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DISPOSITION OF [^{3}H]FLUVASTATIN FOLLOWING SINGLE ORAL DOSES IN BEAGLE DOGS AND RHESUS MONKEYS WITH BILE FISTULAE

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ABSTRACT

The disposition of [^{3}H]fluvastatin was examined following single oral doses in dogs (12·4 mg kg^{-1}) and monkeys (0·48 and 45·5 mg kg^{-1}) with bile fistulae. Serial plasma and complete urine, feces, and bile were collected at designated intervals for 3 or 4 d, and were analyzed for total radioactivity and unchanged fluvastatin. In the dog, peak radioactivity concentrations (C_{max}) averaged 7260 ng equiv. mL^{-1} and the mean time to peak (t_{max}) was ~9 h. In the monkey, the mean radioactivity t_{max} values were ~5 and 13 h following the low and high doses, the respective C_{max} values being 116 and 10 400 ng equiv. mL^{-1}. The mean AUC of total radioactivity was proportional to the dose while that of fluvastatin was overproportional to dose, suggesting dose independent absorption but saturable first-pass effect. The AUC ratio of unchanged fluvastatin versus total radioactivity was approximately 63% in the dog, and 9% and 13% for the low and high doses, respectively in the monkey. The bile was the major excretory route of radioactivity (dog, 56%; low-dose monkey, 73%; high-dose monkey, 69%) whereas the renal pathway accounted for < 5% of the dose in both species. Approximately 12% of the biliary radioactivity in the dog was due to intact fluvastatin, compared with 0% and 7·5% after the low and high doses in the monkey. These results showed a smaller extent of fluvastatin metabolism in the dog than in the monkey, and suggested that metabolism in the monkey was saturable in the dose range studied.

KEY WORDS: fluvastatin; disposition; dog; monkey; bile fistula

INTRODUCTION

Fluvastatin (Lescol®, Sandoz), [R*, S*-(E)]-(±)-sodium-3, 5-dihydroxy-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indole-2-yl]-hept-6-enoate, is the first synthetic inhibitor of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate limiting enzyme in cholesterol biosynthesis.[1] Fluvastatin is structurally distinct from the other HMG-CoA reductase inhibitors lovastatin, simvastatin, and pravastatin, all of which are derived from fungal metabolites and are analogs of compactin.

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The pharmacokinetics of fluvastatin have been studied in a number of laboratory species[2,3] and in humans.[4,5] Oral doses of the drug were almost completely absorbed, and absorption was unaffected by the presence of food. However, fluvastatin was subject to extensive presystemic hepatic extraction, followed by direct excretion in the bile, thus yielding high liver/peripheral tissue concentration gradients of drug derived materials. The bioavailability of the parent drug increased with dose, apparently because of saturation of the first-pass effect. Fluvastatin was $\geqslant 98\%$ bound to proteins in rat, dog, and human plasma.[6] The drug was partially metabolized before excretion,[7] the extent of metabolism being smallest in the dog and greatest in the mouse.[2] The half-life of intact fluvastatin ranged from approximately 30 min in humans to 4–7 h in dogs.

Fluvastatin is excreted predominantly in feces, with the renal route accounting for as little as 2–3% of a radiolabeled dose in the dog and monkey. In the dog, approximately 29% and 23% of the fecal radioactivity was attributed to unchanged fluvastatin at dose levels of 0·95 and 34 mg kg^{-1}, respectively. In the monkey, parent drug accounted for only 5% of fecal radioactivity after a 0·57 mg kg^{-1} dose and 15% after a 46 mg kg^{-1} dose.[2] Part or all of the parent drug found in feces appears to represent fluvastatin excreted in bile rather than unabsorbed drug, since similar results were observed even after intravenous dosing.[2]

In order to provide direct evidence of biliary excretion of absorbed fluvastatin in the dog and monkey, the present study examined the disposition of the drug following singe oral doses in bile duct cannulated dogs and in monkeys with cannulated gallbladders. Because of the apparent dose dependence of biliary unchanged drug content in the monkey,[2] two dose levels were tested in this species whereas only a single dose was used in the dog.

MATERIALS AND METHODS

Radiolabeled compound

[^{3}H]Fluvastatin (Isotope Laboratory, Sandoz Research Institute, East Hanover, NJ) was synthesized with the tritium label at the 3 position of the *p*-fluorophenyl moiety. The radiochemical purity of each dilution batch was $>98\%$ and the specific activities ranged from 0·44 to 51·4 μCi mg^{-1}. In this report, the doses are expressed as the equivalent weights of fluvastatin free acid.

Animals

Dogs. Four mature, male beagle dogs weighing 11·6–14·6 kg (13·1 ± 1·32 (SD) kg) were used. The common bile duct of each dog was cannulated. Before the operation, the dog was given a prophylactic, IM injection of penicillin (20 000 U kg^{-1}) and a subcutaneous dose of acepromazine (0·1 mg kg^{-1}).

Anesthesia was then induced with 2% thiamylal sodium (8·8 mg kg^{-1}, IV). The dog was intubated and maintained on halothane gas. Under aseptic surgical conditions, a midline incision was made, exposing the abdominal cavity. The liver and gallbladder were located and the common bile duct was isolated. A 1 cm incision was made into the duct, approximately 0·5 cm proximal to its entrance into the duodenum. One free end of an 8 French Latex T-tube was inserted cranially into the duct, followed by insertion of the second free end caudally. The duct was sutured closed over and around the T-tube, and was then ligated just proximal to its entrance into the duodenum. The remaining arm of the T-tube was passed through a small stab incision in the abdominal wall. The tube was then brought out to the subcutaneous level and tunneled under the skin to the desired location, where it was exteriorized through a small stab incision in the skin and attached to a collection container. The tubing was secured to the abdominal wall, and the midline incision was then sutured closed. The dog was given a second i.m. injection of penicillin within 24 h post-operation. The dogs were housed inividually in metabolism cages and were fed once daily. They were adapted to and maintained on a low-fat diet during the study. Water was provided *ad libitum*.

Monkeys. Eight mature, male rhesus monkeys weighing 6·40–9·45 kg (7·76 ± 0·94 (SD) kg) were used. The gallbladder of each monkey was cannulated. Prior to surgery, the monkeys were anesthetized with ketamine/xylazine (5/1, v/v) at a dose of 0·1 mL kg^{-1}, IM. Anesthesia was maintained by halothane gas delivered via an endotracheal tube. Under aseptic surgical conditions, a right subcostal incision was made. The gallbladder was exposed, and a small incision into the fundus of the gallbladder was made. The gallbladder was then cannulated with a Silastic® medical grade tubing (0·078" ID × 0·125" OD, Dow Corning, Midland, MI). The cannula was secured in the lumen of the body of the gallbladder with a purse string suture. The common bile duct was ligated just proximal to its juncture with the pancreatic duct. The tubing was then routed through a small stab incision in the abdominal muscle wall and tunneled under the skin of the thorax to the lower back of the animal, where it was exteriorized through a small incision in the skin. The tubing was secured to the abdominal wall and the subcostal incision was sutured closed. The monkey was placed in a restraining chair. The distal end of the cannula was covered with a sterile dressing. Bile was allowed to drip into a collection container. The monkeys remained seated in restraining chairs for the duration of the study. They were offered certified Purina monkey chow and fresh fruit several times daily. Water was provided *ad libitum*.

Dosing and sample collection

The tested doses were 12·4 mg kg^{-1} for the dogs and 0·48 and 45·5 mg kg^{-1} for the monkeys. [^{3}H]Fluvastatin was placed in gelatin capsules and

administered after the animal had recovered from surgery (1–2 d) and following an overnight fast. Food was withheld for 4 h postdose. From each dog or monkey, venous blood (2–3 mL) was collected in a heparinized syringe immediately before and at 0·25, 0·5, 1, 2, 3, 4, 6, 8, 12, 24, 32, 48, 56, and 72 h after dosing. An additional blood sample was collected from the dog only at 96 h. Plasma was separated by centrifugation and immediately stored frozen at −20 °C. Bile was collected quantitatively in designated intervals for 3 d postdose, with an additional 72–96 h sampling from the dog only. Urine and feces were collected in 24 h intervals for 3 (monkey) or 4 (dog) d postdose. The weight of each bile, urine, and feces specimen was measured. After the final collection, the dog cage and the monkey chair and collection pan were rinsed with ethanol and water, and the weight of the wash was determined. All samples were stored frozen until analysis.

Analysis of radioactivity

Radioactivity was measured in a liquid scintillation spectrometer (Model 460, Packard Instrument Co., Downers Grove, IL). Plasma, urine, and cage/chair wash were assayed by directly counting aliquots in Formula-989 scintillant (NEN® Research Products, Boston, MA). Bile and fecal homogenates were air dried and combusted in a sample oxidizer (System 387, Packard) before counting. Radioactivity concentrations are given as nanogram equivalents of fluvastatin free acid per milliliter or gram.

Analysis of unchanged fluvastatin

Plasma concentrations of fluvastatin were determined using a high performance liquid chromatographic (HPLC) method with fluorescence detection.[8] The lower limit of quantification was 5·17 ng mL^{-1} for both the dog and the monkey studies. Cumulative bile, urine, and fecal samples, pooled for individual animals, were analyzed for the parent drug using HPLC with radioactivity monitoring.[7]

RESULTS

Plasma concentrations

The plasma concentrations of total radioactivity and parent drug in the dog and monkey are summarized in Table 1. In the dog, measurable levels of radioactivity and fluvastatin were observed in plasma at 0·5 h postdose. The peak radioactivity concentrations (C_{max}) averaged 7260 ng equiv. mL^{-1} and the mean time to peak (t_{max}) was ~9 h. For the first 4 h postdose plasma radioactivity consisted almost entirely of the unchanged drug. Subsequently

Table 1. Plasma concentrations of total radioactivity and fluvastatin

	Dog		Monkey			
	12·4 mg kg^{-1}		0·48 mg kg^{-1}		45·5 mg kg^{-1}	
	Radioactivity	Fluvastatin	Radioactivity	Fluvastatin	Radioactivity	Fluvastatin
Concentration (ng mL^{-1})[a] at						
0·25 h	0±0	0±0	2·33±2·33	0±0	218±145	106±92·5
0·5 h	17·0±10·4	24·4±12·7	22·0±22·0	19·4±19·4	812±620	586±510
1 h	752±675	999±880	62·9±51·1	46·9±38·6	1680±1190	1040±795
2 h	3050±2610	3240±2740	86·5±52·6	43·7±25·0	5030±2970	2980±1950
3 h	4620±3170	5830±4250	99·2±42·9	37·0±13·5	4120±2430	1610±900
4 h	5950±2330	6320±2810	98·5±31·6	24·0±1·05	4880±3310	2080±1460
6 h	3660±1170	2930±965	87·3±34·7	19·9±5·40	4560±2580	1180±505
8 h	2650±800	1990±590	82·3±7·60	15·7±10·1	3750±1450	866±92·5
12 h	1270±159	710±108	63·5±12·0	4·85±4·85	3740±965	1000±305
24 h	817±431	463±433	49·0±3·25	0±0	7060±2180	1060±650
32 h	344±61	44·6±42·5	35·7±0·85	0±0	4740±1320	260±197
48 h	249±58	2·46±2·46	32·0±5·35	0±0	3320±960	82·4±36·5
56 h	227±67	0±0	23·9±0·05	0±0	2380±560	38·3±17·3
72 h	204±67	0±0	23·2±3·00	0±0	2040±420	28·8±11·3
96 h	182±62	0±0	—	—	—	—
t_{max} (h)	9·3±5·0	8·8±5·1	5·5±2·5	4·5±3·5	13·3±6·3	9·8±5·4
C_{max} (ng mL^{-1})	7260±2500	7850±3670	116±26·1	55·7±29·9	10400±1980	4020±1600
AUC (ng h mL^{-1})	66900±9200	42000±11700	3090±10·0	285±17·5	281000±36800	37100±5350
AUC/dose	5410±775	3410±980	6440±20·0	593±36·5	6180±820	817±113

[a]Mean±SEM, $N=4$ (dog and high-dose monkey studies) or 2 (low-dose monkey study). Radioactivity levels are given as ng equiv. fluvastatin mL^{-1}.

Table 2. Excretion of radioactivity after a single oral dose of [^{3}H]fluvastatin

Time interval (h)	Radioactivity excreted (% of dose)[a]								
	Dog, 12·4 mg kg^{-1}			Monkey, 0·48 mg kg^{-1}			Monkey, 45·5 mg kg^{-1}		
	Urine	Feces	Bile	Urine	Feces	Bile	Urine	Feces	Bile
0–24	1·6±0·67	13·7±9·1	50·6±7·3	3·6±0·72	0±0	65·4±1·7	2·6±0·38	0±0	50·7±14·9
24–48	0·15±0·06	9·0±5·3	5·4±4·4	0·75±0·39	0±0	5·8±3·4	1·3±0·79	2·2±2·2	14·5±6·5
48–72	0·04±0·02	9·8±7·6	0·22±0·13	0·20±0·03	1·1±1·1	2·2±0·64	0·34±0·17	4·3±2·6	3·4±1·2
72–96	0·05±0·04	0·22±0·18	0·08±0·03	—	—	—	—	—	—
Total 0–96	1·8±0·65	32·7±4·9	56·3±5·8	4·6±0·30	1·1±1·1	73·4±5·7	4·3±0·99	6·5±3·9	68·5±3·7

[a]Mean±SEM, $N=4$ (dog and high-dose monkey studies) or 2 (low-dose monkey study).

fluvastatin concentrations declined more rapidly than total radioactivity and were below the quantification limit after 56 h, while measurable radioactivity levels were observed throughout the 96 h sampling period. The area under the concentration–time curve (AUC), calculated using the linear trapezoidal rule, for the parent drug was approximately 63% of that for total radioactivity.

One monkey in the low-dose group was misdosed and another exhibited partially obstructed bile flow; both were deleted from the study. In the monkey, plasma radioactivity and fluvastatin were observed at the first sampling time, 0·25 h, after both the low and high oral doses. The average peak time was 4–5 h following the low dose but was prolonged (10–13 h) and more variable following the high dose. The mean peak concentrations of radioactivity were 116 ng equiv. mL^{-1} after the low dose and 10 400 ng equiv. mL^{-1} following the high dose, while the respective values for unchanged fluvastatin were 55·7 and 4020 ng mL^{-1}. At both dose levels, the plasma decay of parent drug was more rapid than that of total radioactivity. The AUC ratio of parent drug versus total radioactivity was 9% for the low dose and 13% for the high dose. The mean values of dose normalized AUC (AUC/dose) of total radioactivity were similar after the low and high doses. In contrast, AUC/dose of the parent drug increased with dose.

Excretion

The excretion of radioactivity in the dog and monkey is summarized in Table 2. In bile duct cannulated dogs, 56·3% of administered radioactivity was excreted in bile, 1·8% in urine, and 32·7% in feces. Total recovery averaged ~ 91% during 0–96 h, with 0·2% found in the cage wash. The low and high oral doses in monkeys with bile fistulae yielded similar radioactivity recoveries in bile (68·5–73·4% of dose) and urine (4·3–4·6% of dose), but the high dose resulted in a greater mean recovery (6·5%) in feces than the low dose (1·1%). Approximately 79% of either dose was recovered during 0–72 h, with less than 0·02% in the chair wash.

As shown in Table 3, 11·9% of the biliary radioactivity in the dog was due to intact fluvastatin, compared with 0% and 7·5% after the low and high doses in the monkey. These figures represented 6·9% of the dose in the dog and 5·2% of the high dose in the monkey. Little or no parent drug was found in the urine of either species. In contrast, parent drug accounted for 16·0% and 9·5% of the fecal radioactivity in the dog and the monkey (only measured in the high-dose group), respectively.

DISCUSSION

Compared with previous data in intact animals,[2] the absorption of fluvastatin in dogs and monkeys with bile duct cannulation was more prolonged,

Table 3. Cumulative excretion of fluvastatin after a single oral dose of [^{3}H]fluvastatin

Species	Dose (mg kg^{-1})	Percentage of radioactivity in sample[a]			Percentage of dose[a]		
		Urine	Feces	Bile	Urine	Feces	Bile
Dog	12·4	NA[b]	16·0±2·2	11·9±2·0	NA	5·2±1·1	6·9±1·8
Monkey	0·48	NA	NA	0±0	NA	NA	0±0
Monkey	45·5	0±0	9·5±1·5	7·5±1·6	0±0	1·2±0·05	5·2±1·4

[a]Mean±SEM, $N=4$ (dog and high-dose monkey studies) or 2 (low-dose monkey study).
[b]Not analyzed; total radioactivity in each sample was < 5% of dose.

apparently as a result of depletion of bile salts. However, the extent of absorption, as reflected in the drug concentrations in plasma, appeared to be unaffected. In the dog, radioactivity concentrations in plasma agreed with projections from previous data after a 0·95 mg kg^{-1} dose assuming a linear dose–concentration relationship.[2] Parent drug levels in the present study were greater than the projected values, indicating an overproportionality to dose, which also was observed previously and attributed to saturable first-pass effect.[2] In the monkey, the high dose yielded radioactivity and parent drug AUC values similar to those reported previously,[2] although C_{max} values were understandably smaller owing to slow absorption in the present study. The AUC/dose for radioactivity was similar between low and high doses whereas that for fluvastatin increased with dose, suggesting dose independent absorption but saturable first-pass metabolism, as were observed in the dog. Comparison of the dog and monkey data also confirmed the previous finding that a greater portion of the plasma radioactivity was due to unchanged fluvastatin in the dog than in the monkey.[2]

The excretion data in the present study are in good agreement with previous results in intact animals.[2] The bile was the major excretory route of administered radioactivity whereas the renal pathway only accounted for less than 5% of the dose in both the dog and the monkey. A greater percentage of the biliary radioactivity in the dog was due to parent drug compared with that in the monkey, thus confirming a smaller extent of metabolism in the former as previously reported.[2] In the monkey, the fluvastatin:radioactivity ratio in bile increased with increasing dose (Table 3), suggesting saturable metabolism.

It is interesting to note that, in bile duct cannulated dogs and monkeys, unchanged fluvastatin accounted for only 16% and 9·5%, respectively, of the fecal radioactivity. This finding would imply either intestinal metabolism or extrabiliary excretion (exsorption) of metabolites into the gastrointestinal tract, which has been reported for a number of drugs.[9–13]

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