking the effect of red wine polyphenolics. Apo B100 production was significantly attenuated by 45% with the polyphenolic compounds resveratrol and quercertin. In addition, dealcoholized and alcoholized red wine and atorvastatin significantly increased 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase mRNA and LDL receptor binding activity relative to controls. Dealcoholized

red wine also increased LDL receptor gene expression. Collectively, this study suggests that red wine polyphenolics regulate major pathways involved in lipoprotein metabolism.

Effect of red wine and wine polyphenol resveratrol on endothelial function in hypercholesterolemic rabbits.

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Int J Mol Med. 2003 Mar;11(3):317-20

The effect of red wine and wine polyphenol resveratrol on endothelial function was investigated in experimental hypercholesterolemic rabbits. Endothelial function as measured by flow-mediated dilation (FMD) in the femoral artery was 19.28+/-2.81% in control animals fed a regular diet. In contrast, rabbits fed a high-cholesterol (1.5%) diet showed a reduced endothelial function, as revealed by a 25% reduction in the measured FMD. Intragastric feeding of resveratrol (3 mg/kg/day), red wine (4 ml/kg/day), dealcoholized red wine (4 ml/kg/day), for 12 weeks in hypercholesterolemic rabbits significantly mitigated the reduction in endothelial function, and resulted in FMD values of 14.52+/-0.60, 18.95+/-2.30, 17.58+/-1.43, and 18.80+/-3.94%, respectively. Measurement of plasma endothelin 1 (ET-1) and nitric oxide (NO) levels showed that feeding a high-cholesterol diet significantly increased plasma ET-1 levels (from 51.4 +/-17.6 to 96.9+/-24.3 pg/ml), and decreased plasma NO concentration (from 104.6 +/-18.5 to 67.7+/-16.1 pg/ml). With administration of resveratrol, red wine, or dealcoholized red wine, plasma ET-1 levels statistically decreased, in parallel with a significant elevation in NO levels. These results provide in vivo evidence suggesting that resveratrol and red wine improve endothelial function, which may be one of the mechanisms by which this red wine polyphenol exerts its alcohol-independent cardioprotective effects.

Effect of resveratrol on platelet aggregation in vivo and in vitro.

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Chin Med J (Engl). 2002 Mar;115(3):378-80

OBJECTIVE: Low or moderate consumption of red wine has a greater benefit than the consumption of other beverages in the prevention of atherosclerosis and coronary heart disease and this is increasingly attributed to the polyphenol compounds in red wine, such as resveratrol. In the present study, we investigated the effect of resveratrol on platelet aggregation in vitro and in vivo. METHODS: Platelet aggregation in rabbits and normal subjects was measured using Born's method. RESULTS: Resveratrol, at 10 - 1000 micromol/L, significantly inhibited platelet aggregation in vitro induced by collagen, thrombin, and ADP in healthy subjects. The inhibitory effect was concentration-dependent. Hypercholesterolemia induced by high-cholesterol diet enhanced ADP-induced platelet aggregation. Resveratrol 4 mg x kg(-1) x d(-1) inhibited ADP-induced platelet aggregation in vivo despite no changes in serum lipid levels. CONCLUSIONS: Resveratrol inhibits platelet aggregation both in vitro and in vivo. This may be one of the mechanisms by which resveratrol prevents atherosclerosis.

Effects of red wine and wine polyphenol resveratrol on platelet aggregation in vivo and in vitro.

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Int J Mol Med. 2002 Jan;9(1):77-9

Low to moderate consumption of red wine reportedly has a relatively greater benefit than other alcoholic beverages in the prevention of atherosclerosis and coronary heart disease (CHD). This beneficial effect is increasingly attributed to the polyphenol resveratrol, present in red wine. In the present study, we investigated the effects of resveratrol and red wine on aggregation of platelets isolated from healthy, normotensive male volunteers and in rabbits with experimental hypercholesterolemia. Platelet aggregation rate (PAR) was measured using Born's method. The results showed that aggregation of platelets from healthy subjects induced in vitro by collagen (5 microg/ml), thrombin (0.33 units/ml), and ADP (4 microM) was significantly inhibited by 10-1000 microM resveratrol, in a concentration-dependent manner. Hypercholesterolemic rabbits showed enhanced ADP-induced platelet aggregation; the average PAR increased from 39.5+/-5.9% in normal animals to 61.0+/-7.0% in the high-cholesterol fed group (n=8, p<0.001). Resveratrol (4 mg/kg/day) inhibited ADP-induced platelet aggregation in vivo by maintaining the PAR at 35.7+/-6.3% (vs. 39.5+/-5.9% for control rabbits, n=8, p=0.228), but had no effect on serum lipid levels. Similarly platelet aggregation in hypercholesterolemic rabbits was also inhibited when animals received intragastrically Chinese red wine (with or without

alcohol, 4 ml/kg/day). These results suggest that resveratrol can inhibit platelet aggregation both in vitro and in vivo, which conceivably could be one of the mechanisms by which this red wine polyphenol exerts its cardioprotective effects.

Comparison of the antioxidant effects of equine estrogens, red wine components, vitamin E, and probucol on low-density lipoprotein oxidation in postmenopausal women.

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Menopause. 2001 Nov-Dec;8(6):408-19

OBJECTIVE: Oxidized low-density lipoprotein (LDL) seems to play an important role in the etiology of atherosclerosis. To further study this, we performed two studies: (1) we determined the ability of 10 estrogen components of the drug, conjugated equine estrogen (CEE), trans-resveratrol (t-resveratrol) and quercetin (red wine components), trolox (vitamin E analog), and probucol (a serum cholesterol-lowering drug) to delay or prevent the oxidation of plasma LDL isolated from untreated postmenopausal women, and (2) we assessed the effect of long-term (>1 year) estrogen replacement therapy and hormone replacement therapy on LDL oxidation by ex vivo methods. DESIGN: For the in vivo study, three groups of postmenopausal women were selected based on whether they were on long-term CEE therapy (group A: 0.625 mg CEE; n = 21), on combination CEE plus progestogen therapy (group B: 0.625 mg CEE + 5.0 mg medroxyprogesterone acetate, 10 days; n = 20), or not on any hormone therapy (group C; n = 37). For the in vitro study, only LDL samples obtained from group C were used. The kinetics of LDL oxidation were measured by continuously monitoring the formation of conjugated dienes followed by determination of the lag time. RESULTS: All compounds tested protected the LDL from oxidative damage. The relative antioxidant potency of estrogen components was generally greater than that of the other compounds. The minimum dose (nmoles) required to double the lag time from the control lag time of 57 +/- 2 min was 0.47 for 17beta-dihydroequilenin, 17alpha-dihydroequilenin, Delta 8 -estrone; 0.6 to 0.7 for Delta 8 -17beta-estradiol, equilenin, and quercetin; 0.9 for 17beta-dihydroequilin and 17alpha-dihydroequilin; 1.3 for equilin, estrone, 17beta-estradiol, 17alpha-estradiol; 1.4 for trolox; 1.9 for probucol; and 3.0 for t-resveratrol. The data from the in vivo study indicate that after long-term estrogen replacement therapy (group A) and hormone replacement therapy (group B), the LDL was significantly ($p < 0.01$) protected (higher lag time) against oxidation compared with the control (group C). There was no difference between groups A and B. CONCLUSIONS: The oxidation of LDL isolated from postmenopausal women is inhibited differentially by various estrogens and other antioxidants. The unique ring B unsaturated estrogen components of CEE were the most potent, and t-resveratrol, the red

wine component, was the least potent. Long-term CEE or CEE + medroxyprogesterone acetate administration to postmenopausal women protects the LDL against oxidation to the same extent. These combined data support the hypothesis that some of the cardioprotective benefits associated with CEE therapy and perhaps red wine consumption may be due to the ability of their components to protect LDL against oxidative modifications.

Inhibitory effect of resveratrol on proteinuria, hypoalbuminemia and hyperlipidemia in nephritic rats.

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Life Sci. 2001 May 11;68(25):2845-52

The effect of resveratrol, a polyphenolic compound present in grapes and other plants, on proteinuria, hypoalbuminemia and hyperlipidemia was studied in rats with glomerulonephritis. The nephritis was induced by an intravenous injection of anti-rat kidney glomerular basement membrane rabbit antiserum. Nephritic rats were given oral intubation of resveratrol (5 mg/day/100 g body weight) for 14 days, while control nephritic rats as well as normal ones were similarly given vehicle alone. By resveratrol treatment, enlargement in liver and kidney due to nephritis induction was significantly reduced, together with partial restoration of nephritis-induced reduction in body weight gain. Both proteinuria and hypoalbuminemia, characteristic symptoms to nephrotic syndrome, were significantly remedied, that is, urinary protein excretion was suppressed and serum albumin concentration was increased by resveratrol treatment. Resveratrol also suppressed significantly hyperlipidemia incident to nephritis, the hypotriglyceridemic action being more prominent than the hypocholesterolemic one. From these results, resveratrol is suggested to be a potent anti-glomerulonephritic food factor capable of suppressing proteinuria, hypoalbuminemia and hyperlipidemia at the same time.

Experimental study of resveratrol and flavonoids in red wine with regard to their possible hypolipemic effects

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Vnitr Lek. 2000 Dec;46(12):856-60.

Resveratrol (trans-3,5,4'-trihydroxystilben) is a polyphenol (phytoalexin) naturally found in wine and different therapeutic plants. It is a substance with an antioxidant and estrogenic effect and the ability to inhibit the growth of some tumours. Some studies mention its possible antiaggregation, neuroprotective and antiallergic effect. In the submitted pilot study the authors investigated the effect of resveratrol and flavonoids (anthocyanins, catechins) on serum lipid levels, in particular total cholesterol and liver enzymes in the laboratory rat. In the experiments healthy animals were used (fed a standard diet) as well as hypercholesterolemic animals (fed a special sugar diet) and treated animals. The investigated parameters were total cholesterol, HDL-cholesterol, aminotransferase aspartate (AST) and alanine aminotransferase (ALT). The conclusions of the investigation indicate that resveratrol and flavonoids (anthocyanins, catechins) found in red wine significantly reduce the total cholesterol level in the hypercholesterolemic rat. The resultant effect of resveratrol and flavonoids on liver enzymes in our experiment is not unequivocal.

Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids.

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Life Sci. 1999;64(26):2511-21

Wine polyphenols were examined for their capacity to protect the lipid and protein moieties of porcine low density lipoproteins (LDL) during oxidation. The efficiency of resveratrol (3, 4', 5, trihydroxystilbene) and defined flavonoids was compared to that of a wine extract (WE) containing 0.5 g/g proanthocyanidols. The efficiency of resveratrol for protecting polyunsaturated fatty acids (PUFA) was higher than that of flavonoids in copper-induced oxidation and lower in AAPH (radical initiator)-induced oxidation. The LDL receptor activity was evaluated by flow cytometry using LDL labeled with fluorescein isothiocyanate (FITC) and Chinese hamster ovary cells (CHO-K1). The incubation of CHO-K1 with FITC-LDL oxidized for 16 h reduced the proportion of fluorescent cells from 97% to 4%. At a concentration of 40 microM, resveratrol and flavonoids completely restored the uptake of copper-oxidized LDL and AAPH-oxidized LDL respectively. Total fluorescence could also be obtained with 20 mg/L of WE with both oxidation systems. These data are consistent with previous findings relative to the formation of degradative products from PUFA. They confirm that resveratrol was more effective than flavonoids as a chelator of copper and less effective as a free-radical scavenger. Moreover, they show that WE, which contained monomeric and oligomeric forms of flavonoids and phenolic acids, protected LDL by both mechanisms.

Beyond alcohol: beverage consumption and cardiovascular mortality.

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Clin Chim Acta. 1995 Jun 15;237(1-2):155-87

This paper reviews epidemiological investigations which have identified an inverse relationship between alcohol consumption and death from coronary heart disease: evidence from studies of mixed populations as well as of single-sex populations have, on the whole, demonstrated that this relationship is independent of sex or age. This 'cardioprotective effect' of alcohol can be explained, at least in part, by ethanol-related increases in high density lipoprotein cholesterol and reduced platelet coagulability. With certain beverages, especially red wine, phenolic compounds may provide additional protection by altering eicosanoid metabolism in favour of increased prostacyclin and decreased thromboxane synthesis, as well as antioxidant functions which prevent the peroxidation of low-density lipoprotein. Trans-resveratrol, a tri-hydroxy stilbene present in the skins of specific grape cultivars, is a constituent of certain red wines which may play a crucial role in modulating lipoprotein metabolism, eicosanoid synthesis, oxidation and coagulation. Preliminary studies using the human hepatoma cell line HepG2 are described, demonstrating that this compound has no effect upon cell viability or overall protein synthesis in these cells, and at high concentrations DNA synthesis as measured by radioactive thymidine incorporation is enhanced. Reduced intracellular concentration and secretion of apolipoprotein B have been shown to occur in response to resveratrol although a clear dose-dependency has not yet been demonstrated. The mechanisms underlying these changes as well as the effects upon the synthesis and secretion of other apolipoproteins are under active investigation in our laboratory.

Antimicrobial activity

Antimicrobial effect of resveratrol on dermatophytes and bacterial pathogens of the skin.

Biochem Pharmacol. 2002 Jan 15;63(2):99-104.

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The phytoalexin resveratrol is commonly found in food and drinks, including red wine, grapes, and peanuts. Many studies have shown that this compound has anti-inflammatory properties, and it has been ascribed as having health benefits that help to prevent cancer and coronary heart disease. A treatment that combines antimicrobial and anti-inflammatory actions may be desirable for alleviating many skin conditions that range in severity. Therefore, we evaluated the antimicrobial activity of resveratrol against bacteria and dermatophytes that are major etiologic agents of human skin infections. Using the broth microdilution protocol of the National Committee for Clinical Laboratory Standards (NCCLS) M7-A5, growth of the bacterial species Staphylococcus aureus, Enterococcus faecalis, and Pseudomonas aeruginosa was inhibited at 171-342 microg/mL of resveratrol in the solvent dimethyl sulfoxide. Using the NCCLS protocol M38-P, activity against the fungal species Trichophyton mentagrophytes, Trichophyton tonsurans, Trichophyton rubrum, Epidermophyton floccosum, and Microsporum gypseum was also tested. The growth of dermatophytes was inhibited at 25-50 microg/mL of resveratrol. Thus, this study indicates a novel application for resveratrol, a molecule of plant defense, to combat human fungal pathogens. Resveratrol and its analogs may have wide application to skin conditions that afflict a significant portion of our population, and may also have promising clinical potentials in diabetic wounds.

Fungicidal effect of resveratrol on human infectious fungi.

Arch Pharm Res. 2005 May;28(5):557-60

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Resveratrol, a phenolic antioxidant found in grapes, has been known to mediate various biological activities on the human body. In the present study, we tested the antifungal

activity of resveratrol against human pathogenic fungi before carrying out further studies to elucidate the antifungal mechanism(s) of resveratrol. Resveratrol displayed potent antifungal activity against human pathogenic fungi at concentration levels of 10-20 microg/mL. Furthermore, time-kill curve exhibited fungicidal effect of resveratrol on C. albicans, but the compound had no hemolytic activity against human erythrocytes. The destruction of C. albicans cells by resveratrol was confirmed by scanning electron microscopy. These results suggest that resveratrol could be employed as a therapeutic agent to treat fungal infections of humans.

Resveratrol and curcumin reduce the respiratory burst of Chlamydia-primed THP-1 cells.

Biochem Biophys Res Commun. 2005 Jul 22;333(1):21-7

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The intracellular bacterium Chlamydia pneumoniae is involved in the inflammation process of atherosclerosis. We previously demonstrated that C. pneumonia infected monocytes (THP-1 cells) responded to stimulation by an increased respiratory burst linked to an increased NADPH oxidase (NOX) activity. We now tested agents acting on the assembly of the NOX subunits or on protein kinase C, a trigger of NOX activity. Apocynin, resveratrol, rutin, quercetin, curcumin, and tocopherols were tested. The cells were pre-incubated with Chlamydia and the agent for 19 h, and then stimulated with phorbol myristate acetate. The NOX activity was monitored by measuring the hydrogen peroxide production. Resveratrol and curcumin (10(-4)-10(-6) M) were better inhibitors than apocynin. alpha-Tocopherol was inactive, and gamma-tocopherol inhibitor at 10(-4) M only. Quercetin was inactive, and rutin a moderate but significant inhibitor. The inhibition by resveratrol was increased by 10(-6) M rutin or quercetin. Resveratrol and curcumin thus appeared to be interesting for atherosclerosis treatment.

Resveratrol and red wine extracts inhibit the growth of CagA+ strains of Helicobacter pylori in vitro.

Am J Gastroenterol. 2003 Jun;98(6):1440-1.

Mahady GB, **Pendland SL**, **Chadwick LR**.

PMID: 12818294 [PubMed - indexed for MEDLINE]

Resveratrol and CNS activity

Resveratrol-induced activation of the mitogen-activated protein kinases, ERK1 and ERK2, in human neuroblastoma SH-SY5Y cells.

Neurosci Lett. 1999 Apr 2;264(1-3):141-4

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Phosphorylation of the mitogen-activated protein (MAP) kinases, extracellular signal-regulated kinase 1 (ERK1) and extracellular signal-regulated kinase 2 (ERK2), induced by resveratrol, a natural antioxidant present in grapes and wine, has been studied in vitro on undifferentiated and differentiated (induction by retinoic acid) SH-SY5Y human neuroblastoma cells. In undifferentiated cells resveratrol 1 microM induced phosphorylation of ERK1 and ERK2, which was already evident at 2 min, peaked at 10 min and persisted at 30 min. A wide range (from 1 pM to 10 microM) of resveratrol concentrations were able to induce phosphorylation of ERK1 and ERK2, while higher concentrations (50-100 microM) inhibited MAP kinases phosphorylation. In retinoic acid (RA) differentiated cells resveratrol (1 microM) induced an evident increase in ERK1 and ERK2 phosphorylation. This study demonstrates that resveratrol, even at very low concentrations, may have a biological effect on neuron-like cells.

Red wine ingredient resveratrol protects from beta-amyloid neurotoxicity.

Gerontology. 2003 Nov-Dec;49(6):380-3.

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BACKGROUND: beta-Amyloid peptide (Abeta), a neutrotoxic substance, has been implicated to a great degree in cell death during the course of AD. Resveratrol, a natural polyphenol mainly found in red wine, has been shown to be cardioprotective and chemoprotective. Since a moderate wine intake correlates with a lower risk for Alzheimer disease (AD), an additional neuroprotective effect has been postulated for resveratrol. OBJECTIVE: The present study aimed at elucidating the possible neuroprotective effects of resveratrol against Abeta-induced neurotoxicity. METHODS: The neuroprotective

capacity against Abeta-related oxidative stress was studied in a cell culture model suitable for studying such potentially neuroprotective substances. RESULTS: Resveratrol maintains cell viability and exerts an anti-oxidative action by enhancing the intracellular free-radical scavenger glutathione. CONCLUSION: Our findings suggest that red wine may be neuroprotective through the actions of resveratrol.

Anti-apoptotic effect of trans-resveratrol on paclitaxel-induced apoptosis in the human neuroblastoma SH-SY5Y cell line.

Neurosci Lett. 2001 Apr 13;302(1):41-4

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Paclitaxel, an anticancer drug, induces apoptosis in human neuroblastoma cell line SH-SY5Y. The addition of trans-resveratrol, a natural antioxidant present in grapes and red wine, to SH-SY5Y cultures exposed to paclitaxel significantly reduces cellular death. The neuroprotective action of trans-resveratrol is due neither to its antioxidant capacity nor to interference with the polymerization of tubulin induced by paclitaxel. However, trans-resveratrol is able to inhibit the activation of caspase 7 and degradation of poly-(ADP-ribose)-polymerase which occur in SH-SY5Y exposed to paclitaxel. Resveratrol, therefore, exerts its anti-apoptotic effect by modulating the signal pathways that commit these neuronal-like cells to apoptosis.

Potential mechanism by which resveratrol, a red wine constituent, protects neurons.

Ann N Y Acad Sci. 2003 May;993:276-86; discussion 287-8

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Polyphenolic compounds, such as resveratrol, are naturally present at high concentration in grape skin, seeds, and red wine. Resveratrol is present in cis and trans isoforms and the

major trans isomer is the biologically active one. Epidemiologic studies have revealed a reduced incidence of cardiovascular risk associated with consumers of red wine; this has been popularized as the French paradox. Resveratrol has been shown to have significant antioxidant properties in a variety of in vitro and in vivo models. It can reduce ischemic damage in heart ischemia reperfusion injury and also in brain ischemia/reperfusion in rodent models. Due to the high rate of oxygen consumption in the brain, and especially low levels of antioxidant defense enzymes, this organ is particularly susceptible of free radical damage. Most of the protective biological actions associated with resveratrol have been associated with its intrinsic radical scavenger properties. We have investigated the possibility of other indirect pathways by which resveratrol can exert its neuroprotective abilities. We have specifically tested whether heme oxygenase neuroprotective enzyme could be stimulated after resveratrol treatment. Using primary neuronal cultures, resveratrol was able to significantly induce heme oxygenase 1, whereas vehicle control showed no effect. No detectable toxicity was quantified. It is well established that after stroke significant levels of intracellular heme levels increase. The source of free heme comes mainly from several heme-containing enzymes. Heme (iron-protoporphyrin IX) is a pro-oxidant and its rapid degradation by heme oxygenase is believed to be protective. Moreover, the generation of heme metabolites can also have their own intrinsic cellular properties. All together, increased heme oxygenase activity by resveratrol is a unique pathway by which this compound can exert its neuroprotective actions.

Brain protection by resveratrol and fenofibrate against stroke requires peroxisome proliferator-activated receptor alpha in mice.

Neurosci Lett. 2003 Dec 11;352(3):203-6

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Peroxisome proliferator-activated receptors (PPARs) are ligand-dependent transcription factors which belong to the nuclear receptor family. We examined whether PPARalpha agonists and resveratrol, a polyphenol contained in grapes, protect the brain against ischemia. To investigate whether resveratrol activates PPARs, we performed a cell-based transfection activity assay using luciferase reporter plasmid. PPARalpha and PPARgamma were activated by resveratrol in primary cortical cultures and vascular endothelial cells. Resveratrol (20 mg/kg, 3 days) reduced infarct volume by 36% at 24 h after middle cerebral artery occlusion in wild-type mice. The PPARalpha agonists fenofibrate (30 mg/kg, 3 days) and Wy-14643 (30 mg/kg, days) exerted similar brain protection. However, resveratrol and fenofibrate failed to protect the brain in PPARalpha

knockout mice. The data indicate that PPARalpha agonists protect the brain through PPARalpha.

Resveratrol and aging

Resveratrol is a class IA phosphoinositide 3-kinase inhibitor.

Fröjdö S, **Cozzone D**, **Vidal H**, **Pirola L**.

Biochem J. 2007 Jun 6;

Resveratrol, a polyphenol found in fruits, possesses chemopreventive and chemotherapeutic properties and has been shown to increase lifespan in yeast and metazoans, including mice. Genetic evidence and in vitro enzymatic measurements indicate that the deacetylase Sir2/SIRT1, an enzyme promoting stress-resistance and aging, is the target of resveratrol. Similarly, downregulation of insulin-like pathways - of which phosphoinositide 3-kinase (PI3K) is a key mediator - promotes longevity and is an attractive strategy to fight cancer. We show here that resveratrol inhibits, in vitro and in cultured muscle cell lines, class IA PI3K and its downstream signalling at the same concentration range at which it activates sirtuins. Our observations define class IA PI3K as a target of resveratrol that may contribute to the longevity-promoting and anticancer properties and identify resveratrol as a natural class-specific PI3K inhibitor.

Evidence for a trade-off between survival and fitness caused by resveratrol treatment of Caenorhabditis elegans.

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Ann N Y Acad Sci. 2007 Apr;1100:530-42

Resveratrol is a naturally occurring polyphenolic compound commonly found in plant-derived products, including red wine. A large number of beneficial effects including anticarcinogenic action and protection from atherosclerotic disease have been attributed to resveratrol. Increased resveratrol intake has been suggested as an explanation for the beneficial effects of moderate red wine consumption. Resveratrol also consistently extends the mean and maximum life span in model organisms including nematode worms. It has been suggested that resveratrol exerts its life-span-extending effect through calorie restriction or hormesis mimetic effects. We have characterized the effect of resveratrol on stress resistance, developmental rate, growth, and fecundity in the nematode worm Caenorhabditis elegans in order to determine whether the beneficial effects of resveratrol on life span are associated with trade-offs in terms of early life fitness in nematodes. We find that resveratrol treatment increases stress resistance,

specifically to oxidative stress, and causes a small but significant decrease in fecundity early in life without affecting overall fecundity. Resveratrol increased mean and maximum life span by delaying the onset of the exponential increase in mortality characterizing the "dying phase" in C. elegans, but did not affect the dying phase itself, suggesting that it did not act by directly affecting metabolism.

Resveratrol stimulates AMP kinase activity in neurons.

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Proc Natl Acad Sci U S A. 2007 Apr 24;104(17):7217-22. Epub 2007 Apr 16

Resveratrol is a polyphenol produced by plants that has multiple beneficial activities similar to those associated with caloric restriction (CR), such as increased life span and delay in the onset of diseases associated with aging. CR improves neuronal health, and the global beneficial effects of CR have been postulated to be mediated by the nervous system. One key enzyme thought to be activated during CR is the AMP-activated kinase (AMPK), a sensor of cellular energy levels. AMPK is activated by increases in the cellular AMP:ATP ratio, whereupon it functions to help preserve cellular energy. In this regard, the regulation of dietary food intake by hypothalamic neurons is mediated by AMPK. The suppression of nonessential energy expenditure by activated AMPK along with the CR mimetic and neuroprotective properties of resveratrol led us to hypothesize that neuronal activation of AMPK could be an important component of resveratrol activity. Here, we show that resveratrol activated AMPK in Neuro2a cells and primary neurons in vitro as well as in the brain. Resveratrol and the AMPK-activating compound 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR) promoted robust neurite outgrowth in Neuro2a cells, which was blocked by genetic and pharmacologic inhibition of AMPK. Resveratrol also stimulated mitochondrial biogenesis in an AMPK-dependent manner. Resveratrol-stimulated AMPK activity in neurons depended on LKB1 activity but did not require the NAD-dependent protein deacetylase SIRT1 during this time frame. These findings suggest that neuronal activation of AMPK by resveratrol could affect neuronal energy homeostasis and contribute to the neuroprotective effects of resveratrol.

Increased mitochondrial H2O2 production promotes endothelial NF-{kappa}B activation in aged rat arteries.

Ungvari ZI, **Orosz Z**, **Labinskyy N**, **Rivera A**, **Xiangmin Z**, **Smith KE**, **Csiszar A**.

Am J Physiol Heart Circ Physiol. 2007 Apr 6; [Epub ahead of print]

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Previous studies have shown that the aging vascular system undergoes pro-atherogenic phenotypic changes, including increased oxidative stress and a pro-inflammatory shift in endothelial gene expression profile. To elucidate the link between increased oxidative stress and vascular inflammation in aging the carotid arteries and aortas of young and aged (24 month old) F344 rats were compared. In aged vessels there was an increased NF-kappaB activity (assessed by luciferase reporter gene assay and NF-κB binding assay), which was attenuated by scavenging H2O2. Aging did not alter the vascular mRNA and protein expression of p65 and p50 subunits of NF-kappaB. In endothelial cells of aged vessels there was an increased production of H2O2 (assessed by DCF fluorescence), which was attenuated by the mitochondrial uncoupler FCCP. In young arteries and cultured endothelial cells antimycin A plus succinate significantly increased FCCP-sensitive mitochondrial H2O2 generation, which was associated with activation of NF-kappaB. In aged vessels inhibition of NF-kappaB (by PDTC, resveratrol) significantly attenuated inflammatory gene expression and inhibited monocyte adhesiveness. Thus, increased mitochondrial oxidative stress contributes to endothelial NF-kappaB activation, which contributes to the pro-inflammatory phenotypic alterations in the aged vaculature. Our model predicts that by reducing mitochondrial H2O2 production and/or directly inhibitingNF-kappaB novel anti-aging pharmacological treatments (e.g. calorie restriction mimetics) will exert significant anti-inflammatory and vasoprotective effects. Key words: aging, senescence, endothelial cell, resveratrol, inflammation.

Antioxidative effects of plant polyphenols: from protection of G protein signaling to prevention of age-related pathologies.

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Ann N Y Acad Sci. 2007 Jan;1095:449-57

The antioxidant potency of three natural polyphenols, resveratrol, curcumin, and genistein, was compared by using the two human models: oxymodified with H(2)O(2) and homocysteine (Hcy) G proteins in the postmortem frontal cortex (FC) membranes of age-matched control and Alzheimer's disease (AD) subjects; and Cu(2+)-induced oxidation of plasma low-density lipoproteins (LDL). In Co, 3-10 microM polyphenols dose-dependently depressed the G protein 25% stimulation induced by 10 microM H(2)O (2) or 500 microM Hcy. Resveratrol revealed significantly higher antioxidativity than

curcumin or genistein. In AD, the antioxidativity of polyphenols showed no significant differences. Polyphenols (1 microM) significantly increased the LDL oxidation lag time (oxyresistance) as compared with control, the effect of resveratrol being most potent. Due to the dual antioxidant mechanism, the investigated polyphenols, particularly resveratrol, should have preferences for the preventive-therapeutic use in age-related oxidative stress-based pathologies.

SIRT1 and neuronal diseases.

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Mol Aspects Med. 2007 Feb 16;

SIRT1 is the mammalian homologue of yeast silent information regulator (Sir)-2, a member of the sirtuin family of protein deacetylases which have gained much attention as mediators of lifespan extension in several model organisms. Induction of SIRT1 expression also attenuates neuronal degeneration and death in animal models of Alzheimer's disease and Huntington's disease. SIRT1 induction, either by sirtuin activators such as resveratrol, or metabolic conditioning associated with caloric restriction (CR), could be neuroprotective in several ways. It could promote the non-amyloidogenic cleavage of the amyloid precursor protein, enhance clearance of amyloid beta-peptides, and reduced neuronal damage through potential inhibition of neuroinflammatory signaling pathways. In addition, increased SIRT1 activity could alter neuronal transcription profiles to enhance anti-stress and anti-apoptotic gene activities, and has been proposed to underlie the inhibition of axonal degeneration in the Wallerian degeneration slow (Wld(s)) phenotype. As neuronal degeneration is a major pathophysiological aspect of human aging, understanding the mechanism of SIRT1 neuroprotection promises novel strategies in clinical intervention of neurodegenerative diseases.

An anti-aging drug today: from senescence-promoting genes to anti-aging pill.

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Drug Discov Today. 2007 Mar;12(5-6):218-24.

Numerous mutations increase lifespan in diverse organisms from worms to mammals. Most genes that affect longevity encode components of the target of rapamycin (TOR) pathway, thus revealing potential targets for pharmacological intervention. I propose that one target, TOR itself, stands out, simply because its inhibitor (rapamycin) is a non-toxic, well-tolerated drug that is suitable for everyday oral administration. Preclinical and clinical data indicate that rapamycin is a promising drug for age-related diseases and seems to have anti-tumor, bone-sparing and calorie-restriction-mimicking 'side-effects'. I also discuss other potential anti-aging agents (calorie restriction, metformin, resveratrol and sirtuins) and their targets, interference with the TOR pathway and combination with antioxidants.

Vertebrate aging research 2006.

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Aging Cell. 2007 Apr;6(2):135-8. Epub 2007 Feb 28

This Hot Topics review, the first in a projected annual series, discusses those articles, published in the last year, which seem likely to have a major impact on our understanding of the aging process in mammals and the links between aging and late-life illnesses. The year's highlights include studies of oxidation damage in the very-long-lived naked mole-rat, and of caloric restriction in monkeys, humans, and growth hormone-unresponsive mice. Two studies of resveratrol, one showing its ability to extend lifespan in a short-lived fish, the other demonstrating beneficial effects in mice subjected to a diet high in fat, may well be harbingers of a parade of intervention studies in the coming decade.

Resveratrol in cell fate decisions.

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J Bioenerg Biomembr. 2007 Feb;39(1):59-63

Resveratrol, a polyphenolic phytoalexin, is one of the most extensively studied natural products, with wide ranging biological activity and tremendous clinical potential. First identified from fruits and plants, in particular grapes and wines, its positive effects on a variety of disease states have been unraveled over the past decade or so. Most noticeable are its anti-thrombogenic, anti-inflammatory, cardio-protective, neuro-protective, anti-aging, and cancer preventive and therapeutic activities. Recent data also indicate that depending upon the concentration/dose, resveratrol can trigger or block cell death signaling in tumor cells. Considering the heightened interest in this compound, here we present a short review on the biological activity of this remarkable compound, with a specific focus on its effects on cell survival and death signals.

Resveratrol increases vascular oxidative stress resistance.

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Am J Physiol Heart Circ Physiol. 2007 May;292(5):H2417-24

Epidemiological studies suggest that Mediterranean diets rich in resveratrol are associated with reduced risk of coronary artery disease. However, the mechanisms by which resveratrol exerts its vasculoprotective effects are not completely understood. Because oxidative stress and endothelial cell injury play a critical role in vascular aging and atherogenesis, we evaluated whether resveratrol inhibits oxidative stress-induced endothelial apoptosis. We found that oxidized LDL and TNF-alpha elicited significant increases in caspase-3/7 activity in endothelial cells and cultured rat aortas, which were prevented by resveratrol pretreatment (10(-6)-10(-4) mol/l). The protective effect of resveratrol was attenuated by inhibition of glutathione peroxidase and heme oxygenase-1, suggesting a role for antioxidant systems in the antiapoptotic action of resveratrol. Indeed, resveratrol treatment protected cultured aortic segments and/or endothelial cells against increases in intracellular H(2)O(2) levels and H(2)O(2)-mediated apoptotic cell death induced by oxidative stressors (exogenous H(2)O(2), paraquat, and UV light). Resveratrol treatment also attenuated UV-induced DNA damage (comet assay). Resveratrol treatment upregulated the expression of glutathione peroxidase, catalase, and heme oxygenase-1 in cultured arteries, whereas it had no significant effect on the expression of SOD isoforms. Resveratrol also effectively scavenged H(2)O(2) in vitro. Thus resveratrol seems to increase vascular oxidative stress resistance by scavenging H(2)O(2) and preventing oxidative stress-induced endothelial cell death. We propose that the antioxidant and antiapoptotic effects of resveratrol, together with its previously described anti-inflammatory actions, are responsible, at least in part, for its cardioprotective effects.

SIR2: a potential target for calorie restriction mimetics.

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Trends Mol Med. 2007 Feb;13(2):64-71. Epub 2007 Jan 4

Calorie restriction (CR) extends lifespan in a wide variety of species and mitigates diseases of aging in mammals. Here, we describe the evidence that the silent information regulator 2 (SIR2) gene, which encodes a nicotinamide adenine dinucleotide (NAD)-dependent deacetylase, regulates lifespan and mediates CR in lower species such as Saccharomyces cerevisiae and Caenorhabditis elegans. We discuss the emerging roles of mammalian SIR2 homologs in regulating physiological changes triggered by CR and their potential connections to diseases of aging. We conclude with the recent advances on small molecules that activate the enzymatic activity of SIR2 as potential CR mimetics. The SIR2 family represents an evolutionarily conserved lifespan regulator. Modulating the activity of SIR2 might provide effective CR mimetics to combat diseases of aging.

In vino veritas: a tale of two sirt1s?

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Cell. 2006 Dec 15;127(6):1091-3

Resveratrol increases life span in lower organisms by activating the NAD(+)-dependent histone deacetylase Sirt1. Studies by and now show that resveratrol promotes longevity and improves glucose homeostasis in mice by stimulating the Sirt1-mediated deacetylation of the transcriptional coactivator PGC-1alpha.

Research on resveratrol's mechanism of immunity in anti-aging

[Article in Chinese]

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Zhong Yao Cai. 2006 May;29(5):464-7

OBJECTIVE: To research resveratrol's mechanism of immunity in anti-aging and explore the new clinical use of resveratrol. METHODS: The sub acute aging model was made by continuous subcutaneous injection of D galactose to mice inducing reactive oxygen species (ROS). Meanwhile, the resveratrol was given and its effect on anti-aging was

observed. RESULTS: The resveratrol could increase the content of SOD and decrease the content of MDA in serum. There was no change in spleen index, but thymus index increased obviously. There was no change in the quantity of CD4+, but the quantity of CD8+ increased and the ratio of CD4+/CD8+ was decreased. The serum IL-6 and IL-8 level were decreased obviously. CONCLUSION: The resveratrol possessed the function to anti-aging and the applications foreground in anti-aging.

Resveratrol attenuates TNF-alpha-induced activation of coronary arterial endothelial cells: role of NF-kappaB inhibition.

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Am J Physiol Heart Circ Physiol. 2006 Oct;291(4):H1694-9

Epidemiological studies suggest that Mediterranean diets rich in resveratrol are associated with reduced risk of coronary artery disease. However, the mechanisms by which resveratrol exerts its cardioprotective effects are not completely understood. Because TNF-alpha-induced endothelial activation and vascular inflammation play a critical role in vascular aging and atherogenesis, we evaluated whether resveratrol inhibits TNF-alpha-induced signal transduction in human coronary arterial endothelial cells (HCAECs). We found that TNF-alpha significantly increased adhesiveness of the monocytic THP-1 cells to HCAECs, an effect that could be inhibited by pretreatment with resveratrol and the NF-kappaB inhibitor pyrrolidine dithiocarbamate. Previously, we found that TNF-alpha activates NAD(P)H oxidases, and our recent data showed that TNF-alpha-induced endothelial activation was prevented by the NAD(P)H oxidase inhibitor apocynin or catalase plus SOD. Resveratrol also inhibited H(2)O(2)-induced monocyte adhesiveness. Using a reporter gene assay, we found that, in HCAECs, TNF-alpha significantly increased NF-kappaB activity, which could be inhibited by resveratrol (>50% inhibition at 10(-6) mol/l) and pyrrolidine dithiocarbamate. Resveratrol also inhibited TNF-alpha-induced, NF-kappaB-driven luciferase expression in rat aortas electroporated with the reporter gene construct. In TNF-alpha-treated HCAECs, resveratrol (in the submicromolar range) significantly attenuated expression of NF-kappaB-dependent inflammatory markers inducible nitric oxide synthase, IL-6, bone morphogenetic protein-2, ICAM-1, and VCAM. Thus resveratrol at nutritionally relevant concentrations inhibits TNF-alpha-induced NF-kappaB activation and inflammatory gene expression and attenuates monocyte adhesiveness to HCAECs. We propose that these anti-inflammatory actions of resveratrol are responsible, at least in part, for its cardioprotective effects.

Botanical antioxidants in the prevention of photocarcinogenesis and photoaging.

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Exp Dermatol. 2006 Sep;15(9):678-84

Exposure of the skin to ultraviolet (UV) radiation, particularly its UV-B component (280-320 nm), from the sun results in erythema, edema, hyperplasia, hyperpigmentation, sunburn cells, immunosuppression, photoaging, and skin cancer. Amongst these various adverse effects of UV-B radiation, skin cancer and photoaging are of great concern. More recent changes in lifestyle have led to a significant increase in the amount of UV-B radiation people receive leading to a surge in the incidence of skin cancer and photoaging. As these trends are likely to continue in the foreseeable future, the adverse effect of UV-B has become a major human health concern. Therefore, development of novel strategies to reduce the occurrence of skin cancer and delay the process of photoaging are highly desirable goals. One approach to reduce their occurrence is through photochemoprevention, which we define as the use of agents capable of ameliorating the adverse effects of UV-B on the skin. Photochemoprevention via use of botanical antioxidants, present in the common diet of human have gained considerable attention as photochemopreventive agents for human use. Many such agents have also found a place in skin care products. This review will focus on the effects of selected botanical antioxidants in the prevention of photocarcinogenesis and photoaging.

Therapeutic potential of resveratrol: the in vivo evidence.

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Nat Rev Drug Discov. 2006 Jun;5(6):493-506. Epub 2006 May 26

Resveratrol, a constituent of red wine, has long been suspected to have cardioprotective effects. Interest in this compound has been renewed in recent years, first from its identification as a chemopreventive agent for skin cancer, and subsequently from reports that it activates sirtuin deacetylases and extends the lifespans of lower organisms. Despite scepticism concerning its bioavailability, a growing body of in vivo evidence indicates that resveratrol has protective effects in rodent models of stress and disease. Here, we

provide a comprehensive and critical review of the in vivo data on resveratrol, and consider its potential as a therapeutic for humans.

Resveratrol and the pharmacology of aging: a new vertebrate model to validate an old molecule.

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Cell Cycle. 2006 May;5(10):1027-32. Epub 2006 May 15

The natural phytoalexin resveratrol, found in grapes and red wine, recently rose to public fame for its positive effects on longevity in yeasts, worms and flies. Resveratrol anti-cancer and anti-inflammatory in vitro action on mammalian cell cultures also suggest a possible positive effect on human health and life-expectancy. To study the effects of resveratrol on vertebrate aging is obviously a particularly relevant question. We have studied resveratrol effects in a very short-lived vertebrate: the annual fish Nothobranchius furzeri. Resveratrol treatment prolonged lifespan and delayed the onset of age-related dysfunctions in this fish. This result identifies resveratrol as the first molecule which consistently retards aging in organisms as diverse as yeast, worm, fly and fish, but it also reveals the potential of this short-lived fish as an animal model for pharmacological research. Moreover, being related to stickleback (Gasterosteus aculeatus) the "pufferfishes" Takifugu and Tetraodon, and even more closely related to medaka (Oryzias latipes), it can greatly beneficiate from the recent development of genomic resources for these fish models and in the future become a complete model system for the aging research community.

Vascular and anti-angiogenic activity

Antiangiogenic and vascular-targeting activity of the microtubule-destabilizing trans-resveratrol derivative 3,5,4'-trimethoxystilbene.

Mol Pharmacol. 2005 May;67(5):1451-9. Epub 2005 Feb 9

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Neovascularization plays an important role in neoplasia and angioproliferative diseases. Two major modalities have been developed so far to affect neovascularization: its prevention by antiangiogenic compounds, and immature vessel disruption by vascular-targeting agents. trans-Resveratrol, found in grapes and wine, exerts antioxidant, antineoplastic, and antiangiogenic activities. Here, among various synthetic trans-resveratrol derivatives tested, 3,5,4'-trimethoxystilbene was an antiangiogenic agent 30 to 100 times more potent than parent compound in inhibiting endothelial cell proliferation, sprouting, collagen gel invasion, and morphogenesis (ID50 = 0.3-3.0 microM). In addition, 3,5,4'-trimethoxystilbene acts as a vascular-targeting agent by causing microtubule disassembling and tubulin depolymerization and by impairing the repositioning of the microtubule organization center and the formation of membrane ruffles in migrating endothelial cells. In keeping with a vascular-targeting ability, 3,5,4'-trimethoxystilbene induced apoptosis only in subconfluent endothelial cells and apoptotic regression of immature vessels in the ex vivo rat aorta ring assay. In vivo, 3,5,4'-trimethoxystilbene caused the rapid stasis of blood flow and regression of intersegmental vessels in the trunk of zebrafish embryos. In addition, it inhibited blood vessel growth and caused the disappearance of pre-existing blood vessels in the area vasculosa of the chick embryo. In conclusion, 3,5,4'-trimethoxystilbene associates an antiangiogenic profile to a significant vascular-targeting activity.

trans-3,4,5'-Trihydroxystibene inhibits hypoxia-inducible factor 1alpha and vascular endothelial growth factor expression in human ovarian cancer cells.

Clin Cancer Res. 2004 Aug 1;10(15):5253-63

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trans-3,4,5'-Trihydroxystibene (resveratrol) is a natural product commonly found in the human diet and has been shown recently to have anticancer effects on various human cancer cells. However, the molecular basis for its anticancer action remains to be elucidated. In this study, we investigated the effect of resveratrol on hypoxia-inducible factor 1alpha (HIF-1alpha) and vascular endothelial growth factor (VEGF) expression in human ovarian cancer cells A2780/CP70 and OVCAR-3. We found that although resveratrol did not affect HIF-1alpha mRNA levels, it did dramatically inhibit both basal-level and growth factor-induced HIF-1alpha protein expression in the cells. Resveratrol also greatly inhibited VEGF expression. Mechanistically, we demonstrated that resveratrol inhibited HIF-1alpha and VEGF expression through multiple mechanisms. First, resveratrol inhibited AKT and mitogen-activated protein kinase activation, which played a partial role in the down-regulation of HIF-1alpha expression. Second, resveratrol inhibited insulin-like growth factor 1-induced HIF-1alpha expression through the inhibition of protein translational regulators, including M(r) 70,000 ribosomal protein S6 kinase 1, S6 ribosomal protein, eukaryotic initiation factor 4E-binding protein 1, and eukaryotic initiation factor 4E. Finally, we showed that resveratrol substantially induced HIF-1alpha protein degradation through the proteasome pathway. Our data suggested that resveratrol may inhibit human ovarian cancer progression and angiogenesis by inhibiting HIF-1alpha and VEGF expression and thus provide a novel potential mechanism for the anticancer action of resveratrol.

Inhibition of vascular endothelial growth factor-induced angiogenesis by resveratrol through interruption of Src-dependent vascular endothelial cadherin tyrosine phosphorylation.

Mol Pharmacol. 2003 Nov;64(5):1029-36

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Resveratrol, a polyphenolic compound found in grapes and other fruits, has been reported to inhibit angiogenesis with an as yet elusive mechanism. Here, we investigate the detailed mechanism by which resveratrol inhibits vascular endothelial growth factor (VEGF)-induced angiogenic effects in human umbilical endothelial cells (HUVECs). Exposure of HUVECs to 1 to 2.5 muM resveratrol significantly blocked VEGF-mediated migration and tube formation but not cell proliferation. Under the same concentrations, resveratrol failed to affect VEGF-stimulated activation of VEGF receptor, extracellular

signal-regulated protein kinase 1/2, p38 mitogen-activated protein kinase, and Akt. Of interest, resveratrol, at the dose of 1 or 2.5 muM, effectively abrogated VEGF-mediated tyrosine phosphorylation of vascular endothelial (VE)-cadherin and its complex partner, beta-catenin. This inhibitory effect of resveratrol reflected on the retention of VE-cadherin at cell-cell contacts as demonstrated by immunofluorescence. Src kinase assay showed that VEGF-induced endogenous Src kinase activation was strongly inhibited by 1 and 2.5 muM resveratrol. Supportively, inhibition of Src activity by overexpression of Csk resulted in attenuation of the tyrosine phosphorylation of VE-cadherin and endothelial cell (EC) tube formation. Again, transfection with v-Src, an active form of Src, could reverse resveratrol inhibition of VE-cadherin tyrosine phosphorylation and EC tube formation. Reactive oxygen species (ROS) has been shown to be involved in VE-cadherin phosphorylation and its related functions. Flow cytometric analysis showed that VEGF stimulated an evident increase of peroxide, which was strongly attenuated by resveratrol. In addition, antioxidant N-acetyl-cysteine was demonstrated to strongly inhibit VEGF-mediated Src activation, VE-cadherin tyrosine phosphorylation, and HUVEC tube formation. Together, our data suggest that resveratrol inhibition of VEGF-induced angiogenesis was mediated by disruption of ROS-dependent Src kinase activation and the subsequent VE-cadherin tyrosine phosphorylation.

Anti-angiogenic activity of resveratrol, a natural compound from medicinal plants.

J Asian Nat Prod Res. 2005 Jun;7(3):205-13

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Resveratrol (3,4',5-trihydroxy-trans-stilbene), a naturally occurring phytoalexin found in grapes and wine, possesses cancer-preventive activity. Angiogenesis is a crucial step in the growth and metastasis of cancers. We have investigated the effect of resveratrol on angiogenesis in vitro and ex vivo, and found that resveratrol directly inhibited human umbilical vein endothelial cell growth and decreased the gelatinolytic activities of matrix metalloproteinase-2. Tube formation was inhibited by treatment with resveratrol after plating endothelial cells on Matrigel. Resveratrol treatment also inhibited endothelial cell attachment to basement membrane components fibronectin and laminin, and displays similar behavior on cell chemotaxis. In addition, resveratrol has been found to be an angiogenesis inhibitor in the rat aorta matrix culture model. Therefore, inhibition of angiogenesis associated with cancer may be a novel mechanism for the anticancer activity of resveratrol.

Resveratrol inhibits TNF alpha-induced endothelial cell activation.

Therapie. 2001 Sep-Oct;56(5):613-6

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Resveratrol, a phytoalexin found in grapes and wine, has been shown to have anti-inflammatory properties. Since endothelium is activated during inflammation by some cytokines released by macrophages and many other cells, we tested whether resveratrol could modulate endothelial cell activation. We studied the effect of resveratrol treatment in vitro on the expression of vascular cell adhesion molecule-1 by tumour necrosis factor alpha-stimulated human umbilical vein endothelial cells. In addition, we studied the effect of resveratrol treatment in vivo (in a murine experimental model) on the modulation of tumour necrosis factor alpha-induced vascular permeability. Resveratrol, used at the concentrations present in human plasma following moderate wine consumption, was demonstrated to be an inhibitor of the adhesion molecule expression by tumour necrosis factor alpha-stimulated endothelial cells. In addition, resveratrol significantly prevented the cytokine-induced vascular leakage. Our results (both in vitro and in vivo) may explain some aspects of the anti-inflammatory effects of resveratrol.

Profound negative regulatory effects by resveratrol on vascular smooth muscle cells: a role of p53-p21(WAF1/CIP1) pathway.

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We investigated the role of resveratrol, a polyphenol rich in red wine, in cell cycle progression and apoptosis of vascular smooth muscle cells (VSMCs). Resveratrol inhibited the growth of human aortic VSMCs at concentrations as low as 1 microM. This was due to the profound dose-dependent inhibition of DNA synthesis by resveratrol. DNA synthesis was more effectively inhibited when cells were pretreated with

resveratrol. Resveratrol caused a dose-dependent increase in intracellular p53 and p21 (WAF1/CIP1) levels. At lower concentrations (6.25-12.5 microM), resveratrol effectively blocked cell cycle progression of serum-stimulated VSMCs without inducing apoptosis, while the higher concentration of resveratrol (25 microM) selectively induced apoptosis in the same VSMCs. Intriguingly, however, the same high concentration of resveratrol could not induce apoptosis in quiescent VSMCs. These differential biological effects of resveratrol on quiescent and proliferating VSMCs suggest that resveratrol may be capable of selectively eliminating abnormally proliferating VSMCs of the arterial walls in vivo.

Summary

Extensive research from all over the globe continues to confirm the benefits of this interesting compound. While it is important to point out that a lot of the research on this extract has been done only in test tubes or rodents, the sheer volume and extraordinary consistency of results suggest that resveratrol is one of the most versatile and effective plant compounds discovered so far. Resveratrol represents a novel solution to many common serious and debilitating health problems encountered by humans.

Nearly 500 newspapers reported on the resveratrol story, and virtually every major TV news department followed. The news media heralded a study which showed that high-dose resveratrol, known as a red wine molecule, maintained the quality of life of laboratory mice (balance, freedom of disease, endurance and coordination) as they aged, despite a high-fat diet, and the high-fat fed mice lived 31% longer when given oral resveratrol.

Resveratrol became the first-ever supplement known to activate a longevity gene when its Sirt1, 2,3,4 and 6 activation properties were documented.

The research on resveratrol is so voluminous that it's not practical to cover it in one article. Of note are the positive reports in the Oxford Journal and the Federation of American Societies for Experimental Biology. These reports indicate that the benefits of resveratrol to modern medicine and human health are multi-faceted. The singular absence of observed adverse effects at doses, the human equivalent of which, would be almost impossible to consume, is virtually unprecedented for any compound shown to produce the range of positive health effects reported by researchers at major universities and research organisations. If one assesses resveratrol safety by the accepted conservative standard of the precautionary principle the judgement would seem to militate in favor of informed and judicious use of this compound as a human health aid as opposed to the restriction of its use. Give the fact that this compound exists in varying concentrations in over 70 commonly consumed foods and no significant hazards have been yet observed related to its consumption in these foods the compound itself has been proven safe at the amounts contained in these foods. Higher concentrations by several orders of magnitude have been shown to be safe in mammals however amounts greater than 500mg per day have not been formally tested for toxicity in humans.

No compound can be considered entirely safe or free of potential adverse effects; however when a large body of research shows that a high probability has been scientifically established for a compound to produce a wide range of important beneficial effects vis-a-vis lethal diseases and conditions which have so far eluded the existing prevention and treatment modalities, and this compound has shown a very low probability for serious adverse effects, its judicious and informed use is considered as justified. Additional human studies are on going and the authors of this paper are currently designing the protocols which will define a study of resveratrol's anti-cancer properties at the Mumbai Centre for Advanced Cancer Research, Education and Treatment.

In terms of its potential contribution to human health and disease prevention and treatment, in our opinion, the body of studies on resveratrol since 1994 compares to Alexander Fleming's discovery of penicillin or Louis Pasteur's use of heat to destroy pathogenic bacteria. Only time will tell if this assessment is accurate however the evidence continues to accumulate at an accelerating pace; and as more funding becomes available additional resources will be focused on the investigation of this compound.

"If Penicillin can be recognized as the magic bullet of the 20th century, will Resveratrol be recognized as the 21st century's magic bullet?"

Common synonyms/names: (In the majority of instances when the term resveratrol is used the author/researcher is actually referring to the Trans-resveratrol isomer, not to cis-resveratrol, Polydatin, emodin or the glucuronides of resveratrol. These compounds have been the subject of substantial investigation in their own right)

3,4',5-trihydroxystilbene, *trans*-3,4',5-trihydroxystilbene, 5-methoxy-(E)-resveratrol 3-O-rutinoside, , grape seed proanthycyanidin extract (GSPE), grape skin, Heyneanol A (a resveratrol tetramer), hydroxystilbene, lyophilized grape powder (LGP), , RESV, resverol, Resveratrol 3-O-beta-D-glucopyranoside, , *trans*-piceid, *trans*-resveratrol.

Resveratrol and its analogs are naturally present in *Belamcanda chinensis*, *Cissus quadrangularis*, *Elephantorrhiza goetzei*, epsilon-Viniferin (a dimer of resveratrol), *Erythrophleum lasianthum, Gnetum montanum* - gnetin H (a resveratrol analog), *Polygonum cuspidatum*, *Reynoutria japonica*, *Scutellaria barbata* D. Don (Lamiaceae), *Sophora moorcroftiana* Benth., *Sophora tomentosa* L.- suffruticosol B (a resveratrol analog), *Vaticapauciflora, Vatica rassak* (Dipterocarpaceae), vaticanol C (a resveratrol tetramer), *Veratrum taliense*, viniferin (a resveratrol analog), *Vitis vinifera* L.

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