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IN VITRO FEBANTEL TRANSFORMATION BY SHEEP AND CATTLE RUMINAL FLUIDS AND METABOLISM BY HEPATIC SUBCELLULAR FRACTIONS FROM DIFFERENT ANIMAL SPECIES

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Abstract—Febantel and one of its main metabolites, febantel sulphoxide, are chemically modified to only a slight extent when incubated *in vitro* with sheep and cattle ruminal fluids; other major metabolites, fenbendazole and oxfendazole, are respectively, oxidized to oxfendazole and reduced to fenbendazole. Febantel is negligibly metabolized by hepatic cytosol fractions but microsome preparations effect more extensive metabolic transformations.

Important differences in this respect were found between microsome preparations from rat, horse, pig, cattle, sheep, chicken and trout livers.

Febantel, [N-2-(2,3-bis-(methoxycarbonyl)-guanidino)-5-5(phenylthio)-phenyl-2-methoxy-acetamide], has been widely investigated because of its broad spectrum activity (at 5.0–10.0 $mg \cdot kg^{-1}$ or higher doses) against nematodes and cestodes parasitizing horses, cattle, sheep, goats, swine, poultry and wild animals [1].

When administered orally to monogastric and ruminant mammals the drug (or its metabolites FBZ or OXF)§ is almost equiactive against both intestinal and lung worms: a fact suggesting a number of intriguing questions about the pharmacokinetics of the compound which have not been resolved despite a long series of studies devoted to this end [2–7]. One of these [8] described the fast metabolism of febantel to fenbendazole in mice treated with high doses of the drug and suggested the possible modification of the guanidino structure to a benzimidazole one (in agreement with a similar pathway hypothesized for thiophanate [9]) prior to its absorption by the gastrointestinal system.

In another study [7] the blood assay for FBT and its metabolites was performed too long after administration to sheep to permit a distinction between possible early chemical modification and metabolism subsequent to absorption. Another interesting fact emerging from these pharmacokinetic–metabolic studies was that F.SUL was detected in cattle plasma but not in sheep plasma (up to a threefold therapeutic dose) [7].

The possibility that FBT may be cyclized in the forestomach of ruminants raises interesting questions concerning its and fenbendazole's oxidative metabolism to the active sulphoxide and probably inactive sulphone derivatives.

These sulphoxidative pathways, once believed to be mediated by liver cytochrome P-450-dependent monooxygenases [5] and recently attributed to FAD-dependent oxygenases [10] were considered indispensable for the anthelmintic activity both of FBT and FBZ since they resulted in the formation of OXF [11]. This hypothesis was supported by findings of Averkin *et al.* [12] which could also account for some toxicological properties of these substances, i.e. their theratogenic activity [13–15]. The *in vitro* experiments reported in this paper were designed to shed light on some of these questions: to determine whether or not FBT is cyclized to FBZ in cattle and sheep ruminal fluids and to investigate quantitatively the production of the three main metabolites FBZ, F.SUL and fenbendazole sulphoxide (OXF) in liver microsome and cytosol preparations from various animal species: this last also as part of an important comparative evaluation of hepatic metabolic activity towards a xenobiotic compound in various animal species.

MATERIALS AND METHODS

Chemicals. NADPH and NADH were purchased from Boehringher Biochemia Robin (Italy) and the solvents (analytical grade) from Bracco Merck (Italy).

Febantel (FBT), its sulphoxide (F.SUL) and fenbendazole (FBZ) were kindly supplied by Bayer Ltd.

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§ Abbreviations: FBT, febantel, i.e. [[2-[(methoxyacetyl)amino]-4-(phenylthio) phenyl] carbonimidoyl] biscarbamic acid dimethyl ester; FBZ, fenbendazole, i.e. [5-(phenylthio)-1Hbenzimidazol-2-yl]carbamic acid methyl ester; F.SUL, febantel sulphoxide, i.e. the oxidized derivative of febantel; OXF, oxfendazole, i.e. the oxidized derivative of fenbendazole; DMF, dimethylformamide.

(Leverkusen, F.R.G.); and oxfendazole (OXF) by Vetem SpA (Agrimont, Milan, Italy). Prior to use they were dissolved in dimethylformamide (DMF) 1.0 mg·ml^{-1}.

Ruminal fluids assays. Fresh sheep and cattle ruminal fluids were collected by means of an oral probe from clinically healthy animals and subsequently always kept at 37°. After pH measurement (sheep = 6.6; cattle = 7.3) the fluids were filtered through a sterile gauze, analyzed for their protein content (biuret method [16]) and kept saturated with pure N_2. To 2 ml aliquots were added either 0.0896 μmoles of FBT, or 0.0668 μmoles of FBZ, or 0.0432 μmoles of F.SUL, or 0.0634 μmoles of OXF. These were incubated under anaerobic conditions in a shaking water-bath at 37° for 60 and 360 min. Blank samples were prepared with boiled (100° for 60 min) ruminal fluids.

Hepatic subcellular fraction assays. Samples of liver tissue from five albino rats (Wistar, males, 250.00 g mean body weight), five horses (cross breed, males and females, 480 kg m.b.wt), five cattle (Limousine, males and females, 600 kg m.b.wt), seven sheep (Italian breed Bergamasca, males, 15 kg m.b.wt), four pigs (cross breed, males, 12.0 kg m.b.wt), eight chickens (Hubbard, females, 0.85 kg m.b.wt) and twelve rainbow trout (*Salmo gairdneri*, 0.45 kg m.b.wt) were collected immediately after slaughter.

Because of the small size of chicken and trout livers, in each case four pooled samples were prepared from the combined tissues of two or three animals respectively. After weighing, the tissue was cut into small pieces with scissors, washed three times with ice-cold 1.15% KCl (to remove haemoglobin), suspended in 2 vol. of a 0.1 M phosphate buffer (pH 7.45) and 1.15% KCl solution (1:1, v:v) and finally homogenized (in an Elvehjem Potter apparatus with a Teflon pestle) for 30 sec.

The homogenate was centrifuged (4°) at 9000 *g* for 30 min; the supernatant was centrifuged again at 105,000 *g* to obtain microsome and cytosol fractions.

The fractions were stored under liquid N_2 pending metabolic assay (within 1 month). For the assay they were defrosted at 37° (trout samples were also defrosted at 15°) and suspended in 0.1 M, pH 7.2, phosphate buffer to give a protein concentration (biuret method [16]) of about 4 mg·ml^{-1}. The microsome fractions were checked for purity using the method of Omura *et al.* [17] which assayed cytochrome P-450 concentration.

The metabolic assays were carried out in aerobic conditions (O_2 atmospheric pressure) by incubating in a shaking water-bath, for 15 and 30 min respectively, 2.0 mg of protein (either microsomal or cytosol), 0.0896 μmoles of FBT with either 1.2 μmoles of NADPH (microsomes) or NADH (cytosol), 2.5 μmoles of $MgCl_2$, 3.1 μmoles of $FeCl_2$ (according to Douch and Buchanan [18]) and phosphate buffer (pH = 7.2) up to a final volume of 1 ml.

Blank samples containing either a single substance or all four substances under study (but in the absence of any cofactor) were prepared following the same procedure and extracted just before and immediately after incubation, to serve as recovery controls and to check for the possible occurrence of spontaneous reactions.

Extraction and analyses. After incubation, the samples were extracted twice with 10 ml ether for 10 min and the combined organic phases were evaporated to dryness under N_2.

Each residue obtained was dissolved in 1 ml of DMF and analyzed (double or triple replications) in a Perkin Elmer series 4 HPLC system. FBT, F.SUL, FBZ and OXF were detected according to the method of Delatour *et al.* [19] modified as follows: column: 100 RP 8 (Merck), 5 μm, length 12.5 cm, diameter 0.4 cm equipped with a Hibar precolumn (Merck), 10 μm, length 2.5 cm, diameter 0.4 cm; injection volume: 20 μl; solvents: (A) acetonitrile, (B) AnalaR water + 0.1% (v:v) orthophosphoric acid (85%); elution mixture: 7 min linear gradient from 75% to 30% B followed by an isocratic elution at the final concentration for 3 further min; detector:

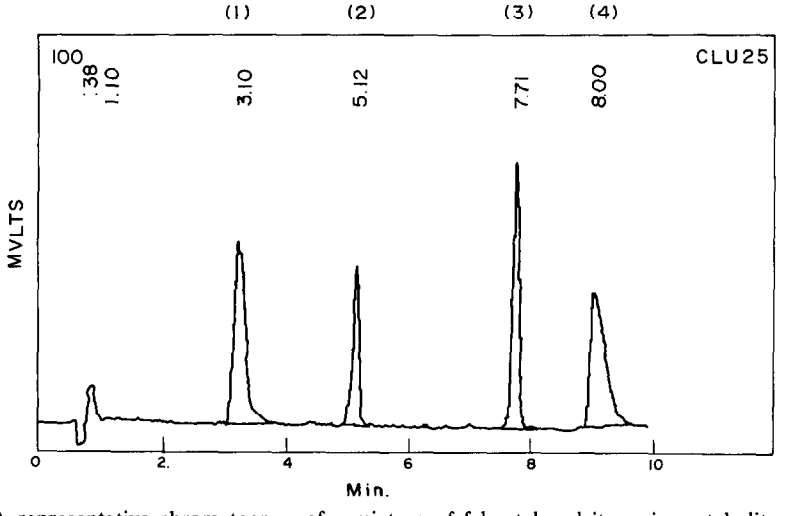

Fig. 1. A representative chromatogram of a mixture of febantel and its main metabolites: (1) fenbendazole sulphoxide, i.e. oxfendazole (OXF); (2) febantel sulphoxide (F.SUL); (3) febantel (FBT); (4) fenbendazole (FBZ). Detection limit: <50 pg.

Table 1. Incubation of FBT with cattle and sheep ruminal fluids

Ruminal fluid	Incubation time (min)	% consumption of FBT added to r.f.	Products (as % of consumed FBT) F.SUL	FBZ	OXF
Cattle	60	4.29	75.00	25.00	0.00
	360	9.49	100.00	0.00	0.00
Sheep	60	10.95	88.36	8.33	3.31
	360	7.46	31.72	68.20	0.00

The figures represent the mean values of three replications and have been calculated as referred to 1 mg of protein of the biological fluids.

LC 95 (Perkin Elmer) at 290 nm; flow: 2 ml·min^{-1}; detection limit: <50 pg. The quantitative determinations were always carried out using three point reference curves.

RESULTS

A representative chromatogram of a standard mixture of OXF, F.SUL, FBT and FBZ is exhibited in Fig. 1.

The retention times for these substances were 186 ± 3, 305 ± 6, 462 ± 6 and 535 ± 15 sec respectively. It was observed that FBZ retention time decreased (with a maximum variation of 3%) with increasing concentration.

The mean extraction recoveries (±SE) were as follows: OXF (N = 9) 87.38 ± 3.25; F.SUL (N = 9) 80.03 ± 3.11; FBT (N = 8) 89.82 ± 2.33 and FBZ (N = 9) 91.23 ± 2.36% as calculated in preliminary tests. Boiled ruminal fluids lost both their oxidizing and reducing properties but retained some ability to cyclize F.SUL into OXF.

Preliminary assays performed using the above methods revealed that (a) microsomes were metabolically active only in the presence of NADPH, and cytosol only with NADH, (b) no spontaneous reactions ever occurred, and (c) by doubling or halving protein and cofactor concentrations the reaction rates showed linear correlations (less than 10% variations).

Cattle and sheep ruminal fluids (r.f.)

FBT was chemically modified to only a slight extent by sheep and cattle r.f.; a maximum of 10% was altered after incubation for 360 min. Modification occurred at a faster rate in sheep but at a slowly increasing rate in cattle (Table 1).

Cattle r.f. was able to cyclize FBT to FBZ and to oxidize it to F.SUL, but was not able to produce OXF (Table 1). Sheep r.f. revealed higher sulphoxidative capacities with respect to both F.SUL and OXF production together with important and slowly increasing ability to cyclize FBT to FBZ (Table 1).

During the long incubation (360 min) F.SUL transformation (Table 2) was greater in cattle (about 40%) than in sheep r.f. (about 28%). This transformation was directed mainly towards FBT production (92% and 73% of consumed F.SUL in cattle and sheep respectively) while FBZ production reached a maximum concentration at 60 min (26%) in sheep followed by a gradual reduction.

OXF production reached levels of less than 10% of the incubated F.SUL at 60 min (7% in cattle and about 9% in sheep) and decreased to lower values during the following 5 hr of incubation (Table 2). Results with boiled r.f. suggest this might be due to a spontaneous cyclization.

Upon incubation of FBZ and OXF with sheep and cattle r.f. it was observed that: (a) in cattle steady state concentrations of FBZ and OXF were obtained after incubation with FBZ for 60 min. But when OXF was the initial substance the reverse reaction occurred to a much greater extent, but a steady state had not been reached after 360 min, thus revealing a more potent reducing activity than oxidizing activity (Table 3). (b) Sheep r.f. was less active in reducing OXF to FBZ but more potent in bringing about the initial oxidation of FBZ to OXF (by a factor of 4 at 60 min).

Hepatic microsome preparations

The mean values (±SE) for FBT consumption by hepatic microsomal preparations from the animal species examined are represented in Fig. 2.

Table 2. Incubation of F.SUL with sheep and cattle ruminal fluids

Ruminal fluid	Incubation time (min)	% consumption of F.SUL added to r.f.	Products (as % of consumed FBT) FBT	FBZ	OXF
Cattle	60	25.74	72.49	19.97	7.54
	360	39.03	92.08	6.53	1.39
Sheep	60	12.99	73.28	17.68	9.04
	360	27.79	73.45	26.55	0.00

The figures represent the mean values of three replications and have been calculated as referred to 1 mg of protein of the biological fluids.

Table 3. Incubation of FBZ or OXF with cattle or sheep ruminal fluids

Ruminal fluid	Incubated with (dose = 100)	Found (as % of the added compound) at 60 min	360 min
Cattle	FBZ = 100	94.72	93.44
	OXF = 0	5.28	6.56
	OXF = 100	59.80	43.65
	FBZ = 0	40.20	56.36
Sheep	FBZ = 100	80.39	99.25
	OXF = 0	19.61	0.75
	OXF = 100	85.69	65.61
	FBZ = 0	14.31	34.39

The figures represent the mean values of three replications and have been calculated as referred to 1 mg of protein of the biological fluids.

The histograms clearly show that sheep preparations exhibited the strongest consumption activity, being, for example, 10 times (at 15 min) and 5 times (at 30 min) more active than cattle. Other significant differences between the species were observed: rapid attainment of steady-state concentrations in rats, pigs, sheep and trout with slow and time-dependent reactions in horses, cattle and poultry.

The fate of metabolized FBT, as determined by assays for its cyclized, sulphoxidized and/or cyclized-sulphoxidized or sulphoxidized-cyclized metabolites (FBZ, F.SUL and OXF respectively) is summarized in Figs 3–5.

The data in Fig. 3 show that pig hepatic microsomes displayed the highest cyclizing activity (as ng of FBZ produced/mg of microsomal protein) followed by those of trout (at both assay temperatures), sheep, poultry, cattle, horse and rat.

Since the amounts of F.SUL and OXF produced following incubation of FBT with microsomes have been expressed in ng of F.SUL and OXF/nmole of cytochrome P-450, a table (Table 4) has been included to list the mean values (±SE) of cytochrome P-450 found in the microsomal preparations.

Oxidation of FBT into F.SUL increased with time in horses, cattle, sheep and pigs. Quantitatively this metabolic pathway was of major importance in sheep and of decreasing importance respectively in poultry, pigs, horses, rats, cattle and trout (at both incubation temperatures) (Fig. 4).

Pig microsomes exhibited the maximum level of OXF production, whereas those from sheep, trout (at 37°), poultry and horse were less active. This last metabolic pathway proved to be time-related in almost all species studied, with the exception of the rat and cattle where it was practically absent (Fig. 5).

Hepatic cytosol preparations

The data reported in Table 5 show that the FBT consuming activities of hepatic cytosol from the different animal species investigated were very low, reaching a maximum level of 3.23% of the amount of FBT incubated for 30 min (37°) in the trout. FBT consumption by hepatic cytosol appeared moreover to be definitely time-related in trout (37°) and in sheep but not in the other species examined.

As far as F.SUL, FBZ and OXF production is concerned, the results in Table 6 clearly demonstrate

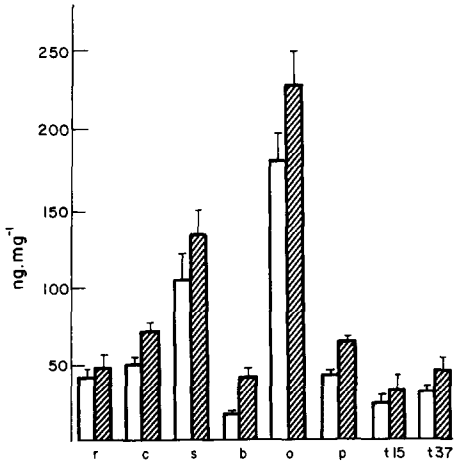

Fig. 2. FBT consumption (ng/mg of microsomal protein) by hepatic microsomes from rats (r), horses (c), pigs (s), cattle (b), sheep (o), chickens (p) and trouts (t). The numbers beside t indicate the two different incubation temperatures (15° and 37°). Data are expressed as mean values (±SE). White columns: at 15 min; hatched columns: at 30 min.

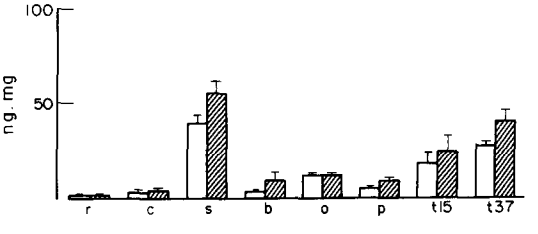

Fig. 3. Production of FBZ (ng/mg of microsomal protein) from incubated FBT by hepatic microsomes from rats (r), horses (c), pigs (s), cattle (b), sheep (o), chickens (p) and trouts (t). The figures beside t indicate the two different incubation temperatures examined. Data are expressed as mean values (±SE). White columns: at 15 min; hatched columns: at 30 min.

Table 4. Cytochrome P-450 found in hepatic microsomes from the different species examined

Species	nmoles Cytochrome P-450/ mg protein
Rat (N = 5)	0.582 ± 0.148
Horse (N = 5)	0.446 ± 0.026
Pig (N = 4)	0.342 ± 0.021
Cattle (N = 5)	0.421 ± 0.106
Sheep (N = 7)	0.599 ± 0.043
Poultry (N = 5)	0.249 ± 0.009
Trout (N = 4)	0.226 ± 0.020

Data are expressed as mean values (±SE). The figures within the brackets indicate the number (N) of replications performed.

that the cytosol from none of the investigated species was able to generate OXF from incubated FBT.

Although the levels of F.SUL and FBZ detected (Table 6) were very low, horse, sheep and trout cytosol (15°) exerted their main FBT metabolizing activity in the direction of F.SUL, while those from the pig and trout (37°) did so in the direction of FBZ production. The FBT cyclizing activity exhibited by trout cytosol at 37° appeared moreover to be time-related.

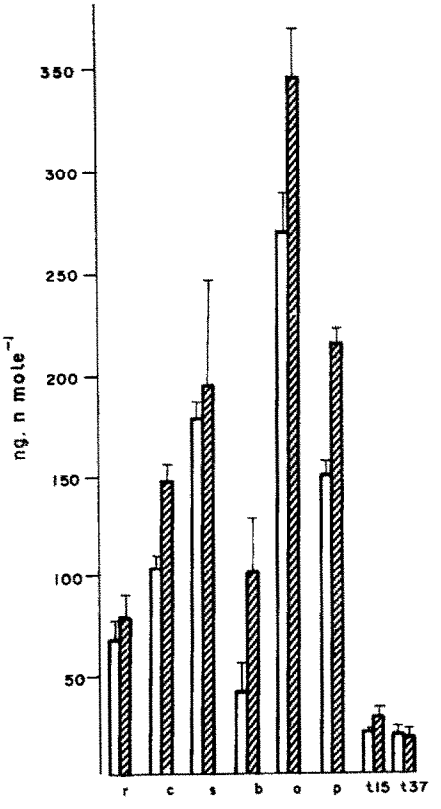

Fig. 4. Production of F.SUL (ng/nmoles of microsomal cytochrome P-450) from incubated FBT by hepatic microsomes from rats (r), horses (c), pigs (s), cattle (b), sheep (o), chickens (p) and trouts (t). The figures next to t indicate two different incubation temperatures. Data are expressed as mean values (±SE). White columns: at 15 min; hatched columns: at 30 min.

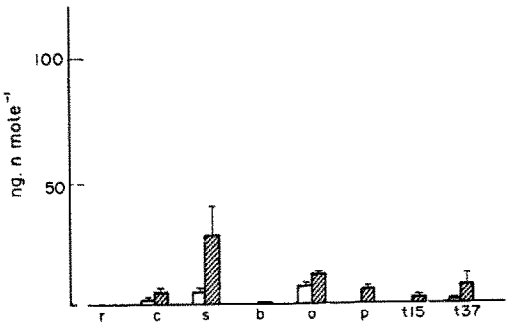

Fig. 5. Production of OXF (ng/nmoles of microsomal cytochrome P-450) from incubated FBT by hepatic microsomes from rats (r), horses (c), pigs (s), cattle (b), sheep (o), chickens (p) and trouts (t). The figures beside t indicate the two different incubation temperatures examined. Data are expressed as mean values (±SE). White columns: at 15 min; hatched columns: at 30 min.

DISCUSSION

The fate of probenzimidazole drugs such as FBT, thiophanate as well as Netobimin [20], which are believed to generate benzimidazole derivatives through metabolic or other reactions within the animal body, remains incompletely known but is germane to the prediction of the anthelmintic efficacy and the teratogenic properties of these drugs.

The experimental results obtained by investigating the *in vitro* chemical modifications of FBT incubated with sheep and cattle ruminal fluid have revealed that these transformations were very slight under the experimental conditions employed, and were mainly directed to F.SUL production in cattle and to an early F.SUL production succeeded by a significant shift towards FBZ production in sheep.

The amounts of OXF produced were very low (sheep at 60 min) or undetectable (cattle).

The levels of consumption of F.SUL incubated with both cattle and sheep ruminal fluids were greater than those of FBT. F.SUL was reduced back to FBT or transformed to FBZ, but its transformation to OXF was always less than 10%.

It is concluded that sheep ruminal fluid exhibits a greater cyclizing activity than cattle but that this activity is exerted preferentially on FBT rather than on F.SUL. The oxidation of FBZ into OXF by both ruminal fluids never exceeded 20% of incubated FBZ, while reduction of OXF into FBZ occurred to a greater extent in both cattle (about 56%) and in sheep (about 34%).

Whenever the presence of OXF was detected (by incubating either FBT or F.SUL or FBZ) it was observed that its production occurred rapidly and was followed by a decay due to a further oxidation, either towards fenbendazole sulphone (detected but not measured) or to a backward reduction to FBZ.

The experiments performed by incubating OXF or FBZ with the ruminal fluids showed lastly that these biological fluids were able both to oxidize and reduce these benzimidazole molecules and that their reducing activity was greater than their oxidizing activity.

The metabolic transformations undergone by

Table 5. FBT consumption (ng/mg of cytosolic protein) when incubated for 15 and 30 min with hepatic cytosol from different animal species under the conditions described

Incubation time (min)	Animal species							
	Rat	Horse	Pig	Cattle	Sheep	Poultry	Trout (15°)	Trout (37°)
15	0.92 ± 1.037 (0.23%)	2.43 ± 0.493 (0.61%)	4.32 ± 0.500 (1.08%)	3.13 ± 0.559 (0.78%)	6.72 ± 0.670 (1.68%)	1.60 ± 0.387 (0.40%)	8.28 ± 1.150 (2.07%)	6.56 ± 0.535 (1.64%)
30	0.21 ± 1.340 (0.05%)	2.10 ± 0.356 (0.53%)	7.32 ± 1.760 (1.83%)	2.24 ± 0.722 (0.56%)	10.77 ± 1.980 (2.69%)	2.93 ± 0.582 (0.73%)	8.72 ± 1.420 (2.18%)	12.74 ± 1.011* (3.23%)

Data are expressed as mean values (±SE). The figures within the brackets indicate the % values as related to the starting amount of incubated FBT considered = 100.

* 15 versus 30 min comparison significant by the Student *t*-test: $P < 0.01$.

Table 6. F.SUL, FBZ and OXF productions (ng/mg of protein) by hepatic cytosol from different animal species after incubation of FBT for 15 and 30 min under the conditions described

Incubation time (min)	Metabolites	Animal species							
		Rat	Horse	Pig	Cattle	Sheep	Poultry	Trout (15°)	Trout (37°)
15	F.SUL	0.06 ± 0.06	2.15 ± 0.31	0.00	0.98 ± 0.98	4.25 ± 1.05	1.60 ± 0.39	5.96 ± 0.72	0.56 ± 0.45
	FBZ	0.86 ± 1.03	0.29 ± 0.18	4.32 ± 0.50	2.15 ± 0.70	2.48 ± 0.50	0.00	2.32 ± 1.09	5.97 ± 0.54
	OXF	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	F.SUL	0.00	2.10 ± 0.36	0.00	0.00	5.16 ± 0.74	1.97 ± 0.21	6.50 ± 0.47	0.96 ± 0.41
	FBZ	0.21 ± 0.04	0.00	7.32 ± 1.76	2.24 ± 0.72	5.61 ± 1.24	0.97 ± 0.63	2.22 ± 0.97	11.79 ± 0.71
	OXF	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Data are expressed as means values (±SE).

FBT, when incubated with liver subcellular fractions, were different in the various species investigated, in the experimental *in vitro* conditions reported here.

The results indicate first of all (Table 5) that FBT metabolizing activity by hepatic cytosol is negligible in all animal species investigated, except sheep and trout (at both 15° and 37°).

It is believed in fact that this low activity may be due to the presence of small amounts of microsomal residues. This suggestion is reasonable not only for sheep but also for horse hepatic cytosol because of their F.SUL producing activity from FBT, while in all other cases the small amounts of FBT consumed were always directed towards FBZ production through cyclizing reactions unattributable to microsomal monooxygenases.

Trout hepatic cytosol behaves quite differently, suggesting the existence of a true cytosol oxidase function, very active at 15° (towards F.SUL production), in addition to an FBT cyclizing function (towards FBZ production) whose activity proved to be temperature-dependent (Table 6).

The FBT metabolizing activities by hepatic microsomes from different animal species were examined under fixed protein concentrations in order to standardize the assays, but which were inconsistent with the natural protein content of liver microsomes from the different species investigated [21, 22]. Under these conditions sheep microsomes showed the greater effect: twice as great as pigs, 3–4 times greater than horses and poultry, 5–8 times greater than cattle and trout.

A thorough investigation of these FBT metabolizing activities revealed that in pig microsomes the cyclizing pathway leading to a FBZ production predominated: a predominance exhibited, though in a lesser degree and in a temperature-dependent way, also by trout microsomes. In the remaining species investigated (i.e. sheep, cattle, poultry and horses), the FBT to FBZ reaction was either negligible or took place to very small extent (e.g. rats) (Fig. 3). Sulphoxidation of FBT to F.SUL occurred very intensely in sheep and actively in poultry and pig, and was less important in horse, cattle and rat microsomes. Trout microsomes were practically lacking in this last activity (Fig. 5).

OXF production from FBT (Fig. 6) was remarkable in pig, noticeable in sheep, slight in horse, poultry and trout and negligible in cattle and rat microsomes.

An important fact emerging from these studies is that the sulphoxidative reactions producing F.SUL or OXF (subsequent to FBZ production) occur to widely different extents in the different animal species investigated.

The oxidative pathway leading to OXF seems to be consistent with the amount of FBZ previously generated from the incubated FBT. Only a direct incubation of FBZ with hepatic microsomes from the various species examined (presently being investigated) could, however, substantiate this suggestion. The considerable amounts of F.SUL detected following microsomal incubation with FBT were not related to the cytochrome P-450 content of the preparations (Table 5). This may be explained by two different hypotheses: either (a) the monooxygenase-dependent sulphoxidations vary in the different species according to species-specific isozymes, or (b) in some animal species this pathway is not mediated by cytochrome P-450 but is catalyzed by NADPH-dependent FAD oxygenases, in agreement also with a recent suggestion in the literature [10].

Finally the present results suggest that chemical modification of FBT by sheep and cattle ruminal fluids is insignificant and that the liver microsomes of the different animal species studied revealed important differences, both qualitatively and quantitatively in their modes of metabolizing these drugs.

This last point is reinforced by the observation that there are differences in the natural estate of animal livers with respect to their microsomal protein, cytochrome P-450 [21, 22] and their FAD oxygenase contents, and which were intentionally neglected in our experiments.

The qualitative and quantitative variations revealed by the present comparative investigations suggest that the recommended therapeutic doses of FBT deserve a revision in order to avoid under- or overdosage in the different animal species to be treated and suggest moreover that its teratogenic hazard cannot be inferred by the usual teratogenic assays carried out in rats.

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