COMMUNICATIONS

The disposition of FPL 55712 acid (7-[3-(4-acetyl-3-hydroxy-2-propyl-phenoxy)-2-hydroxypropoxy)-4-oxo-8 propyl-4H-benzopyran-2-carboxylic acid) in rat and dog

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FPL 55712 (7-[3-(4-acetyl-3-hydroxy-2-propyl-phenoxy)-2-hydroxypropoxy]-4-oxo-8 propyl-4H-benzopyran-2 carboxylic acid I), has been widely referred to since its discovery as a selective antagonist of SRS-A (Augstein et al 1973) and the literature has been reviewed (Chand 1979). However, there are no reports describing the disposition or pharmacokinetics of the compound. The results of experiments conducted in the rat and dog as part of a limited programme of preclinical safety studies are therefore described here.

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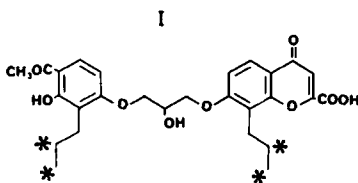
I. The structure of FPL 55712 acid. The position of the radiolabel is as indicated*.

Excretion and metabolic fate

The excretion and metabolic fate of FPL 55712 was investigated in male and female beagle dogs (n = 6) and male COBS Wistar rats (n = 3) after intravenous administration. Animals received ³H-labelled FPL 55712LL (lysine salt) by bolus injection (10 mg kg⁻¹). The animals were housed in metabolism cages and urine and faeces collected, as discrete daily collections, for seven days. Radioactivity present in urine samples was determined by liquid scintillation spectrometry. Radioactivity present in faeces was determined after their homogenization in distilled water (10·0% w/v), combustion of aliquots in a Packard B306 sample oxidizer (Caversham, Berkshire, UK) and liquid scintillation spectrometry. The results from dog and rat were very similar. Faecal excretion represented the predominant excretory route suggesting extensive biliary elimination. The recovery of radioactivity in the faeces of beagle dogs represented 97 ± 1% of the total radioactivity recovered (83±8% of the administered dose). Peak excretion of radioactivity occurred 24–72 h after administration of the dose, and no sex difference was detectable. Similarly, faecal excretion accounted for 96±1% of the total recovery in male rats (104±3% of the administered dose). Peak excretion of radioactivity in the rat occurred within 24 h after administration of the dose. Urinary

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excretion accounted for the remaining radioactivity recovered (3% of total recovered in dogs, 4% of total recovered in rats).

Samples of urine and faecal homogenates representative of peak excretion were acidified to pH 1 by dropwise addition of concentrated hydrochloric acid and extracted three times with equal volumes of ethyl acetate. This procedure extracted greater than 80% of the radioactivity present in any sample. The extracts were concentrated under reduced pressure and applied to Merck 200 × 200 × 0·5 mm silica gel F$_{254}$ thin layer chromatography plates. Chromatography was with chloroform–methanol–acetic acid (7:2:1 v/v) and chloroform–methanol–diisopropylamine (15:4:1 v/v).

Radioactivity was detected using a radiochromatogram scanner (Panax Nucleonics, Redhill, Surrey, UK). All of the radioactivity extracted from excreta chromatographed with an R_F identical to authentic FPL 55712 indicating that little if any metabolism of the compound occurred.

Biliary excretion

Biliary excretion of FPL 55712 was confirmed by bile collection experiments with male rats anaesthetized with pentobarbitone sodium. In animals (n = 3), with cannulated bile ducts, 50±17% of an intravenous bolus dose of FPL 55712LL (10 mg kg⁻¹) was recovered in the bile within 2 h. The rate of biliary excretion of FPL 55712 by the rat is illustrated in Fig. 1. Direct applications of aliquots (10–20 μl) of bile samples to thin layer chromatography plates and development in the solvent systems described above confirmed that FPL 55712 was eliminated without detectable metabolism.

Table 1. Binding of FPL 55712 with plasma from rat and dog.

Concentration of FPL 55712	Percentage plasma protein binding with s.d. (n = 3)			
μg ml⁻¹	Dog		Rat	
1	98·9	s.d. 0·2	99·5	s.d. 0·1
10	98·7	0·1	97·9	0·1
50	98·9	0·2	97·2	0·1
150	98·5	0·3	97·2	0·5
300	95·4	0·6	76·3	2·3
1000	50·0	10·0	50·9	2·4

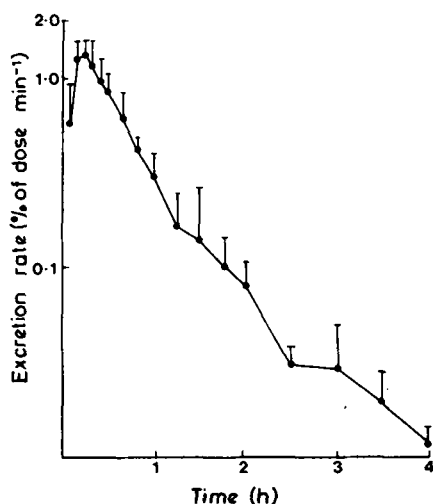Binding was measured by equilibrium dialysis as described in text.

FIG. 1. Rate of biliary excretion of FPL 55712 by anaesthetized male rats as represented by total radioactivity (see text) after bolus intravenous administration of ^{3}H-labelled FPL 55712LL (10 mg kg^{-1}).

Plasma protein binding

Plasma protein binding of FPL 55712 was investigated by equilibrium dialysis using a Dianorm apparatus (MSE, Crawley, Surrey, UK). Dog and rat plasma was dialysed against Tris-HCl pH 7·4 (0·1 M) buffer containing ^{3}H-labelled FPL 55712LL for 2 h. Radioactivity was assayed by liquid scintillation spectrometry. Binding of FPL 55712 to rat and dog plasma is shown in Table 1. Binding was greater than 97% over the concentration range of 1–150 µg ml^{-1} FPL 55712 but decreased at concentrations above this.

Intravenous pharmacokinetics

The intravenous pharmacokinetics of FPL 55712 were investigated in dogs (3 male, 3 female) and rats (3 male, 2 female). As no metabolism of FPL 55712 was detected, total radioactivity was used as a measure of FPL 55712 concentration. Animals received ^{3}H-labelled FPL 55712LL (10 mg kg^{-1}) by bolus intravenous injection, blood

Table 2. Kinetic constants (min^{-1}) for FPL 55712 obtained when mean plasma concentration data from rat and dog are fitted to a two compartment model in which elimination occurs from the central (a) or peripherical compartment (b).

	Dog		Rat	
	a	b	a	b
K_{12}	0·056	0·141	0·066	0·014
K_{21}	0·008	0·003	0·020	0·010
Kel	0·103	0·004	0·050	0·009
α	0·139		0·115	
β	0·006		0·007	
Vc	107		703	

Vc = volume of central compartment (ml kg^{-1}).

samples (2 ml, dog; 0·2 ml, rat) were withdrawn from the cephalic vein of dogs and the caudal vein of rats and plasma obtained by centrifugation. Radioactivity was determined as described above for urine samples. The mean plasma concentrations of FPL 55712 in dogs and rats are illustrated in Fig. 2. No apparent difference was detected for either species between the sexes. Plasma clearance calculated by the relationship dose/area under plasma concentration time curve was 9·3 ± 2·3 and 30·0 ± 5·7 ml min^{-1} kg^{-1} in dog and rat respectively. As the drug is extensively protein-bound and little is associated with red blood cells, these values are approximately half the value that would be calculated for blood clearance. The species difference in clearance can be correlated with reported liver blood flow values of 66 to 105 ml min^{-1} kg^{-1} and 28 to 45 ml min^{-1} kg^{-1} for the unanaesthetized rat (Altman & Dittmer 1974; Denis et al 1975) and dog (Altman & Dittmer 1974) respectively.

The plasma concentration data from both species were fitted to two compartment models using linear least squares regression analysis and conventional equations (Gibaldi & Perrier 1975). Table 2 lists the individual rate constants calculated from the data when fitted to two compartment models reflecting elimination from the central compartment or from the peripheral compartment (Gibaldi & Perrier 1975). In the latter model the liver would be assumed to represent the peripheral compartment. The model with elimination from the central compartment is more applicable with the rat, since, when the biliary excretion data (Fig. 1) are considered the rate constant for excretion of FPL 55712 into bile, during the first 2 h of the
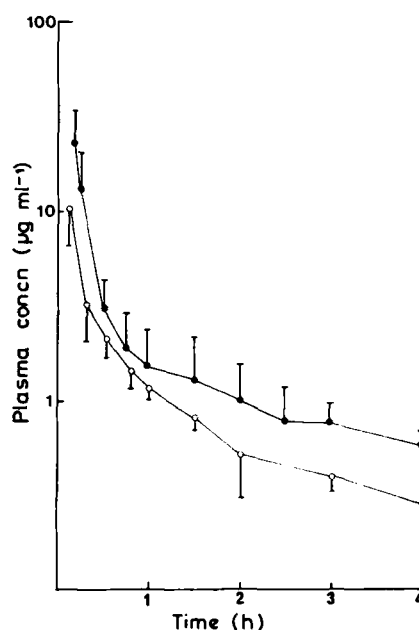

FIG. 2. Plasma concentrations of FPL 55712 as represented by total radioactivity (see text) in dog ● and rat ○ after bolus intravenous administration of ^{3}H-labelled FPL 55712LL (10 mg kg^{-1}).

experiment, is approximately 0·035 min⁻¹. This value would be expected to be closer to the β rate constant (0·007 min⁻¹, Table 2) if the model with elimination from the peripheral compartment was more appropriate. No choice of model can be made for the dog with the data available, however, the large differences in the volumes of the central compartment (Table 2) may indicate that different models are applicable to the two species.

In summary FPL 55712 is eliminated rapidly in rat and dog by biliary clearance of unchanged drug. The high biliary clearance is consistent with the structure of the compound since it conforms closely to the parameters necessary for biliary clearance described by Smith (1973): in particular its molecular weight (498), acidity (pK_a 1·8), and its amphipathic nature. Despite the acidity of the compound little urinary excretion occurred. This may be related to both the protein binding of the compound and its inherent lipophilicity as indicated by its high distribution coefficient (Scherrer & Howard 1977) (log D of 1·92 ± 0·01 at pH7·4 in octanol–aqueous buffer systems). The species differences in clearance seen between rat and dog can be correlated with differences in liver blood flow in these species.

It is possible therefore that the clearance values in the two species reflect to a large extent differences in the blood flow rather than differences in the ability of the liver to extract the compound.

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