Distribution of lovastatin to bone and its effect on bone turnover in rats

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Abstract

Statins, the widely used lipid-lowering drugs, are inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase, which catalyses a rate-limiting step in the biosynthesis of cholesterol. Many previous reports show that statins can act both as bone anabolic and as anti-resorptive agents but their beneficial effects on bone turnover are still controversial. Considering their high liver specificity and low oral bioavailability, the distribution of statins to the bone microenvironment is questionable. In this study, the distribution of lovastatin and its active metabolites to bone, with respect to plasma and liver compartments, was examined after oral and intravenous administration in female rats. As compared with oral administration, the distribution of lovastatin to the bone compartment was significantly enhanced after intravenous administration. Further, the effect of lovastatin on bone turnover was studied in-vitro and in-vivo to assess its anti-osteoporotic potential. Lovastatin acid but not lovastatin was found to inhibit parathyroid-hormone-induced bone resorption in an in-vitro chick embryo bone assay. Oral, as well as intravenous, short-term lovastatin treatment significantly reduced the serum total cholesterol, serum total alkaline phosphatase and urinary crosslinks in ovariectomized rats. In accordance with its increased distribution to the bone compartment, intravenously administered lovastatin was more effective in reducing the ovariectomy-induced increase in markers of bone metabolism, especially urinary crosslinks. The findings of this study suggest that statins inhibit bone resorption and that their anti-resorptive efficacy can be increased by administering them by routes other than oral so as to achieve their enhanced concentration in bone.

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Introduction

Postmenopausal and senile osteoporosis occurs because the amount of bone removed from the skeleton by bone-resorbing osteoclasts exceeds that laid down by osteoblasts. Restoring the balance between bone resorption and formation is therefore a key goal of therapeutic intervention in osteoporosis. Inhibitors of osteoclastic bone resorption, such as bisphosphonates, oestrogen or selective oestrogen receptor modulators, are already widely used in the treatment and prevention of osteoporosis. These agents reduce the incidence of fractures but lack the ability to replace the amount of bone, which may be substantial, that has already been lost by the time osteoporosis is detected clinically (Baylink et al 1999; Rosen & Bilezikian 2001; Meunier 2001). Drugs that stimulate new bone formation would therefore be a good alternative to treat osteoporosis.

Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme (HMG-CoA) reductase to decrease cholesterol biosynthesis in the liver, thereby reducing serum cholesterol concentration. They are the most widely used drugs in the treatment of atherosclerosis and other cardiovascular disorders (LaRosa et al 1999). Recent findings indicating their bone anabolic action as one of their pleiotropic effects have generated much interest among researchers and clinicians. Various in-vitro and in-vivo preclinical studies suggest that statins increase the bone mass by enhancing bone morphogenetic protein-2 (BMP-2)-mediated osteoblast differentiation (Mundy et al 1999; Sugiyama et al 2000; Maeda et al 2001; Oxlund et al 2001; Oxlund & Andreassen 2004). Statins also inhibit osteoclast activity and can act as anti-resorptive agents (Baumann et al 2001; Staal et al 2003). A few case–control studies have also observed that statins may have the potential to reduce the risk of fractures (Meier et al 2000; Wang

et al 2000; Rejnmark et al 2004). Nevertheless, this issue remains controversial with many studies arguing their beneficial effect on bone and fracture reduction (Maritz et al 2001; van Staa et al 2001). One possible reason for the discrepancy in the results of preclinical, as well as clinical, studies is the liver-specific nature of statins. Lipophilic statins like lovastatin and simvastatin are characterized by low oral bioavailability and low peripheral distribution (Corsino et al 1999; Reinoso et al 2002). They are lactone prodrugs and undergo first-pass metabolism in liver where they get converted to active acid forms. Even the active transport system responsible for the uptake of hydrophilic statins in liver is absent in bone (Mundy 2001). Due to their liver-specific pharmacokinetic behaviour, distribution of statins to the bone microenvironment in optimum concentration is questionable. An important consideration in evaluating the potential for a skeletal effect of statins is the extent of their uptake into bone (Jadhav & Jain 2006). The aim of this study was to determine the distribution of lovastatin, a prototype statin, to the bone compartment and to examine its dose- and route of administration-dependent effects on biochemical markers of bone turnover in rats.

Materials and Methods

Chemicals

DL-[3-^{14}C]Hydroxymethyl glutaryl-CoA (57 mCi mmol^{-1}), ^{45}CaCl$_2$ (specific activity: 0.185–1.85 GBq mg^{-1} Ca) and ACSII scintillation fluid were purchased from Amersham Biosciences (UK); BGJ$_b$ bone culture medium, parathyroid hormone (PTH, aa 1–34, MW 4117.7), bovine serum albumin (fraction V), HEPES, streptomycin, penicillin, methyl cellulose, Tween-80, heptafluorobutyric acid, NADP, glucose-6-phosphate dehydrogenase and cholestyramine were procured from Sigma (USA) and alkaline phosphatase and cholesterol estimation kit were from Roche (Germany). Cellulose solid phase extraction cartridges, crosslink calibration standards and internal standard were procured from Chromsystems GmbH (Germany). All other chemicals and reagents were of analytical grade. Lovastatin and lovastatin acid were kind gifts from M/s. Biocon India Ltd, Bangalore.

Tissue distribution of lovastatin

All animal experiments were performed with prior approval from the animal ethics committee of the institute. Female Sprague-Dawley rats, ~120 g, were obtained from the institute's animal colony and were maintained under conditioned environment with alternate 12-h light–dark periods supplemented with standard rat diet (Lipton India, Bangalore). They were divided into two groups and lovastatin was administered orally (10 mg kg^{-1}, in 0.5% methylcellulose suspension) or intravenously (1 mg kg^{-1}, in dimethyl acetamide–Tween-80–physiological saline; 30:8:62). In a sparse sampling setup, the rats were bled by cardiac puncture and blood was collected in heparinized tubes, at different time points (0.16, 0.5, 1, 2, 4, 8, 12 and 24 h). Plasma was collected after centrifugation at 3000 g for 15 min and stored at −70°C until assayed. The rats

were sacrificed by excessive ether inhalation to collect the liver and tibia, which were then stored at −70°C until assayed. The thawed organs were trimmed of excess tissue and homogenized in potassium phosphate buffer (pH 7.0). Livers were homogenized using a motor-driven, tight-fitting glass-Teflon Potter-Elvehjem homogenizer. The whole tibiae were homogenized with the help of mortar and pestle using liquid nitrogen until a homogenous powder was formed. The homogenized tissues were extracted with 2 × 2 mL ethyl acetate. After evaporation of ethyl acetate, the extracts were reconstituted in HPLC-grade methanol and were assayed for inhibitory activity against soluble HMG-CoA reductase microsomal preparation.

HMG-CoA reductase enzyme inhibition assay

Soluble rat liver HMG-CoA reductase was prepared as describe previously (Kleinsek et al 1981) from the liver of male Sprague-Dawley rats, ~200 g, fed with 5% cholestyramine powdered rat chow for 4 days. The assay mixture contained potassium phosphate buffer (pH 7.0, 2 μM dithiothreitol, 2 μM glucose-6-phosphate, 0.5 μM NADP+, 0.15 μM DL-[3-^{14}C] HMG-CoA, 1.25 U glucose-6-phosphate dehydrogenase and 5 μL of inhibitor in the final 100 μL of reaction volume. The reaction mixture was incubated at 37°C for 10 min. Soluble rat-liver HMG-CoA reductase was added and reaction was further carried out at 37°C for 10 min. The reaction was terminated by the addition of 50 μL of 2.4 M HCl, and the sample was incubated at 37°C for 30 min to allow the formation of mevalonolactone. The [^{14}C] mevalonolactone formed was separated from the unreacted [^{14}C] HMG-CoA by passing the reaction mixture through columns containing 2.0 mL of anion exchange resin (Biorex-5,100–200 mesh, chloride form; BioRad, USA) slurry prepared by mixing it with TritonX-100 (Liu et al 2003). Each column was then washed twice with 2 mL of Triton X-100. The eluted solutions from each resin column were mixed with 10 mL scintillation cocktail in a 20-mL scintillation vial separately and counted on scintillation counter. The level of active drug equivalents formed in plasma, liver and tibia was determined by comparing the amount of inhibition with different standard curves constructed by spiking lovastatin acid to normal rat plasma, liver and tibia, respectively. The assay procedure was validated for calibration range and linearity (1–160 ng mL^{-1} or ng (g tissue)$^{-1}$), intra and inter-day variation, recovery and stability of analyte in different biomatrices using three quality control (QC) samples (1, 20 and 160 ng mL^{-1} or ng (g tissue)$^{-1}$). Since the assay procedure involved many steps and no internal standard was used, acceptable assay precision was maintained by assaying each blank, standard, QC and study sample in duplicate. When the deviation between the two values was smaller than 10%, the average of the two duplicate values was reported as the final concentration for the sample. If the deviation between the two duplicate values was greater than 10% for a particular sample, then the assay result for that sample was considered not acceptable and samples were re-analysed. Before each analysis, a fresh standard curve for each tissue was constructed. When a calculated concentration of HMG-CoA reductase inhibitors exceeded the upper limit of quantitation,

the sample was diluted with corresponding control matrix (drug free) and reanalysed. The plasma, liver and bone (tibia) concentration of lovastatin equivalents were normalized on the basis of administered dose. Area under curve up to the last measured time point (AUC_{0-24}) was calculated using the trapezoidal rule and expressed as % of dose h mL^{-1} or % of dose h (g tissue)$^{-1}$.

In-vitro anti-resorptive assay

An in-vitro anti-resorptive assay was performed as described previously (Arshad et al 2004) with some modifications. Briefly, femur bones isolated from chick embryos on day 11 post-ovulation were cleared of adhering connective tissue by carefully rotating each bone on dry Whatman (I) filter paper. Each femur was placed in a drop of phosphate-buffered saline (PBS) before culturing in 300 μL of BGJ_b medium (pH 7.3) supplemented with penicillin (0.075 mg mL^{-1}), streptomycin (0.05 mg mL^{-1}), HEPES (2.382 mg mL^{-1}) and BSA (1 mg mL^{-1}) in a sterile 96-well plate. Bones were then transferred to BGJ_b culture medium containing $^{45}CaCl_2$ (0.5 $\mu Ci/300\,\mu L$ medium) and incubated for 3 h at 37°C under 5% CO_2 in air and then washed thoroughly with BGJ_b medium. Labelled bones were then transferred to BGJ_b medium containing PTH (0.4 μM) and chase cultured for 96 h in the presence or absence of test agents or vehicle (ethanol; final concentration 0.01%) in 1 mL of BGJ_b medium. The contralateral femur of each fetus served as a corresponding control. Culture medium with respective treatment in each well was changed after 48 h. On termination of the culture, bones were transferred to 0.1 M HCl for 24 h. Radioactivity due to ^{45}Ca in spent medium collected at 48 and 96 h of culture and HCl extract was quantified by a Liquid Scintillation Spectrophotometer (Beckman Counter, USA) in 10 mL of scintillation fluid. Anti-resorptive activity was expressed as T/C ratio and % ^{45}Ca resorption as shown below.

$$\text{T/C Ratio} = \frac{(^{45}Ca \text{ resorption in presence of PTH} + \text{test agent})}{(^{45}Ca \text{ resorption in presence of PTH} + \text{vehicle})} \quad (1)$$

$$\%\,^{45}Ca \text{ release} = \frac{(^{45}Ca \text{ released into the medium}) \times 100}{(^{45}Ca \text{ released into the medium} + {}^{45}Ca \text{ remaining in bone HCl extract})} \quad (2)$$

Bone-sparing activity of lovastatin in ovariectomized rats

Female Sprague-Dawley rats, ~120 g, were randomly divided in six groups. One group consisted of intact females (non-ovariectomised) that served as intact control and received only the vehicle. Five groups were bilaterally ovariectomized and rested for two weeks. The treatment was started two weeks after ovariectomy and continued daily for five days. Three groups received lovastatin daily orally at a dosage of 10, 20 or 50 mg kg^{-1} formulated in 0.5% methylcellulose suspension. One group received lovastatin intravenously at a dose of 1 mg kg^{-1} formulated in solution containing dimethyl acetamide–Tween-80–saline (30:8:62) and the fifth group served as ovarietomized control. During this period, rats were

allowed free access to food and water. After dosing on the fifth day, rats were fasted and urine was collected in metabolic cages over 24 h. Blood samples were collected by cardiac puncture, allowed to clot at 4°C and serum was separated by centrifugation.

Estimation of serum cholesterol and alkaline phosphatase

Serum total cholesterol was analysed using commercially available kit (No. 1489232; Roche, Germany) and was based on determination of cholestenone after enzymatic cleavage of the cholesterol ester by cholesterol esterase, conversion of cholesterol by cholesterol oxidase and subsequent measurement by the Trinder reaction of the hydrogen peroxide formed at 546 nm.

Serum total alkaline phosphatase activity was estimated by commercially available kits (No. 396494; Roche, Germany). The assay is based on colorimetric estimation of p-nitrophenol formed after dephosphorylation of p-nitrophenylphosphate by alkaline phosphatase. Absorbance was read at 405 nm using a pre-programmed semiautomatic photometer (model 5010; Boehringer Mannheim, Germany).

Measurement of urinary cross-links

A fluorometric HPLC method was developed and validated for the analysis of urinary crosslinks, pyridinoline (PYD) and deoxypyridinoline (DPD) as described previously (Black et al 1988). Briefly, a sample of urine spiked with internal standard was subjected to acid hydrolysis at 110°C for 16 h and the crosslinks were extracted from the hydrolysate. After washing, the cellulose solid phase extraction (SPE) cartridges with a buffer containing n-butanol–glacial acetic acid–water (4:1:1), the crosslinks were eluted with 1 mL 2 mM heptaflurobutyric acid. The final sample containing the crosslinks was analysed by HPLC using a RP-18 (4×250 mm, 5 μm column) (Lichrosphere, Merck). The PYD and DPD were isocratically separated using acetonitrile–2 mM heptaflurobutyric acid (5:95) as a mobile phase at a flow rate of 1.2 mL min^{-1}. The column effluent was monitored by fluorescence detector with excitation at 295 nm and emission at 395 nm. Final concentrations of PYD and DPD were expressed relative to urinary creatinine levels determined by standard Jaffe's method.

Statistical analysis

Student's t-test was used to compare data between two groups, and analysis of variance followed by Newman–Keuls multiple comparison test was used for more than two groups. Data are expressed as mean ± s.e.m.

Results

Tissue distribution of lovastatin equivalents

A specific and reliable enzyme inhibition assay was developed and validated for the determination of lovastatin-derived active HMG-CoA reductase inhibitor concentrations in rat

plasma, liver and bone homogenate. The % CV measured at three quality control samples (1, 20 and 160 ng mL^{-1}) for intra-day and inter-day variation was found to be 4% and 6%, respectively. The mean recovery of active inhibitors measured at three quality control samples was 93.64%, 90.57% and 84.27% in plasma, liver and bone (tibia) homogenate, respectively. The active HMG-CoA reductase inhibitors were found to be stable after 2 h room temperature bench-top storage and also after three freeze–thaw cycles. The lower limit of quantitation was found to be 1 ng equivalent per mL or per g of tissue.

After oral administration, lovastatin was rapidly absorbed, reaching peak concentrations within 2 h, followed by rapid decrease in the concentration over a period of 24 h in all three biomatrices (i.e. plasma, liver and bone). The maximum concentration achieved in plasma was 4.5×10^{-4}% of dose/mL as opposed to 1.26×10^{-2}% in liver after oral administration (Figure 1). Tibia achieved a very low amount of drug (4.75×10^{-5}% of dose/g) as compared with plasma and liver and it could not be detected at 24 h (Figure 1). Even after intravenous administration, liver accumulated higher concentrations than plasma and tibia (Figure 2). The AUC_{0-24} (expressed as % of dose) values for plasma and tibia were increased after intravenous administration in comparison with oral administration, while it decreased in the case of liver (Table 1).

In-vitro anti-resorptive activity

Lovastatin (100 μM) was found to be inactive in this assay with a T/C ratio 1.08. Lovastatin acid inhibited PTH-induced resorption of ^{45}Ca from chick fetal femur bones in a concen-

tration-dependent fashion and the inhibition was of the order of 30, 50 and 75% with a T/C ratio of 0.70, 0.50 and 0.25 at 50, 100 and 250 μM concentration, respectively (Figure 3). Inhibition of ^{45}Ca resorption at 100 μM concentration of raloxifene (standard) was 32% with a T/C of 0.68.

Effect of lovastatin in ovariectomized rats

Body weight and serum cholesterol
There was a significant increase in the body weight after ovariectomy (Table 2). Lovastatin treatment for 5 days significantly reduced this ovariectomy-induced increase in body weight. The effect of oral treatment was dose dependent with the 50 mg kg^{-1} dose being maximally effective. The effect of the 1 mg kg^{-1} intravenous dose was comparable with that of the 50 mg kg^{-1} oral dose and reduced the body weight to the intact control level. Ovariectomy produced a two-fold increase in serum total cholesterol (95 ± 10 vs intact control 54 ± 10; $P < 0.01$). This increase in cholesterol was prevented by lovastatin administration. Although a trend of dose dependency was evident, the results within the oral treatment group were not statistically significant. Intravenous treatment appeared to be less efficacious in reducing serum cholesterol but the effect was not significantly different from that of oral treatment (Figure 4).

Serum total alkaline phosphatase
Ovariectomy caused a marked increase in serum total alkaline phosphatase (413.8 ± 59.28 vs intact control 183.56 ± 29.31; $P < 0.01$). Five days of treatment with lovastatin significantly reduced ovariectomy-induced increase in serum total alkaline
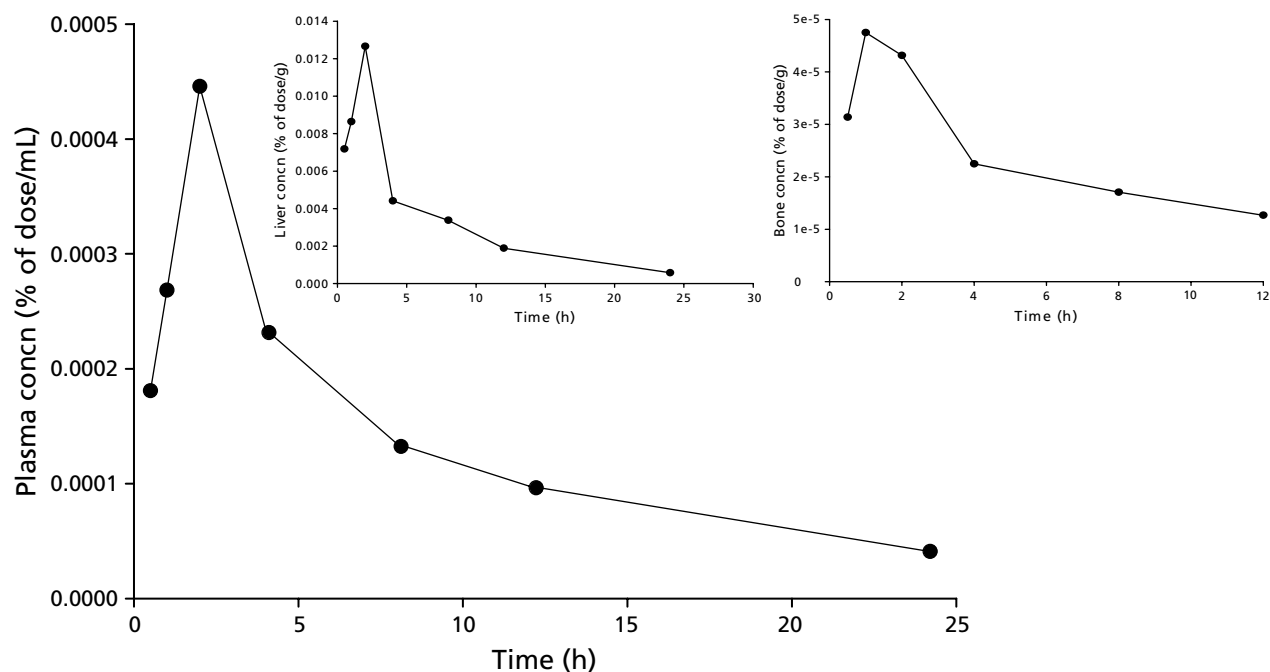

Figure 1   Concentration-time profile of lovastatin equivalents in plasma, liver and bone after oral (10 mg/kg) administration.
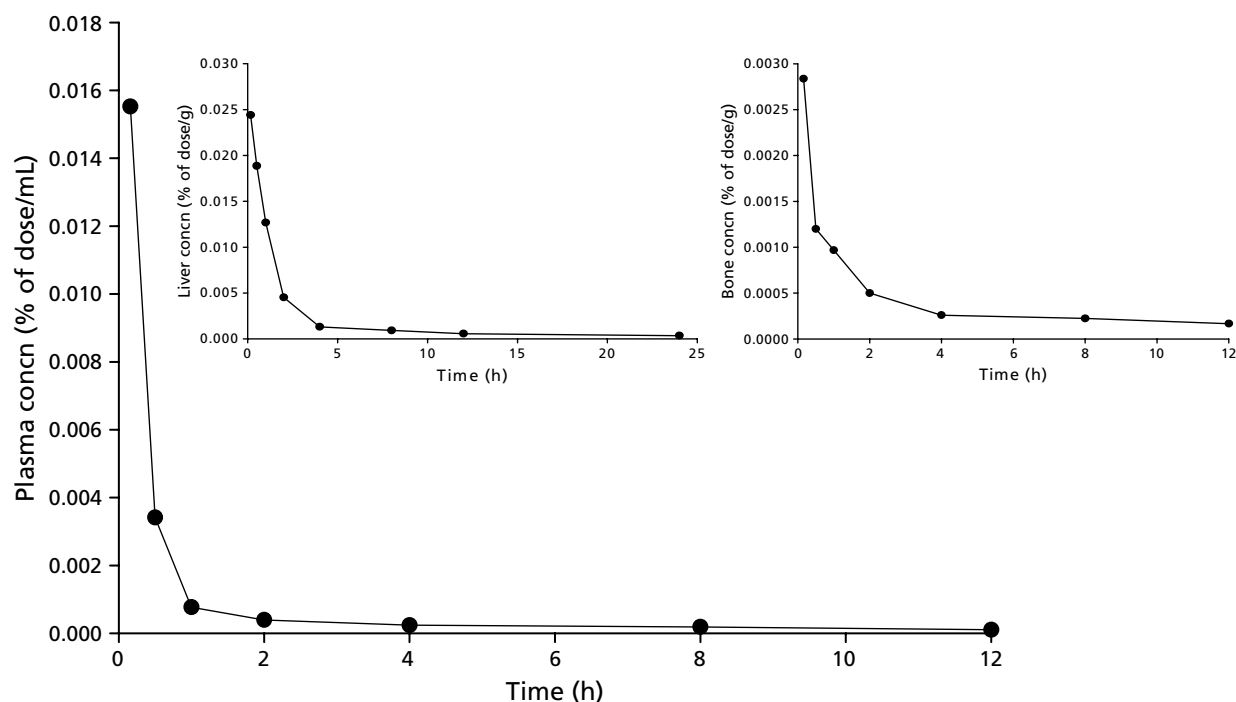
Figure 2   Concentration-time profile of lovastatin equivalents in plasma, liver and bone after intravenous (1 mg/kg) administration.

Table 1 AUC_{0-24} (area under curve) of active inhibitors of lovastatin (lovastatin equivalents) in rat plasma, liver and bone (tibia) after oral (10 mg kg^{-1}) and intravenous (1 mg kg^{-1}) administration

Administration route	AUC_{0-24} (% of dose h mL^{-1} or % of dose h g^{-1})				
	Plasma	Liver	Bone	Ratio liver/ plasma	Ratio bone/ plasma
Oral	3.2×10^{-3}	7.4×10^{-2}	3.5×10^{-4}	23.12	0.11
Intravenous	8.7×10^{-3}	4.4×10^{-2}	5.7×10^{-3}	5.05	0.65

AUC_{0-24} is expressed as % of administered dose. There is significant increase in AUC_{0-24} ratio between bone and plasma after intravenous administration. Each value represents the mean of three rats (n = 3).

phosphatase (Figure 5). There was no significant difference between oral and intravenous treatments in reducing serum alkaline phosphatase levels.

Urinary crosslinks
A simple, sensitive HPLC analytical method was developed for determination of total collagen crosslinks in rat urine. The retention times for PYD, DPD and internal standard were 11 min, 12 min and 14 min, respectively. The mean intra-assay variations were 4.1% and 3.8%, for total DPD and PYD, respectively. The mean inter-assay variations were 9.1% and 8.2% for total deoxypyridinoline and pyridinoline, respectively. The mean recoveries were 89% for total deoxypyridinoline and 103% for total pyridinoline. Significant increase in levels of urinary crosslinks PYD and DPD was observed 15 days after ovariectomy. Oral lovastatin treatment decreased the level of these markers in a dose-dependent

fashion with maximum effect being observed at the 50 mg kg^{-1} dose. The intravenous treatment was found to be more effective than oral administration at reducing the levels of these markers (Table 3).

Discussion

Due to their liver specificity and poor peripheral distribution, distribution of statins to the bone microenvironment is very low. The inadequate exposure of statins to the bone compartment after oral dosing may be one of the reasons for various conflicting in-vivo results as there were differences in dosages, method of administration and duration of exposure in these studies (Maritz et al 2001; von Stechow et al 2003; Oxlund & Andreassen 2004). To our knowledge, none of the previous studies verified the actual presence of statins in
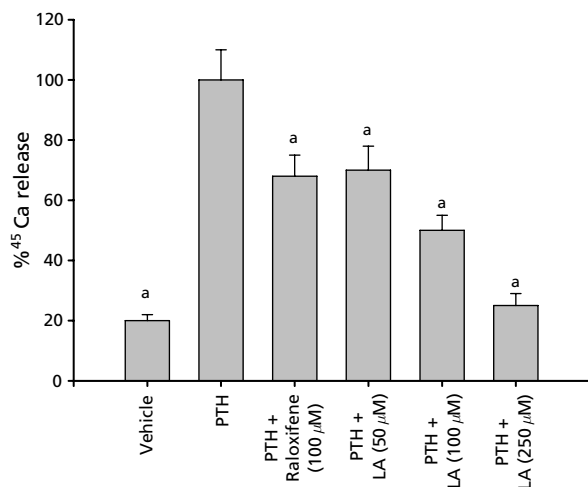
Figure 3 Inhibition of PTH stimulated bone-resorption by lovastatin (LA) acid in chick embryo bones in-vitro. Values are mean ± s.d., n = 6 per group; [a]$P < 0.05$ versus PTH.

Table 2 Effect of lovastatin administration on body weight of rats

Treatment group	Body weight (g)
Intact control	122.85 ± 6.16[a]
OVX + placebo	220.00 ± 5.71
OVX + lovastatin (per oral)	
0 mg kg^{-1}	145.40 ± 5.71[a,b]
20 mg kg^{-1}	142.50 ± 3.09[a,b]
50 mg kg^{-1}	125.00 ± 4.28[a]
OVX + lovastatin (intravenous)	
1 mg kg^{-1}	133.66 ± 3.32[a]

OVX, bilaterally ovariectomized. Values are mean ± s.e.m., n = 6 per group; [a]$P < 0.001$, versus OVX + placebo group; [b]$P < 0.05$, versus intact control. Both oral and intravenous treatment significantly reduced ovariectomy-induced increase in body weight.
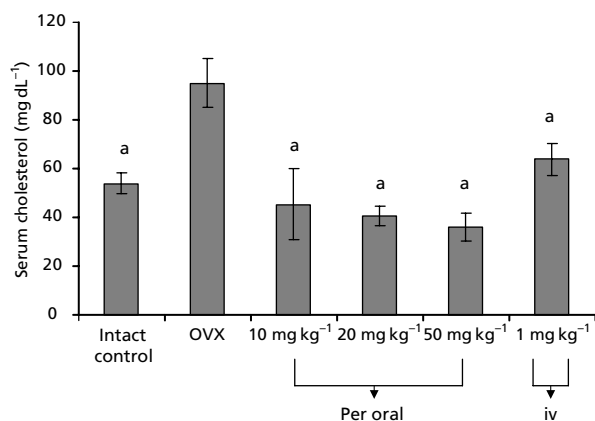

Figure 4 The effect of lovastation on serum total cholesterol. OVX, bilaterally ovariectomized. Values are mean ± s.e.m., n = 6 per group; [a]$P < 0.05$ versus OVX + placebo group.
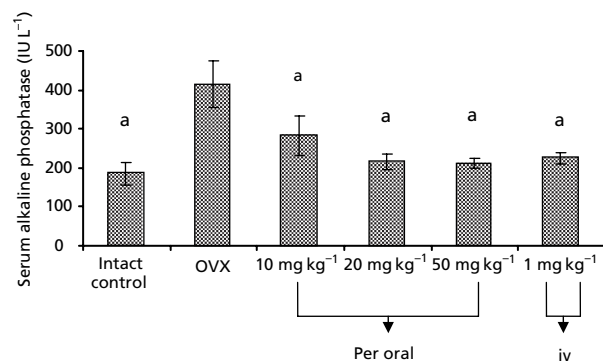

Figure 5 The effect of lovastatin on serum total alkaline phosphatase. OVX, bilaterally ovariectomized. Values are mean ± s.e.m., n = 6 per group; [a]$P < 0.05$, versus OVX + placebo group.

Table 3 Effect of lovastatin on urinary crosslinks in rats

Treatment group	Deoxypyridinoline (pmol/μmol creatinine)	Pyridinoline (pmol/μmol creatinine)
Intact control	10.50 ± 0.89[a]	14.20 ± 0.80[a]
OVX + placebo	26.01 ± 0.68	30.97 ± 0.36
OVX + lovastatin (per oral)		
10 mg kg^{-1}	22.49 ± 0.94[a]	24.73 ± 0.54[a]
20 mg kg^{-1}	15.42 ± 1.64[a]	22.65 ± 1.02[a]
50 mg kg^{-1}	15.14 ± 1.22[a]	21.88 ± 1.37[a]
OVX + lovastatin (intravenous)		
1 mg kg^{-1}	10.17 ± 1.58[a,b]	15.05 ± 1.72[a,b]

OVX, bilaterally ovariectomized. Values are mean ± s.e.m., n = 6 per group; [a]$P < 0.001$, versus OVX + placebo group; [b]$P < 0.001$, versus oral treatment group. Intravenous treatment with lovastatin was found to be more potent in reducing ovariectomy-induced increase in urinary pyridinoline and deoxypyridinoline.

bone. It was therefore considered of interest to study the distribution of lovastatin, a representative statin, to the bone compartment after oral and intravenous administration. The HMG-CoA reductase enzyme inhibition assay, which measures the overall inhibitory activity from all active metabolites, was used for this purpose (Germershausen et al 1989; Lilja et al 1998; Liu et al 2003). After intravenous administration, significantly high distribution of lovastatin to bone was evident (Table 1). The presence of a high concentration of lovastatin equivalents in bone after intravenous administration suggests that the change in route of administration, so as to increase its concentration in the bone compartment, might enhance its bone-sparing potential.

Based on the above results, we evaluated the bone-sparing effect of lovastatin in in-vitro and in-vivo experiments. In the chick embryo bone resorption assay, lovastatin lactone was found to be inactive but lovastatin acid significantly reduced PTH-induced bone resorption. Lovastatin is a prodrug and is converted in-vivo by chemical (pH) and enzymatic (esterases) hydrolysis to the active β-hydroxy acid form, which is responsible for the inhibition of HMG-CoA reductase

enzyme and lipid-lowering activity. This anti-resorptive effect of lovastatin is in agreement with other reports (Baumann et al 2001; Staal et al 2003). Further, effects of lovastatin on markers of bone turnover were assessed in an ovariectomized rat model. Both serum total alkaline phosphatase and urinary crosslinks, along with body weight and serum total cholesterol, were significantly elevated two weeks after ovariectomy. The body weight of ovariectomized rats in our experiments was extremely high. However, in a separate study conducted to establish an animal model for osteoporosis, the Sprague-Dawley rats of the institute's animal colony showed almost 3–4 times change in body weight than reported elsewhere (Sengupta et al 2005).

The ovariectomy-induced increase in body weight and serum total cholesterol levels were restored to normal after five days of lovastatin treatment. The seemingly low efficacy of intravenous treatment in reducing cholesterol levels may be due to the reduction in the ratio of AUCs between liver and plasma (Table 1). Upon intravenous administration, although the major fraction of administered lovastatin is accumulated in liver, an enhanced peripheral distribution was detected.

Short-term lovastatin treatment significantly reduced the ovariectomy-induced increase in serum total alkaline phosphatase. There was no significant difference in the potency of oral and intravenous treatments. Both liver and bone isoforms contribute to serum total alkaline phosphatase and analysis of the bone specific isoform is required to reflect the change in bone metabolism, particularly in situations where liver metabolism is altered. Urinary crosslinks are sensitive and reliable markers of bone metabolism. Orally administered lovastatin significantly inhibited the ovariectomy-induced increase in the concentration of total PYD and DPD in urine collected over a period of 24 h (Table 3). Intravenous treatment was found to be more effective than oral treatment in reducing the urinary crosslinks. To our knowledge, this is the first report that has evaluated the effect of statin treatment on urinary crosslinks in rats.

It has been shown that nitrogen-containing bisphosphonates (e.g. alendronate) exert their anti-resorptive effects through the inhibition of farnesyl pyrophosphate synthase, a key enzyme required for the formation of isoprenoids in the mevalonate pathway. This results in inhibition of prenylation of GTP binding proteins, inducing osteoclast apoptosis (Benford et al 1999; Fisher et al 1999; van Beek et al 1999). They also possess a beneficial effect in-vivo on plasma lipid levels and the atherosclerotic process (Luckman et al 1998; Dunford et al 2001). Statins, by inhibiting the rate-limiting step of the same mechanistic pathway, may exhibit similar osteoclast inhibition and anti-resorptive activity to that of aminobisphophonates. Our results support this opinion, whereby effects of lovastatin are attributed to its active metabolite, lovastatin acid. Mundy et al (1999), in his pioneering study, had also observed reduction in osteoclast numbers after simvastatin administration in rats.

In our study, the bone-sparing effects of lovastatin are pronounced with only five days of treatment. This finding is in accordance with some earlier reports where other than oral routes have been used to deliver statins for their effects on bone. Dermal application of lovastatin to rats caused greater increases in bone formation, which was evident within 5 days of treatment (Gutierrez et al 2000, 2001). Topical or transdermal application results in sustained blood levels and increased bioavailability of lovastatin, thereby increasing its distribution to the bone compartment. Crawford et al (2001) studied the effect of lovastatin after local bone marrow injection to 6-week old male rats and observed that cortical bone area and cortical thickness were significantly increased. Incidentally, Mundy et al (1999) had shown the bone anabolic effect of statins in murine calvarial bone culture after 3–7 days. Although oral administration significantly reduced the markers of bone turnover in our study, a 10 mg kg^{-1} dose was much less effective in reducing the level of these markers. Higher peripheral exposure of statins, particularly to muscles, after intravenous or dermal administration may increase the incidence of myopathy, a typical side effect of HMG-CoA reductase, but no untoward or toxic reaction was observed in our experiment. Again, the dose selection in our study was based on previous reports (Mundy et al 1999; Reinoso et al 2002). We support the opinion that the oral route is not an attractive way of administering statins for their effect on bone. Alternative routes of administration, so as to increase their bioavailability and achieve their maximum concentration in the bone compartment, should be explored for statins if they are to be used for the management of osteoporosis.

Conclusion

One of the most important pleiotropic actions of statins is their effect on bone metabolism. Many previous reports show that statins can act both as bone anabolic and as anti-resorptive agents. Liver specific pharmacokinetics of these drugs obstructs their distribution to bone. In this study, distribution of lovastatin to bone in comparison to liver was assessed after oral and intravenous administration in rats. Although maximum amount of lovastatin equivalents were observed in liver after both oral and intravenous administration, their concentration in bone was significantly increased after intravenous administration in comparison with oral treatment. Intravenously administered lovastatin was more effective in reducing ovariectomy-induced increase in serum total alkaline phosphatase and urinary crosslinks in accordance with its increased distribution to bone. The effects on bone turnover markers were evident only after 5 days of treatment and, especially, the effect on urinary crosslinks was more pronounced. Both in-vitro and in-vivo results support the anti-resorptive effect of lovastatin and this efficacy can be increased without compromising its cholesterol-reducing activity by using routes other than the oral route of administration.

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