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Disposition of pravastatin sodium, a tissue-selective HMG-CoA reductase inhibitor, in healthy subjects

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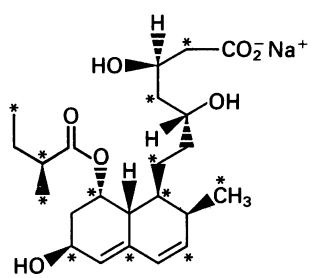
Pravastatin sodium, a competitive inhibitor of HMG-CoA reductase, is a new orally effective hypocholesterolaemic agent. In a two-way crossover study, eight healthy male subjects each received an intravenous and an oral dose of [^{14}C]-pravastatin sodium. The oral absorption of [^{14}C] activity from pravastatin sodium was about 34% and the oral bioavailability was about 18%, suggesting first-pass metabolism of pravastatin. After the intravenous dose, the recovery of radioactivity averaged 60% and 34% in urine and faeces, respectively. Corresponding values were 20% (urine) and 71% (faeces) for the oral dose. The estimated average plasma elimination half-life of pravastatin was 0.8 and 1.8 h for the intravenous and oral routes, respectively. The average values for total and renal clearances were 13.5 and 6.3 ml min^{-1} kg^{-1}, respectively, and the steady-state volume of distribution averaged 0.5 l kg^{-1}. These results suggest that both kidney and liver are important sites of elimination for pravastatin.

Keywords pravastatin sodium HMG-CoA reductase inhibitor hypocholesterolaemic agent pharmacokinetics absorption bioavailability metabolism

Introduction

Pravastatin sodium (SQ 31,000, CS-514), a competitive inhibitor of 3-hydroxy-3-methyl-glutaryl CoA reductase (HMG-CoA reductase), the rate-limiting enzyme in cholesterol biosynthesis, is a new hypocholesterolaemic agent structurally related to lovastatin and compactin. Clinical trials have demonstrated that pravastatin sodium (hereafter referred to as pravastatin) is a safe and potent orally effective cholesterol-lowering agent (Nakaya *et al.*, 1987; Yoshino *et al.*, 1986). Animal studies have demonstrated that pravastatin is a tissue-selective inhibitor of HMG-CoA reductase (Tsujita *et al.*, 1986). Since the major site of cholesterol synthesis is the liver, this tissue-selective property of pravastatin may be a desirable clinical feature.

Pravastatin sodium is designated chemically as [1S-[1α,(βS-δS),2α,6α,8β-(R),8aα]]-1,2, 6,7, 8,8a-hexahydro-β,δ,6-trihydroxy-2-methyl-8-(2 -methyl-1-oxobutoxy)-1-naphthalene heptanoic acid, monosodium salt (Figure 1). The primary



*denotes sites labeled with ^{14}C

Figure 1 The structure of [^{14}C]-pravastatin sodium.

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objectives of this study in healthy human volunteers were to estimate the oral absorption and bioavailability of pravastatin and to evaluate the pharmacokinetics of pravastatin after its intravenous administration.

Methods

Eight healthy Caucasian subjects ranging in age from 21 to 39 years (mean = 27 years) were studied. They weighed between 64.0 and 83.4 kg (mean = 73.8 kg), and ranged in height from 172 to 188 cm (mean = 178 cm). The study protocol was reviewed and approved by the Institutional Review Committee and all subjects gave written informed consent.

In this open, balanced, randomised, two-way crossover study, each of the eight subjects received a single 19.2 mg dose of oral solution (100 μCi) and a 9.9 mg intravenous dose (25 μCi) of [^{14}C]-pravastatin sodium over a 2 min period. The oral dose was within the therapeutic dosing range for pravastatin. The radiochemical purity of [^{14}C]-pravastatin sodium was greater than 97%. The oral dose was approximately double the intravenous dose because animal studies had demonstrated that oral absorption of pravastatin was less than 50%.

Samples of venous blood (10 ml) were drawn just before drug administration and at frequent times over the 96 h interval after dosing. Each blood sample was centrifuged under refrigeration for 15 min and the plasma was collected. Urine and faeces were collected for 4 days after drug administration. All samples were frozen at −20° C until analysed.

The radioactivity in plasma samples (2 ml) was extracted into methanol (6 ml of methanol three times). Aliquots (2 ml) of the combined methanol extracts were analyzed for total radioactivity, and the remainder was concentrated to dryness *in vacuo* at 25° C. The dried residue was then reconstituted in 0.6 ml of methanol containing nonradiolabelled pravastatin (0.6 mg ml^{-1}) and the radioactive components were measured using thin-layer radiochromatography (t.l.r.c.). Aliquots (50 μl) of urine samples and aliquots (100–150 μl) of the reconstituted extracts of plasma were spotted on silica gel GF (Analtech Inc., Newark, DE) plates and developed in a chloroform/glacial acetic acid/methanol (9:1:1 v/v) solvent system. Nonradioactive pravastatin sodium was used as a reference standard and was detected using short-wave ultraviolet light; the R_F value for pravastatin was about 0.55 in this system.

Control plasma and urine samples, collected from untreated healthy volunteers, were spiked with appropriate amounts of [^{14}C]-pravastatin sodium to prepare samples at several different concentrations covering the range of concentrations of pravastatin found in the plasma and urine samples from this study. These spiked control samples were used for validation of the t.l.r.c. assay and for determination of the stability of pravastatin in plasma and urine during storage; these samples were stored for up to 4 months, and were analysed in a manner identical to that of the samples of the study.

. Additional 10 ml aliquots of venous blood withdrawn from each subject at 2 and 4 h after dosing were used for analysis of radioactivity in blood, plasma, and protein-free filtrate (PFF). For preparation of PFF, a 1.0 ml aliquot of plasma was transferred to a Centrifree® Micropartition System (Amicon Corp., Lexington, MA) and centrifuged at 1000 *g* for at least 10 min to obtain about 200 μl of the PFF.

The scintillation cocktail of Anderson & McClure (1973) was used to count all samples. Soluene 350® (Packard Instrument Co.) was used to solubilise blood, plasma, and urine samples. Faeces were homogenized with water, and samples were counted after digestion with soluene 350. All samples were counted in either a Packard Tri-Carb® or an Intertechnique Model SL-4200 liquid scintillation spectrometer. Counting efficiencies were determined using automatic external standardisation.

Concentrations of total radioactivity and individual radioactive components in plasma were expressed as equivalents of pravastatin. The concentration of pravastatin in plasma was calculated by multiplying the concentration of total radioactivity in plasma (pravastatin equivalents) by the recovery of radioactivity in the methanol extract and by the relative amount of radioactivity in the methanol extract that corresponded to pravastatin.

For pravastatin, the area under the plasma concentrations *vs* time curve from time zero to time t (AUC(0,t)) was calculated by the integration method of Lagrange (Yeh & Kwan, 1978). The AUC from time zero to infinity was calculated by adding AUC(0,t) and $C(t)/\lambda_2$, where C_t was the last measurable concentration of pravastatin and λ_2 was the slope of the terminal portion of the log drug concentration–time curve.

In all subjects, plasma concentrations of pravastatin declined in an apparent biexponential fashion after the i.v. dose, and the concentrations of pravastatin in plasma at any time (C) were fitted by the following equation:

$$C = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t}$$

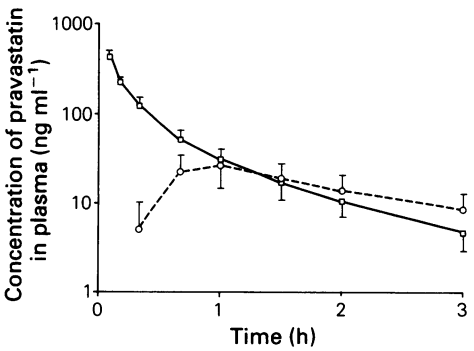

Figure 2 Mean (± s.d.) concentrations of pravastatin in plasma after intravenous administration of 9.9 mg (□) and oral administration of 19.2 mg (○) of [^{14}C]-pravastatin to eight healthy subjects.

The best fits were obtained when all plasma concentrations were weighted by $1/C^2$. Model-independent pharmacokinetic parameters (mean residence time, mean absorption time, and steady-state volume of distribution) of pravastatin were calculated using standard procedures (Gibaldi & Perrier, 1982). Total clearance (CL) was calculated by dividing the i.v. dose by AUC. Renal clearance (CL_R) was calculated by multiplying the fraction of the dose excreted in urine as unchanged pravastatin by CL. The time-averaged fraction of total radioactivity in plasma corresponding to pravastatin (f_r) was calculated as AUC for pravastatin divided by the AUC(0, 48 h) for total radioactivity.

Results

The t.l.r.c. assay for pravastatin was validated by performing replicate assays of plasma and urine samples containing known concentrations of [^{14}C]-pravastatin. For plasma, the measured concentrations were calculated by preparation of a standard curve. For urine, the t.l.r.c. data for pravastatin were corrected for recovery, which averaged 88%. Pravastatin was stable in both plasma (126 days) and urine (63 days) under the conditions of storage in the present study.

The assay for pravastatin in plasma and urine showed excellent linearity, accuracy, and reproducibility within the range of concentrations studied. For plasma, the coefficient of variation was less than 12% at concentrations of 4 and 11 ng ml^{-1} and less than 3% at 55 to 1071 ng ml^{-1}, and the maximum average deviation was 8% at all concentrations. For urine, the coefficient of variation was less than 5% and the average deviation, after correction for recovery, was also less than 5%. At the specific activity used, the lowest limits of assay of pravastatin were 4.3 ng ml^{-1} and 0.3 μg ml^{-1} for plasma and urine, respectively.

The specificity of the assay was confirmed by comparing pravastatin concentrations in selected plasma and urine samples measured by a specific gas chromatographic/mass spectrometric procedure to those measured by the t.l.r.c. method. The t.l.r.c. system resolved pravastatin from several structural analogues, some of which have been identified as authentic metabolites of pravastatin in man.

Over 96 h after the i.v. administration, an average of 94% of the dose was recovered in the excreta with 60% in urine and 34% in faeces. After p.o. administration, recovery of radioactivity in the excreta averaged 91% of the dose with 20% in urine and 71% in faeces.

The 0 to 48 h urinary excretion of intact pravastatin averaged 47% and 8% of the i.v. and p.o. doses, respectively. The urinary excretion of total radioactivity and intact pravastatin was rapid, with more than 80% occurring within the first 12 h after dosing.

The mean concentrations of pravastatin in plasma after p.o. and i.v. administration are shown in Figure 2. Mean values for drug bioavailability are shown in Table 1. Maximum concentrations (C_{max}) of pravastatin and total radioactivity in plasma after the p.o. dose were 27 ng ml^{-1} and 96 ng equivalents ml^{-1}, respectively; the mean time to attain maximum concentrations (t_{max}) of pravastatin and total radioactivity was 0.9 h.

The average binding of radioactivity to plasma proteins at 2 and 4 h after dosing by both routes of administration ranged from 43 to 48%. The association of radioactivity with cellular elements of whole blood was negligible after administration by both routes.

A comparison of the p.o. and i.v. areas under the drug concentration-time (0 to 12 h) curves (AUC(0, 12)) for total radioactivity in plasma gave an estimate of absolute absorption of 54.1%. However, the absorption averaged 33.5% based on comparisons of the cumulative urinary excretion of radioactivity from 0 to 96 h after the dose. The absorption estimate derived from urine data appears to be more reliable, since a comparison of AUC values for total radioactivity in plasma after the two routes of administration gave variable results depending upon the time interval used for calculation of AUC values. These variable results may have been due to the presence of varying amounts of

Table 1 Mean (± s.d.) pharmacokinetic parameters for pravastatin after oral (19.2 mg) and intravenous (9.9 mg) administration of [^{14}C]-pravastatin sodium to eight healthy subjects

Parameter (units)	*Intravenous dose*	*Oral dose*
Total clearance (ml min^{-1} kg^{-1})	13.5 ± 2.4	—
Renal clearance (ml min^{-1} kg^{-1})	6.3 ± 1.0	—
$t_{½,1}$ (h)	0.09 ± 0.02	—
$t_{½,2}$ (h)	0.78 ± 0.18	1.77 ± 0.75
V_{ss} (l kg^{-1})	0.46 ± 0.04	—
V (l kg^{-1})	0.88 ± 0.14	—
Mean residence time (h)	0.59 ± 0.11	2.96 ± 0.94
Mean absorption time (h)	—	2.37 ± 0.95
C_{max} (ng ml^{-1})	—	27.4 ± 10.7
t_{max} (h)	—	0.88 ± 0.16
AUC (ng ml^{-1} h)	171.2 ± 28.7	66.2 ± 32.1
f_r (%) *a*	25.4 ± 5.72	5.69 ± 2.21

a Time-averaged fraction of total radioactivity in plasma corresponding to intact pravastatin (see text for details).

metabolites in plasma with different volumes of distribution.

The absolute bioavailability of pravastatin after oral administration averaged 19.1 and 17.3% of the dose, based on plasma AUC and urinary .excretion data for intact-pravastatin, respectively.

The mean pharmacokinetic parameters for pravastatin are shown in Table 1. Total and renal clearance values averaged 13.5 and 6.3 ml min^{-1} kg^{-1}, respectively. The volume of distribution at steady-state (V_{ss}) and during the elimination phase (V) averaged 0.46 and 0.88 l kg^{-1}, respectively, indicating that pravastatin undergoes extensive extravascular distribution. The elimination half-life of pravastatin was 0.8 and 1.8 h after i.v. and p.o. administration, respectively; corresponding mean residence times were 0.6 and 3.0 h. The mean absorption time for the oral dose was 2.4 h.

Pravastatin was well tolerated by all subjects. There were no adverse effects and no significant changes attributable to the drug were noted on physical, electrocardiographic, or clinical laboratory examination.

Discussion

The low oral bioavailability of pravastatin (about 18%) was due partly to incomplete absorption (about 34%) and partly to a greater extent of biotransformation of pravastatin after oral administration compared with systemic administration (first-pass effect). However, the poor systemic availability may not be undesirable for an HMG-CoA reductase inhibitor because the site of action is primarily in the liver. The occurrence of first-pass metabolism is supported by the fact that the time-averaged fraction of total radioactivity in plasma corresponding to intact pravastatin (f_r) was 6% and 25% after p.o. and i.v. dosing, respectively (Table 1).

As a result of the rapid systemic elimination of pravastatin, the rate of elimination after oral administration was apparently limited by the absorption rate ('flip-flop' kinetics). Although absorption was rapid, as reflected by an average mean absorption time (MAT) of about 2.4 h and an average t_{max} of less than 1 h, it nonetheless became rate-limiting relative to the extremely rapid elimination rate of pravastatin.

There was substantial biliary excretion of pravastatin since an average of 34% of the i.v. dose was recovered in the faeces. After i.v. administration, approximately 47% of the total clearance was through renal excretion of intact pravastatin. The average renal clearance of greater than 400 ml min^{-1}, is much higher than glomerular filtration rate (130 ml min^{-1}), indicating that tubular secretion is a predominant mechanism in the renal excretion of pravastatin.

These results indicate that both the kidney and the liver are important sites of elimination for pravastatin in man. Thus, there appears to be the potential for significant elimination of pravastatin in patients with impairment of either renal or hepatic function.

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