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-inflammation effects and inhibited cyclooxygenase and hydroperoxidase functions (antipromotion activity); and it induced human promyelocytic leukemia cell differentiation (antiproliferation 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 cancer (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 decades, 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 the active principle (5). The compound also functioned as an antimutagen, as illustrated by its dose-dependent inhibition of the mutagenic response induced by treatment of Salmo nella typhimurium strain TM677 with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (6). In addition, resveratrol inhibited the progression stage of carcinogenesis (Fig. 1) (7)

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 (8). 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 (Fig. 2A). This activity correlates with antitumor promotion. Although its inhibitory activity was less than that of certain NSAIDs, such as indomethacin (ED50 = 2.3 μM) (Fig. 2A), it was much greater than that mediated by compounds such as aspirin (ED50 = 880 μM). Also, unlike indomethacin and most other NSAIDs, resveratrol inhibited the hydroperoxidase activity of COX-1 (ED50 = 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 (ED50 = 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 basal 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 tumorigenesis.

Resveratrol was also found to inhibit events associated with tumor initiation. For example, resveratrol inhibited, in a dose-dependent manner, free-radical formation (ED50 = 27 μM) when human promyelocytic leukemia (HL-60) cells were treated with 1,2-D-tetradeconeophosphol-13-acetate (TPA) (8). The compound also functioned as an antimutagen, as illustrated by its dose-dependent reduction in the mutagenic response induced by treatment of Salmonella typhimurium strain T677 with 1,2-dimethylbenz(a)anthracene (DMBA) (ED50 = 4 μM) (9). In addition, resveratrol induced quinone reductase activity with cultured mouse hepatoma (Hepa 1c1c7) cells (24 μM) (10), which is relevant because phase II enzymes, such as quinone reductase, are capable of metabolically detoxifying carcinogens (11).

We also tested the ability of resveratrol to inhibit the progression stage of carcinogenesis. In this model, resveratrol inhibited the progression stage of carcinogenesis (Fig. 1) (12).

14. Video sequences of Figs. 1 and 3, as well as other sequences showing MT movement in melanophore fragments, can be seen at http://borisy.rocklabs.wisc.edu/.
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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. 2. (A) Effects of indomethacin (C) on COX-1 activity, and resveratrol on COX-1 (\(\square\)) or COX-2 (\(\triangle\)) 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 (C) hydroperoxidase activity, and resveratrol on COX-1 (\(\square\)) or COX-2 (\(\triangle\)) hydroperoxidase activity. Hydroperoxidase activity was determined by spectrophotometry. Reaction mixtures contained 0.1 M tris-HCl (pH 8.5), 1.2 \(\mu\)M hemin, 0.24 mM \(N\)\(N\)\'\(N\)\'\(N\)'-tetramethyl-p-phenylenediamine (TMPD), COX-1 (36 \(\mu\)g of protein) or COX-2 (45 \(\mu\)g of protein), and test compounds. \(H_2O_2\) (250 \(\mu\)M) 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 \(\pm\) 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.

Fig. 3. Effects of resveratrol (\(\square\), 3 mg per kilogram of body weight; \(\bigtriangleup\), 8 mg/kg), phenylbutazone (\(\triangle\)), or indomethacin (\(\bigtriangledown\)) on carrageenan-induced inflammation in rats (22). Percent reduction (\(\pm\) SD) was obtained by comparing the paw volume in the control group (\(\square\)) (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 \(\mu\)mol of DMBA in 0.2 ml of acetone on the shaved dorsal region (23). One week later, the mice were treated with 5 \(\mu\)mol of TPA in 0.2 ml of acetone alone (\(\square\)) or together with 1 (\(\bigtriangleup\)), 5 (\(\triangle\)), 10 (\(\bigtriangledown\)), or 25 (\(\bigtriangleup\)) \(\mu\)mol 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.
relative to control plates that were treated with DMSO only.
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 residu- ence time within the blood circulation and relevant tissues (D. M. Goldberg, Clin. Chim. Acta, 14 (1996)). Resveratrol concentrations in other food products, such as grape juice, pomace, and pur- es are provided in B. J. Ector, J. B. Magee, C. P. Hegwood, and M. J. Coign [Am. J. Enol. Vitic. 47, 57 (1996)].
22. K. Slowing, E. Carretoro, A. Villar, J. Ethnopharma- col. 43, 9 (1994). Female Wistar rats (150 to 200 g body weight) were divided into groups of seven ani- mals each. All rats received 0.1 ml of Freund’s com- plete 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 (5 or 6 mg per gram of body weight or reference drugs including phenylbutazone (80 mg/ kg) and indomethacin (5 mg/kg), the rats were inject- ed with 0.1 ml of a 2% (w/v) suspension of carragee- anan in saline solution into the left hind paw. For the control group, a 1:1 mixture of Tween 80 and water (0.2/3, 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 (Latica, model L17500) before the adjuvant injection and, again, 6 days later, before the injection of car- rageenan. Paw volumes were determined within 5 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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