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Cancer Chemopreventive Activity of Resveratrol, a Natural Product Derived from Grapes

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Resveratrol, a phytoalexin found in grapes and other food products, was purified and shown to have cancer chemopreventive activity in assays representing three major stages of carcinogenesis. Resveratrol was found to act as an antioxidant and antimutagen and to induce phase II drug-metabolizing enzymes (anti-initiation activity); it mediated anti-inflammatory effects and inhibited cyclooxygenase and hydroperoxidase functions (antipromotion activity); and it induced human promyelocytic leukemia cell differentiation (antiprogression activity). In addition, it inhibited the development of preneoplastic lesions in carcinogen-treated mouse mammary glands in culture and inhibited tumorigenesis in a mouse skin cancer model. These data suggest that resveratrol, a common constituent of the human diet, merits investigation as a potential cancer chemopreventive agent in humans.

Cancer is the largest single cause of death in both men and women, claiming over 6 million lives each year worldwide. Chemoprevention, the prevention of cancer by ingestion of chemical agents that reduce the risk of carcinogenesis (*1*), is one of the most direct ways to reduce morbidity and mortality. Cancer chemopreventive agents include nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin, aspirin, piroxicam, and sulindac, all of which inhibit cyclooxygenase (COX) (*2*). This inhibitory activity is relevant to cancer chemoprevention because COX catalyzes the conversion of arachidonic acid to pro-inflammatory substances such as prostaglandins, which can stimulate tumor cell growth and suppress immune surveillance (*3*). In addition, COX can activate carcinogens to forms that damage genetic material (*4*).

In searches for new cancer chemopreventive agents over the past several years, hundreds of plant extracts have been evaluated for their potential to inhibit COX. An extract derived from *Cassia quinquangulata* Rich. (Leguminosae), collected in Peru, was identified as a potent inhibitor, and on the basis of bioassay-guided fractionation, resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) (Fig. 1) was identified as the active principle (*5*).

The process of chemical carcinogenesis can be divided into three general stages, and chemopreventive agents have been categorized according to the stage that they inhibit (*6*). Resveratrol inhibits cellular events associated with tumor initiation, promotion, and progression. As noted above, the compound was identified on the basis of its ability to inhibit the cyclooxygenase activity of COX-1 (median effective dose ED_{50} = 15 μM) (Fig. 2A), and this activity correlates with antitumor promotion. Although its inhibitory activity was less than that of certain NSAIDs, such as indomethacin (ED_{50} = 2.3 μM) (Fig. 2A), it was much greater than that mediated by compounds such as aspirin (ED_{50} = 880 μM). Also, unlike indomethacin and most other NSAIDs, resveratrol inhibited the hydroperoxidase activity of COX-1 (ED_{50} = 3.7 μM) (Fig. 2B). Resveratrol-mediated inhibition was specific for the cyclooxygenase activity of COX-1 because there was no discernable activity when oxygen uptake was assessed with COX-2 (Fig. 2A), an inducible form of the enzyme associated with responses such as inflammation (*7*), and inhibition of the hydroperoxidase activity of COX-2 (ED_{50} = 85 μM) (Fig. 2B) was greatly reduced relative to the activity observed with COX-1.

On the basis of these results, we investigated the anti-inflammatory activity of resveratrol. In the carrageenan-induced model of inflammation in rats, resveratrol significantly reduced pedal edema both in the acute phase (3 to 7 hours) and in the chronic phase (24 to 144 hours). The edema-suppressing activity of resveratrol was greater than that of phenylbutazone and was similar to that of indomethacin (Fig. 3). Overall, these data demonstrate the potential of resveratrol to inhibit tumor promotion.

Resveratrol was also found to inhibit events associated with tumor initiation. For example, resveratrol inhibited, in a dose-dependent manner, free-radical formation (ED_{50} = 27 μM) when human promyelocytic leukemia (HL-60) cells were treated with 12-O-tetradecanoylphorbol-13-acetate (TPA) (*8*). The compound also functioned as an antimutagen, as illustrated by its dose-dependent inhibition of the mutagenic response induced by treatment of *Salmonella typhimurium* strain TM677 with 7,12-dimethylbenz(*a*)anthracene (DMBA) (ED_{50} = 4 μM) (*9*). In addition, resveratrol induced quinone reductase activity with cultured mouse hepatoma (Hepa 1c1c7) cells (concentration required to double activity, 21 μM) (*10*), which is relevant because phase II enzymes, such as quinone reductase, are capable of metabolically detoxifying carcinogens (*11*). An identical response profile was observed with cultured BP^rC1 hepatoma cells (a derivative of Hepa 1c1c7 cells that is incapable of phase I enzyme induction), indicating that resveratrol is a monofunctional inducer.

We also tested the ability of resveratrol to inhibit the progression stage of carcino-

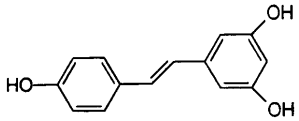

Fig. 1. Structure of resveratrol.

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genesis by treating cultured HL-60 cells (*12*) with resveratrol. Under normal culture conditions, these cells have unlimited proliferative capacity. In a dose-dependent manner, resveratrol induced expression of nitroblue tetrazolium reduction activity, a marker of granulocyte formation (ED_{50} = 11 μM), and nonspecific acid esterase activity, a marker of macrophage (monocyte) formation (ED_{50} = 19 μM). Concurrently, incorporation of [^{3}H]thymidine was inhibited (ED_{50} = 18 μM), indicative of terminal differentiation to a nonproliferative phenotype.

To assess more directly the cancer chemopreventive activity of resveratrol, we investigated its effects in a mouse mammary gland culture model of carcinogenesis (*13*). Resveratrol inhibited, in a dose-dependent manner, the development of DMBA-induced preneoplastic lesions (ED_{50} = 3.1 μM) (Fig. 2C). No signs of toxicity were observed, as judged by morphological examination of the glands. Finally, we studied tumorigenesis in the two-stage mouse skin cancer model in which DMBA was used as initiator and TPA as promoter. During an 18-week study mice treated with DMBA-plus TPA developed an average of two tumors per mouse with 40% tumor incidence (Fig. 4A). Application of 1, 5, 10, or 25 μmol of resveratrol together with TPA twice a week for 18 weeks reduced the number of skin tumors per mouse by 68, 81, 76, or 98%, respectively, and the percentage of mice with tumors was lowered by 50, 63, 63, or 88%, respectively (Fig. 4B). No overt signs of resveratrol-induced toxicity were observed, as judged by visual inspection of the skin, gross morphological examination of major organ systems, or change in body weights, relative to controls.

The physiological function of resveratrol in plants is not well defined. The compound is thought to be a phytoalexin, one of a group of compounds that are produced during times of environmental stress or pathogenic attack. Resveratrol has been found in at least 72 plant species (distributed in 31 genera and 12 families), a number of which are components of the human diet, such as mulberries, peanuts, and grapes. Relatively high quantities are found in the latter, possibly because of the response of *Vitis vinifera* (Vitaceae) to fungal infection (*14*). Fresh grape skin contains about 50 to 100 μg of resveratrol per gram, and the concentration in red wine is in the range of 1.5 to 3 mg/liter (*15*). Appreciable amounts are also found in white and rosé wines (*16*).

The results of several epidemiological studies have suggested that coronary heart disease mortality can be decreased by moderate consumption of alcohol, especially red wine (*17*). It is conceivable that resveratrol plays a role in the prevention of heart disease (*18*) because it has been reported to inhibit platelet aggregation and coagulation, alter eicosanoid synthesis, and modulate lipoprotein metabolism (*19*).

Our results suggest that resveratrol merits further investigation as a cancer chemopreventive agent in humans. In light of the adverse health effects of long-term alcohol consumption, however, foods and nonalcoholic beverages derived from grapes (*20*) should be considered as alternative dietary sources.

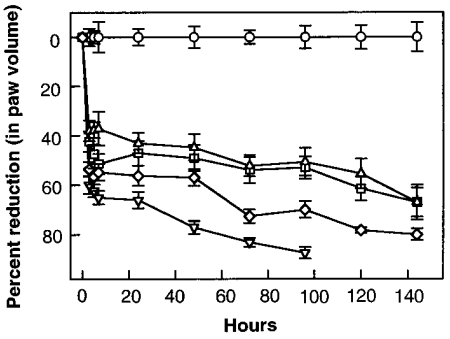

Fig. 3. Effects of resveratrol (□, 3 mg per kilogram of body weight; ◇, 8 mg/kg), phenylbutazone (△), or indomethacin (▽) on carrageenan-induced inflammation in rats (*22*). Percent reduction (± SD) was obtained by comparing the paw volume in the control group (○) (treated with carrageenan only) with that in the drug-treated group. Dosing was repeated daily for 7 days. Hours refers to hours after carrageenan injection. The data for the indomethacin group at 120 hours and 140 hours were not reliable because of the induction of secondary lesions.

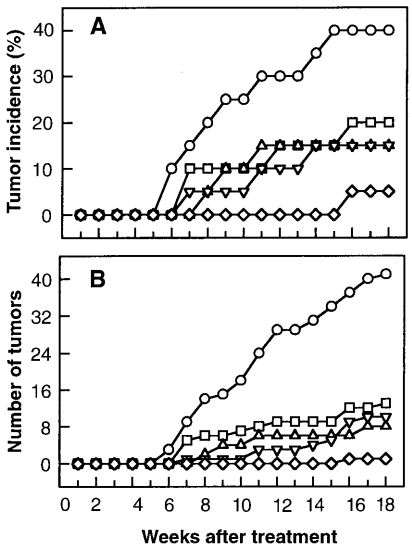

Fig. 4. Effect of resveratrol on tumorigenesis in the two-stage mouse skin model. Six groups of 20 female CD-1 mice (4 to 5 weeks old) were treated topically with 200 μmol of DMBA in 0.2 ml of acetone on the shaved dorsal region (*23*). One week later, the mice were treated with 5 μmol of TPA in 0.2 ml of acetone alone (○) or together with 1 (□), 5 (△), 10 (▽), or 25 (◇) μmol of resveratrol in 0.2 ml of acetone, twice a week for 18 weeks. Animals were weighed weekly and observed for tumor development once every week. (**A**) Percent incidence of observable skin tumors; (**B**) total number of observable skin tumors.

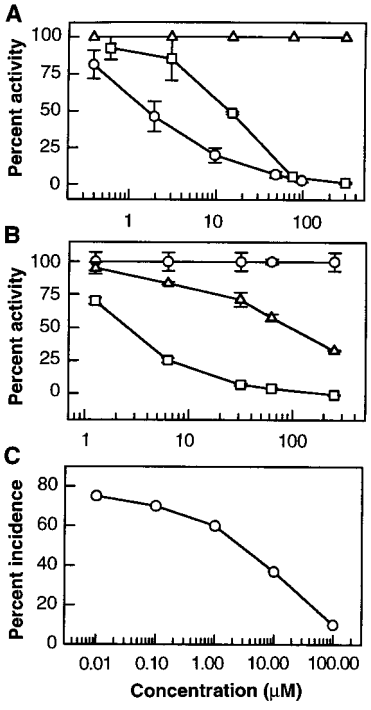

Fig. 2. (**A**) Effects of indomethacin (○) on COX-1 activity, and resveratrol on COX-1 (□) or COX-2 (△) activity. COX activity was measured by assessing oxygen consumption at 37°C (*21*). Reactions were started by adding 0.6 mM arachidonic acid to a mixture containing 0.1 M sodium phosphate (pH 7.4), 1.0 mM phenol, and 0.01 mM hemin; microsomes (0.2 mg of protein) derived from sheep seminal vesicles as a crude source of COX-1 or recombinant human COX-2 (0.1 mg of protein); and the test compound. (**B**) Effect of indomethacin on COX-1 (○) hydroperoxidase activity, and resveratrol on COX-1 (□) or COX-2 (△) hydroperoxidase activity. Hydroperoxidase activity was determined by spectrophotometry. Reaction mixtures contained 0.1 M tris-HCl (pH 8.5), 1.2 μM hemin, 0.24 mM *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD), COX-1 (36 μg of protein) or COX-2 (45 μg of protein), and test compounds. H_2O_2 (250 μM) was used to initiate the reaction, and changes in absorbance at 595 nm were measured. Inhibitory activity was calculated by comparing the initial rate of change in absorbance in the presence of test compounds with that observed with solvent (DMSO) only. Each point represents the mean ± SD of two determinations. (**C**) Inhibition of DMBA-induced preneoplastic lesions in mouse mammary gland culture by treatment with resveratrol. Mammary glands were incubated with resveratrol for 10 days and DMBA for 24 hours on day 3 (*13*). Percent incidence of mammary lesions was determined after an additional 14 days of incubation. The data from resveratrol-treated groups were compared with control groups and the results expressed as a percentage.

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20. Daily consumption of two to five glasses (or a maximum of 375 ml/day) of red wine may deliver a sufficient amount of resveratrol to alter arachidonic acid metabolism or other physiological responses, depending on absorption, metabolism, and residence time within the blood circulation and relevant tissues [D. M. Goldberg, *Clin. Chem.* **41**, 14 (1995)]. Resveratrol concentrations in other food products, such as grape juice, pomace, and purees are provided in B. J. Ector, J. B. Magee, C. P. Hegwood, and M. J. Coign [*Am. J. Enol. Vitic.* **47**, 57 (1996)].
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22. K. Slowing, E. Carretero, A. Villar, *J. Ethnopharmacol.* **43**, 9 (1994). Female Wistar rats (150 to 200 g body weight) were divided into groups of seven animals each. All rats received 0.1 ml of Freund's complete adjuvant (Difco; Sigma) by intradermal injection into the tail. Animals were used 7 days after injection of adjuvant. One hour after oral administration of resveratrol (3 or 8 mg per kilogram of body weight) or reference drugs including phenylbutazone (80 mg/kg) and indomethacin (5 mg/kg), the rats were injected with 0.1 ml of a 2% (w/v) suspension of carrageenan in saline solution into the left hind paw. For the control group, a 1:1 mixture of Tween 80 and water (0.2/3.3, v/v) and 1% (w/v) methylcellulose was used as a vehicle. The left hind paw volume of each rat was measured by water plethysmography (Letica, model L17500) before the adjuvant injection and, again, 6 days later, before the injection of carrageenan. Paw volumes were determined within 3 to 144 hours after injection of carrageenan. Inhibition of edema was calculated relative to the mean edema of the vehicle-treated control group.
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Synaptic Depression and Cortical Gain Control

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Cortical neurons receive synaptic inputs from thousands of afferents that fire action potentials at rates ranging from less than 1 hertz to more than 200 hertz. Both the number of afferents and their large dynamic range can mask changes in the spatial and temporal pattern of synaptic activity, limiting the ability of a cortical neuron to respond to its inputs. Modeling work based on experimental measurements indicates that short-term depression of intracortical synapses provides a dynamic gain-control mechanism that allows equal percentage rate changes on rapidly and slowly firing afferents to produce equal postsynaptic responses. Unlike inhibitory and adaptive mechanisms that reduce responsiveness to all inputs, synaptic depression is input-specific, leading to a dramatic increase in the sensitivity of a neuron to subtle changes in the firing patterns of its afferents.

Cortical neurons transmit information by responding selectively to changes in the spatial and temporal pattern of presynaptic action potentials arriving at about 10,000 synapses. Extracting meaningful information from such a large and complex set of inputs presents a severe challenge. Presynaptic afferents fire action potentials at a wide variety of different rates, and signals carried by slowly firing afferents may be masked by random fluctuations in the activity of afferents firing at high rates. This problem can be avoided if cortical neurons monitor slowly firing afferents at high gain while reducing the gain for high-rate inputs. Such gain control cannot be achieved through fixed synaptic weights, because afferent firing rates change over time. We propose that short-term synaptic depression provides an automatic, dynamic gain-control mechanism. By balancing contributions

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