

Cancer Chemoprevention and Therapy by Monoterpenes

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Monoterpenes are found in the essential oils of many plants including fruits, vegetables, and herbs. They prevent the carcinogenesis process at both the initiation and promotion/progression stages. In addition, monoterpenes are effective in treating early and advanced cancers. Monoterpenes such as limonene and perillyl alcohol have been shown to prevent mammary, liver, lung, and other cancers. These compounds have also been used to treat a variety of rodent cancers, including breast and pancreatic carcinomas. In addition, *in vitro* data suggest that they may be effective in treating neuroblastomas and leukemias. Both limonene and perillyl alcohol are currently being evaluated in phase I clinical trials in advanced cancer patients. The monoterpenes have several cellular and molecular activities that could potentially underlie their positive therapeutic index. The monoterpenes inhibit the isoprenylation of small G proteins. Such inhibitions could alter signal transduction and result in altered gene expression. The results of a new gene expression screen—subtractive display—have identified or confirmed several up- or downregulated genes in regressing mammary carcinomas. For example, these regressing tumors overexpress the mannose 6-phosphate/IGF II receptor. The product of this gene both degrades the mammary tumor mitogen IGF II and activates the cytostatic factor TGF- β . These and other alterations in the gene expression of mammary carcinomas lead to a G₁ cell cycle block, followed by apoptosis, redifferentiation, and finally complete tumor regression in which tumor parenchyma is replaced by stromal elements. It is likely that monoterpenes prevent mammary cancer during their progression stage by mechanisms similar to those that occur during therapy. In contrast, prevention of mammary cancer by polycyclic hydrocarbons such as 7,12-dimethylbenz[*a*]anthracene occur by the induction of detoxifying phase II hepatic enzymes. — *Environ Health Perspect* 105(Suppl 4):977–979 (1997)

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Anticancer agents have traditionally been classified as those that are targeted either at the prevention or treatment of cancer. Prevention agents are classified as those that block initiation or suppress promotion/progression. Cancer therapeutic drugs today are mainly cytotoxic. They act to directly or indirectly kill cancer cells. More recently cytostatic agents that block cell division with or without stimulating differentiation are being used in and developed

for use in cancer patients. Based on this classification it is possible for agents to bridge cancer prevention and therapy.

An example of a group of such anticancer agents are the monoterpenes. Monoterpenes are naturally occurring hydrocarbons composed of the condensation of two isoprenes. They are widely distributed in the plant kingdom and are best known in plant essential oils. Two monoterpenes that have efficacy in both cancer prevention and therapy are limonene and perillyl alcohol. These monocyclic monoterpenes prevent several forms of cancer by blocking initiation (1). For example, limonene has been shown to block mammary cancer induced by 7,12-dimethylbenz[*a*]anthracene (DMBA) in rats (2). In addition to this blocking activity, limonene and perillyl alcohol have been shown to have suppressing activity in both mammary (3,4) cancer models. When given post-initiation, they prevent the progression of cancer in these organs following carcinogen

exposure. Finally, both limonene and perillyl alcohol have been shown to be effective agents for treating established rodent breast (5,6) and pancreatic (7) cancer. For example, advanced rat breast cancer treated with limonene or perillyl alcohol shows up to an 80% response rate, most of which are complete regressions. We feel that the monoterpenes likely prevent cancer at the progression stage by altering very small masses of nondetectable early cancer cells. Thus we postulate that the monoterpenes are an example of a potential new class of anticancer agents with utility that bridges prevention and therapy. In this review, examples of this bridging activity will be given, together with an attempt to shed light on the cellular and molecular mechanisms underlying these anticancer activities.

Monoterpene Suppressing Activity

Limonene prevents both mammary and liver cancers at the promotion/progression stage. When mammary cancers were initiated in rats by either the direct acting carcinogen *N*-methyl-*N*-nitrosourea or the indirectly acting carcinogen DMBA, they could be prevented from developing if the carcinogen-exposed rats were fed limonene starting 2 weeks after carcinogen dosing (3). Mammary cancer with a noncarcinogen etiology could also be prevented by limonene treatment. Mammary carcinomas can be induced by the direct transduction of activated *ras* genes into ductal epithelial cells using retroviral vectors. If limonene is given to such *ras*-transduced rats, the development of mammary carcinomas is greatly diminished (8).

To test if the ability of monoterpenes to prevent carcinogenesis during promotion/progression was confined to mammary tissue, as it is for example with tamoxifen prevention, we explored its activity in a liver carcinogenesis model. Rats were treated with multiple exposures to the hepatic carcinogen diethylnitrosamine. Two weeks following the final exposure, they were fed perillyl alcohol until the termination of the experiment. At necropsy, the treated rats had a 90% reduction in tumor mass compared to controls (4).

Monoterpene Tumor Therapy

In several early chemoprevention–mammary cancer experiments, we noticed a greater than expected frequency of complete

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Abbreviations used: Co-Q, ubiquinone; DMBA, 7,12-dimethylbenz[*a*]anthracene; M6P, mannose 6-phosphate/IGF II; TGF- β , transforming growth factor beta.

regression of mammary tumors in the terpene-fed group. While such an observation could have several explanations, we first tested the possibility that terpenes could act as therapeutic molecules for rat mammary carcinomas. We first tested the ability of limonene to induce the regression of chemically induced small mammary carcinomas, which occasionally spontaneously regress. We found that a large but nontoxic dose of limonene could cause the complete regression of the majority of treated mammary tumors. We also observed that advanced (> 10 mm diameter) chemically induced mammary tumors, which only rarely spontaneously regress (5), completely regressed when treated with limonene. Even though the large amount (10% of diet) of limonene used in these pair feeding studies was not toxic, we felt that it was important to increase the potency of the terpene to be used. Following screening of a large variety of natural and synthetic monoterpenes (9), we chose to focus further studies on the hydroxylated monocyclic monoterpene perillyl alcohol.

Perillyl alcohol was found to be approximately five times more potent than limonene, but it had a similar excellent therapeutic index at efficacious doses. Both perillyl alcohol and limonene were very rapidly metabolized to the same metabolites—perillic acid and dihydroperillic acid. However, rats chronically fed perillyl alcohol at 2% in the diets had more than twice the serum metabolite levels than rats fed diets containing 10% limonene (6). If indeed the biological activity of the terpenes resides in their metabolites, i.e., they are prodrugs, it is likely that perillyl alcohol is more potent than limonene due to differences in their pathways of metabolism and their pharmacokinetics. Yet, as in most cases, this interpretation may represent only part of the mechanistic basis for differential potency because the relative activities of these two compounds cannot totally be accounted for by the ratio of their chronic serum metabolite levels (6).

Finally, as was the case for prevention, the therapeutic efficacy of monoterpenes is not restricted to mammary carcinomas of the rat. Recently, Stark et al. (7) showed that perillyl alcohol was effective in a transplantable pancreatic tumor model in the hamster. They showed that nontoxic dietary levels of perillyl alcohol could both reduce the growth rate of these tumors and cause the complete regression of about a quarter of these cancers. Thus it is likely that the

therapeutic activity of perillyl alcohol is not species or site specific.

Cellular and Molecular Activities

The monoterpenes have been shown to have a large number of diverse cellular and molecular effects both *in vitro* and *in vivo*. However, it is not yet apparent which if any of these activities are mechanistically related to the anticancer effects of the monoterpenes.

We first examined the ability of the plant monoterpenes to alter the mevalonate-lipid metabolic pathway in mammalian cells because monoterpenes are produced in plants but not in mammals. These terpenes have only minor activities at central early parts of this pathway (MN Gould and Z Ren, unpublished data). However, they inhibit the isoprenylation of certain proteins (10), the synthesis of ubiquinone (Co-Q), and the conversion of lathosterol to cholesterol (11). The latter two activities have the potential to modify both cell structure and cell energy production. Furthermore, a reduction in Co-Q could reduce the rate at which free radicals are detoxified.

The inhibition of protein isoprenylation, which involves the addition of either farnesyl or geranyl-geranyl to the carboxyl end of selected proteins, could potentially modify protein function. We found in cultured cells that the monoterpenes inhibited both farnesylated and geranyl-geranylated proteins. However, this activity was confined to proteins with molecular weights between 20 and 26 kD. Most prenylated proteins in this size range are small G proteins such as the ras, rac, and rho proteins. It has been shown that the inhibition of prenylation reduces the physiologic functioning of these proteins. While these inhibitions of prenylation occur in cell culture as well as in enriched enzyme preparations, both perillyl alcohol and its metabolites perillic acid and dihydroperillic acid have IC₅₀ that approach 1 mM (12). While this is only slightly higher than the serum levels of terpene metabolites in rats chronically fed perillyl alcohol, it does raise the important question of whether the inhibition of prenylation from monoterpenes is an important *in vivo* mechanism in the anticancer activities of these compounds. This question is currently under active investigation.

Whether via change in prenylation or via other signaling related activities, monoterpene treatment alters gene expression in treated tumors *in vivo*. In monoterpene-treated regressing tumors we have shown changes in the cellular level of several gene

products. The most well characterized to date is the increase in the mannose 6-phosphate/IGF II (M6P) receptor and in the tumor levels of the transforming growth factor beta (TGF- β). Increases in both these proteins can be clearly seen by immunohistochemical analysis (13). In addition, the mRNA for M6P but not TGF- β increases in treated regressing tumors. In limonene tumors that did not respond to treatment, no changes in the mRNA level for M6P were observed (13).

Both these proteins are important in the growth control of rodent and human breast cancers. The M6P receptor has a dual function. First, it helps to degrade IGF II, which is a potent mitogen for breast cancer. Removal of this mitogen potentially slows tumor growth. Second, this receptor is involved in the activation of the secreted latent form of TGF- β (14). Activated TGF- β is both growth inhibitory and differentiating in breast cancer. When a regressing tumor is histopathologically analyzed it is apparent both that cell division is almost completely absent and that the tumor is redifferentiating and remodeling via an apoptotic process (Ariazi et al., unpublished data).

Toward Clinical Testing

Based on the favorable therapeutic ratio observed in tumor-bearing rodents as described above, we believe that the monoterpenes are potentially useful chemotherapeutic agents that warrant testing in human trials. Limonene is currently completing phase I clinical trials under the direction of C. Coombes in London (15). Because of the low potency of this compound, it is unlikely that a dose comparable to the rat dose of 10% of diet will be achieved. Thus in the United States, our laboratory, together with the National Cancer Institute, is beginning to examine perillyl alcohol for clinical efficacy. At the University of Wisconsin, we have begun clinical testing of perillyl alcohol in advanced cancer patients on a dose-escalating protocol. This phase I toxicology trial will help establish the maximum tolerated dose to be used for future trials in which the efficacy of this compound will be tested for cancer therapy. If efficacy is seen in a therapeutic setting, it will likely trigger further development and testing of the monoterpenes for cancer prevention. Monoterpenes are thus an example of the development of agents that will bridge the areas of prevention and therapy while, we hope, more efficiently benefiting both areas of anticancer research.

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