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Mammary carcinoma regression induced by perillyl alcohol, a hydroxylated analog of limonene

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Abstract. The monoterpene perillyl alcohol has been shown to induce the regression of 81% of small mammary carcinomas and up to 75% of advanced mammary carcinomas initiated by 7,12-dimethylbenz(a)anthracene (DMBA) in the Wistar-Furth rat. Dietary perillyl alcohol was greater than 5 times more potent than the monoterpene limonene at inducing tumor regression. Perillyl alcohol is rapidly metabolized in the rat, as is limonene. Rats chronically fed perillyl alcohol had the same circulating plasma metabolites as rats fed limonene; however, the levels of these metabolites found in the plasma were higher for perillyl alcohol-fed rats. For example, rats given a 2% perillyl alcohol diet for 10 weeks had plasma levels of terpene metabolites of 0.82 m*M* whereas those fed a 10% limonene diet for the same period had blood levels of 0.27 m*M*. It thus appears that the increased potency of perillyl alcohol over limonene in causing tumor regression may be due at least in part to differences in the pharmacokinetics of these two monoterpenes. We feel that perillyl alcohol is a good candidate for clinical testing of anticancer efficacy in humans.

Key words: Mammary carcinoma – Monoterpene – Drug metabolism – Cancer therapy – Cancer prevention

Introduction

Dietary administration of the monoterpene d-limonene is effective in preventing the formation and causing the regression of chemically induced rat mammary carcinomas. It is currently in phase I therapeutic trials [14]. As a chemopreventive agent, dietary limonene is capable of increasing tumor latency and decreasing tumor multiplicity when fed at the initiation or promotion/progression stages of 7,12-dimethylbenzanthrene (DMBA)-induced rat mammary carcinogenesis [5, 7]. In the nitrosomethylurea (NMU)-induced rat tumor model, these same effects are observed when limonene is fed during the promotion/progression stage only [12]. As a therapeutic agent, dietary limonene is capable of causing the regression of both DMBA- and NMU-induced rat mammary carcinomas [6, 9]. When rats with advanced chemically induced mammary cancer were fed a 10% limonene diet, regression was observed in approximately 80% of the tumors. The majority of these regressions were complete and were associated with no major toxicity [9]. Although limonene has an excellent therapeutic ratio, the amount of limonene needed for maximal carcinoma regression in the rat is almost 10 g/kg per day. We thus sought to identify more potent analogs of limonene so as to reduce the administration quantity while maintaining limonene's favorable therapeutic ratio.

We have shown that limonene inhibits the isoprenylation of small G-proteins in the 21- to 26-kDa range, including ras-p21 [1]. Both natural and synthetic analogs of limonene were assayed for their ability to inhibit protein isoprenylation in cultured 3T3 cells [4]. This endpoint was chosen because the inhibition of protein isoprenylation has been postulated to be a potential mechanism by which limonene induces tumor regression [1]. Perillyl alcohol, a hydroxylated analog of limonene, was found to be >5 times more effective at inhibiting the isoprenylation of small G-proteins than were limonene and the two major rat plasma metabolites of limonene, perillic acid and dihydroperillic acid [4]. In the present study we tested the ability of perillyl alcohol to cause the regression of chemically induced rat mammary carcinomas. We also characterized the circulating plasma metabolites of perillyl alcohol following acute and chronic administration. Plasma metabolite levels were then quantitated and compared in rats chronically fed either perillyl alcohol or limonene at dietary levels capable of inducing similar mammary tumor-regression rates.

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Materials and methods

Tumor induction. Virgin female Wistar-Furth (WF) rats were obtained from Harlan Sprague-Dawley, Inc., (Madison, Wis.). Arriving at 43–48 days of age, the rats were housed four per cage in wire-bottom metal cages and were provided with Teklad Lab Blox chow and acidified water ad libitum. All rats were maintained on a light/dark cycle of 12 h.

At the age of 50–55 days, all rats were given DMBA (Eastman Kodak, Rochester, N. Y.) as a single gastric intubation at a dose of 50 mg/kg body weight. The DMBA was dissolved in a stock solution of 20 mg DMBA/ml sesame oil, heated, and allowed to cool to room temperature before administration.

Perillyl alcohol – tumor-regression studies. Perillyl alcohol (>96% pure as determined by gas-chromatographic analysis; Aldrich, Milwaukee, Wis.) was added directly to Teklad 4% mouse/rat diet meal, mixed for 15 min, and stored at –20° C. Fresh diets were prepared every 7–10 days. All rats were provided fresh diet daily. For pair-feeding, the quantity of diet consumed by each 2.5% perillyl alcohol-fed rat was measured every 24 h, and the assigned partner was pair-fed a control diet accordingly for the duration of the study.

In the first experiment (pair-feeding study), a group comprising 70 rats was treated with DMBA. The rats were weighed and palpated weekly beginning at 4 weeks after carcinogen administration. Upon palpation of the first mammary tumor(s) (diameter, ≥3 mm), rats were randomly assigned to a control or 2.5% (w/w) perillyl alcohol diet and were pair-fed. All palpable mammary tumors in this study were classified as either primary, i.e., the first tumor(s) palpated with a minimal diameter of 3 mm, or secondary, i.e., a palpable tumor arising after initial diet assignment. At diet assignment, some rats had more than one primary tumor. All rats in the pair-feeding study were followed for a minimum of 10 weeks after diet assignment for tumor growth or regression at primary tumor sites and all other mammary glands.

In the second experiment (dose-response study), DMBA was given to a total of 215 rats. After 4 weeks, all rats were palpated and weighed weekly. Upon palpation of the first mammary tumor(s) with a diameter of ≥10 mm, rats were randomly assigned to a 0%, 0.5%, 1%, 1.5%, and 2% perillyl alcohol diet and were allowed to feed ad libitum. Some rats had more than one tumor at diet assignment. The rats were individually housed, and food consumption was monitored daily for 14 days using 10 randomly selected rats at each treatment level. All rats were followed for a minimum of 15 weeks after diet assignment.

For both studies, complete regression of a tumor was defined as nonpalpability for a minimum of 3 consecutive weeks. For the dose-response study, partial regression of the large tumors was defined as palpable regression to half or less of the original diameter measured at diet assignment. Rats were removed from the studies and necropsied if they became moribund or if tumor ulceration occurred. Complete necropsies were performed on all rats at the termination of the study. All of the tumors remaining at the autopsy date were diagnosed as mammary carcinomas on the basis of gross and histopathologic criteria [17].

Statistics methodology. Food intake and weight gain were compared at days 0, 4, 8, 12, and 14 using one-way analysis of variance separately at each time point. Comparison of proportions was done using Storer's approximate unconditional test [16]. Estimates of the 25% quantile of the time-to-regression distribution were obtained from Kaplan-Meier estimates of the survival curves.

Gas-chromatographic analysis of plasma following terpene administration. For identification of the circulating plasma metabolites of perillyl alcohol, female WF rats were given a single dose of limonene or perillyl alcohol (1 g/kg) mixed 1:1 (v:v) in sesame oil by oral intubation. Control animals received oil alone. At the chosen time points, rats were anesthetized with ether and blood was collected into heparinized tubes (Heparin, Sigma, grade II; 20 mg/ml in 0.9% saline). Plasma was removed following centrifugation and stored at –20° C. Terpenes remain stable under these conditions for over 1 year.

For quantitation of chronic plasma metabolite levels of perillyl alcohol and limonene, female WF rats were housed individually and fed fresh diet daily. The afternoon before blood collection, the rats, which had been consuming terpene diet for 3, 5, or 10 weeks, were given approximately 20 g of the appropriate diet and allowed to eat ad libitum for 18 h. Food-consumption data were determined the next morning, and the rats were bled within 2 h of food removal.

Perillyl alcohol, limonene, and their metabolites were extracted from plasma by the method of McClean et al. [13]. Briefly, 5 µl of a 10-m*M* solution of perillaldehyde in methanol was added to 50 µl plasma as an internal standard for quantitation. The plasma was mixed with an equal volume of *n*-butanol: acetonitrile (1:1, v/v), after which another volume of saturated dibasic potassium phosphate was added. After vortexing, the samples were centrifuged and the organic layer was removed from the top. Of the organic extract, 2 µl was the injection volume used for all samples.

Plasma extracts were analyzed using a Hewlett-Packard 5891 gas chromatograph (GC) equipped with a flame-ionization detector (FID) and a 30-m, 0.32-mm (inside diameter), 0.25-µm (film thickness) Supelco SPB-5 column. The inlet liner used was a Hewlett-Packard RP-mixing chamber, silanized using dichlorodimethylsilane. The injector and detector temperatures were 250° C and 300° C, respectively. The temperature program was 90° C for 4 min, followed by increases of 10° C/min, followed by 15 min at 275° C. The split ratio was 1:10 and the linear velocity was 30 cm/s at 90° C. The flow rates of the air, hydrogen, and makeup gases were 35, 300, and 20 ml/min, respectively.

Standard curves were generated for perillyl alcohol, limonene, and the synthesized metabolites [2] in rat plasma at concentrations ranging from 0.20 to 1.5 m*M*, using perillaldehyde as an internal standard. Perillaldehyde was chosen as an internal standard due to its extraction efficiency and to the observation that its retention time under the GC-FID conditions used did not overlap that of any of the observed metabolites of either limonene or perillyl alcohol for any of the time points analyzed. The ratio of the terpene peak area/perillaldehyde peak area versus the concentration of terpene in the sample was plotted. The slope of this line was used to determine the concentration of each terpene in the plasma of rats fed perillyl alcohol or limonene. The extraction efficiency was >90% for all terpenes tested. The slopes of the standard curves for all terpenes quantitated were near-linear, with correlation coefficients (r) ranging from 0.98 to 1.0. The limits of detection ranged from 10- to 30 µ*M*, depending on the terpene. For terpene levels quantitated below 200 µ*M*, standard addition was used to confirm the identity of the terpene and the measured levels.

A Hewlett-Packard 5890 chromatograph equipped with a 5971A mass-selective detector was also used for obtaining mass spectra and verifying the identity of metabolites observed following perillyl alcohol administration. All conditions were identical to those used for GC-FID. The electron-impact ion source was set at 70 eV.

Table 1. Complete regression of DMBA-induced rat mammary carcinomas by dietary perillyl alcohol – pair-feeding study

	Complete primary tumor regression (%)[a]	Time to regress (weeks)[b]
2.5% Perillyl alcohol diet	22/27 (81)*	3.0*
Pair-fed control	9/29 (31)	9.0

* Significantly different from the control value ($P < 0.01$)
[a] Number of rats per group = 26 (some rats had more than 1 tumor)
[b] Kaplan-Meier estimate of the time when 25% of tumors will have regressed

Results

Tumor regression – pair-feeding study

DMBA-induced primary carcinomas (≥3 mm in diameter) in perillyl alcohol-treated rats had a complete regression rate of 81% (22/27) as compared with 31% (9/29) for pair-fed controls ($P < 0.01$; Table 1)). The time required for a primary tumor to regress to a nonpalpable mass in the perillyl alcohol-treated group was shorter than the time required for spontaneous regression in the pair-fed controls. Perillyl alcohol also prevented the development of secondary tumors arising after the initial diet assignment (Fig. 1).

At the level of 2.5% perillyl alcohol in the diet, toxicity was observed as a weight loss in perillyl alcohol-fed rats (Fig. 2). The perillyl alcohol-fed rats exhibited some initial food aversion during the 1st week; therefore, both the perillyl alcohol-fed rats and the pair-fed controls experienced initial weight loss. As food consumption increased, a weight gain was observed in both groups; however, the perillyl alcohol-fed rats could not achieve weights similar to those reached by controls. For this reason, this experiment was terminated at 10 weeks after diet assignment. In dietary toxicity studies (data not shown), a 2.5% perillyl alcohol diet corresponded to the maximal nonlethal dose tolerated by the animals. An increase of 0.5% to a 3% total perillyl alcohol diet resulted in several deaths. For the dose-response study, the maximal dose of perillyl alcohol given was 2% so as to minimize weight loss.

Tumor regression – dose-response study

In this study, dietary perillyl alcohol was used to treat advanced DMBA-induced rat mammary tumors (≥10 mm in diameter). In the control group, no spontaneous regression of primary tumors was observed. As the dose of perillyl alcohol in the diet increased, the rates of both complete and partial regression also increased (Table 2). Significant levels of regression, both complete and partial, of mammary carcinomas were observed at all dietary levels tested; however, diets with a minimum of 1% perillyl alcohol were required to show a significant level of complete tumor regression.

Rats fed dietary perillyl alcohol at all levels in this experiment exhibited some food aversion at the beginning of feeding; however, by approximately 2 weeks after the initial feeding, no significant difference in food intake was measured between the perillyl alcohol-fed rats and the non-pair-fed control rats (Table 3). The weight data shown in Fig. 3 indicate that rats fed 2% perillyl alcohol had slightly lower body weights at the end of the experiment. With the exception of this weight loss, there was no other observable toxicity. If we use the food-consumption (Table 3) and weight data obtained at week 2 after initial diet assignment

Table 2. Regression of DMBA-induced rat mammary carcinomas by dietary perillyl alcohol – dose-response study

% Perillyl alcohol	Complete + partial regression of tumors (%)[a]	Complete regression of tumors (%)	Partial regression of tumors (%)[b]
0	0/23 (0)	0/23 (0)	0/23 (0)
0.5	5/20 (25)*	2/20 (10)	3/20 (15)
1.0	11/20 (55)*	7/20 (35)*	4/20 (20)
1.5	13/21 (62)*	8/21 (38)*	5/21 (24)
2.0	15/20 (75)*	10/20 (50)*	5/20 (25)*

* Significantly different from the control value ($P < 0.01$)
[a] Number of rats per group = 20 (some rats had more than 1 tumor)
[b] Partial regression defined as regression to half or less of the original diameter

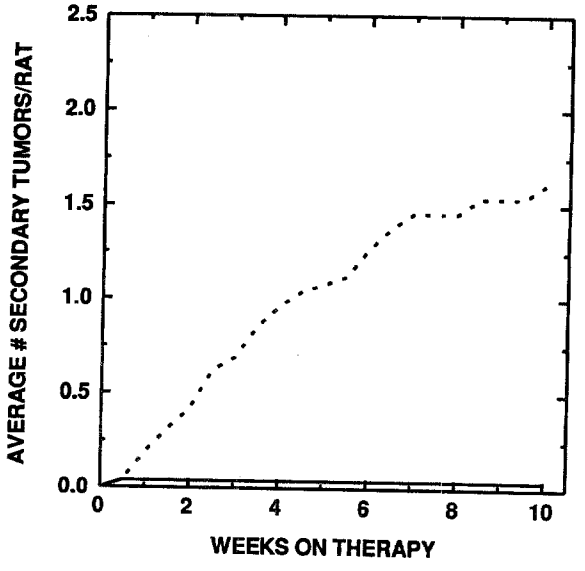

Fig. 1. Prevention of secondary tumors arising in perillyl alcohol-fed rats. The average number of palpated secondary tumors is plotted versus the number of weeks after diet assignment for rats fed 2.5% perillyl alcohol (—, $n = 26$) and pair-fed control rats (---, $n = 26$)

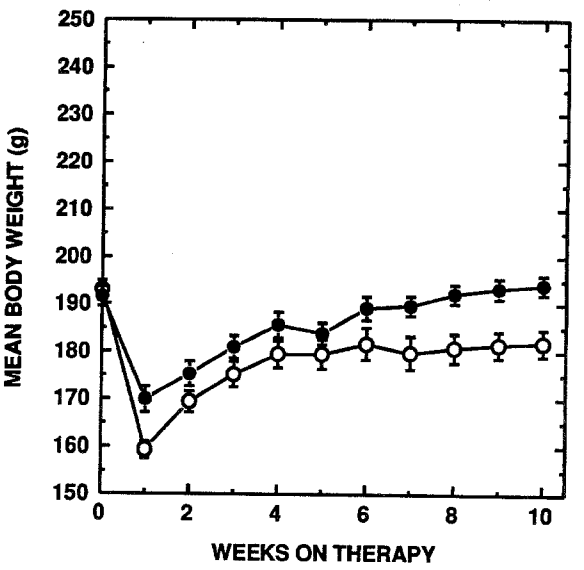

Fig. 2. Effect of dietary perillyl alcohol on weight gain. The mean body weight (in g) ± SEM is plotted versus the number of weeks after diet assignment for rats fed a 2.5% perillyl alcohol diet (○, $n = 26$) and pair-fed control rats (●, $n = 26$)

Table 3. Food-consumption data in rats fed perillyl alcohol

% Diet	Amount of diet consumed/rat (g)[a]				
	Day 0	Day 4	Day 8	Day 12	Day 14
0	13.2±1.2	14.5±0.9	14.5±1.1	16.0±1.3	16.6±1.6
0.5	6.9±1.2*	10.1±1.0*	11.9±1.6	14.6±1.5	16.2±1.0
1.0	3.0±0.6*	9.1±0.6*	12.5±0.6	16.4±0.3	17.0±1.0
1.5	2.1±0.3*	6.1±0.5*	12.9±1.3	14.9±0.6	15.6±0.6
2.0	2.4±0.4*	4.8±0.7*	12.9±1.0	14.4±1.6	14.1±0.9

Data represent mean values ± SEM
* Statistically significantly different from the control value ($P < 0.01$)
[a] Number of rats per group = 10

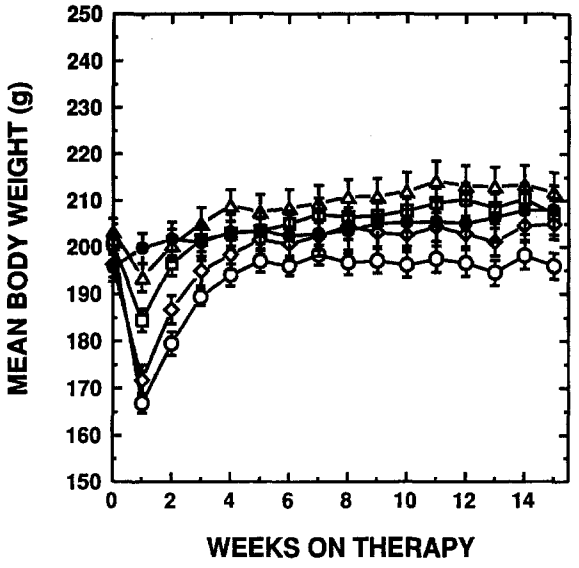

Fig. 3. Weight gain observed in rats fed different levels of perillyl alcohol diet ad libitum. No perillyl alcohol, ● ($n = 20$); 0.5% perillyl alcohol diet, △ ($n = 20$); 1% perillyl alcohol diet, □ ($n = 20$); 1.5% perillyl alchol diet ◇ ($n = 20$); 2.0% perillyl alcohol diet, ○ ($n = 20$)

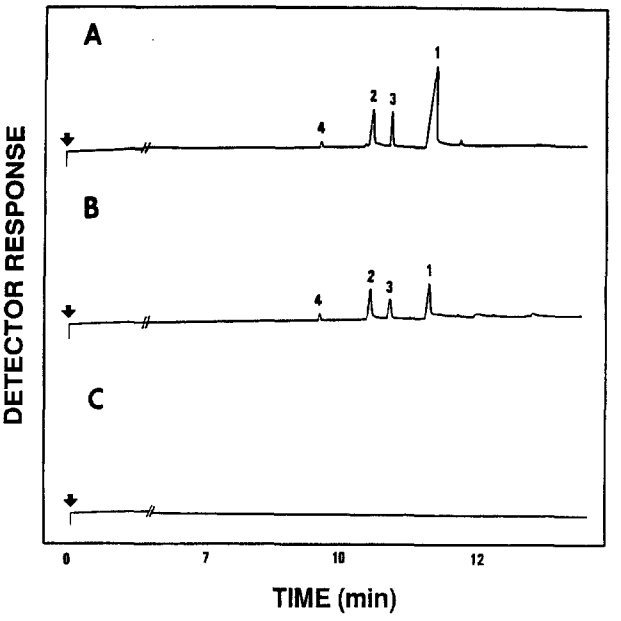

Fig. 4A–C. Gas chromatograms obtained using flame-ionization detection of plasma extracts from rats chronically fed **A** a 2% perillyl alcohol diet, **B** a 10% limonene diet, and **C** a control diet at 10 weeks after the initial feeding. Plasma was collected after rats had been allowed to eat ad libitum for 18 h. Metabolites are identified as perillic acid (*1*), dihydroperillic acid (*2*), perillic acid methyl ester (*3*), and dihydroperillic acid methyl ester (*4*). The *arrow* indicates sample injection (time, 0 min). The chromatograms have been reduced, eliminating the 1–5 min interval, including the solvent front. The retention times for the four metabolites were (*1*) 11.3 min, (*2*) 10.3 min, (*3*) 10.7 min, and (*4*) 9.6 min

(Fig. 3), it is possible to estimate the maximal dose of perillyl alcohol consumed by the rats at each treatment level. For rats consuming a 0.5%, 1.0%, 1.5%, and 2.0% perillyl alcohol diet, the maximal intake of perillyl alcohol was approximately 0.41, 0.88, 1.28, and 1.61 g/kg per day, respectively. It should be noted that these values represent the maximal intake of perillyl alcohol because of the evaporation of perillyl alcohol from the diet. Over the normal feeding period of 24 h, a 2% perillyl alcohol diet loses 30%–50% of its terpene content as determined by the weight loss from the diet noted after its exposure to air and also by GC analysis of the diet (data not shown).

Histopathology

At necropsy, all primary tumor locations were examined for the presence of the initial mammary tumor. Sections from all primary tumors or regressed mammary tumor sites were taken, fixed, sectioned, and examined histologically. Similar to results previously published for limonene-induced tumor regression, the sites at which perillyl alcohol-induced complete tumor regression occurred were predominantly composed of stromal tissue [9].

Identification of plasma metabolites of perillyl alcohol in the rat

For identification of the plasma metabolites of perillyl alcohol, rats were gavaged with an acute dose (1 g/kg) of either limonene or perillyl alcohol and the plasma was collected at 4 h postgavage. The chromatograms of plasma from perillyl alcohol-fed rats were compared with those from limonene-fed rats, whose metabolites have previously been identified [2]. Identification of the metabolite peaks

Table 4. Quantitation of the circulating plasma metabolites in rats acutely or chronically fed limonene or perillyl alcohol

	Rats (*n*)	Treatment	m*M* LIM or POH (% total)	m*M* PA (% total)	m*M* DHPA (% total)	m*M* PAME (% total)	m*M* DHPAME (% total)	Total m*M*
Acute	7	1 g/kg LIM	0.09±0.02 (13)	0.27±0.04 (40)	0.20±0.03 (30)	0.08±0.01 (12)	0.03±0.00 (4)	0.67
4 h	7	1 g/kg POH	0.00±0.00 (0)	1.13±0.20 (71)	0.08±0.01 (5)	0.38±0.09 (24)	0.01±0.00 (<1)	1.60
Chronic	6	10% LIM	0 (0)	0.10±0.02 (53)	0.06±0.01 (32)	0.02±0.01 (11)	0.01±0.00 (5)	0.19
Week 3	6	2% POH	0 (0)	0.40±0.04 (69)	0.11±0.01 (19)	0.06±0.01 (10)	0.01±0.00 (2)	0.58
Chronic	4	10% LIM	0 (0)	0.14±0.03 (50)	0.09±0.02 (32)	0.04±0.01 (14)	0.01±0.00 (4)	0.28
Week 5	4	2% POH	0 (0)	0.39±0.09 (62)	0.16±0.02 (25)	0.06±0.01 (10)	0.02±0.00 (3)	0.63
Chronic	10	10% LIM	0 (0)	0.13±0.01 (48)	0.12±0.01 (44)	0.02±0.00 (7)	Trace (<1)	0.27
Week 10	10	2% POH	0 (0)	0.48±0.04 (59)	0.23±0.02 (28)	0.08±0.01 (10)	0.03±0.00 (4)	0.82

Data represent mean values ± SEM. LIM, limonene; POH, perillyl alcohol; PA, perillic acid; DHPA, dihydroperillic acid; PAME, perillic acid methyl ester; DHPAME, dihydroperillic acid methyl ester

was made by comparison of the retention times of unique peaks in terpene-fed rats with the retention times of synthetic metabolites of limonene. These unique peaks were further identified by comparing the mass spectra of unknowns with those of the synthetic metabolites. The same major limonene metabolites, perillic acid and dihydroperillic acid, were identified as the major plasma metabolites of perillyl alcohol given as an acute dose, with minor levels of these acids' methyl esters also being present (data not shown). These metabolites were also found in the plasma of rats chronically fed perillyl alcohol or limonene (Fig. 4).

Quantitation of terpene metabolite levels in the rat

Although the plasma metabolites of rats given a single acute dose of limonene have been identified and quantitated [2], the plasma metabolite levels of chronically fed rats given either dietary limonene or perillyl alcohol have not previously been evaluated. We chose to compare a chronic 2% perillyl alcohol diet with a 10% limonene diet due to their abilities to induce similar regression rates in this rat model [11]. In addition, the metabolite levels resulting from acute gavage of equivalent doses of limonene or perillyl alcohol (1 g/kg) were also quantitated and compared. The data are summarized in Table 4. Rats chronically fed a 2% perillyl alcohol diet had 2–3 times greater total terpene levels as compared with rats fed a 10% limonene diet. The major metabolites of limonene, perillic acid and dihydroperillic acid, were found at approximately 1:1 ratios in both chronically and acutely limonene-fed rats. In contrast, in perillyl alcohol-fed rats, the ratio of these metabolites was greater than 10:1 in acutely fed rats and greater than 2:1 in chronically fed rats. Neither limonene nor perillyl alcohol was detected in the plasma of chronically fed rats.

Discussion

We have previously reported that dietary limonene is capable of inducing the complete regression of both early [6, 9] and advanced [11] chemically induced rat mammary carcinomas. However, the dose of limonene required to induce these regressions is high. Thus, it would be useful to identify compounds that may be given at lower doses to produce the same, if not a better, therapeutic ratio. Herein we show that dietary perillyl alcohol is much more potent than limonene in causing tumor regression of both early and advanced mammary carcinomas. For example, 1%–2.5% perillyl alcohol diets achieved approximately the same therapeutic effect as a 10% limonene diet [9, 11]. In addition, a 2.5% perillyl alcohol diet had a dramatic effect in preventing the development of secondary tumors, indicating its potential chemopreventive capability.

Limonene has previously been shown to be rapidly metabolized to two major and, possibly, two minor metabolites that also make up the predominant terpene components of the plasma in chronically fed rats and, thus, likely mediate the biological activity of limonene. Therefore, it was important to characterize the circulating plasma metabolites of perillyl alcohol. Perillyl alcohol was found to be rapidly metabolized to the same metabolites as was limonene. These include the two major metabolites, perillic acid and dihydroperillic acid, and lesser amounts of their methyl esters. It is important to reiterate that as we previously reported [2], the methylation of perillic acid and dihydroperillic acid is observed when these synthesized acids (>98% pure) are incubated in plasma, extracted, and analyzed by GC. It thus remains unclear whether the acids' methyl esters are true circulating plasma metabolites or are produced during analysis.

From additional acute-gavage studies (data not shown), limonene was found in the plasma in acutely fed rats for up to 4 h and appeared to be metabolized at that time to equal amounts of perillic and dihydroperillic acid. By 24 h postadministration, no terpene was detectable in the plasma. In contrast, perillyl alcohol was more rapidly converted to metabolites than was limonene, with perillic acid being the major metabolite detected. Perillyl alcohol was not detected at any time point, including 15 min postgavage, whereas perillic and dihydroperillic acids were detectable at 24 h postadministration in rats acutely fed perillyl alcohol. The total plasma levels of terpene determined following an acute administration of perillyl alcohol were 3 times higher than those of limonene at 4 h postgavage. The same trend in total plasma metabolite levels was found in the chronically fed rats. Rats fed a 2% perillyl

alcohol diet had total terpene levels that were approximately 3 times higher than those of rats fed a 10% limonene diet.

The initial motivation for evaluating perillyl alcohol for its ability to treat advanced mammary carcinomas resulted from its ability to block protein isoprenylation in 3T3 cells more efficiently than limonene and other related terpenes [4]. 3T3 cells cannot metabolize terpenes and cellular effects are thus a direct result of the added terpene. In that perillyl alcohol is rapidly converted to the same metabolites as is limonene in the rat, it is unlikely that the differential effects of the tested compounds in inhibiting protein isoprenylation are the underlying mechanisms for the greater potency of perillyl alcohol in causing tumor regression. More likely, perillyl alcohol is more potent than limonene because its chronic administration results in more than a 10-fold increase in the plasma levels of all limonene-related metabolites per dose. The underlying mechanism that results in this abundance of metabolites following perillyl alcohol administration is not known. It is unlikely to be due to differential absorption from the gastrointestinal tract, since limonene has been shown to be extremely well absorbed [2, 10]. Although the chronic profiles of limonene and perillyl alcohol metabolites are quite similar, the pathway to these metabolites may be different. It appears that limonene is rapidly converted to both perillic acid and dihydroperillic acid. In contrast, perillyl alcohol appears to be initially converted to perillic acid. This acid may possibly later be converted to dihydroperillic acid. Whether this difference in metabolism is responsible for the higher plasma terpene levels present following perillyl alcohol administration as compared with limonene feeding remains to be determined.

Perillyl alcohol has been shown to be a potent therapeutic agent for advanced rat mammary cancer. Tumor regression occurs at the cost of little toxicity, if any. The mechanism underlying this excellent therapeutic ratio is unknown; however, we have reported several monoterpene-associated activities that could explain the tumor regression seen following perillyl alcohol administration. The monoterpenes limonene, perillyl alcohol, and their metabolites have been shown to inhibit selectively the isoprenylation of small G-proteins, including ras-p21 [1, 4]. This inhibition of the isoprenylation of small G-proteins has the potential to alter differentiation and inhibit cell cycling. It is likely that the terpenes affect isoprenylation at the level of both farnesyl and geranylgeranyl transferase [1]. In addition to inhibiting these enzymes, perillyl alcohol has also been shown to inhibit two additional branches of the mevalonate-lipid pathway. Perillyl alcohol as well as other terpenes have been shown to inhibit the synthesis of ubiquinone (CoQ) and the conversion of lathesterol to cholesterol [15]. The former may contribute to the positive therapeutic ratio in that many tumor cells are deficient in oxidative phosphorylation and may rely on glycolysis for energy generation [15].

Dietary administration of monoterpenes has also been associated with increased levels of growth factors and their receptors that may regulate the growth of mammary carcinomas. Terpene-treated carcinomas have increased levels of the mannose-6 phosphate/insulin-like growth factor, (IGF) II receptors and of transforming growth factor-β (TGFβ) [11]. The former acts both to degrade the mammary growth factor IGF II and to facilitate the activation of the inhibitory factor TGFβ. TGFβ has the potential to inhibit the growth of breast cancer cells [11]. Together, these various cellular activities associated with monoterpene administration may contribute to the therapeutic efficacy of perillyl alcohol and its metabolites. The relative contribution of each of these diverse activities to the favorable therapeutic ratio induced by perillyl alcohol in advanced rat mammary carcinomas is currently under investigation.

In summary, the present study shows that dietary perillyl alcohol is more potent than dietary limonene in causing the regression of both early and advanced rat mammary carcinomas. This increased potency of perillyl alcohol is likely to be due to its differential metabolism, which results in levels of limonene-associated metabolites higher than those seen after limonene administration. We have shown that limonene is metabolized in humans in a manner similar to that observed in rats [3], and it is thus possible that perillyl alcohol metabolism in humans will parallel these findings in rodents. If this is the case, then it is possible to project the amounts of perillyl alcohol that would be necessary to achieve similar circulating metabolite levels in humans. Rats fed a 1% diet of perillyl alcohol have a regression rate of 55%. We estimate that they consume a maximum of 0.88 g/kg per day of perillyl alcohol. This converts to 5.2 g/m^2 [8]. The latter dose is the equivalent of approximately 10 g/day for a 70-kg human adult [8], which is within the acceptable range for human administration. We conclude that the monoterpene perillyl alcohol is an excellent potential candidate for therapeutic trials for human cancers, including those of the breast.

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