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Pharmacokinetics and oral bioavailability of pravastatin in dogs

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Abstract

Pravastatin sodium, an antihypercholesterolemic HMG-CoA reductase inhibitor, has recently been shown to significantly reduce the incidence of myocardial infarction and death from cardiovascular causes, without adversely affecting the risk of death from non-cardiovascular causes in men with moderate hypercholesterolemia. Because the dog is used as an animal model for pharmacology/toxicology studies, a pharmacokinetic study was carried out in dogs to provide the basis for interspecies comparisons to humans and also to guide dosage selection, so as to provide clinically-relevant exposure in the dog. Values for total body clearance, renal clearance, elimination $t_{1/2}$, and oral bioavailability of pravastatin in dogs are different than those in humans. Based on observed systemic exposure of pravastatin in dogs and literature values for humans, there is approximately a 2.6-fold greater exposure in dogs than in humans at equivalent oral doses.

Keywords: Dogs; Intravenous; Oral; Pharmacokinetics; Pravastatin

Pravastatin sodium (hereafter pravastatin), an HMG-CoA reductase inhibitor, is an effective antihypercholesterolemic agent (McTavish and Sorkin, 1991; Illingworth and Tobert, 1994). In the West of Scotland Coronary Prevention Study, treatment with pravastatin significantly reduced the incidence of myocardial infarction and death from cardiovascular causes without adversely affecting the risk of death from non-cardiovascular causes in men with moderate hypercholesterolemia and no history of myocardial infarction (Shepherd et al., 1995).

Recently, several investigators have used the beagle dog to carry out pharmacology and toxicology studies with pravastatin (Tarumi et al., 1989; Ichihara et al., 1993; Sliskovic et al., 1992; Schmidt et al., 1991; Parker et al., 1990). Although the oral bioavailability and pharmacokinetic profile of pravastatin, with a

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Table 1
Mean ± S.D. ($n = 4$) recovery of radioactivity (% of dose) in excreta after intravenous and oral administration of 1.0-mg/kg doses of [^{14}C]-pravastatin to dogs

Time (h)	Intravenous			Oral		
	Urine	Feces	Total	Urine	Feces	Total
0–24	27.9 ± 5.2	49.4 ± 3.4	77.4 ± 6.6	10.8 ± 5.2	71.9 ± 5.2	82.7 ± 5.4
0–48	28.9 ± 4.8	60.0 ± 3.8	88.8 ± 3.8	11.5 ± 1.8	78.2 ± 2.2	89.7 ± 1.6
0–96	29.3 ± 4.8	60.8 ± 3.8	90.0 ± 3.8	11.6 ± 1.8	78.8 ± 1.8	90.4 ± 1.8

chemically-specific assay, has been described in healthy human subjects (Singhvi et al., 1990) these parameters have not been reported for dogs. Plasma levels of total radioactivity after an oral dose of [^{14}C]pravastatin (Komai et al., 1992) and levels of HMG-CoA reductase inhibition in plasma in dogs after an oral dose (Stubbs et al., 1990) have been previously reported. Our objective was to perform a pharmacokinetic study in dogs with a chemically-specific assay to provide an interspecies comparison and to guide dosage selection in dogs to obtain clinically-relevant exposure.

[^{14}C]-Pravastatin (SQ-31 000; Batch NN-020-IL) was synthesized by fermentation and was uniformly labeled with ^{14}C (Sankyo Corp., Japan). This material had a specific activity of 3.94 μCi/mg and a radiochemical purity of 95% (HPLC). TX-114 scintillation cocktail was purchased from National Diagnostics (NJ) and Hydrocount® cocktail was purchased from Baker Chemical (Phillipsburg, NJ). Soluene-350® was purchased from Packard Instruments (Downers Grove, IL). All other reagents were purchased from Fisher Scientific (Springfield, NJ) and were reagent grade or better.

Four adult male beagle dogs (9.5–12.2 kg) were used; clinical laboratory tests for these animals were in the normal range. The dosing vehicle was prepared on the morning of dosing as an aqueous solution in distilled water. After an overnight fast, each dog received a single 1.0-mg/kg intravenous dose of [^{14}C]-pravastatin and 1 week later they each received the same dose orally. The dogs were fed 8 h after dosing and had free access to water throughout the study. Serial, heparinized blood samples were collected up to 96 h and plasma was prepared. Urine and feces were quantitatively collected in a metabolism cage for up to 96 h after dosing. All samples were stored at −20°C until analyzed.

Each plasma, urine, and fecal homogenate sample was analyzed for total radioactivity as follows. Aliquots were mixed with Soluene-350®, neutralized, and then mixed with 15 ml of scintillation cocktail. Fecal samples were bleached with 20% benzoyl peroxide in toluene prior to counting. All samples were counted in a Model 3375 or 3380 Tri-Carb® liquid scintillation spectrometer (Packard Instruments). Counting efficiency was determined with automatic external standardization.

Plasma and urine samples were also analyzed by a specific thin-layer chromatography (TLC) assay. The specificity of the TLC assay was confirmed with mass spectrometry (Funke et al., 1989). For assay of pravastatin in plasma, a 1 ml aliquot was extracted three times with 3 ml of methanol and the combined extracts were dried in vacuo. The dry residue was reconstituted with 0.4 ml of methanol containing 0.6 mg/ml of non-radiolabeled pravastatin as carrier. The entire reconstituted sample was spotted on 0.25-ml thick silica gel GF plates (Analtech, Newark, DE) and developed in: chloroform/glacial acetic acid/methanol (9:1:1, by vol.). Aliquots of urine (50 μl) were spotted directly and chromatographed as described above. Migration of pravastatin ($R_f \approx 0.6$) was visualized with short wavelength UV light. Three zones were removed from the plate (pravastatin zone, a more polar zone, and less polar zone), mixed with 2 ml of methanol/water (1:1, v/v), and 15 ml of TX-114 scintillation cocktail. The assay was accurate, reproducible, and linear over the range of 3.3–852 ng/ml (plasma) and

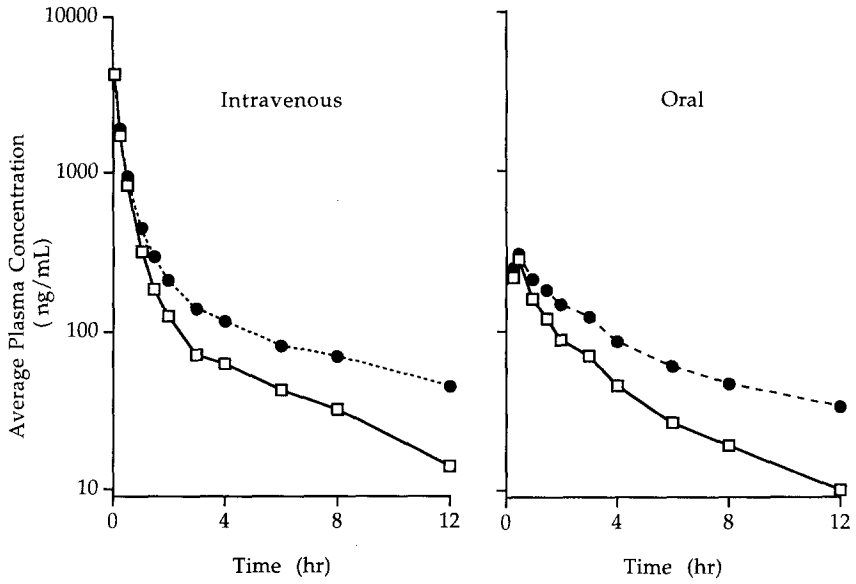

Fig. 1. Average concentration (mean; $n = 4$) of total radioactivity (●) and unchanged pravastatin (□) in plasma after administration of intravenous and oral doses of [^{14}C]-pravastatin to dogs (1.0 mg/kg).

0.92 to 9.2 μg/ml (urine). Standard curves were prepared and analyzed daily. Samples were stored for less than 10 days (plasma) or 17 days (urine) and stability of pravastatin was confirmed by analysis of spiked samples that were stored along with the study samples.

Pharmacokinetic parameters were determined with non-compartmental methods (Gibaldi and Perrier, 1982). The terminal elimination half-life was estimated with log-linear regression of the data between 3 and 12 h after dosing. The area under the curve up until the last detectable time point was determined by LaGrange integration and was extrapolated to infinity with individual terminal slope values. Following the i.v. dose, renal clearance (Cl_R) was estimated by multiplying the fraction of the dose excreted in the 0–48-h urine as unchanged pravastatin by the total clearance (Cl_T). Non-renal clearance was calculated as $Cl_T - Cl_R$. The $AUC_{0\text{ to }96\text{-h}}$ of total radioactivity and the $AUC_{0\text{ to }\infty}$ of pravastatin in plasma after oral and iv doses were used to estimate the absolute oral absorption and absolute bioavailability of pravastatin, respectively. In addition, absorption and bioavailability were also estimated from the urinary recovery of total radioactivity (0–96 h) and unchanged pravastatin (0–48 h), respectively.

Total recovery of the radioactive dose in 0–96-h urine and feces averaged 90% (Table 1). Recovery of the radioactive dose in urine averaged 29% (i.v.) and 12% (p.o.); based on TLC analysis of the 0 to 48-h urine samples, about 18% (i.v.) and 6.5% (p.o.) of the administered dose corresponded to unchanged pravastatin.

The average concentrations of total radioactivity and unchanged pravastatin in plasma are shown in Fig. 1. Concentrations of pravastatin in plasma declined below the quantifiable limit of the assay (3.3 ng/ml) in samples collected after 12 h. The pharmacokinetic parameters for pravastatin are summarized in Table 2. After the i.v. dose, the $t_{1/2}$ averaged 4 h, which is considerably longer than the value reported previously after an i.v. dose in humans (0.8 h; Singhvi et al., 1990). However, after an oral dose in humans (typical clinical route), there is apparent absorption-rate limited kinetics such that the $t_{1/2}$ averaged about 3 h. The Vdss averaged 1 l/kg, which is slightly greater than the value for total body water. The

Table 2
Pharmacokinetic parameters of pravastatin in dogs after intravenous and oral administration of [^{14}C]pravastatin (1.0 mg/kg)

Parameter		Units	Mean ± S.D. ($n = 4$)
Intravenous route	Cl_T	ml/min/kg	8.5 ± 2.2
	Cl_R	ml/min/kg	1.5 ± 0.2
	Cl_{NR}	ml/min/kg	7.1 ± 2.2
	$t_{1/2}$	h	4.0 ± 0.6
	MRT	h	2.1 ± 0.2
	Vd^{ss}	l/kg	1.0 ± 0.2
Oral route	C_{max}	ng/ml	276 ± 133
	$t_{1/2}$	h	3.5 ± 0.8
	MAT	h	2.1 ± 1.4
	Absorption (plasma)[a]	% of dose	50.5 ± 3.0
	Absorption (urine)[b]	% of dose	40.0 ± 5.6
	Bioavailability (plasma)[c]	% of dose	32.3 ± 9.4
	Bioavailability (urine)[d]	% of dose	37.2 ± 10.6

[a]$AUC_{0-96\text{-h}}$ of total radioactivity in plasma for oral route divided by the corresponding $AUC_{0-96\text{-h}}$ for the i.v. route.
[b]Recovery of the radioactive dose in 0–96-h urine for oral route divided by the corresponding recovery for the i.v. route.
[c]$AUC_{0-\infty}$ of unchanged pravastatin in plasma for oral route divided by the corresponding $AUC_{0-\infty}$ for the iv route.
[d]Recovery of unchanged pravastatin in 0–48-h urine for oral route divided by the corresponding recovery for the i.v. route.

total body clearance (Cl_T) of pravastatin in dogs (8.5 ml/min/kg) was about 40% lower than the corresponding value in humans. The renal clearance of pravastatin in dogs (1.5 ml/min/kg) is less than GFR (6.1 ml/min/kg; Davies and Morris, 1993), suggesting that pravastatin undergoes net reabsorption in dogs. In humans on the other hand, the renal clearance (6.3 ml/min/kg) significantly exceeds GFR (1.8 ml/min/kg; Davies and Morris, 1993), suggesting that pravastatin undergoes net tubular secretion. This difference in renal handling of pravastatin apparently accounts for the difference in systemic pharmacokinetics since the non-renal clearance values for these two species are identical (ca. 7 ml/min/kg). A recent report indicates that pravastatin is a substrate for the organic anion transporter (Tamai et al., 1995); it is possible that interspecies differences in the transporter might account for the interspecies difference in renal clearance. Limited in vitro studies indicated that pravastatin is about 50% bound to human plasma proteins (Singhvi et al., 1990) and about 60% bound to dog plasma proteins. Thus, this minor difference in plasma protein binding does not explain the interspecies difference observed in the in vivo pharmacokinetic parameters.

After oral administration, the absorption of the radioactive dose was estimated to be between 40 and 50%; this estimate is in good agreement with a previous report in bile-duct cannulated dogs (Komai et al., 1992). The oral bioavailability of unchanged pravastatin averaged about 32–37% (Table 2). Thus, the oral bioavailability of pravastatin in dogs is about 2-fold greater than in humans (about 18%; Singhvi et al., 1990). Because the clearance of pravastatin in dogs is slower than in humans and an oral dose is more bioavailable in dogs, there is about 2.6-fold higher exposure in dogs relative to humans at equivalent oral doses (based on mg/kg) (Table 3). It is possible, but not very likely, that the apparent differences in pharmacokinetics between dogs and humans are due to non-linear kinetics since the dose given to dogs was about 4-fold higher than that in humans. Although the linearity of kinetics has not been demonstrated in dogs as a function of dose, it seems unlikely that the dose difference, i.e. 1 mg/kg for dogs, and 0.26 mg/kg for humans, accounts for the observed interspecies pharmacokinetic differences based on: (1) the fact that pravastatin disposition in humans is linear throughout the therapeutic dose range (Pan, 1991) and (2) there was no indication of non-linear

Table 3
Comparison of exposure of dogs and humans to oral doses of pravastatin

Parameter		Units	Dogs	Humans[a]	Ratio dogs/humans
	Dose	mg	10.5	20	—
		mg/kg	1.0	0.26	—
	AUC_{∞}	ng × h/mL	671	66.2	—
Normalized	AUC_{∞}	ng × h/ml per mg/kg	671	255	2.6
	C_{max}	ng/ml	277	27.4	—
Normalized	C_{max}	ng/ml per mg/kg	277	105	2.6

[a]Data from eight human subjects in Singhvi et al. (1990).

kinetics for the concentration decay curves in dogs.

The values for total body clearance, renal clearance, elimination $t_{1/2}$, and oral bioavailability of pravastatin in dogs are different compared to those in humans. For evaluation of pharmacology/toxicology of this HMG-CoA reductase inhibitor in dogs, this interspecies difference should be considered. Based on observed systemic exposure of pravastatin in dogs (C_{max} and AUC) and literature values for humans, there is about 2.6-fold greater exposure in dogs than in humans at equivalent oral doses.

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