Prevention and Therapy of Cancer by Dietary Monoterpenes

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ABSTRACT Monoterpenes are nonnutritive dietary components found in the essential oils of citrus fruits and other plants. A number of these dietary monoterpenes have antitumor activity. For example, d-limonene, which comprises >90% of orange peel oil, has chemopreventive activity against rodent mammary, skin, liver, lung and forestomach cancers. Similarly, other dietary monoterpenes have chemopreventive activity against rat mammary, lung and forestomach cancers when fed during the initiation phase. In addition, perillyl alcohol has promotion phase chemopreventive activity against rat liver cancer, and geraniol has in vivo antitumor activity against murine leukemia cells. Perillyl alcohol and d-limonene also have chemotherapeutic activity against rodent mammary and pancreatic tumors. As a result, their cancer chemotherapeutic activities are under evaluation in Phase I clinical trials. Several mechanisms of action may account for the antitumor activities of monoterpenes. The blocking chemopreventive effects of limonene and other monoterpenes during the initiation phase of mammary carcinogenesis are likely due to the induction of Phase II carcinogen-metabolizing enzymes, resulting in carcinogen detoxification. The post-initiation phase, tumor suppressive chemopreventive activity of monoterpenes may be due to the induction of apoptosis and/or to inhibition of the post-translational isoprenylation of cell growth-regulating proteins. Chemotherapy of chemically induced mammary tumors with monoterpenes results in tumor redifferentiation concomitant with increased expression of the mannose-6-phosphate/insulin-like growth factor II receptor and transforming growth factor β1. Thus, monoterpenes would appear to act through multiple mechanisms in the chemoprevention and chemotherapy of cancer. J. Nutr. 129: 775S–778S, 1999.

KEY WORDS: • chemoprevention • rats • hamsters • chemotherapy • isoprenoid

Monoterpene biochemistry. Monoterpenes are nonnutritive dietary components found in the essential oils of citrus fruits, cherry, mint and herbs. They function physiologically as chemoattractants or chemorepellents (McGarvey and Croteau 1995), and they are largely responsible for the distinctive fragrance of many plants. These 10 carbon isoprenoids are derived from the mevalonate pathway in plants but are not produced by mammals, fungi or other species. In citrus fruits (Chayet et al. 1977), peppermint and other plants, d-limonene (p-mentha-1,8-diene; Fig. 1) is formed by the cyclization of geranylpyrophosphate in a reaction catalyzed by limonene synthase (Alonso et al. 1992, Kjonaas and Croteau 1983). Limonene then serves as a precursor to a host of other oxygenated monocyclic monoterpenes such as carveol, carvone, menthol, perillyl alcohol and perillaldehyde (Karp et al. 1990, McGarvey and Croteau 1995; Fig. 1).

Some specific dietary sources of monoterpenes include d-limonene in orange and other citrus peel oils, caraway and dill; perillyl alcohol in cherry and spearmint; carvone in caraway and spearmint; and geraniol in lemongrass oil, an ingredient in herbal teas. In addition, d-limonene is a prevalent flavoring agent for fruit juices, soft drinks, baked goods, ice cream and puddings [National Toxicology Program (NTP) 1990]. Orange oil, naturally consisting of 90–95% d-limonene, is a commercially available food flavoring agent. Furthermore, because of its pleasant citrus fragrance, d-limonene is commonly added to cosmetics, soaps and other cleaning products. Thus, human exposure to monoterpenes through the diet or environment is widespread. Yet, limited information is available for the estimation of monoterpene intake. The d-limonene content of orange juice is ~100 ppm, or 0.01% (NTP 1990).

Monoterpene metabolism and disposition. The metabolism of the monoterpenes d-limonene and perillyl alcohol by mammals has been investigated. These monoterpenes exhibit a very high degree of oral bioavailability in mammals (Kodama et al. 1974 and 1976, Phillips et al. 1995). In rats, the appearance of limonene and its metabolites is evident within 20 min after its administration by gavage (Crowell et al. 1992b). Limonene and/or its metabolites are detectable in serum, liver, lung and many other tissues (Igimi et al. 1974), with higher concentrations detected in adipose tissue and mammary gland than in less fatty tissues (Crowell et al. 1992b). The half-life of limonene in both rats and humans has been estimated to be 12–24 h (Crowell et al. 1992b), and excretion occurs primarily through the urine (Igimi et al. 1974, Kodama et al. 1974 and 1976).

Limonene is metabolized to oxygenated metabolites in rats and in humans. In rats, the two major serum metabolites of limonene are perilllic acid and dihydroperilllic acid (Crowell et al. 1976). The metabolism of these active metabolites in humans is not known. However, some of the metabolites of limonene, such as isopulegol, isomenthol and menthol, have been detected in human urine and exhaled breath (Kodama et al. 1974, Phillips et al. 1995, Igimi et al. 1974).
Limonene
Perillyl alcohol
Geraniol
Carveol
Carvone

**FIGURE 1** Examples of oxygenated monocyclic monoterpenes for which limonene serves as a precursor.

Humans produce these two serum metabolites as well as limonene-1,2-diol (Crowell et al. 1994a). These metabolic reactions have, in some cases, been recapitulated in in vitro assays of limonene metabolism by liver microsomes (Watabe et al. 1980). In addition, glycine and glucuronide conjugates of perillie acid and urotropernol (p-mentha-8,9-diol) have been detected in the urine of many limonene-fed mammals (Kodama et al. 1974 and 1976, Regan and Bjeldanes 1976), suggesting that limonene is metabolized by both Phase I and Phase II enzymes.

The metabolism of perillyl alcohol (Fig. 1) is similar to that of limonene. Metabolites, but not the parent drug, are detectable within 10 min after oral administration of perillyl alcohol (Phillips et al. 1995). The predominant serum metabolite of perillyl alcohol in rats and in dogs is perillie acid, and dihydroperillie acid is detectable in lesser amounts (Haag and Gould 1994, Phillips et al. 1995). Interestingly, higher serum concentrations of perillie acid have been observed in rats fed 2% perillyl alcohol than in rats fed five times as much limonene, suggesting a possible difference in pharmacokinetics between the two drugs (Haag and Gould 1994). The half-life of perillyl alcohol in dogs given 250 mg/kg body weight is 3.2 h. At this dose, perillyl alcohol causes no observable toxicity, and serum perillie acid concentrations are present in excess of those reported for rats receiving chemotherapeutic doses of the drug (Haag and Gould 1994, Phillips et al. 1995).

**Antitumor activity of monoterpenes.** A number of dietary monoterpenes have antitumor activity, exhibiting not only the ability to prevent the formation or progression of cancer, but to regress existing malignant tumors. Limonene has well-established chemopreventive activity against many cancer types. Limonene has been shown to inhibit the development of spontaneous neoplasms in mice receiving 1200 mg/kg orally (NTP 1990); dietary limonene also reduces the incidence of spontaneous lymphomas in p53−/− mice (Hursting et al. 1995). Furthermore, when administered either in pure form or as orange peel oil (95% d-limonene), limonene inhibits the development of chemically induced rodent mammary (Elegbede et al. 1984, Elson et al. 1988, Maltzman et al. 1989, Wattenberg 1983), skin (Elegbede et al. 1986b), liver (Di- etrich and Swenberg 1991), lung and forestomach (Wattenberg et al. 1989 and 1991) cancers (reviewed in Crowell and Gould 1994; Elson and Yu 1994, Elson 1995). In rat mammary carcinogenesis models, the chemopreventive effects of limonene are evident during the initiation phase of 7,12-dimethylbenz(a)anthracene (DMBA)-induced cancer (Elson et al. 1988) and during the promotion phase of both DMBA- and nitrosomethylurea (NMU)-induced cancers (Elson et al. 1988, Maltzman et al. 1989). Dietary limonene also inhibits the development of rat oncogene-induced mammary carcinomas in rats (Gould et al. 1994). Recently, Kawamori et al. (1996) reported that the development of azoxymethane-induced aberrant crypt foci in the colon of rats was significantly reduced when they were given 0.5% limonene in the drinking water.

Caraway seed oil, and its principal monoterpenes, carvone, prevent chemically induced lung and forestomach carcinoma development when administered before the carcinogen (Wattenberg et al. 1989). In addition, carvone (Crowell et al. 1992a) and menthol (Russin et al. 1989) have chemopreventive activity against DMBA-induced rat mammary cancers when fed as 1% of the diet only during the initiation phase. Geraniol, an acyclic dietary monoterpenes, has in vivo antitumor activity against murine leukemia, hepatoma and melanoma cells (Shoff et al. 1991, Yu et al. 1995) when administered before and after tumor cell transplantation. In addition, perillyl alcohol has promotion phase chemopreventive activity against chemically induced liver cancer in rats (Mills et al. 1995) and is very effective at preventing tumor recurrences or secondary tumors in animals treated in a chemotherapy regimen (Haag and Gould 1994).

Dietary monoterpenes have promising chemotherapeutic activity against established rodent pancreatic and mammary tumors. Both limonene (Elegbede et al. 1986a, Haag et al. 1992) and perillyl alcohol (Haag and Gould 1994) have chemotherapeutic activity against rat mammary tumors, causing the complete regression of >80% of established DMBA- or NMU-induced mammary carcinomas. Chander et al. (1994) reported that combination chemotherapy of NMU-induced rat mammary tumors with limonene and the aromatase inhibitor 4-hydroxyandrostenedione was more effective than either drug alone. Perillyl alcohol has chemotherapeutic activity against pancreatic cancer at doses that cause little toxicity to the host (Stark et al. 1995). Perillyl alcohol reduced the growth of transplanted hamster pancreatic tumors to less than half that of controls. Moreover, a significant portion of perillyl alcohol–treated pancreatic tumors completely regressed, whereas none of the control tumors regressed (Stark et al. 1995). Phase I clinical trial testing of the cancer chemotherapeutic activity of limonene (McNamee 1993) and perillyl alcohol (Phillips et al. 1995) is in progress.

**Mechanisms of action of monoterpenes.** Chemopreventive agents may have cancer blocking and/or suppressing activity (Wattenberg 1983 and 1992). Blocking chemopreventive agents act during the initiation phase of carcinogenesis to prevent the interaction of chemical carcinogens with DNA, e.g., by modulating carcinogen metabolism to less toxic forms. Suppressing

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2 Abbreviations used: DMBA, 7,12-dimethylbenz(a)anthracene; HMG, 3-hydroxy-3-methylglutaryl; NMU, nitrosomethylurea; TGF, transforming growth factor.
chemopreventive agents, on the other hand, act during the promotion phase of carcinogenesis to prevent the outgrowth of initiated cells. The blocking chemopreventive effects of limonene and other monoterpenes during the initiation phase of mammalian carcinogenesis are likely due to the induction of Phase I and Phase II carcinogen-metabolizing enzymes, resulting in carcinogen detoxication. Chemopreventive doses of dietary limonene induce total cytochrome P450 (Ariyoshi et al. 1975, Austin et al. 1988, Maltzman et al. 1991). The specific cytochrome P450 isoforms induced by limonene include CYP 2B1 and CYP2C (Maltzman et al. 1991). Both limonene and sobrerol, another chemopreventive monoterpene (Crowell 1992a), induce epoxide hydrolase as well as plasmalemma ATPase (Maltzman et al. 1991). Interestingly, a comparative analysis of DMBA metabolism in control, limonene-, and sobrerol-treated rats revealed that more of the proximate carcinogen is produced in monoterpenes-treated animals than in controls (Maltzman et al. 1991). However, these effects are overcome by the induction of glutathione-S-transferase and UDP-glucuronyl transfersase by limonene and sobrerol (Elegbede et al. 1993). The net result is that fewer DMBA-DNA adducts are formed in monoterpenes-treated rats than in controls, and more DMBA is excreted in the urine (Maltzman et al. 1991). These results correlate well with the reduction in tumor incidence and multiplicity observed in rats treated with dietary limonene or sobrerol during the initiation phase of DMBA-induced mammary cancer (Crowell et al. 1992a, Elson et al. 1988).

The cancer suppressing chemopreventive activity of monoterpenes during the promotion phase of mammary and liver carcinogenesis may be due to inhibition of tumor cell proliferation, acceleration of the rate of tumor cell death and/or induction of tumor cell differentiation (Morse and Stoner 1993). The chemopreventive activity of perillyl alcohol during the promotion phase of liver carcinogenesis is associated with a marked increase in tumor cell death by apoptosis, or programmed cell death (Mills et al. 1995). Under the same conditions, there is no detectable effect of perillyl alcohol on BrDU incorporation into DNA, a measure of cell proliferation (Mills et al. 1995). Chemotherapy of chemically induced mammary tumors with monoterpenes results in tumor remodeling or redifferentiation (Haag et al. 1992, Haag and Gould 1994). Similarly, perillyl alcohol promotes the differentiation of cultured neuro2A cells (Shi and Gould 1995). Expression of the mannose-6-phosphate-insulin-like growth factor II receptor and transforming growth factor \( \beta \) (TGF\( \beta \)) is increased in the limonene-treated, regressing mammary tumors, but not in the small number of tumors that are unresponsive to limonene (Jirtle et al. 1993), suggesting that limonene-induced tumor cell remodeling/redifferentiation is TGF\( \beta \)-dependent.

Monoterpenes have multiple pharmacologic effects on metabolic pathways; some of these effects may account for their tumor suppressive activity (Elson 1995). Some monoterpenes, including limonene and menthol, inhibit hepatic 3-hydroxy-3-methylglutaryl CoA reductase activity (Clegg et al. 1980 and 1982) and reduce serum cholesterol (Qureshi et al. 1988). More recently, perillyl alcohol has been shown to inhibit ubiquinone and cholesterol biosynthesis in cultured NIH3T3 cells (Ren and Gould 1994). In this cell system, the inhibition of cholesterol biosynthesis occurred at the conversion of lathosterol to cholesterol, i.e., downstream of HMG CoA reductase.

Many monoterpenes, including limonene, perillyl alcohol and their active serum metabolites inhibit protein isoprenylation (Crowell et al. 1991 and 1994b, Gelb et al. 1995, Kawata et al. 1994, Schultz et al. 1994). Protein isoprenylation involves the post-translational modification of a protein by the covalent attachment of a lipophilic farnesyl or geranylgeranyl isoprenoid group to a Cys residue at or near the carboxyl terminus (Clarke 1992). Isoprenoid substrates for prenylprotein transferase enzymes include farnesylpyrophosphate and geranylglyceranylpyrophosphate, two intermediates in the mevalonate pathway (Goldstein and Brown 1990). Monoterpenes can directly inhibit prenyl-protein transferases in vitro (Gelb et al. 1995) at doses that are attainable in vivo (Crowell et al. 1992b and 1994a, Haag and Gould 1994, Phillips et al. 1995), suggesting that the inhibition of protein prenylation by monoterpenes occurs at the level of prenyl-protein transferase enzymes.

Known mammalian prenylated proteins include Ras-related small GTP-binding proteins, heterotrimeric G proteins and nuclear lamins (Clarke 1992). Prenylation of Ras enables it to associate with the plasma membrane, which is required for its oncogenic activity (Kato et al. 1992). Many prenylated proteins regulate cell growth and/or transformation (Clarke 1992), and impairment of the prenylation of one or more of these proteins might account for the antitumor activity of monoterpenes. However, recent studies suggest that the main target of these effects may not be Ras itself. Ruch and Sigler (1994) found that growth inhibition of ras-transformed liver epithelial cells was attained at doses that do not affect the association of Ras with membranes. In addition, Gould et al. (1994) reported that limonene prevented the formation of mammary tumors expressing normal or oncogenic Ras genes with equal efficiency. Together, these data suggest that either the antitumor activity of monoterpenes is due to prenylation-independent mechanisms or, alternatively, that prenylation of proteins other than Ras may be affected by monoterpenes. Evidence for the latter hypothesis includes the observation that the prenylation of many proteins, in addition to Ras, is affected by monoterpenes in a variety of cell types (Crowell et al. 1991 and 1994b, Kawata et al. 1994, Schultz et al. 1994). Moreover, recent evidence indicates that, like Ras proteins, the prenylated proteins TC21 (Graham et al. 1994), Rho (Perona et al. 1993) and the PRL-1/PTPCAAX tyrosine phosphatases (Crowell et al. 1996) can be oncogenic as well, suggesting that they may be important cellular targets of antitumorogenic protein prenylation inhibitors such as monoterpenes.

In summary, a variety of dietary monoterpenes have been shown to be effective in the chemoprevention and chemotherapy of cancer. Now, monoterpenes research is progressing into human clinical trials for chemotherapeutic activity. Monoterpenes also possess many characteristics of ideal chemopreventive agents, namely, efficacious antitumor activity, comparable effectiveness in cells, and excretion in the urine (Maltzman et al. 1991). The specific cytochrome P450 isozymes induced by limonene include CYP 2B1 and CYP2C (Maltzman et al. 1991). The effect of terpenoid compounds on cytochrome P-450 enzymes in rat liver. Biochem. Pharmacol. 37: 2223–2229.

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