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ORIGINAL ARTICLE

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Pharmacodynamic effects of high dose lovastatin in subjects with advanced malignancies

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Abstract Lovastatin, an inhibitor of the rate-limiting enzyme in the cholesterol biosynthetic pathway, hydroxymethylglutaryl coenzyme A reductase, has shown interesting antiproliferative activities in cell culture and in animal models of cancer. The goal of the current study is to determine whether lovastatin bioactivity levels, in a range equivalent to those used in in vitro and preclinical studies, can be safely achieved in human subjects. Here we present the findings from a dose-escalating trial of lovastatin in subjects with advanced malignancies. Lovastatin was administered every 6 h for 96 h in 4-week cycles in doses ranging from 10 mg/m^2 to 415 mg/m^2. Peak plasma lovastatin bioactivity levels of 0.06–12.3 μM were achieved in a dose-independent manner. Cholesterol levels decreased during treatment and normalized during the rest period. A dose-limiting toxicity was not reached and there were no clinically significant increases in creatine phosphokinase or serum hepatic aminotransferases levels. No antitumor responses were observed. These results demonstrate that high doses of lovastatin, given every 4 h for 96 h, are well-tolerated and in select cases, bioactivity levels in the range necessary for antiproliferative activity were achieved.

Keywords Hydroxymethylglutaryl coenzyme A reductase inhibitors · Cholesterol · Isoprenoid · Malignancy · Phase I

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Introduction

Lovastatin, a potent competitive inhibitor of hydroxymethylglutaryl coenzyme A reductase (HMGR), is widely used to treat hypercholesterolemia. By blocking the synthesis of mevalonate, lovastatin inhibits cholesterol biosynthesis. This results in upregulation in hepatic low-density lipoprotein (LDL) receptors and a reduction of plasma LDL-cholesterol. In addition, treatment with lovastatin also results in the depletion of nonsterol isoprenoids, such as ubiquinone and the 15-carbon farnesyl pyrophosphate (FPP) and 20-carbon geranylgeranyl pyrophosphate (GGPP) moieties required for protein isoprenylation. Isoprenylated proteins account for approximately 1% of proteins [20] and play diverse cellular roles including regulation of proliferation, differentiation, cytoskeletal organization, mitosis, and intracellular trafficking.

The potential use of lovastatin as an anticancer agent has been suggested from a large number of studies demonstrating cytotoxic effects. Lovastatin, and other statins, have been shown to be cytotoxic or to induce apoptosis in a variety of leukemia cell lines [11, 37, 47], prostate cancer cells [39, 45], glioblastoma [48], neuroblastomas [38], medulloblastomas [35], colon cancer cells [1], malignant mesothelioma cells [49], and pancreatic cancer cell lines [44]. The concentration of lovastatin required to induce apoptosis varies among cell types, however these effects have been observed in cells that have been treated with 0.3–30 μM lovastatin for 36–96 h with the majority of cells requiring >5 μM.

Animal studies have also demonstrated the anticancer effects of statins. In a model system involving nude mice implanted subcutaneously with human EJ bladder carcinoma cells, i.p. administration of high dose lovastatin (50 mg/kg/day) for 12 days was shown to significantly inhibit the growth of the tumor [51]. In addition, inhibition of Ras membrane association, as a marker of isoprenylation, was demonstrated [51]. Higher doses of lovastatin (100 mg/kg/day for 3 days) resulted in complete inhibition of tumor growth, however four of the six

mice died within 8 days [51]. In a murine skin cancer model, i.p. administration of lovastatin (25 mg/kg/day) given on alternate days for 3 weeks resulted in significant decrease in tumor growth [27]. Infusion of lovastatin (5 mg/kg/h via subcutaneous osmotic pump) for 7 days significantly inhibited the growth of neuroblastoma cells injected subcutaneously in mice without altering serum cholesterol levels [38]. Additionally, lovastatin has been shown to reduce tumor growth and metastasis in mouse mammary tumor [5], mouse melanoma [24], rat lymphoma [42], and rat fibrosarcoma [41].

It should be noted that the concentrations required to limit isoprenylation in vitro are significantly higher (500-fold) than the concentrations that inhibit cholesterol biosynthesis (IC_{50} 10 nM) [52]. Therefore under standard dosing regimens (˜1 mg/kg/day) for hypercholesterolemia, which result in serum drug levels of ˜0.1 μM [46], cholesterol synthesis is inhibited but protein isoprenylation is conserved. As mentioned earlier, the concentrations required for cytotoxicity are in the range of 5 μM. The observation that nonsterols, in particular mevalonate and GGPP, can prevent lovastatin-induced apoptosis [3] suggests that inhibition of isoprenylation is a key mechanism underlying lovastatin-induced anticancer activity. Thus higher dosing regimens are required in order to achieve these effects in vivo.

There are limited data available regarding whether the concentrations required in vitro for anticancer activity can be safely achieved in humans. In a prior Phase 1 study, lovastatin, in doses ranging from 2 mg/kg/day to 45 mg/kg/day, was given to cancer patients [57]. In this trial, lovastatin was administered orally four times a day for 7-day courses given monthly [57]. Peak lovastatin bioactivity levels were found to be in the range of 0.10–3.9 μM [57]. The dose-limiting toxicity (DLT) was determined to be myopathy [57]. Other toxicities included gastrointestinal distress, and elevation of hepatic aminotransaminases and creatine phosphokinase levels [57]. As the majority of these toxicities occurred after 7 days of treatment [57], we hypothesized that a shorter course length might be advantageous. Thus we performed a dose-escalating clinical trial in which subjects with advanced malignancies received 4-day courses of lovastatin given monthly. The objectives of this study included determining the maximally tolerated dose (MTD) and the DLT of lovastatin given every 6 h for 96 h, the peak bioactive lovastatin serum level achieved, select pharmacodynamic measures of HMGR inhibition, and to observe any response in subjects treated with lovastatin for cancer.

Methods

Subject population

Adults (age > 8) with a histological diagnosis of malignancy and without an available life-prolonging therapy for that malignancy were eligible for the trial. Additional eligibility criteria included: no obstruction of GI tract or evidence of malabsorption, not pregnant, and no evidence of serious cataract formation prior to the start of therapy. Adequate hematologic and organ function were defined as hemoglobin concentration > 10 mg/dl, white blood cell count $> 4{,}000/mm^3$, platelet count > 100,000/mm^3, serum creatinine concentration < 1.5 mg/dl, normal creatine phosphokinase (CPK), and bilirubin, AST, ALT, GGT, and LDH levels all less than 1.5×normal. Subjects were ineligible if they had a history of problems with alcohol-related liver disease or alcohol abuse within the previous 5 years. Subjects were not permitted to take other cholesterol-lowering agents while in the study. Initially, only ECOG performance score 0–1 patients were to be entered, but later performance score 2 patients were entered. All subjects signed an informed consent document approved by the UIHC Institutional Review Board.

Each subject underwent an initial evaluation on day 1 consisting of a complete history and physical assessment of performance status, and pretreatment laboratory analysis including a complete blood count with platelet count, liver enzymes (AST, ALT, LDH, alkaline phosphatase, bilirubin), basic metabolic panel, calcium, magnesium, BUN, CPK, total protein, albumin, cholesterol, and urinalysis. Repeat laboratory studies were performed on days 3, 5, and 14 of each cycle. At a minimum, patients were evaluated after every two cycles. Radiological studies were performed as needed to assess tumor progression. Drug levels from subjects taking phenytoin, phenobarbital, and carbamazepine were monitored. Concomitant therapy with CYP3A4 inhibitors was limited.

Drug administration and dose escalation

Lovastatin was administered p.o. every 6 h for 96 h followed by a 24-day rest period. Subjects were admitted to the UIHC Clinical Research Center for the duration of the lovastatin administration. A standard phase I design was used for dose escalation. The starting dose was 10 mg/m^2. The dose was escalated for subsequent groups of three subjects to 20, 33, 50 and 70 mg/m^2. Thereafter, the dose was increased by 33%. At least three subjects were treated at each dose level prior to dose escalation. If one of the subjects experienced a DLT, then three additional subjects would be added at the dose level below that at which a DLT was observed. If no DLT was observed at that level, then that would be the MTD. DLT was defined during the first 28-day cycle as any grade 3 or 4 toxicity using the NCI Expanded Common Toxicity Criteria. As CPK is not defined in the cooperative group criteria, the following criteria were employed: grade 1 toxicity is an increase of up to 1.5×baseline, grade 2 toxicity is an increase of 1.5–2.5×baseline, grade 3 toxicity is an increase of 2.5–5×baseline, and grade 4 toxicity is an increase in CPK > 5×baseline. Subjects were taken off the study if there

was evidence of cancer progression, development of DLT, or if there were clinical changes in the subject that the investigator deemed unsuitable for further treatment (Table 1).

Pharmacokinetic sampling

During the first cycle, blood samples were collected to measure lovastatin bioactivity at baseline and 0.5, 1, 1.5, 2, 4, 6, 12, 24, 36, 48, 72, and 96 h. For five subjects, consecutive 6-h urine samples were collected during the 96 h of lovastatin treatment.

Measurement of plasma lovastatin bioactivity levels

Plasma samples were obtained before and after lovastatin dosing. Plasma lovastatin bioactivity is measured as the degree of inhibition of a standard amount of HMGR present in a rat microsomal preparation [4, 29]. For this assay HMGR was obtained from hepatic microsomes of male rats (Sprague–Dawley) that were fed a 10% cholestyramine diet for 1 week. The Lowry protein assay was used to determine the protein content of the microsomal fraction [34]. An enzyme rich supernatant was prepared by diluting microsomes in Kyrolysing buffer (50 mM potassium phosphate buffer pH 7.4, 5 mM EDTA, 0.2 M potassium chloride, 0.25% Kyro EOB) to yield 1.5 μg of total protein/μl total volume. Samples were subsequently vortexed, incubated (10 min, 37°C), and centrifuged (10,000 *g*, 90 s, 4°C). Lovastatin standards were prepared in a range of 5–5,000 nM lovastatin. Enzymatic reactions, involving 50 μl of the patient plasma sample or lovastatin standard, 75 μg of microsomal protein, 100 μl of substrate solution (2.5 mM NAD^+, 5 mM dithiothreitol, 20 mM glucose-6-phosphate, 0.7 unit glucose-6-phosphate dehydrogenase, and 100 mM potassium phosphate) and 30 μM ^{14}C-HMG CoA (5.5 mCi/mmol) [6, 26] were performed based on the work of Alberts et al. [4]. After the initial incubation of sample or standard, microsomal lysate, and substrate solution (10 min, 37°C), ^{14}C-HMG CoA was added and incubated (1 h, 37°C). The reactions were terminated after 1 h with the addition of HCl.

Table 1 Distribution of subjects and cycles per dose level

Lovastatin dose (mg/m^2)	Subjects (*n*)	Cycles (*n*)
10	3	6
20	3	5
33	3	3
50	4	5.5
70	3	10
100	3	4
133	3	6
177	3	4
235	3	5
312	3	3
415	1	1

RS-[5−^{3}H (N)]-mevalonolactone (20 μCi/mmol) was added to the samples as an internal standard. To ensure lactonization, the samples were vortexed and incubated (15 min, 37°C). The samples and standard were loaded onto AG1-X8 formate columns (BioRad, Hercules, CA, USA) for anion exchange separation. Scintillation spectoscopy was used to quantify the amount of radiolabel. The plasma lovastatin levels were determined by extrapolation from the standard curve. This assay has been shown to be linear in the range of 5–5,000 nM using lovastatin standards [29]. Samples with concentrations higher than 1,000 nM were diluted and rerun. A standard curve was generated for each patient. All samples were run in triplicate with one of the samples serving as a control due to the addition of HCl prior to isotope addition. The limit of detection is 1 nM lovastatin bioequivalents [29].

Measurement of urinary mevalonic acid excretion

Urinary mevalonate was measured by capillary gas chromatography/negative ion-chemical ionization mass spectrometry (methane) using deuterated mevalonate as an internal standard and monitoring selected ions m/z = 291 and 294 for endogenous and trideuterated mevalonate ,respectively. Mevalonate was analyzed as the pentaflurobenzyl ester (bis) trimethylsilyl ether, after selective extraction and purification by solid phase and thin layer chromatography in a similar fashion to another urinary lactone, 2,3-dinor-6-keto-$BGF_{1\alpha}$ [15].

Assessment of response

A complete response was defined as the disappearance of all measurable disease, signs, symptoms, and biochemical changes related to the tumor for >4 weeks. Partial response was defined as reduction of $>50\%$ in the sum of the products of the perpendicular diameters of all measurable lesions lasting >4 weeks, during which time new lesions do not appear and existing lesions do not enlarge. Stable disease was defined as a $<50\%$ reduction and $<25\%$ increase in the sum of the products of two perpendicular diameters of all measured lesions, and the appearance of no new lesions for >8 weeks. Progressive disease included a $>25\%$ increase in the product of the perpendicular diameters of any measured lesion compared to the size present upon entry into the study, the appearance of new areas of malignancy, or a 2-step deterioration in performance status. The study was carried out prior to the use of RECIST criteria.

Statistical analysis

Descriptive analysis included means and standard deviations. Regression analyses were utilized to generate the correlations between lovastatin dose, plasma HMGR

inhibitory activity, and changes in plasma cholesterol. Plasma lovastatin bioactivity measurements were analyzed using non-compartmental methods [19]. The maximum plasma concentration (C_{max}) and time to maximum concentration (t_{max}) were observed values. The area under the plasma concentration–time curve from 0 h to 6 h ($AUC_{0–6}$) was calculated using the linear trapezoidal method. Pharmacokinetic analysis was performed using Microsoft Excel software. Statistics were performed using JMP software (SAS Institute, Cary, NC, USA).

Results

Subject characteristics

Thirty-three subjects were enrolled in the trial beginning in June 1993 and thirty-two of those subjects received lovastatin. The characteristics of the subjects are shown in Table 2. The most common malignancies were primary CNS tumors and colorectal carcinomas. The majority of the subjects had undergone prior therapy.

Plasma lovastatin bioactivity

High doses of lovastatin given every 6 h for 96 h resulted in highly variable plasma lovastatin bioactivity levels. As shown in Fig. 1, doses of 10–100 mg/m^2 resulted in plasma bioactivity levels at 24 h that ranged from 0.06 μM to 1.9 μM. At doses of 133–412 mg/m^2 the levels variably increased as the dose increased and ranged from 0.2 μM to 12.3 μM. Multiple blood draws were obtained during the 96-h treatment regimen and changes in plasma lovastatin bioactivity were determined. There is limited further accumulation of bioactive lovastatin beyond 24 h. Figure 2 depicts four

Table 2 Subject characteristics

Characteristic	*n*
Total subjects treated	32
Male	15
Female	17
Age	
Median	59
Range	27–77
ECOG performance status	
Grade 0	2
Grade 1	15
Grade 2	14
Grade 3–4	1
Primary disease site	
Primary CNS	8
Anaplastic astrocytoma	5
Glioblastoma multiforme	3
Colorectal	7
Lung	4
Kidney	3
Pancreas	3
Unknown	2
Melanoma	1
MDS	1
Esophagus	1
Ovary	1
Thyroid	1
Prior treatments	
Chemotherapy	21
Surgery	20
Radiation	17
None	5

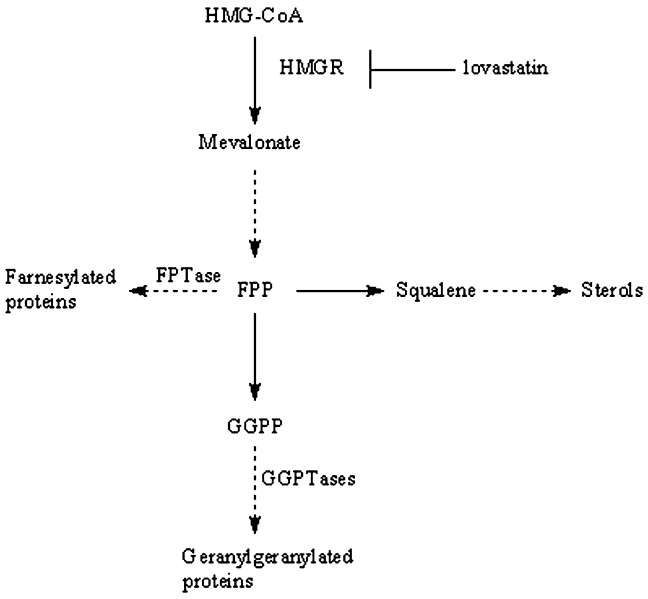

Fig. 1 Comparison of peak plasma lovastatin bioactivities versus lovastatin dose. Peak plasma lovastatin bioactivities $C_{max\ (0–96)}$ were measured by comparing the level of HMGR inhibition in the samples to a set of lovastatin standards as described in the Methods section

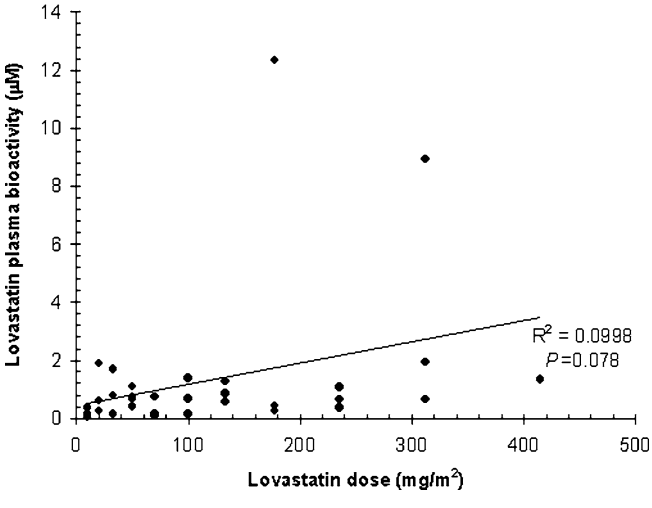

Fig. 2 Examples of changes in plasma lovastatin bioactivity levels over time with different doses of lovastatin in four different subjects. Serial samples were obtained throughout the 96-h treatment period during the first cycle. Plasma lovastatin bioactivity levels were determined as described in the Methods section

Table 3 Pharmacokinetic analysis of lovastatin plasma bioactivity levels during the first 6 h of treatment

Dose (mg/m^2)	C_{max} (μM equivalents) (range)	t_{max} (h) (range)	AUC_{0-6} (nM equivalents × h) (range)
10 ($n=3$)[a]	0.094 (0.058–0.118)	2.67 (2–4)	406 (257–568)
20 ($n=3$)[a]	0.338 (0.255–0.436)	2.67 (2–4)	1,105 (708–1,337)
33 ($n=3$)[a]	0.458 (0.138–0.636)	2.67 (2–4)	1,948 (665–2,612)
50 ($n=3$)[a]	0.468 (0.161–0.633)	2.75 (1–4)	1,802 (558–2,786)
70 ($n=3$)[a]	0.292 (0.100–0.607)	1.5 (−0.5–2)	739 (280–1,321)
100 ($n=2$)	0.543 (0.470–0.616)	2 (2–2)	2,329 (1,549–3,108)
133 ($n=2$)	0.652 (0.460–0.844)	1.5 (1–2)	1,977 (1,177–2,777)
177 ($n=3$)	2.121 (0.096–6.059)	2.17 (1–4)	10,429 (455–30,061)
235 ($n=3$)	0.402 (0.197–0.784)	2.33 (1–4)	1,373 (739–2,401)
312 ($n=2$)	1.442 (0.225–2.659)	3 (2–4)	6,614 (980–1,2247)

The mean C_{max}, t_{max}, and AUC_{0-6} values are shown as well as their ranges. Lovastatin plasma bioactivity levels were measured as described in the Methods section[a]Sampling was incomplete out to 6 h. Cmax and tmax were the observed values from 2 h to 6 h

representative subjects at varying dose levels. Apparent is the variability in accumulation of plasma lovastatin bioactivity. Table 3 displays a summary of the pharmacokinetic data during the first 6 h. Consistent with literature reports, the t_{max} was approximately 2 h [10]. Lack of association between dose received and AUC_{0-6} is also noted.

Plasma cholesterol levels

The changes in cholesterol levels were followed. At the end of the 96-h of lovastatin treatment in the first cycle all but one subject experienced a reduction in plasma cholesterol. Cholesterol concentrations decreased by 4–47% (baseline 194 ± 43 mg/dl; 96 h 147 ± 47 mg/dl (75 ± 13%, $n=31$)). While there appeared to be a trend suggesting that higher lovastatin doses have greater effects to reduce cholesterol levels (Fig. 3a, $P<0.005$), no such correlation was observed when changes in cholesterol levels were plotted against lovastatin bioactivity levels (Fig. 3b, $P=0.82$). Once off lovastatin, subject cholesterol levels returned to baseline as at day 14 the average cholesterol level was 94 ± 12% (range 80–126%, $n=25$) and at day 28, the level was 100 ± 12% (range 78–145%, $n=17$).

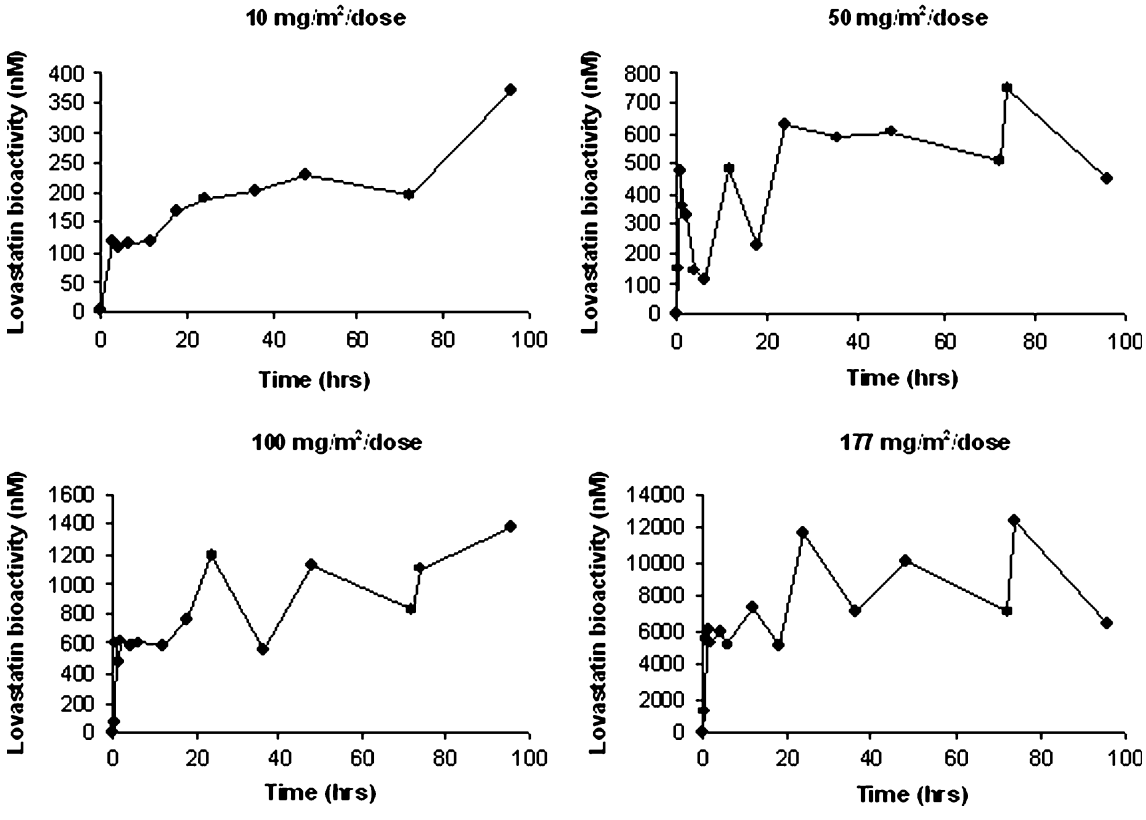

Fig. 3 Comparison of changes in cholesterol levels with lovastatin dosing (**a**) or peak lovastatin bioactivity levels (**b**). Plasma cholesterol levels at the end of the 96-h lovastatin treatment period are expressed as a percent of the control levels obtained prior to initiation of therapy

Table 4 Comparison of lovastatin-induced changes in cholesterol and urinary mevalonate excretion in five patients

Dose (mg/m^2)	Peak lovastatin bioactivity (μM)	Plasma cholesterol (mg/dl)			Urinary mevalonate (nmol/h)		
		Pre	Post	Post/pre	Pre	Post	Post/pre
20	0.59	127	117	0.92	38	28	0.74
20	1.90	131	98	0.75	44	28	0.64
177	12.32	300	247	0.82	51	21	0.41
312	8.92	203	124	0.61	19	15	0.79
415	1.33	236	149	0.63	58	38	0.66

Plasma cholesterol levels and urinary mevalonate excretion were determined prior to initiation of lovastatin treatment ("pre") and at the end of the 96 h of treatment ("post")

Urinary mevalonate excretion

Urine from five subjects was collected over the course of the 96 h of lovastatin treatment and the rate of mevalonate excretion was determined. As shown in Table 4, there is an equivalent (two patients) or greater (three patients) reduction in urinary mevalonate excretion as compared to reduction in plasma cholesterol. Mevalonic acid excretion in these patients by day 4 was 65 ± 15% baseline whereas cholesterol was 75 ± 13%. In addition, the subject with the highest lovastatin bioactivity level (12.3 μM) experienced the greatest decrease in urinary mevalonate excretion (41% of baseline). A plot of peak lovastatin bioactivity level vs. change in urinary mevalonate excretion reveals a correlation coefficient of 0.27 (r^2) and a *P*-value of 0.37 (data not shown).

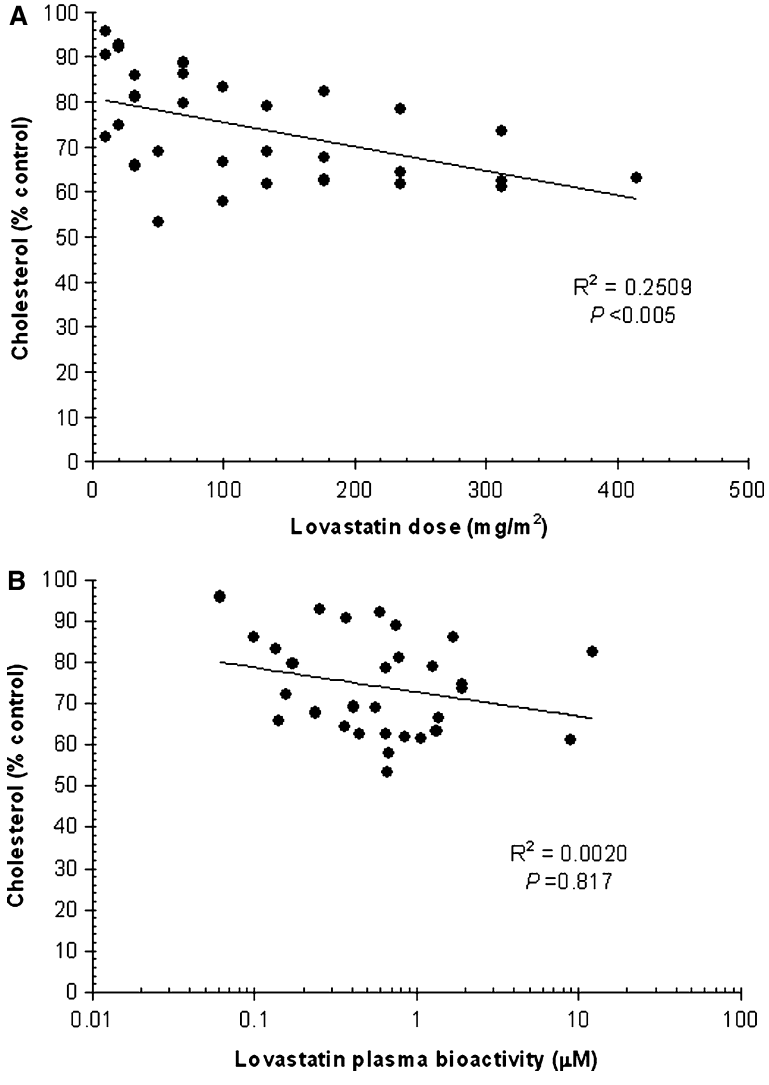

Fig. 4 The isoprenoid biosynthetic pathway. Lovastatin inhibits HMGR, the rate-limiting step in the isoprenoid biosynthetic pathway, leading to inhibition of cholesterol synthesis. In addition, depletion of mevalonate also results in depletion of FPP and GGPP, thereby preventing protein farnesylation and geranylgeranylation, catalyzed by the enzymes farnesyl protein transferase (FPTase) and geranylgeranyl protein transferases (GGPTase), respectively

Toxicities

A DLT was not reached in this trial. The trial was eventually terminated at the 415 mg/m^2 dose level because with the exception of one subject, increasing dosing seemed unlikely to uniformly achieve plasma bioactivity levels in the targeted range based on in vitro studies. One subject with pancreatic cancer was removed from the trial on day 14 because of hemobilia and subsequently died 11 days later. Two subjects died while enrolled in the trial. One subject with metastatic pancreatic cancer suffered a massive pulmonary embolism on day 3 of the first cycle. A subject with metastatic malignant melanoma with brain involvement developed worsening seizures and died on day 8 of the second cycle.

Several subjects experienced transient elevations in liver function tests. One subject had a grade 1 increase in LDH levels on day 5 of treatment. The level returned to within normal parameters by day 14. Four subjects had grade II level increases in alkaline phosphatase levels (but all but one had abnormal levels prior to lovastatin therapy). There were no occurrences of AST or ALT toxicities.

Eight subjects had increases in CPK levels in the grade 1 toxicity level, five subjects had grade 2 level increases, and one subject had a grade 3 level increase at the end of the 96-h treatment period. In all cases the CPK levels remained within normal limits. In all but three cases, the CPK levels returned to baseline by day 14, and two of those normalized by day 28. There were no occurrences of myalgias or muscle weakness although one subject reported leg cramps.

Serum levels of seizure medications were monitored throughout the trial. Three subjects receiving carbamazepine and three subjects receiving phenobarbitol had steady drug levels. One subject receiving phenytoin had persistently low levels and another subject variably low levels.

Responses

No responses (complete or partial) were observed. The majority (27) of the subjects were found to have progressive disease. One subject with colorectal cancer had stable disease after seven cycles of lovastatin therapy. Three other subjects had stable disease after two to three cycles.

Discussion

In this Phase I trial subjects with advanced malignancies received high doses of lovastatin (10–415 mg/m^2/dose) given every 6 h for 96 h. A DLT was not reached. While cholesterol levels decreased, there did not appear to be a significant relationship between either dose levels or bioactive drug levels (Fig. 3). Peak plasma bioactivity levels of HMGR inhibition ranged from 0.06 μM to12.3 μM lovastatin bioequivalents; however, there was no correlation between plasma bioactivity and lovastatin dosing (Fig. 1). No responses to therapy were observed, however one subject had stable disease after seven cycles.

Similar to the Phase I trial performed by Thibault et al. [57], we found that cholesterol levels decreased while on treatment and subsequently returned to baseline values before the start of the next cycle. In addition, similar peak bioactivity levels were obtained, although two subjects in our trial reached >5 μM while the peak level was 3.9 μM in the Thibault trial [57]. It should be noted that unlike the previously described Phase I trial, subject compliance with the very large doses of lovastatin and frequent dosing schedule was assured because the subjects were admitted as inpatients for the 96-h of treatment. Although myopathy was previously found to be the DLT [57], no evidence of myopathy was observed in our trial. The shorter treatment length (4 days) employed in our trial likely explains this difference. While there were slight elevations in CPK levels, these were not clinically significant. Unlike the Thibault trial [57], where 23% of evaluable cycles resulted in increases in serum hepatic aminotransferases, no such increases were observed with the 4-day dosing schedule.

There was considerable variability in peak bioactive levels. There are a number of pharmacological considerations that will impact bioactive serum levels. Lovastatin undergoes extensive first-pass metabolism, thus its bioavailability is limited ($<5\%$) [8]. Lovastatin is a lactone prodrug which must be converted to its active free-acid form in the liver. In addition, several oxidization products also inhibit HMGR [58]. Cytochrome P450 (CYP) 3A4 is responsible for the metabolism of lovastatin [58]. The activity of CYP3A4 is modulated by a large number of drugs. Drugs such as cyclosporin A and antifungals inhibit CYP3A4 and there have been reports of myopathy when taken with statins [8]. Dietary components, including grapefruit juice, are also known to inhibit CYP3A4 and thus increase lovastatin bioavailability [25]. This trial did not control for diet nor were subjects taking drugs which modify CYP3A4 activity excluded. The lack of a clear relationship between lovastatin dose and plasma bioactivity level was also evident when patients who were taking CYP3A4 inducers were excluded from the analyses (data not shown), indicating that there are multiple factors affecting bioactivity levels. Examination of the literature reveals the absence of a direct relationship between lovastatin dosing and C_{max} or AUC in studies with lower dosing of lovastatin [10].

Lovastatin was used in this trial because it is the HMGR inhibitor whose antiproliferative effects have been the best studied in vitro. It is likely that the results obtained from this study are representative of other statins. However, there are some differences among the statins that might influence drug levels and side-effect profiles. As a prodrug, lovastatin is considered to be one

of the more lipophilic statins and is found in high concentrations in the liver while statins such as pravastatin have been shown to have much higher concentrations in peripheral tissues [18]. Lovastatin and simvastatin, another prodrug, have been shown to cross the blood brain barrier while pravastatin does not [8]. Poorer uptake of pravastatin compared with lovastatin and simvastatin by muscle cells has been suggested as the reason for a greater incidence of myotoxicity with the latter two agents [40].

There are several methods with which to assess drug levels and activities. One option is to use an analytical methodology to measure total plasma levels of lovastatin (lactone and free acid) via HPLC [60]. While this method can be readily performed, it does not differentiate between the fraction of lovastatin and lovastatin metabolites which possess HMGR inhibitory activity and the inactive metabolites. We have chosen to measure bioactive levels of lovastatin by assessing HMGR inhibitory activity. The advantage of determining levels of bioactive drug is that it allows for more direct comparison with in vitro studies. The methodology is quite labor-intensive and requires radioactive materials. The use of an LC/MS/MS method for measuring lovastatin levels has revealed that active inhibitors (lovastatin acid and active metabolites) comprise approximately 70% of measured total inhibitors (lovastatin plus lovastatin acid plus latent and active metabolites) [9]. More recently, robotic methods have been developed for the measurement of both active and inactive levels of simvastatin in plasma and urine samples [12, 33]. While those methods will improve the facility with which data are collected, it is not clear if measured plasma bioactivity levels are predictive for either drug activity in tissue or toxicity.

An alternative approach is to measure markers for the effects of lovastatin, including changes in cholesterol, urinary mevalonate excretion, ubiquinone levels, and isoprenylation. Although a dose-dependent decrease in cholesterol levels has been observed in animals up to 180 mg/kg/day [36], no such relationship has been found in humans who do not have primary hypercholesterolemia [43]. Furthermore, it is well-established that inhibition of cholesterol synthesis is not responsible for lovastatin's cytotoxic effects [16, 59]. Urinary mevalonate excretion has been shown to be directly correlated with the rate of cholesterol synthesis [31]. This methodology has the advantage of being noninvasive, however subject compliance is required. Furthermore, urinary excretion may also be affected by dietary sources of mevalonate [30]. As our preliminary data show (Table 4), high doses of lovastatin result in decreased urinary mevalonate excretion, but not in an apparent dose-dependent manner. It has been suggested that the ability of statins to decrease the synthesis of ubiquinone, a polyisoprenylated quinoid which plays a role as a cofactor in the electron transport chain, is related to the development of myotoxicity and some investigators have monitored serum ubiquinone levels [57]. However, it appears that serum levels may not be representative of muscle ubiquinone levels [28]. Furthermore, it is not clear that decreased muscle levels of ubiquinone are associated with statin-induced muscle lesions [17] and depletion of ubiquinone does not appear to be responsible for lovastatin's antiproliferative effects [7]. Finally, while there is strong evidence that inhibition of isoprenylation via depletion of FPP and GGPP contributes to the cytotoxic effects of HMGR inhibitors, the precise targets have yet to be identified. While Ras appeared to be an attractive target, it is now becoming clear that it is not the only isoprenylated protein which plays an important role in cancer development, and more recent work has focused on geranylgeranylated proteins [50]. More work needs to be done to determine the critical targets which are affected by HMGR inhibition and thus identify markers which may be used to monitor drug activity and toxicity(See Fig. 4).

Although statins are unlikely to be used as a single agent, there has been much interest in the use of statins as adjuncts to standard chemotherapeutic agents. Lovastatin and other statins increase the cytotoxicity of cytosine arabinoside [22, 32, 56], paclitaxel [23], 5-fluorouracil (5-FU) [1], cisplatin [1, 13], *N,N*'-bis(2-chloroethyl)- *N*-nitrosurea (BCNU) [53], sulindac [2], butyrate [21], doxorubicin [14], and tumor necrosis factor-α [55] in cell culture. In some cases, these effects also have been observed in animal models [13, 14, 54]. The mechanisms underlying these synergistic interactions have not yet been defined. However, the results obtained from this study have suggested the degree and duration of HMGR inhibition that could be used in subsequent trials that combine HMGR inhibition with standard chemotherapy agents.

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