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Species variation in the taurine conjugation of clofibric acid

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Clofibric acid (4'-chlorophenoxyisobutyric acid) is the pharmacologically active metabolite of the hypolipidaemic agent, clofibrate, and is itself used as a hypolipidaemic. This acid is metabolised by glucuronic acid conjugation in rat (Cayen, Fernandini, Greselin, Robinson & Dvornik, 1977) and man (Houin & Tillement, 1978), and in dog gives rise to a second unknown conjugate (Cayen *et al.*, 1977). We now report a reinvestigation of the metabolism of clofibric acid in six animal species and man. The present study confirms that it forms its ester glucuronide *in vivo*, and shows for the first time its extensive conjugation with taurine in three carnivorous species.

[^{14}C]-Clofibric acid (100 mg/kg; 3 μCi/animal) was administered to rats, guinea pigs, rabbits, cats, dogs and ferrets by intraperitoneal injection dissolved in propane-1,2-diol and their urine collected for up to 3 days. Three healthy male volunteers took [^{14}C]-clofibric acid (500 mg; 1 μCi) by mouth in a hard gelatine capsule and collected their urine for 48 h. The [^{14}C] content of the urines was assayed by liquid scintillation counting, and urinary metabolites determined by solvent extraction, thin-layer chromatography, colour reactions and enzymic and chemical hydrolysis. Quantitative and qualitative results are given in Table 1. The cat, dog and ferret excreted the administered [^{14}C] slowly, with 24–39% of the dose excreted in 24 h and a total of 50–72% recovered in 3 days. Each species excreted as the major metabolite the taurine conjugate of clofibric acid, with smaller amounts of the unchanged acid and its ester glucuronide also present. The taurine conjugate was identified subsequent to its isolation, hydrolysis to clofibric acid and taurine, identified by chromatography as such and as its dansyl derivative, and by its infra red spectrum.

In the rat, rabbit, guinea-pig, mouse and man, at least 60% of the administered dose was recovered in the urine in 24 h, with further amounts excreted up to 3 days (total 66–96%). The only compounds present in the urines of these species were unchanged clofibric acid and its glucuronic acid conjugate. In each species, this was identified as the 1-*O*-acyl glucuronide by comparison of its chromatographic, solvent extraction and hydrolysis properties with a fully characterised sample of this metabolite isolated from rabbit urine (Caldwell & Emudianughe, 1979). Little is known about the chemical and biological factors

Table 1 The metabolism of clofibric acid in man and animals. Drug administration, urine collection and analysis as described in the text. Figures represent the means of at least three experiments

% [^{14}C]-dose in urine	Rat	Guinea Pig	Rabbit	Dog	Cat	Ferret	Man
0–24 h	95.6	73.6	60.4	38.9	23.7	24.0	59.8
0–72 h	—*	—*	73.9	66.4	50.0	71.8	80.0
%0–24 h urinary [^{14}C] *present as*							
Free acid	44	20	9	30	56	23	5
Glucuronide	56	80	91	19	tr	5	95
Taurine Conjugate	n.d.	n.d.	n.d.	51	43	72	n.d.

* 24 h collection only. tr = trace. n.d. = not detected.

determining the taurine conjugation of carboxylic acids, and this is the first report of this reaction occurring with an aryloxyacetic acid in mammalian species. It must be mentioned that the taurine conjugation of arylacetic acids in general occurs at random with certain combinations of acid and species, although it is especially evident in carnivores.

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Differences in toxicity due to species variation in the metabolism of an oral anti-allergy agent

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During the toxicological evaluation of an oral anti-allergy compound FPL 57787 (6,7,8,9-tetrahydro-5-hydroxy-4-oxo-10-propyl-4H-naptho (2,3-b) pyran-2-carboxylic acid) (Augstein *et al.*, 1977) a marked difference was noted in the oral toxicity when the compound was administered to rat and dog. The compound proved severely hepatotoxic to some dogs during 90 days administration at 60 mg kg^{-1} day^{-1}. No toxicity was seen in the rat after 180 days administration at dose levels up to 125 mg kg^{-1} day^{-1} even though absorption was similar (>50%).

The species difference in toxicity corresponded to a variation in the rate of metabolism. The only metabolites of the compound in rat, dog, hamster, rabbit, monkey and man were produced by hydroxylation of the alicyclic ring. The rate of hydroxylation as judged by *in vivo* and *in vitro* evidence was much slower in dog than in other species examined. Microsomes prepared from dog liver metabolised the compound at a rate $<6 \times 10^{-12}$ moles metabolites formed min^{-1} mg $protein^{-1}$. In contrast rat liver microsomal fraction metabolised the compound at a rate of 38×10^{-12} moles min^{-1} mg^{-1}. Analysis for FPL 57787 and its metabolites in the urine, faeces and plasma after administration of FPL 57787 substantiated the *in vitro* data. After oral doses the hydroxylated metabolites were present in the plasmas of man and rat whilst only unchanged compound was present in plasma samples obtained from the dog. Similarly the dog excreted most of the administered dose (either i.v. or oral) as unchanged compound in the faeces, whilst the other species excreted substantial proportions of metabolites in the urine.

Since unchanged FPL 57787 was not cleared renally by any species the slower rate of metabolism of the dog resulted in a dependence on biliary excretion for the elimination of the compound. Transport maximum values for the biliary excretion of FPL 57787 in the dog obtained from animals after biliary cannulation ranged from 0.09–1.25 mg kg^{-1} $hour^{-1}$. The dose levels that produced toxicity in the dog were sufficient to saturate the biliary excretion of the compound and resulted in a very high area under the plasma curve (AUC) (4600 h μg/ml at 60 mg/kg). The species capable of clearance by metabolism had a much lower plasma AUC (e.g. rat – 300 h μg/ml at 80 mg/kg) at corresponding doses.

The dog is widely used as a species for toxicological evaluation. The reported work indicates the value of studying metabolism both from a quantitative as well as the normal qualitative view point when interpreting toxicological data. The inability of the dog to readily hydroxylate alicyclic carbon in a compound extensively metabolised by rat and man may be viewed as unusual. It has, however, been noted in previous publications. (Dayton, Cunningham, Israili & Weiner, 1973; Gros, Dari, Chasseaud & Hawkins, 1974; & Zacchei, Wishousky & Watson, 1978).

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