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ABSORPTION AND DISPOSITION OF FLUVASTATIN, AN INHIBITOR OF HMG-CoA REDUCTASE, IN THE RABBIT

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ABSTRACT

The absorption and disposition of fluvastatin have been studied in the female rabbit. In naive rabbits receiving a single oral dose (1 mg kg^{-1}) of [^{3}H]fluvastatin, absorption was rapid and amounted to *c.* 90 per cent compared with an intravenous reference dose. The drug was subject to considerable first-pass effect, its absolute bioavailability being 46 per cent. The steady-state volume of distribution of fluvastatin was $0{\cdot}29 \pm 0{\cdot}04$ l kg^{-1} while the total body clearance was $0{\cdot}33 \pm 0{\cdot}05$ l h^{-1} kg^{-1}. The administered radioactivity was excreted predominantly in feces, with the renal pathway accounting for 28 ± 4 per cent of the oral dose and 32 ± 3 per cent of the intravenous dose. In pregnant rabbits on a multiple oral dosing regimen, 1 mg kg^{-1} day^{-1} beginning day 6 post-conception (p.c.), steady-state concentrations in maternal blood and tissues were achieved within 5 days. The concentrations in the reproductive organs were *c.* 25–50 per cent of that in maternal blood while those in the kidneys and liver were considerably higher. In contrast, radioactivity levels in the fetuses and amniotic fluid decreased significantly during repeated drug administration. The fetus:placenta and amniotic fluid:placenta concentration ratios declined from 0·92 and 0·97, respectively, on day 10 p.c. to 0·10 and 0·04, respectively, on day 18 p.c., indicating a limited transfer of fluvastatin and/or its metabolite(s) across the placenta at the later stage of pregnancy. After cessation of dosing, radioactivity levels in all tissues and fluids showed a progressive and nearly parallel decline, suggesting no tissue retention of the drug.

KEY WORDS Fluvastatin HMG-CoA reductase Rabbit Absorption Disposition Placental transfer

INTRODUCTION

It is now well documented that lowering plasma levels of low-density lipoprotein (LDL) cholesterol while increasing high-density lipoprotein (HDL) cholesterol levels reduces the risk of coronary heart disease and atherosclerosis.[1,2] Fluvastatin (Sandoz compound XU 62-320), [R*, S*-(E)]-(±)-sodium-3,5-dihydroxy-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indole-2-yl]-hept-6-enoate,

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is a potent inhibitor of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis. Previous studies have shown significant reduction in serum total cholesterol, LDL cholesterol, and serum triglyceride levels in rats, dogs, and monkeys treated with fluvastatin.[3] It was also demonstrated that fluvastatin is more potent than compactin and lovastatin in inhibiting HMG-CoA reductase *in vitro* and cholesterol biosynthesis *in vivo*.[4]

The absorption and disposition of fluvastatin have been studied in the mouse, rat, dog, and monkey using ^{14}C- or ^{3}H-labeled drug.[5] Oral doses of the drug were absorbed at a moderate to rapid rate, the extent of absorption being dose-independent and essentially complete in all species. However, fluvastatin was subject to extensive presystemic hepatic extraction followed by direct excretion via the bile, thus yielding high liver/peripheral tissue concentration gradients of drug-related materials. The bioavailability of the parent drug increased with dose, apparently due to saturation of the first-pass effect. Dose-normalized blood levels of fluvastatin and total radioactivity were higher in the dog than in the other species. Fluvastatin was partially metabolized before excretion, the extent of metabolism being smallest in the dog and greatest in the mouse. The half-life of intact fluvastatin ranged from 1–2 h in the monkey to 4–7 h in the dog. Regardless of the dose or dose route, the administered radioactivity was recovered predominantly in feces, with the renal route accounting for less than 8 per cent of the dose. No tissue retention of radioactivity was observed, and material balance was essentially achieved within 96 h after dosing.

The present study was conducted to examine the absorption, distribution, placental transfer, and excretion of fluvastatin in the female rabbit. This pharmacokinetic information is important for the support of teratogenicity trials in the same species. Knowledge of the transfer characteristics of drug-derived materials across the placental barrier is needed in assessing the potential risk of the use of fluvastatin in pregnant women.

METHODS

Radiolabeled compound

[^{3}H]fluvastatin was synthesized with the tritium label at the 3-position of the *p*-fluorophenyl moiety (Figure 1). Radiochemical purity was determined by radio-thin-layer chromatography and radio-high-performance liquid chromatography to be >95 per cent, and the specific activity was $10{\cdot}4\ \mu Ci\ mg^{-1}$.

Absorption and excretion study

Mature, female New Zealand White rabbits, weighing $4{\cdot}2 \pm 0{\cdot}2$ (SD) kg, were housed individually in metabolism cages with free access to food and water at

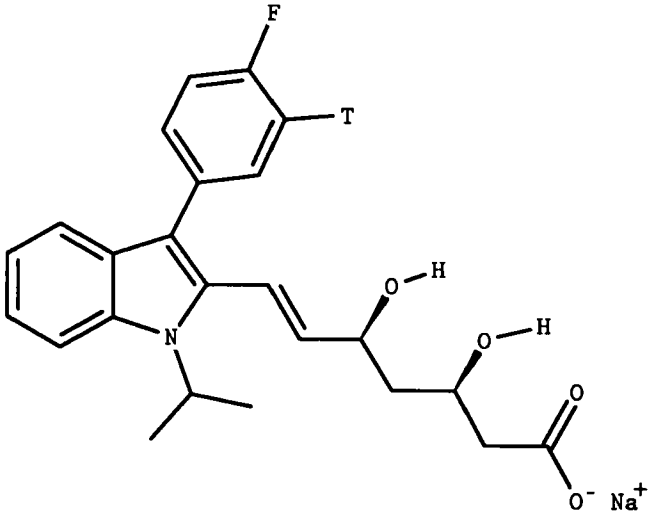

Figure 1. Fluvastatin labeled with ^{3}H (T)

all times. Three rabbits received a single oral dose and another three an intravenous dose of 1 mg kg^{-1} [^{3}H]fluvastatin, equivalent to 0·95 mg kg^{-1} of the free acid. The oral dose was administered by gavage as a suspension in 1 per cent carboxymethylcellulose while the intravenous dose was delivered via a marginal ear vein as an aqueous solution. Venous blood samples (1 ml) were obtained immediately before and at 0·5, 1, 2, 3, 4, 6, 8, 12, 24, 32, 48, 56, 72, and 96 h after dosing. From the intravenously dosed rabbits, additional samples were obtained at 5 and 15 min postdose. Complete urine and feces were collected in 24 h intervals for 4 days postdose. All samples were stored at − 15° until analysis.

Tissue distribution and placental transfer study

Female rabbits were mated with males of the same source and strain. The day after mating is considered day 1 post-conception (p.c.). Each pregnant rabbit received daily oral doses of [^{3}H]fluvastatin, 1 mg kg^{-1} day^{-1}, from day 6 to day 18 p.c. Blood and selected tissues were obtained from groups of three animals sacrificed at 2 h after dosing on days 10 and 14 p.c. and at 2, 24, and 48 h after the final dose on day 18 p.c. The samples were stored at − 15° until analysis.

Analysis of radioactivity

Radioactivity was measured in a liquid scintillation spectrometer (Model 460, Packard Instrument Co., Downers Grove, IL). Urine was assayed by directly

counting aliquots in ACS® scintillant (Amersham Corp., Arlington Heights, IL). Blood and tissue and fecal homogenates were air-dried and combusted in a sample oxidizer (Model 306, Packard Instrument Co.) before counting. Radioactivity concentrations are given as ng equivalents of fluvastatin free acid per ml or g.

Analysis of unchanged fluvastatin

Blood concentrations of fluvastatin free acid were determined using the previously reported high pressure liquid chromatographic (HPLC) method,[5] with slight modifications. In the revised procedure (Kalafsky *et al.*, submitted for publication), fluvastatin and an internal standard, which differs from fluvastatin only by having a methyl group at the 6-position of the heptenoic acid chain, were extracted from buffered (pH 7) blood samples into methyl tertiary butyl ether (MTBE) followed by evaporation of an aliquot of the organic phase. The residue was reconstituted in the mobile phase, methanol–water (6:4, v/v) containing 5 ml of tetrabutylammonium fluoride per liter. The solution was chromatographed on a 150 mm × 4·6 mm i.d. 5 μm Supelcosil® LC-18 column (Supelco Inc., Bellefonte, PA) at 50°.

Fluvastatin and the internal standard were detected by monitoring the intrinsic fluorescence at 380 nm following excitation at 305 nm. A standard curve was prepared by analyzing blood samples containing known quantities of fluvastatin on each day of analysis. The lower limit of quantification was 1 ng ml^{-1}. Using HPLC with radioactivity monitoring, fluvastatin was also determined in selected feces samples.

RESULTS

Absorption and excretion

The blood concentrations and relevant pharmacokinetic parameters in rabbits following a single dose of [^{3}H]fluvastatin are summarized in Table 1. In Table 1, C_{max} is the observed peak concentration, t_{max} is the time to peak concentration, AUC is the area under concentration–time curve determined by the trapezoidal rule, and half-life is calculated by linear regression of the terminal log-linear phase. The terms CL and Vd_{ss} represent, respectively, the total body clearance and the steady-state volume of distribution calculated by the method of Benet and Galeazzi.[6]

Following oral administration [^{3}H]fluvastatin was rapidly absorbed yielding peak blood levels of radioactivity and the parent drug at approximately 45 min postdose. Unchanged fluvastatin represented 94 ± 11 per cent of the total radioactivity concentration initially (0·5 h) but declined more rapidly than the latter, and was below quantification limit at 32 h. In contrast, low but persistent blood levels of radioactivity were still observed at 96 h postdose. The terminal half-lives of fluvastatin and total radioactivity were 4·8 ± 1·1 h and 69 ± 11 h,

Table 1. Blood concentrations and relevant pharmacokinetic parameters (mean ± SD, n = 3) following a single dose (1 mg kg^{-1}) of [^{3}H]fluvastatin

	Oral		Intravenous	
	Radioactivity	Fluvastatin	Radioactivity	Fluvastatin
Concentration (ng ml^{-1})* at				
0·08 h	–	–	3400 ± 587	3210 ± 1380
0·25 h	–	–	2680 ± 592	2430 ± 435
0·5 h	312 ± 126	285 ± 82·4	2020 ± 213	1730 ± 344
1 h	407 ± 61·4	240 ± 108	1200 ± 85·7	760 ± 113
2 h	349 ± 59·4	209 ± 71·5	657 ± 52·6	311 ± 89·8
3 h	320 ± 67·5	165 ± 46·9	454 ± 16·4	170 ± 73·9
4 h	301 ± 64·9	98·3 ± 59·7	364 ± 15·2	39·7 ± 35·2
6 h	274 ± 67·2	73·5 ± 26·3	325 ± 137	BQL†
8 h	217 ± 28·1	45·1 ± 28·8	144 ± 39·3	BQL
12 h	152 ± 31·3	17·8 ± 2·10	91·1 ± 11·8	BQL
24 h	86·0 ± 10·5	4·28 ± 0·27	68·9 ± 17·3	BQL
32 h	55·7 ± 14·2	BQL	41·4 ± 11·5	BQL
48 h	28·9 ± 5·69	BQL	21·4 ± 4·64	BQL
56 h	21·6 ± 8·67	BQL	17·9 ± 0·37	BQL
72 h	17·6 ± 6·43	BQL	11·1 ± 1·64	BQL
96 h	14·2 ± 4·77	BQL	9·12 ± 1·76	BQL
C_{max} (ng ml^{-1})	420 ± 67·8	300 ± 82·5	3400 ± 587	3210 ± 1380
t_{max} (h)	0·8 ± 0·3	0·7 ± 0·3	0·08 ± 0	0·08 ± 0
AUC (ng · h ml^{-1})	8010 ± 1490	1320 ± 377	8550 ± 218	2890 ± 425
Half-life (h)	69 ± 11	4·8 ± 1·1	24 ± 1·2	1·1 ± 0·39
CL (l h^{-1} kg^{-1})	–	–	–	0·33 ± 0·05
Vd_{ss} (l kg^{-1})	–	–	–	0·29 ± 0·04

*Radioactivity levels are given as ng equiv. fluvastatin free acid ml^{-1}.
†Below quantification limit.

respectively. The mean AUC values for total radioactivity and the parent drug were 94 per cent and 46 per cent, respectively, of those obtained from an intravenous dose. After intravenous dosing, 94 ± 17 per cent of the initial blood radioactivity consisted of unchanged fluvastatin, the concentrations of both being more than 10 times greater than the respective values following oral administration. Again, fluvastatin concentrations decreased more rapidly than total radioactivity levels with respective half-lives of 1·1 ± 0·4 h and 24 ± 1·2 h. The steady-state volume of distribution of fluvastatin was 0·29 ± 0·04 l kg^{-1} while the total body clearance was 0·33 ± 0·05 l h^{-1} kg^{-1}.

The excretion of radioactivity following a single dose of [^{3}H]fluvastatin is summarized in Table 2. The major elimination pathway appeared to be via the bile into feces, accounting for 50–60 per cent of the administered radioactivity. Approximately 28 ± 4 per cent of the dose was recovered in urine after an oral

Table 2. Excretion of radioactivity (mean ± SD, $n = 3$) following a single dose (1 mg kg^{-1}) of [^{3}H] fluvastatin

	Radioactivity excreted (% dose)			
	Oral		Intravenous	
Time interval (h)	Urine	Feces	Urine	Feces
0–24	12·8 ± 8·7	19·7 ± 17·0	16·7 ± 14·5	21·5 ± 12·6
24–48	12·3 ± 6·0	32·1 ± 10·7	11·9 ± 12·3	18·3 ± 1·5
48–72	1·8 ± 0·5	6·7 ± 3·4	2·8 ± 3·6	8·9 ± 5·5
72–96	0·8 ± 0·8	1·6 ± 0·4	0·3 ± 0·1	2·8 ± 2·0
Total: 0–96	27·7 ± 3·9	60·0 ± 2·6	31·7 ± 2·6	51·5 ± 3·9

dose compared with 32 ± 3 per cent after intravenous dosing, yielding an oral:intravenous ratio of *c.* 87 per cent. The total recovery of radioactivity during the 96 h collection interval was 88 ± 3 per cent from the oral dose and 83 ± 1 per cent from the intravenous dose. In a pooled feces sample from the orally dosed rabbits, analysis for fluvastatin recovered 2·9 per cent of the dose as the intact parent drug.

Tissue distribution and placental transfer

The concentrations of radioactivity in the tissues and body fluids of pregnant rabbits are summarized in Table 3. At 2 h following the 5th daily dose, on day 10 p.c., the highest radioactivity concentrations were observed in the kidneys and liver with values of 3220 ± 621 and 1570 ± 303 ng equiv. g^{-1}, respectively,

Table 3. Tissue and body fluid concentrations of radioactivity (mean ± SD, $n = 3$) during multiple oral administration of [^{3}H] fluvastatin (1 mg kg^{-1} day^{-1}) in pregnant rabbits

	Concentration (ng equiv. ml^{-1} or g^{-1})				
	Day 10 p.c.	Day 14 p.c.	Days 18–20 p.c. (final dose)*		
Tissue/fluid	2 h	2 h	2 h	24 h	48 h
Blood	450 ± 70·9	489 ± 74·2	484 ± 45·1	253 ± 119	92·0 ± 6·69
Liver	1570 ± 303	1690 ± 423	1540 ± 493	500 ± 93·9	234 ± 48·8
Kidneys	3220 ± 621	2960 ± 721	2120 ± 290	591 ± 151	228 ± 8·41
Amniotic fluid	141 ± 35·3	17·8 ± 4·61	4·03 ± 1·41	3·67 ± 3·68	1·25 ± 2·17
Fetuses	134 ± 10·8	8·38 ± 2·00	9·01 ± 4·15	5·26 ± 0·93	0·94 ± 1·62
Placentas	146 ± 28·7	98·5 ± 16·0	92·8 ± 20·7	55·7 ± 5·29	39·7 ± 5·72
Ovaries	157 ± 26·5	186 ± 31·8	151 ± 15·6	79·0 ± 4·15	50·9 ± 18·5
Corpora lutea	238 ± 21·9	196 ± 29·1	183 ± 48·9	83·0 ± 14·0	47·0 ± 17·3
Uterus	98·6 ± 36·0	96·5 ± 22·5	122 ± 25·3	62·8 ± 8·26	40·3 ± 8·85

*Dosing started on day 6 p.c.

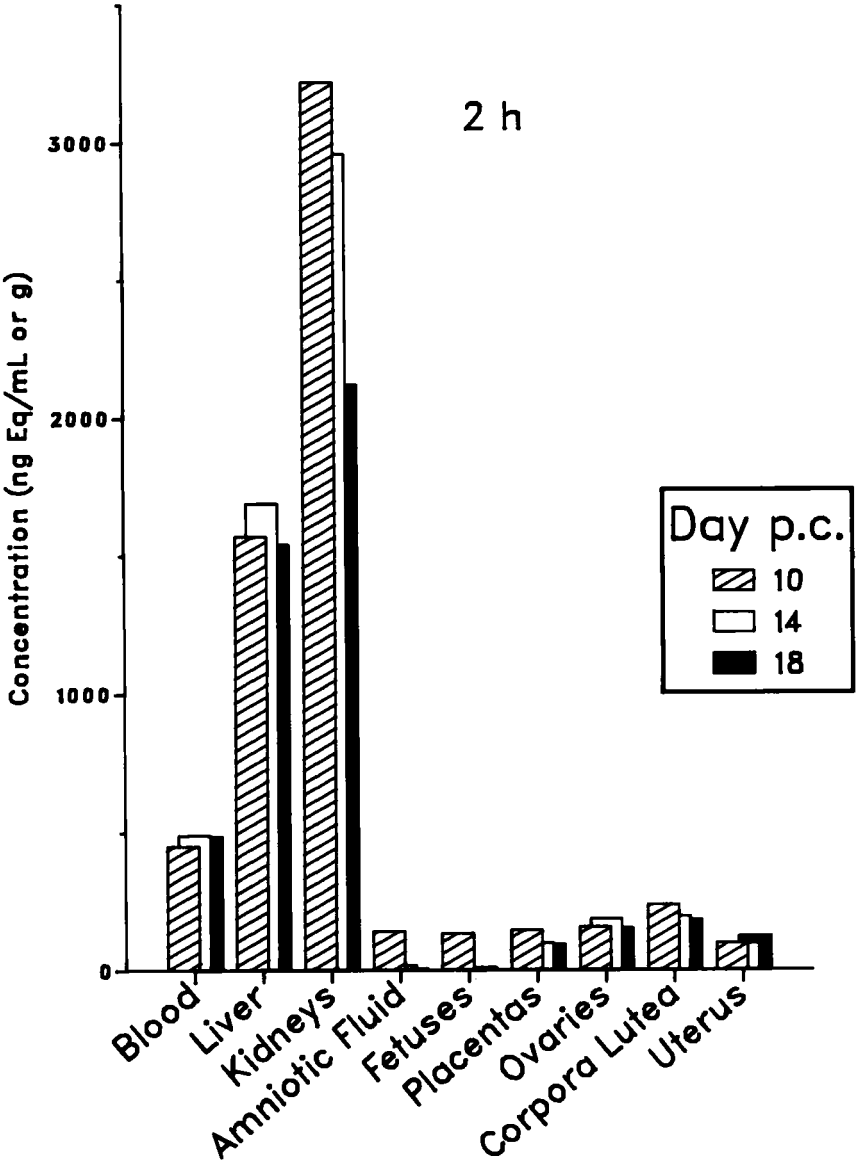

Figure 2. Distribution of radioactivity during multiple oral administration of [^{3}H]fluvastatin (1 mg kg^{-1} day^{-1}) in pregnant rabbits

which were considerably greater than the blood level of 450 ± 70·9 ng equiv. ml^{-1}. The concentrations in the remaining tissues and fluids, all lower than that in blood, ranged from 98·6 ± 36·0 ng equiv. g^{-1} (uterus) to 238 ± 21·9 ng equiv. g^{-1} (corpora lutea).

Comparison of the 2 h data on days 10, 14, and 18 p.c. (Figure 2) showed that the radioactivity levels in maternal blood and tissues were fairly constant while, in contrast, mean concentrations in the fetuses and amniotic fluid decreased by a factor of 15 and 35, respectively, during this period.

Following the final dose, radioactivity levels in all tissues and fluids showed a progressive and nearly parallel decline, as shown in Figure 3. The half-life estimated by log-linear regression analysis of the 2, 24, and 48 h maternal blood concentrations was 19 h.

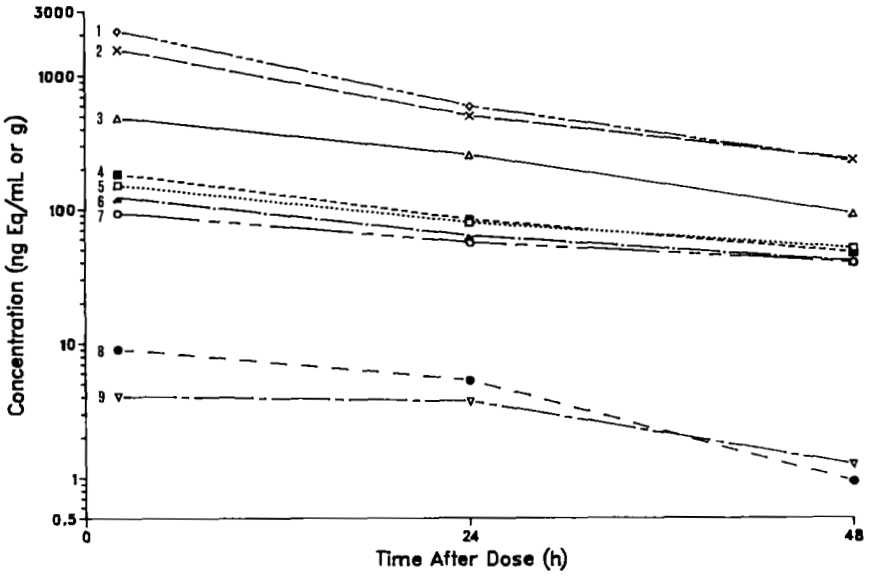

Figure 3. Distribution of radioactivity after the final dose of [^{3}H]fluvastatin (1 mg kg^{-1} day^{-1}, oral) in pregnant rabbits. 1: kidneys, 2: liver, 3: blood, 4: corpora lutea, 5: ovaries, 6: uterus, 7: placentas, 8: fetuses, 9: amniotic fluid

DISCUSSION

As previously observed in other animal species,[5] fluvastatin administered orally in 1 per cent carboxymethylcellulose was rapidly and almost completely absorbed in the rabbit. The extent of absorption estimated by comparing the oral and intravenous radioactivity data in both blood and urine was *c.* 90 per cent. The absolute bioavailability of fluvastatin as indicated by the oral:i.v. AUC ratio of the parent drug was 46 per cent, reflecting a smaller first-pass effect than that reported in the other species.[5]

The steady-state volume of distribution of fluvastatin was $0{\cdot}29 \pm 0{\cdot}04$ l kg^{-1}, smaller than that in the monkey (3·7 l kg^{-1}), mouse (1·0 l kg^{-1}), and dog (0·7 l kg^{-1}), thus suggesting less extensive tissue uptake of the drug in the

rabbit. Radioactivity data in the pregnant rabbits showed highest concentrations in the kidneys and liver, consistent with the function of these organs as well-perfused excretory sites. The greater radioactivity concentrations in kidneys than in the liver were in contrast to previous observations in the rat and mouse,[5] and probably reflected the increased importance of the renal route as an elimination pathway for fluvastatin in the rabbit. After the fifth daily dose (day 10 p.c.), radioactivity concentrations in the reproductive organs were approximately 25–50 per cent of that in maternal blood. The transfer of drug-related materials across the placenta into the fetuses was significant, the fetus:placenta and amniotic fluid:placenta concentration ratios being almost unity (0·92 and 0·97, respectively) at this time. The radioactivity levels in maternal blood and tissues were fairly constant between days 10 and 18 p.c., indicating that steady state had been achieved with the multiple dosing regimen employed. In contrast, the concentrations in the fetuses and amniotic fluid declined considerably during this period. The fetus:placenta and amniotic fluid:placenta concentration ratios on day 18 p.c. were 0·10 and 0·04, respectively, which were 9 and 24 times smaller than the respective values on day 10 p.c. This phenomenon probably can be explained in terms of an increased capability of the fetal excretory organs during the later stage of pregnancy. Increased drug excretion by the fetus would have no discernible effect on drug concentrations in the mother because of the small apparent volume of the fetal compartment, but could appreciably decrease the fetal:maternal drug concentration ratio.[7] Following the final dose, radioactivity levels in all maternal and fetal tissues and fluids showed a progressive and nearly parallel decline, suggesting no tissue retention of the drug.

Although the renal route was a minor elimination pathway in the rabbit, it accounted for 28–32 per cent of the radioactive dose, a significant increase compared with results in the rat, mouse, dog, and monkey (<8 per cent).[5] The remainder of the dose was excreted via the bile into the feces. The small amount (2·9 per cent of dose) of intact fluvastatin recovered in feces after oral dosing probably represented unabsorbed drug. The clearance of fluvastatin was $0{\cdot}33 \pm 0{\cdot}05\ l\,h^{-1}\,kg^{-1}$ while the half-life was $4{\cdot}8 \pm 1{\cdot}1$ h after an oral dose and $1{\cdot}1 \pm 0{\cdot}4$ h following intravenous administration, suggesting no accumulation of parent drug upon multiple daily administration in the rabbit. The apparently longer fluvastatin half-life after oral administration than after intravenous dosing cannot be definitively explained, but could be associated with the rate of absorption from the oral dose. The relatively long half-lives of total radioactivity probably reflected the slower elimination of certain metabolite(s) which would show moderate accumulation following multiple doses. Nonetheless, the administered radioactivity was almost completely recovered within the 96 h collection interval.

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