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ORIGINAL ARTICLE

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A phase I trial of perillyl alcohol administered four times daily for 14 days out of 28 days

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Abstract *Purpose*: Perillyl alcohol (POH) has been shown to have both chemopreventative and chemotherapeutic activities in preclinical studies. The underlying mechanism(s) of action of POH have yet to be delineated but may involve effects on the transforming growth factor β (TGFβ) and/or the Ras signaling pathways. A phase I study of POH for 14 days out of every 28 days in subjects with advanced malignancies was performed to evaluate dose escalation, toxicity, pharmacokinetics, and effects on TGFβ and Ras. *Methods*: POH was administered orally (500 mg capsules containing 250 mg POH) to 20 patients four times a day on a continuous basis for 14 days followed by a 14-day rest period, for up to three courses. The starting dose was 1200 mg/m^2 per dose. A minimum of three patients were treated and evaluated at each escalating POH dose. Pharmacokinetic analysis was performed on days 1 and 14 of course 1 and day 1 of selected later courses. Plasma TGFβ levels were measured on days 1 and 14. Peripheral blood lymphocyte (PBLs) Ras levels were assayed on days 1 and 2 of the first course. *Results*: The 20 patients, of whom 15 were evaluable, received doses between 1200 and 2000 mg/m^2 per dose for a total of 43 courses. The most common observed toxicities were nausea, gastrointestinal distress, and fatigue. Other toxicities included diarrhea or constipation, hypokalemia, and one incidence of acute pancreatitis. Due to these toxicities, four of the patients declined further treatment either during or after the second course. While POH was not detected in plasma, perillic acid (PA) and dihydroperillic acid (DHPA) were detected in plasma, and the peak levels at 2000 mg/m^2 per dose were approximately 600 μM (PA) and 50 μM (DHPA). There was some evidence for linearity in the peak plasma levels and area under the concentration–time curve of the metabolites from the starting dose to the highest dose. Metabolite pharmacokinetics were not significantly affected by ingestion in the fed or fasting state, or repeated exposure to POH. No evidence for an effect of POH on plasma TGFβ or PBL Ras protein was observed. No objective responses were observed. *Conclusions*: In adults with advanced malignancies, an interrupted administration schedule of POH did not reveal significant advantages over continuous dosing schedules.

Keywords Monoterpene · Perillyl alcohol · Phase I

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Introduction

Perillyl alcohol (POH) and limonene, naturally occurring monoterpenes, have been studied and continue to be studied as potential cancer preventive or therapeutic agents [15, 20, 23]. Initial research with POH and limonene showed significant preclinical growth inhibition and significant tumor preventive properties against multiple different tumor cell types [2, 5, 7, 8, 19, 24]. Much of the initial mechanistic focus on monoterpenes was based on their ability to inhibit the incorporation of radiolabeled mevalonate into small GTPase protein pools, i.e., p21^{RAS} [3]. Although these data were initially used to support the hypothesis that these monoterpenes inhibit the isoprenylation of these proteins, it has subsequently been demonstrated that monoterpenes only weakly inhibit the isoprenyltransferases at high concentrations (>1 mM) [9]. Furthermore, we have

demonstrated that the effects of POH on Ras are due to decreased Ras protein synthesis and not to impairment of Ras processing [10, 11]. The actual mechanism(s) of monoterpenes growth inhibition are still unclear. Other purported mechanisms include inhibition of the transition out of the G_1 phase of the cell cycle and increased apoptosis [22]; differential gene regulation, including overexpression of insulin-like growth factor/mannose-6-phosphate II receptor [13]; and activation of the transforming growth factor beta signaling pathway [1].

The initial clinical trials of monoterpenes focused on mirroring the preclinical experience of repeated exposure at the highest tolerable amount. Therefore, most of the clinical development has been with POH, a more potent growth inhibitor than limonene [4, 7]. Early phase I trials employed a three or four times a day continuous dosing scheme. Two studies by Ripple et al. [20, 21] resulted in recommendations of 1600 mg/m^2 per dose three times a day or 1200–1600 mg/m^2 per dose four times a day, continuously. Higher doses were administered but not recommended due to increased gastrointestinal (GI) toxicity and no evidence of increased POH metabolite concentrations above 1600 mg/m^2 per dose. Murren et al. [17] administered POH in three daily doses continuously and discontinued dose escalation due to fatigue and low-grade nausea. They recommended 1600–2100 mg/m^2 per dose three times a day.

In these initial phase I trials of POH, a common theme of low-grade toxicities, which were dose-limiting due to the unremitting nature of the drug administration, were observed. This coupled with the observations of potential synergy between monoterpenes and/or farnesyl transferase inhibitors with cytotoxic agents led to the testing of an interrupted administration schedule of POH. Therefore, we undertook a phase I dose-escalation study of POH administered four times a day for 14 days out of every 28 days in an attempt to achieve higher daily doses or better long term tolerability.

Patients and methods

Patient selection. Individuals with advanced malignancy for whom no effective standard therapy was available and who gave informed written consent according to Food and Drug Administration and institutional guidelines were eligible. Patients were required to have adequate bone marrow function (WBC count $\geq$4000 mm^3, absolute neutrophil count $\geq$1500 mm^3, and platelet count $\geq$100,000 mm^3), renal function (blood urea nitrogen $\leq$ 30 mg/dl, creatinine $\leq$ 1.5 mg/dl), and hepatic function (bilirubin $\leq$ 1.5 mg/dl, aspartate aminotransferase not more than two times the upper limit of normal). Patients with an Eastern Cooperative Oncology Group performance status of >2, a life expectancy of <12 weeks, or brain metastases were ineligible. Patients must not have received any hormonal or immunological therapy within 2 weeks or cytotoxic chemotherapy or radiation therapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) of receiving the drug. Patients were required to have measurable or evaluable disease by physical examination or radiography. Patients were not permitted to take cholesterol-lowering agents, vitamins or other antioxidants while on-study.

Drug formulation. POH was formulated in soft gelatin capsules containing 250 mg POH and 250 mg soybean oil. Capsules were supplied by the Investigational Drug Branch, Division of Cancer Treatment, Diagnosis and Centers, NCI (Bethesda, Md.).

Drug administration and dose escalation. POH was administered orally on a continuous basis for 14 days followed by a 14-day rest period. Patients remained on-study until evidence of cancer progression, the development of irreversible or life-threatening toxicity, patient refusal to continue therapy, or changes in the patient's condition rendering him or her unacceptable for further therapy in the judgment of the investigator.

Dose escalation was carried out according to a standard phase I design. The starting dose was based on experience obtained with POH given on a continuous three-times-daily schedule. A minimum of three patients were treated and evaluated for 4 weeks or more at each dose level prior to dose escalation. If one of the initial three patients experienced a dose-limiting toxicity (DLT), three additional patients were to be added at the same dose level. A DLT was defined as any toxicity of grade 3 or higher according to NCI common toxicity criteria that occurred within the first 4 weeks on-study with the following additions: grade 2 or higher vomiting for 3 days or more, grade 2 or higher diarrhea for 3 days or more, grade 2 or higher creatinine, or patient refusal to continue on therapy due to drug intolerance regardless of the grade of toxicity. The maximum tolerated dose (MTD) was defined as the dose level prior to that at which two or more of six patients experienced DLT. Hematological and non-hematological parameters were monitored weekly during courses 1, 2, and 3, and every 2 weeks thereafter.

During course 1, patients took only one dose of drug on days 1 and 2. Patients were randomized to take their day-1 dose either in a fasting or in a fed state. A patient's day-2 dose was taken in the opposite manner. Patients were hospitalized on the General Clinical Research Center Unit of each institution for these 2 days and received standardized meals and snacks that were controlled for intake of fat and total calories. On the day on which drug was taken with food, patients received three meals in addition to a snack at bedtime. On the day on which drug was taken without food, patients received two meals (lunch and dinner) and a snack. Men received a total of 2400 kcal (2200 on fasting days), and women received 1800 kcal (1600 on fasting days) divided into 15% protein, 40% fat, and 45% carbohydrate.

Pharmacokinetic sampling. During course 1, heparinized blood samples were collected to measure POH and its metabolites at baseline (assay blank) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 23 and 25 h on days 1 and 2. On day 14 of course 1 and day 1 of course 2 or later courses, samples were drawn before the drug was administered and at 0.5, 1, 1.5, 2, 3, 4, and 6 h after ingestion of the first dose. A 24-h urine collection was performed on day 1, and 6-h collections were performed on days 14 and 29 (day 1, course 2).

Analytical methods. POH, perillic acid (PA), and dihydroperillic acid (DHPA) were measured in plasma and urine using the gas chromatographic method of Phillips et al. [18]. Standards for the assay were provided by the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, Diagnosis and Centers, National Cancer Institute. For each single-dose concentration–time data set, pharmacokinetic parameters for PA and DHPA were determined by non-compartmental methods [6]. The AUC for 0–6 h was determined using the linear trapezoidal rule. The C_{max} and t_{max} were determined by direct inspection of the data. The single-dose $t_{1/2}$ was determined by log-linear regression of the terminal portion of the concentration–time curve. PKAnallyst (MicroMath Scientific Software, Salt Lake City, Utah) and Sigma Stat (Jandel Scientific, San Rafael, Calif.) were used to determine the AUC and to perform the linear regression.

Transforming growth factor β (TGFβ). Prior to drug administration on day 1 of each course and day 14 of course 1 only, approximately 5 ml whole blood was collected and handled in a manner to limit platelet injury. Plasma concentrations were measured using a validated R&D Systems (Minneapolis, Minn.) Quantikine human TGFβ1 ELISA kit and read on a Molecular Dynamics Biolumin 960 plate reader.

Detection of Ras. Approximately 5 ml whole blood was collected at four time points from each patient during the first course: prior to drug administration on day 1, 2 h after drug administration on day 1, prior to drug administration on day 2, and 2 h after drug administration on day 2. Mononuclear cells were extracted using Ficoll-Paque according to the manufacturer's protocol (Sigma). Trypan blue (Sigma) exclusion was used to determine cell counts (with a hemocytometer) and viability. Cells were lysed as previously described [10]. Protein content was determined using the method of Lowry et al. [16]. Electrophoresis and immunoblotting for Ras were performed as described previously [10]. The NCC-004 anti-pan RAS antibody [14] was kindly provided by Dr. Setsuo Hirohashi (National Cancer Center, Tokyo). Densitometric analysis was performed using an HP ScanJet II CX/T and ImageJ software (National Institutes of Health).

Results

Patients. A total of 20 patients were entered onto this study and received 49 courses of treatment. Five patients who participated in six courses of therapy were unevaluable for the following reasons. One patient at level 2 who underwent two courses of therapy was later determined to be ineligible due to prestudy and during-study use of vitamins, which was prohibited. Another patient at level 2 was unable to receive all 14 days of therapy with course 1 due to rapid clinical deterioration secondary to progressive cancer. One patient at level 3 was hospitalized on day 15 of course 1 with signs and symptoms of a cerebrovascular accident. Two patients at level 3 declined further therapy secondary to pill compliance after 1 and 6 days, retrospectively, of therapy during course 1. Patient characteristics and treatment courses per level are listed in Tables 1 and 2. The majority of patients had received prior systemic therapy, primarily chemotherapy, but also hormonal and biological therapy.

Toxicity. Table 2 summarizes the predominant toxicities observed during the study. Similar to prior experience, GI toxicity was common and fatigue was observed on occasion. Nausea with rare vomiting was the most common GI toxicity. Also commonly observed was any one of a constellation of GI symptoms including early satiety or abdominal fullness, increased burping or eructation, and abdominal discomfort or pain usually in the epigastric area. The above GI toxicities commonly started during week 1 of therapy and resolved during weeks 3 and 4 when drug was not administered. These same toxicities were again observed starting with day 1 of course 2. Both diarrhea (*grade 1*: two at level 1, one at

Table 1 Patient characteristics at enrollment in the trial

No. of enrolled patients	20
No. of evaluable patients	15
No. of evaluable courses	43
Age (years)	
Median	57
Range	32–82
Male/female	9/11
Performance status, ECOG	
0	9
1	9
2	2
Cancer type	
Colorectal carcinoma	5
Prostate carcinoma	2
Sarcoma	2
Breast carcinoma	2
Non-small-cell lung carcinoma	2
Non-Hodgkin's lymphoma	2
Chronic myelogenous leukemia	1
Pancreatic carcinoma	1
Small-bowel carcinoma	1
Melanoma	1
Renal cell carcinoma	1

Table 2 Dosage levels and toxicity from evaluable courses

Dose level	Dose (mg/m^2/dose)	No. of evaluable courses (total courses)	Nausea/vomiting				Gastrointestinal distress[a]		Fatigue	
			Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 1	Grade 2
1	1200	8 (8)	2	0	1	0	1	2	0	1
2	1600	18 (21)	6	3	2	0	4	0	4	0
3	2000	17 (20)	6	7	2	0	3	0	1	4

[a]Bloating, pain, eructation

level 2, and three at level 3) and constipation (*grade 1*: one at level 2, four at level 3; *grade 2*: one at level 2), usually mild, were also observed. The majority of cases were observed at levels 2 and 3, whether due to dose or the greater number of courses administered at those levels as compared to level 1. There were three instances of greater than grade 1 diarrhea (*level 2*: one at grade 2; *level 3*: one at grade 2, and one at grade 3) that usually occurred prior to day 14 and resolved after drug discontinuation on day 15. Many of the episodes of grade 1 diarrhea or constipation were of questionable relationship to therapy. One patient at level 3 was observed to have grade 1 stomatitis with course 3 despite having no similar toxicity with prior or subsequent courses.

Two episodes of grade 3 hypokalemia occurred in conjunction with mild diarrhea. A patient at level 3 developed grade 2 hypokalemia with course 1 and grade 3 hypokalemia with course 2. A patient at level 2 developed grade 3 hypokalemia with course 2.

A patient with non-Hodgkin's lymphoma at level 2 developed clinical signs (grade 4 increase in amylase and transaminases) and symptoms of acute pancreatitis on day 21 of course 6. These signs and symptoms resolved to baseline over 1–2 weeks and the patient received courses 7 and 8 without toxicity.

The above-mentioned toxicities were significant enough to result in several subjects declining to participate further either during or after course 2: two of eight at level 2 and two of nine at level 3.

Clinical activity. A patient with heavily pretreated and slowly progressive non-Hodgkin's small cell-cleaved lymphoma received eight courses of therapy at level 2. During treatment the treating physician noted a decrease in palpable adenopathy which did not meet response criteria. Eventually, progressive disease was observed after eight courses of therapy. No other evidence for possible clinical activity was observed and no other patients received more than five courses of therapy.

Pharmacokinetics. Plasma pharmacokinetic data for the three dose levels are summarized in Tables 3 and 4. POH was not detected in plasma by our method. Peak plasma concentrations of PA occurred at 1–2.5 h after ingestion; and of DHPA at 2.5–4 h. The mean half-life of PA was 1.0 h (range 0.6–1.4 h) and of DHPA was 1.6 h (1.0–2.8 h). Neither of these parameters appeared to be dose-related. The area under the concentration–time curve (AUC) and peak metabolite plasma concentrations (C_{max}) for both metabolites increased significantly with dose on day 1, although over the small dose range studied, the r values were only approximately 0.5 (day 1, PA AUC $r = 0.570$, $P = 0.0087$; DHPA AUC $r = 0.51$, $P = 0.0073$). Figures 1 and 2 show the dose relationships

Table 3 Single-dose pharmacokinetic parameters for PA. At each dose level, the peak plasma concentration (C_{max}), time to peak plasma concentration (t_{max}), area under the concentration–time curve for the first 6 h (6-h AUC), and plasma half-life ($t_{1/2}$) are shown for PA on days 1 and 2 (in both the fed and fasting state), and days 14 and 29. Values are means ± SD (*NA* not applicable)

Dose level	C_{max} (μ*M*)	t_{max} (h)	0–24 h AUC (μ*M* h)	0–6 h AUC (μ*M* h)	$t_{1/2}$ (h)
Level 1					
Days 1 and 2					
Fed	203 ± 57	1.0 ± 0.5	680 ± 204	663 ± 175	0.6 ± 0.1
Fasting	311 ± 8	2.5 ± 1.3	823 ± 213	813 ± 194	0.6 ± 0.1
Day 14	225 ± 55	1.5 ± 0.5	NA	472 ± 221	NA
Day 29	337 ± 275	1.7 ± 1.2	NA	655 ± 437	NA
Level 2					
Days 1 and 2					
Fed	306 ± 116	2.2 ± 1.2	908 ± 429	871 ± 453	1.4 ± 0.8
Fasting	442 ± 124	1.8 ± 1.0	1329 ± 541	1314 ± 530	0.9 ± 0.3
Day 14	327 ± 119	1.7 ± 0.4	NA	766 ± 256	NA
Day 29	371 ± 193	1.4 ± 0.2	NA	859 ± 347	NA
Level 3					
Days 1 and 2					
Fed	600 ± 207	2.2 ± 1.0	2046 ± 791	2004 ± 759	0.9 ± 0.3
Fasting	604 ± 349	1.8 ± 0.8	2177 ± 1293	2115 ± 1239	1.2 ± 0.6
Day 14	539 ± 115	1.9 ± 0.7	NA	1248 ± 455	NA
Day 29	788 ± 213	1.5 ± 0.4	NA	2010 ± 535	NA

Table 4 Single-dose pharmacokinetic parameters for DHPA. At each dose level, the peak plasma concentration (C_{max}), time to peak plasma concentration (t_{max}), area under the concentration–time curve for the first 6 h (6-h AUC), and plasma half-life ($t_{1/2}$) are shown for DHPA on days 1 and 2 (in both the fed and fasting state), and days 14 and 29. Values are means ± SD (*NA* not applicable)

Dose level	C_{max} (μ*M*)	t_{max} (h)	0–24 h AUC (μ*M* h)	0–6 h AUC (μ*M* h)	$t_{1/2}$ (h)
Level 1					
Days 1 and 2					
Fed	22 ± 11	3.7 ± 2.1	83 ± 51	77 ± 44	1.0 ± 0.1
Fasting	24 ± 11	3.3 ± 0.6	74 ± 34	72 ± 32	1.0 ± 0.1
Day 14	26 ± 8	2.3 ± 0.6	NA	88 ± 31	NA
Day 29	19 ± 9	2.7 ± 0.6	NA	70 ± 26	NA
Level 2					
Days 1 and 2					
Fed	15 ± 5	3.7 ± 2.1	71 ± 33	57 ± 23	1.9 ± 1.1
Fasting	22 ± 11	3.4 ± 1.4	93 ± 41	89 ± 41	1.1 ± 0.5
Day 14	26 ± 22	3.0 ± 1.0	NA	77 ± 47	NA
Day 29	18 ± 5	2.7 ± 1.0	NA	66 ± 23	NA
Level 3					
Days 1 and 2					
Fed	44 ± 21	3.6 ± 1.1	239 ± 160	208 ± 116	1.7 ± 0.4
Fasting	48 ± 29	4.0 ± 1.7	289 ± 214	235 ± 137	2.8 ± 2.5
Day 14	66 ± 48	2.5 ± 0.5	NA	228 ± 198	NA
Day 29	51 ± 29	2.8 ± 1.5	NA	207 ± 79	NA

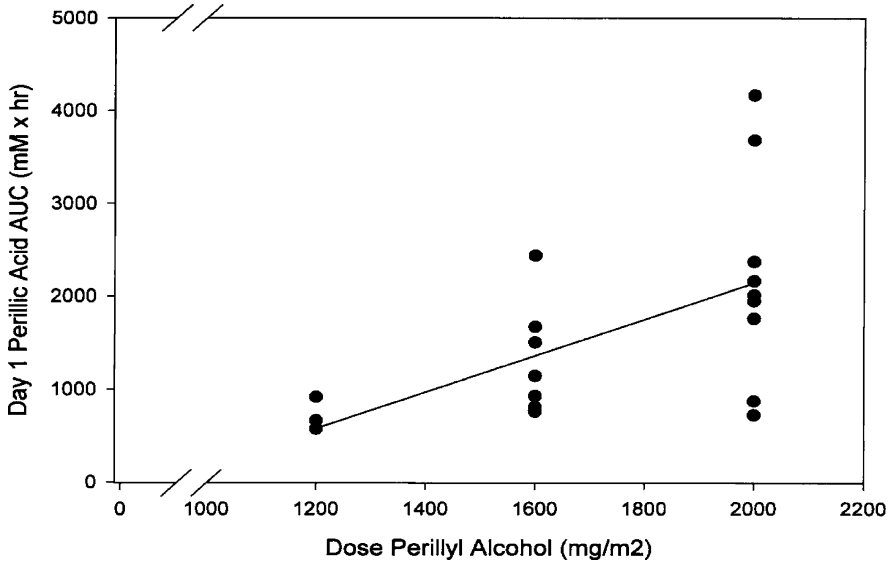

Fig. 1 Relationship between PA AUC and dose of POH

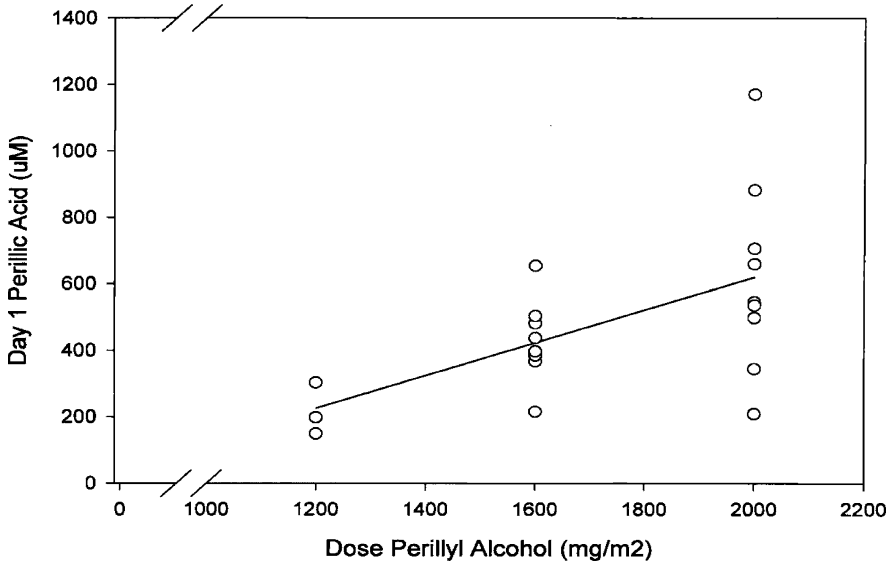

Fig. 2 Relationship between PA C_{max} and dose of POH

to C_{max} and AUC of PA. Level 3 C_{max} and AUC values were significantly different from those at levels 1 and 2 (t-test, $P < 0.05$). No significant differences in C_{max} or AUC were observed between levels 1 and 2.

Using day-1 and day-2 data from all three levels, the effects of food on metabolite concentrations were compared using the paired t-test. Overall a very slight difference in both 24-h AUC and C_{max} was noted for both PA ($P = 0.02$ and 0.031, respectively) and DHPA ($P = 0.031$ and 0.035, respectively). Concentrations without food appeared slightly higher in most cases (see Figs. 3 and 4). However, no significant differences were noted between the fed and fasted states for these parameters at level 3, the highest dose level with the largest number of patients.

The pharmacokinetics from 0 to 6 h were studied on days 14 and 29 (day 1 of course 2). The day-14 AUC (0–6 h) for PA was significantly different from that on day 1; no other pharmacokinetic parameter was significantly different comparing day 14 or 29 with day 1.

An average of 9–10% of the dose ($n = 20$, fed group $9 \pm 5\%$; $n = 20$, fasted group $10 \pm 4\%$) was recovered in the urine in the first 24 h after drug administration on days 1 and 2. There was no significant difference in urinary recovery whether the dose was taken with or without food, neither was urinary recovery

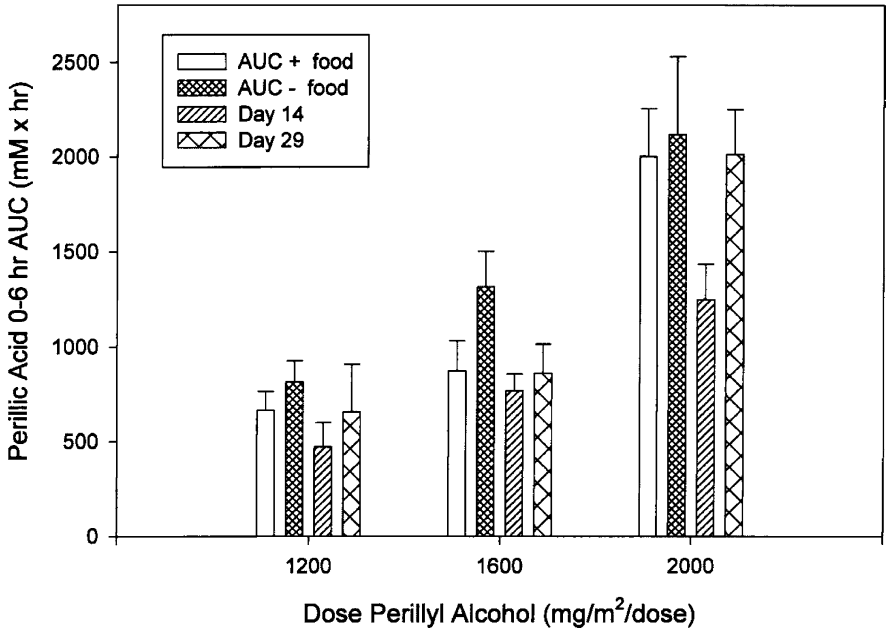

Fig. 3 Effect of food on PA AUC in relation to dose of POH

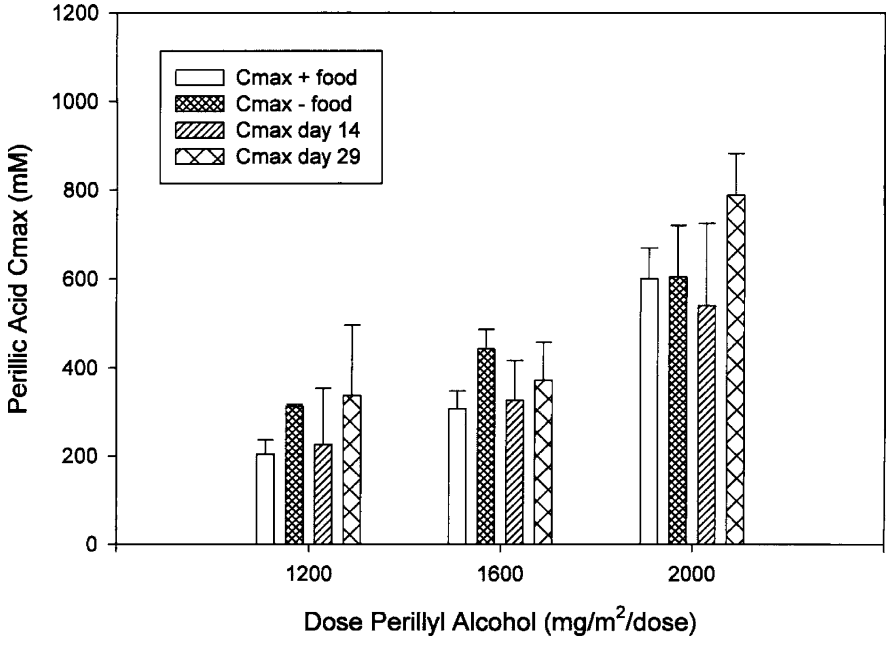

Fig. 4 Effect of food on PA C_{max} in relation to dose of POH

dose-dependent. Between 85% and 87% of the drug in the urine was found to be PA, and less than 1% was the parent POH.

Correlative assays. Plasma TGFβ1 levels did not show any correlation with clinical outcome (toxicity or disease status) or metabolite pharmacokinetics. Day-1 and day-29 levels (mean $\pm$ SD, ng/ml) for each dose level (level 1, $n=3$; level 2, $n=5$; level 3, $n=7$) were as follows: *level 1 day 1* 3.7 ± 3.1, *day 29* 6.7 ± 3.9; *level 2 day 1* 4.0 ± 3.2, *day 29* 3.7 ± 1.4; *level 3 day 1* 13.5 ± 16.8, *day 29* 3.7 ± 1.9. Of note, day-1 values from two level-3 subjects were likely inordinately high (44 and 30 ng/ml) and were considerably lower on day 14 and day 29. If the two high values are not included, the mean concentration for level 3 day 1 was 4.1 ng/ml. Day-14 values were not significantly different than day 1 or day 29. No trend was seen in values obtained monthly in subjects on-study more than 2 months.

Ras levels. Changes in levels of Ras protein were assessed via Western blot analysis. As shown in Table 5, Ras levels varied from a low of 43% to a high of 309% of control (day 1, 0 h). Although the amount of Ras protein did not correlate with dose level, an overall trend of increased Ras levels at later time points across all three dose levels was observed. Unmodified Ras was not detected, indicating a lack of FPTase inhibitory activity.

Discussion

Monoterpenes, and specifically POH, are novel compounds with which there is minimal clinical experience. Consistent with this, there remain many uncertainties as to the optimum dosing and schedule. POH in preclinical testing was administered nearly continuously to animals [7]. Interrupted schedules of administration were not tested preclinically. Another issue of administration is achievable clinical levels of POH metabolites. If the primary goal is inhibition of prenylation, the IC_{50} for FPT and GGT are approximately 1 m*M*. Peak metabolite POH concentrations in prior phase I studies have reached approximately half millimolar concentrations. The current phase I trial of an interrupted administration schedule of POH did not reveal significant advantages over continuous dosing schedules. The interrupted administration schedule did not allow greater drug tolerability leading to substantially higher administered daily doses or to any observed improvement in POH metabolite pharmacokinetics.

Toxicities were found to be very similar to those observed with continuous dosing schedules. GI toxicity, fatigue, and patient concerns over the number of pills ingested daily did not appear to be any less with this schedule as compared to continuous schedules. While patients subjectively and objectively had less toxicity during the 2 weeks off POH, the toxicity rapidly recurred with reinitiation of dosing every 4 weeks. Consistent with this, several patients [two at level 2 and four (two considered unevaluable) at level 3] declined further study participation during or after course 1 or 2 due to their concerns about toxicity (nausea or fatigue). The fact that no increased tolerability was observed with an interrupted administration schedule supports our prior observations that the toxicity does not seem to lessen or worsen over time with continuous daily dosing. As before, measures to ameliorate the toxicity were of little help for some and no help for others. A consistent ''problem'' with POH dosing is the number of capsules required (15–20 capsules four times a day at higher doses). This can only be addressed with a formulation change.

All of these factors led us to halt further dose escalation despite having only one DLT among six evaluable patients at level 3. Hudes et al. [12] performed a similar phase I study except with POH administered three times a day for 14 days out of every 28 days. They observed increasing GI toxicity starting at the initial dose of 1600 mg/m^2 per dose through 2100–2800 mg/m^2 per dose. They determined a dose of 1600–2100 mg/m^2 to be well tolerated. Their findings imply less observed toxicity with similar dosing levels. This is not unexpected since we also observed better tolerability with three-times-daily dosing as compared to four-times-daily.

The pharmacokinetics of POH were very similar to those found in our prior studies, except a significant differences in metabolite AUC and C_{max} were observed

Table 5 Peripheral blood cell Ras levels following POH administration. The data presented are percent of the control (day 1, pretreatment) as determined by densitometric analysis of Western blots

	Dose level 1			Dose level 2			Dose level 3		
	Day 1, 2 h	Day 2, 0 h	Day 2, 2 h	Day 1, 2 h	Day 2, 0 h	Day 2, 2 h	Day 1, 2 h	Day 2, 0 h	Day 2, 2 h
Sample size	2	2	2	8	8	8	7	7	7
Minimum	82.3	89.4	85.2	71.4	42.7	91.4	89.8	78.8	70.6
Maximum	102.9	124.1	149.2	113.2	243.2	309.2	142.3	212.0	297.5
Median	92.6	106.8	117.2	105.5	107.7	137.8	109.9	113.0	119.6
Mean	92.6	106.8	117.2	100.5	120.9	160.3	115.9	122.9	139.9
% <100	50	50	50	37.5	50	37.5	28.6	42.9	42.9

between level 2 (1600 mg/m^2) and level 3 (2000 mg/m^2) [20, 21]. It is possible that our other POH studies did not include sufficient numbers of patients, given the interpatient and intrapatient variability, to detect a difference. Our pharmacokinetic data from multiple studies do not show a definite increase in AUC and C_{max} from 1600 to 2000 mg/m^2. Hudes et al. [12] did report evidence for linear metabolite pharmacokinetics from 1600 to 2800 mg/m^2, but did not find a significant difference in the dose-normalized AUC values. So despite these recent findings it appears most likely that there is a lack of consistent linearity in metabolite pharmacokinetics above a dose of 1600 mg/m^2. We again did not find any definite effects of food ingestion on metabolite pharmacokinetics despite the concern of ingested fat effecting hydrocarbon (POH) absorption.

In addition to assessing plasma TGFβ1 levels, we assessed the effects of POH or its metabolites on Ras and protein farnesylation. Despite preclinical data implicating a possible role for TGFβ in terpene-induced tumor regression, we again found no correlation between plasma TGFβ1 levels and clinical outcome or POH metabolite pharmacokinetics. Assessment of POH on Ras levels revealed varied responses, with some patients having decreased levels of Ras following treatment and others having, in some cases, two- to threefold increases. Interestingly, these preliminary studies showed a trend towards increased Ras levels over time. Inhibition of Ras processing in the peripheral blood cells was not observed, consistent with the weak inhibitory activity of POH and its derivatives against farnesyl protein transferase [9]. Hudes et al. also did not observe any evidence of Ras inhibition in PBLs [12]. A decrease in Ras protein synthesis following treatment with high concentrations of POH has been observed in several leukemia cell lines [10, 11]. That this effect was not observed in the current studies might have been because of differences in Ras regulation between normal cells and cancer cells and/or not achieving sufficient cellular concentrations of POH or metabolites. How crucial these findings are relative to the clinical development of POH is questionable on two points. Actual tumor cell effects have not been examined and may be different than the surrogate tissue and the examined cellular pathways may not be crucial to any potential antitumor effects.

Based on our trial, if this formulation and schedule were to be further explored clinically, we recommend a dose level of 1600 mg/m^2. But, based on this trial and others, we do not find any advantage of this schedule over continuous dosing. Ultimately, we would recommend continuous dosing with a new formulation that might allow higher and more convenient dosing.

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