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Pharmacokinetics of an anthelmintic potential prodrug of a new benzimidazole in gerbil, mouse and sheep

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Summary

N-Methoxycarbonyl-*N'*-2-nitro-4-trifluoromethyl phenyl thiourea (**1**) possesses a good activity against *Echinococcus multilocularis* metacestodes in gerbil. This compound has been synthesized as a potential prodrug of benzimidazole carbamate (**2**). Compound **2** is less active against the parasite. The pharmacokinetics of **1** and **2** have been investigated in sheep, gerbil and mouse. The results indicate that **1** is actually metabolized into **2**. Its metabolization is not complete in rodents. Plasma levels of **2** after its oral administration to gerbils are theoretically high enough for therapeutic action of a benzimidazole compound, but thiourea **1** could also possess a specific activity.

Introduction

Benzimidazoles, especially mebendazole (methyl 5-(6)-benzoylbenzimidazol-2-yl-carbamate), are clinically used against the causative agent of alveolar hydatid disease (AHD), *Echinococcus multilocularis* metacestodes. Long-term treatment and high oral doses are only partly effective, possibly due to the rather poor absorption from the intestinal tract and to rapid inactivation of the drug (Wittassek and Bircher, 1983).

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In order to increase plasma concentrations of active drugs, prodrugs have often been investigated (Douch, 1974; Hennessy et al., 1983; Delatour et al., 1985, 1988). *N*-Methoxycarbonyl-*N'*-2-nitro-4-trifluoromethyl phenyl thiourea **1** has been synthesized as a potential prodrug of a new benzimidazole derivative: methyl-5(6)-trifluoromethylbenzimidazol-2-yl-carbamate **2** (Walchshofer et al., 1990). Compound **1** may undergo cyclization into benzimidazole **2** after reduction of the nitro group by microflora of the intestine (Fig. 1), as already described for other compounds (Dawson and Watson, 1983; Delatour et al., 1986).

Significant morphological damage by *E. multilocularis* metacestodes in gerbil (*Meriones unguiculatus*), a highly susceptible host, was observed after treatment with compound **1** at a daily oral

dose of 50 mg/kg body weight for 18 days (Walchshofer et al., 1990). Compound **2**, administered under the same conditions, was much less active.

We have evaluated the bioavailability of compound **1** as a prodrug of compound **2**. The pharmacokinetics of **2** in gerbil and of **1** in sheep, gerbil and mouse have been investigated after oral administration.

Materials and Methods

Synthesis

N-Methoxycarbonyl-*N'*-2-nitro-4-trifluoromethyl phenyl thiourea **1** was synthesized as described previously (Walchshofer et al., 1990).

Methyl 5-(6)-trifluoromethylbenzimidazol-2-yl-carbamate **2** was synthesized as follows: To a solution of 6.18 g (0.03 mol) of 4-amino 2-nitrobenzotrifluoride in 150 ml concentrated hydrochloric acid was added carefully 18 g of tin shot. The mixture was refluxed for 2 h and then the precipitate was filtered and washed with diethyl ether to give 6.6 g (88%) of 2,4-diaminobenzotrifluoride dihydrochloride.

A solution of 6.3 g (0.023 mol) of *S*-methylisothiourea sulfate in 40 ml of water was stirred in an ice bath and then 2.36 g (0.025 mol) of methylchloroformate were added. A 10% aqueous solution of sodium hydroxide was added dropwise until pH ~ 8. The mixture was stirred for 20 min at room temperature, after which acetic acid was added until pH ~ 5, followed by the addition of 7.5 g (0.03 mol) of 2,4-diaminobenzotrifluoride dihydrochloride and 1.8 g of sodium acetate. The mixture was refluxed for 2 h. The precipitate was filtered, washed with water, then recrystallized from acetonitrile. Yield, 30%; m.p., > 300 °C with decomposition. IR/KBr (Beckman Acculab 3 Spectrometer) ν (cm^{-1}): 3350, 2750, 1710, 1650. ^{1}H-NMR (Me_2SO, Me_4Si, Varian XL 10080 MHz spectrometer): δ (ppm) = 3.6 (s,3H,OCH_3); 7.1–7.7 (m,3H,ArH). Anal.:Calc. for $C_{10}H_3F_3N_3O_2$; C, 46.33; H, 3.09; N, 16.21; F, 22.00. Found: C, 46.24, H, 3.12; N, 16.30; F, 21.90.

Subjects and treatments

The animals selected for the present study were the following: 30 male OF1 mice 20–22 g body weight, raised in SPF conditions were purchased from the Iffa-Credo colony (Saint-Germain-sur-l'Arbresle, France). 40 gerbils (20 for compound **1** studies and 20 for compound **2** studies) of both sexes, about 30 g body weight, were used. For 1 week before the experiment, mice and gerbils were maintained in plastic cages under controlled temperature (20 °C) and illumination (12 h on/12 h off). Three cross-bred ewes, 2–3 years old, 40–50 kg body weight, were housed in laboratory conditions with hay and water ad libitum.

Single dosages of compound **1** were administered as an aqueous suspension in 10% carboxymethylcellulose at levels of 50 mg kg^{-1} (mice and gerbils) or 20 mg kg^{-1} (sheep). Compound **2** was administered in gerbils under the same conditions at a level of 50 mg kg^{-1}.

Plasma collection

Blood samples were taken by decapitation (mice and gerbils) or from the jugular vein (sheep) at standardized intervals for 9–12 h (rodents) or 48 h (sheep) after dosage. Blood was collected in heparinised tubes, immediately centrifuged and plasma was separated and stored at −18 °C until analyses.

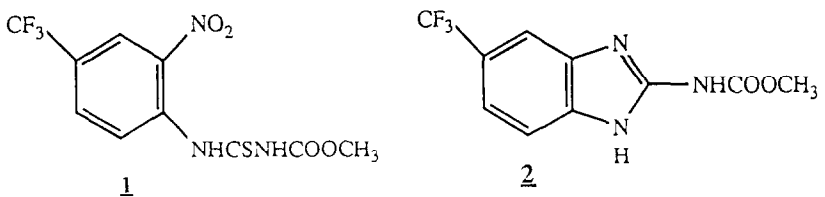

Fig. 1. Molecular structure of compounds **1** and **2**.

Analyses

The plasma concentrations of the parent drug compound **1** and its potential metabolite **2** were determined by high-performance liquid chromatography. The operating conditions were those previously selected for studies concerning similar chemicals (Delatour et al., 1988). Under these conditions, the retention times were 6.5 and 3.9 min for **1** and **2**, respectively.

Results and Discussion

Fig. 2 depicts the plasma profiles of **1** and metabolite **2** after oral administration to gerbils, mice and sheep.

A significant amount of prodrug **1** appears rapidly in the blood of gerbils. The amount of benzimidazole was found to be sufficient for a therapeutic action of **2** (Fig. 3): in infected gerbils, mebendazole plasma levels above 7.4×10^{-2} μg were associated with significant decrease in parasite weights (Van den Bossche et al., 1982).

The disposition of the prodrug in mouse is similar to that in gerbils. The oral standard bioavailability, expressed by the sum of the areas under the curve vs time (AUC) of **1** + **2**, divided by the dose (mg kg^{-1}) is 1.81 and 1.95 μg ml^{-1} h in gerbils and mice, respectively. In contrast, the standard bioavailability of only the benzimidazole metabolite is 0.45 μg ml^{-1} h in sheep plasma. The metabolic capability to change the prodrug **1**

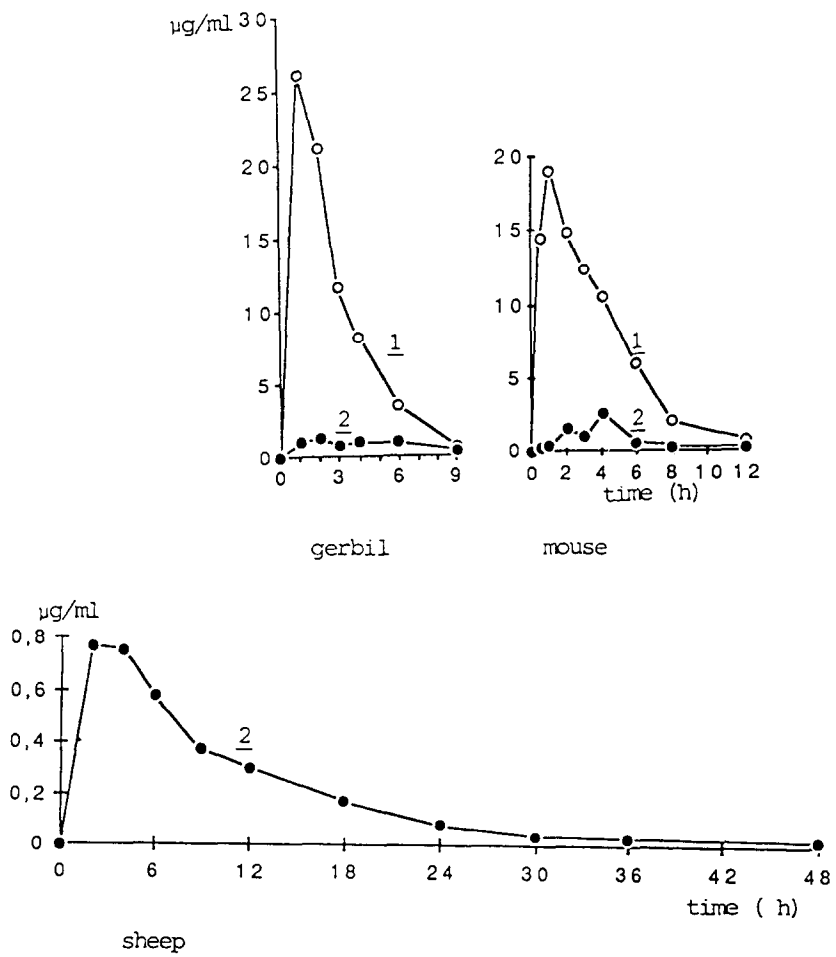

Fig. 2. Plasma levels vs time of **1** and **2** formed from it after oral administration of **1** to gerbils (50 mg/kg), mice (50 mg/kg) and sheep (20 mg/kg).

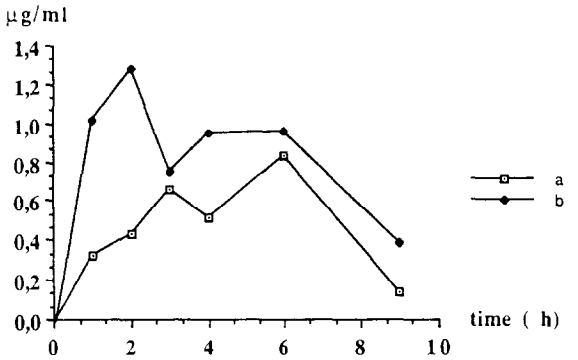

Fig. 3. Plasma levels vs time of **2** after oral administration of (a) **2**, (b) **1** to gerbils (50 mg/kg).

into the benzimidazole metabolite **2** differs from one animal species to another. The ratio AUC of **2**/AUC of **1** is 0.12 in mice and 0.10 in gerbils, while it is not calculable in sheep. Sheep rumen microflora is different from that of rodent intestinal tract; the nitro moiety of prodrug **1** could be reduced more readily in sheep than in rodents, a phenomenon described previously (Delatour et al., 1986) with another nitro prodrug, netobimin.

Fig. 3 shows the plasma levels of **2** in both metabolic situations. There is an increase in the area under the benzimidazole curve when gerbils are dosed with prodrug (AUC = 5.90 μg ml^{-1} h) compared with the corresponding area under the curve when animals are dosed with the benzimidazole (AUC = 3.34 μg ml^{-1} h), which suggests that the prodrug has greater bioavailability than benzimidazole.

The results of these studies indicate that compound **1** is actually metabolized to give benzimidazole **2**, but that this metabolization is not complete in rodents. Activity of **1** against *E. multilocularis* in gerbils could result from the activity of the benzimidazole: plasma levels of **2** after its oral administration are theoretically high enough for a therapeutic action (Van den Bossche et al., 1982). However, morphological damage by *E. multilocularis* in infected gerbils after administration of **2** is much less extensive than that observed after administration of **1** under the same conditions: nitrophenylthioureas like **1** could possess a specific activity against *E. multilocularis* metacestodes.

References

Dawson, M. and Watson, T.R., 4-Amino-3-(3'-methoxycarbonyl-2'-thioureido) Benzophenone, a prodrug of mebendazole. *Eur. J. Drug Metab. Pharmacokinet.*, 8 (1983) 329–334.

Delatour, P., Besse, S. and Romdane, M.N., Pharmacokinetics and anti-dicrocoelium effect of thiophanate and its major metabolite in ruminants. *Ann. Rech. Vet.*, 19 (1988) 119–122.

Delatour, P., Cure, M.C., Benoit, E. and Garnier, F., Netobimin (totabin-SCH): preliminary investigations on metabolism and pharmacology. *J. Vet. Pharmacol. Ther.*, 9 (1986) 230–239.

Delatour, P., Tiberghein, M.P., Garnier, F. and Benoit, E., Comparative pharmacokinetics of febantel and its metabolites in sheep and cattle. *Am. J. Vet. Res.*, 46 (1985) 1399–1402.

Douch, P.G.C., Metabolism of thioureidobenzene fungicides in mice and sheep. *Xenobiotica*, 4 (1974) 457–475.

Hennessy, D.R., Lacey, E. and Prichard, R.K., Pharmacokinetic behaviour and anthelmintic efficacy of 1-n-butylcarbamoyl oxfendazole given by intramuscular injection. *Vet. Res. Commun.*, 6 (1983) 177–187.

Van den Bossche, H., Rochette, F. and Horig, C., Mebendazole and related anthelmintics. *Adv. Pharmacol. Chemother.*, 19 (1982) 67–128.

Walchshofer, N., Delabre-Defayolle, I., Paris, J. and Petavy, A.F., In vivo morphological damage induced by a new benzimidazole prodrug in *Echinococcus multilocularis* metacestodes. *J. Pharm. Sci.*, 79 (1990) 606–608.

Wittasseck, F. and Bircher, J., Chemotherapy of larval echinococcosis with mebendazole: microsomal liver function and cholestasis as determinants of plasma drug level. *Eur. J. Clin. Pharmacol.*, 25 (1983) 85–90.