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Species Differences in the Generation of the Chiral Sulfoxide Metabolite of Albendazole in Sheep and Rats

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ABSTRACT The prochiral anthelmintic drug albendazole was administered orally to sheep and rats. Blood samples were taken at standardized intervals during the time course of the plasma kinetics: 18 h in rats and 48 h in sheep. The enantiomeric ratio of the sulfoxide metabolite was determined by means of HPLC on a chiral stationary phase, the chiral selector of which was a *N*-3,5-dinitrobenzoyl derivative of (S)-tyrosine. Two enantiomers were detected in both animal species but their ratios were inverted in rat vs. sheep. The evolution of the ratio is turned from a racemate at 15 min to 60(−):40(+) at 12 h in rats, while it moved from 23(−):77(+) at 3 h to 4(−):96(+) at 36 h after administration in sheep.

KEY WORDS: enantiomeric separation, sulfoxides, albendazole, metabolism, rat, sheep

INTRODUCTION

Chiral aspects in molecular structure and metabolism are of great concern about the activity and toxicity of drugs.[3,20] All steps of the pharmacokinetics in animal organisms have been described as possibly enantiodependent: intestinal absorption, binding to plasma protein and receptors, biotransformations, and clearances.

Albendazole (ABZ), or (5-(propylthio)-1*H*-benzimidazol-2-yl) carbamic acid methyl ester (MW: 265), is a broad spectrum anthelmintic widely used in domestic animals and man. The ABZ metabolism has been described[11,17] showing that in all species the main metabolites (Fig. 1) are the corresponding sulfoxide (SO.ABZ) and sulfone (SO_2ABZ). The former is considered to be responsible for the activity and the embryotoxicity[6] of the drug.

Rats and sheep are the most sensitive species to the teratogenicity of ABZ and they exhibit a similar pharmacokinetic profile. For these reasons, it is interesting to compare for these animals the chirality of the active sulfoxide metabolite of the prochiral sulfide ABZ.

EXPERIMENTAL

Animals and Treatments

Sprague–Dawley male rats, 140–160 g body weight, raised in specific pathogen-free conditions, were purchased from the IFFA-Credo colony. They were dosed orally (8 h AM) by gastric intubation with an aqueous suspension at the level of 10 mg ABZ/kg. Groups of six animals were sacrificed at the following times: 15 min, 30 min, 1, 2, 3, 6, 9, 12, and 18 h after dosing.

Three clinically healthy cross-bred 1 year old ewes, 40–50 kg body weight, maintained in laboratory conditions with hay and water ad libitum were selected for the study. They were dosed with a 1.9% drench (Valbazen®) at the oral dosage of 5.0 mg ABZ/kg. Blood samples were taken from the jugular vein at 3, 6, 9, 12, 18, 24, 30, 36, and 48 h postdose.

Rat and sheep blood samples were collected into evacuated heparinised tubes and immediately centrifuged. The plasma were separated and stored at −18°C until analysis.

Chromatographic Analysis

Pharmacokinetic profiles

Aliquots of 200 μl of plasma were percolated on cassettes of the "Advanced Automated Sample Processor" (AASP) Varian and subjected to chromatographic analysis on a C_{18} column according to the conditions previously described.[7] In this way, the simultaneous quantification of ABZ and its metabolites SO.ABZ and SO_2ABZ was obtained.

Enantiomeric ratio of SO.ABZ vs. time

Plasma samples of 1–6 ml, depending on SO.ABZ concentration previously determined, were extracted by diethyl oxide, which was subsequently evaporated

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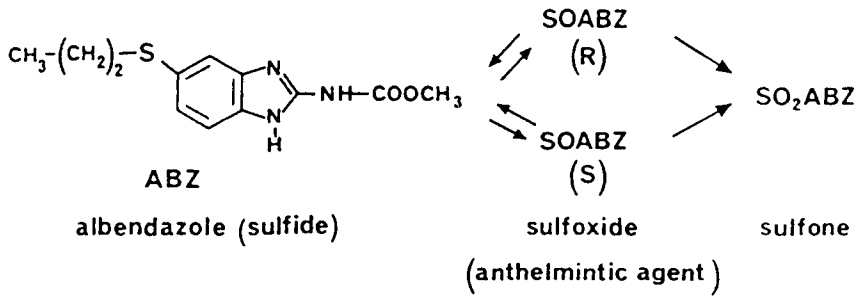

Fig. 1. Schematic metabolic pathway after oral administration of ABZ to rats and sheep.

to dryness under a nitrogen stream at 45°C. This crude extract was then percolated on a cassette of the AASP and chromatographed with the same procedure as described previously for the pharmacokinetic study; the eluates (2–3 ml) corresponding to SO.ABZ (RT: 7.10 min) were collected. The SO.ABZ contained in this fraction was again extracted with diethyl oxide as described above. Finally, the purified residue was dissolved into 40 μl of UV grade methanol. An aliquot (20 μl) was injected onto a chiral column (25 × 0.46 cm i.d.) packed with the (S)-*N*-(3,5-dinitrobenzoyl) tyrosine-*O*-(2-propen-1-yl) *n*-butylamide bonded silica (5 μm)[19] [(S)-thio-DNBTyr-A]. Solvent: hexane:ethanol (90:10 v/v); flow rate: 2 ml/min; detection: UV at 296 nm.

The representative parameters of the chromatograms obtained (Fig. 2) are as follows: the retention times of (−)SO.ABZ and (+)SO.ABZ are 23 and 26 min, respectively. Separation factors $\alpha = 1.12$; resolution factor $R_s = 1.39$. An integrator (HP 3393 A) provided the relative proportions (%) of both peaks directly.

The signs of rotary power have been determined by coupling the chiral column with an optical rotation detector, Chira Monitor, ACS Ltd. (Fig. 3).

RESULTS AND DISCUSSION

The chiral resolution of sulfoxides has been already described. The chiral phases (CSPs) used are either protein immobilized on silica gel such as BSA[1,2] and glycoproteins[13,15] or classical Pirkle type CSP such as (R)-DNBPG.[16,21] Unfortunately, with SO.ABZ, the latter CSP entails no separation. On the other hand, the (S)-thio-DNBTyr-A developed by some of us[19] gives a satisfactory separation[14] ($\alpha = 1.12$, $R_s = 1.39$).

From the raw data obtained in rats and sheep, the following graphs, including means and standard deviations, are obtained: the pharmacokinetics of the plasma metabolites (Fig. 4), the evolution vs. time of

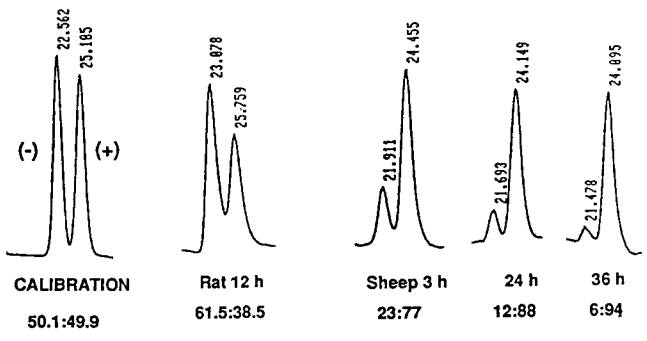

Fig. 2. Chromatograms showing the ratio of the enantiomers of SO.ABZ in rats and sheep after an oral administration of ABZ. Column: 25 cm × 4.6 mm i.d. Stationary phase: (S)-thio-DNBTyr-A. Operating conditions: mobil phase, hexane–ethanol 90–10 (v/v); flow rate, 2 ml/min; temperature, 20°C; UV detection at 296 nm. Injection volume 20 μl.

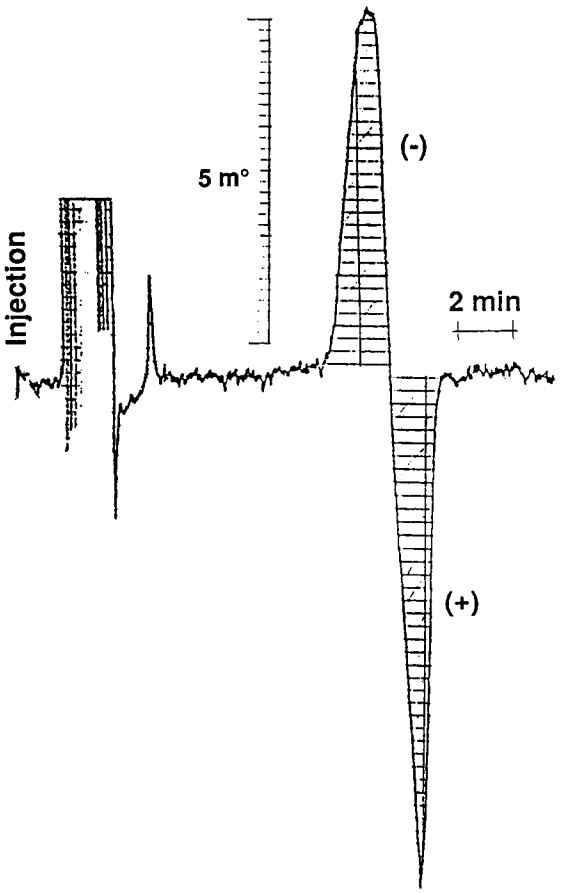

Fig. 3. Separation of SO.ABZ and optical rotation detector (Chira Monitor). Column: 15 cm × 4.6 mm i.d. Stationary phase: (S)-thio-DNBTyrA bonded on spherical silica (Nucleosil 50–5 μm). Operating conditions: mobil phase: hexane–ethanol 80–20 v/v; flow rate, 1 ml/min; temperature, 20°C; optical rotation is measured at 820 nm (Chira Monitor). Injection: 20 μl corresponding to 1 mg of racemate [50 mg of SO.ABZ was dissolved in 1 ml of the chloroform–methanol mixture (50–50 v/v)].

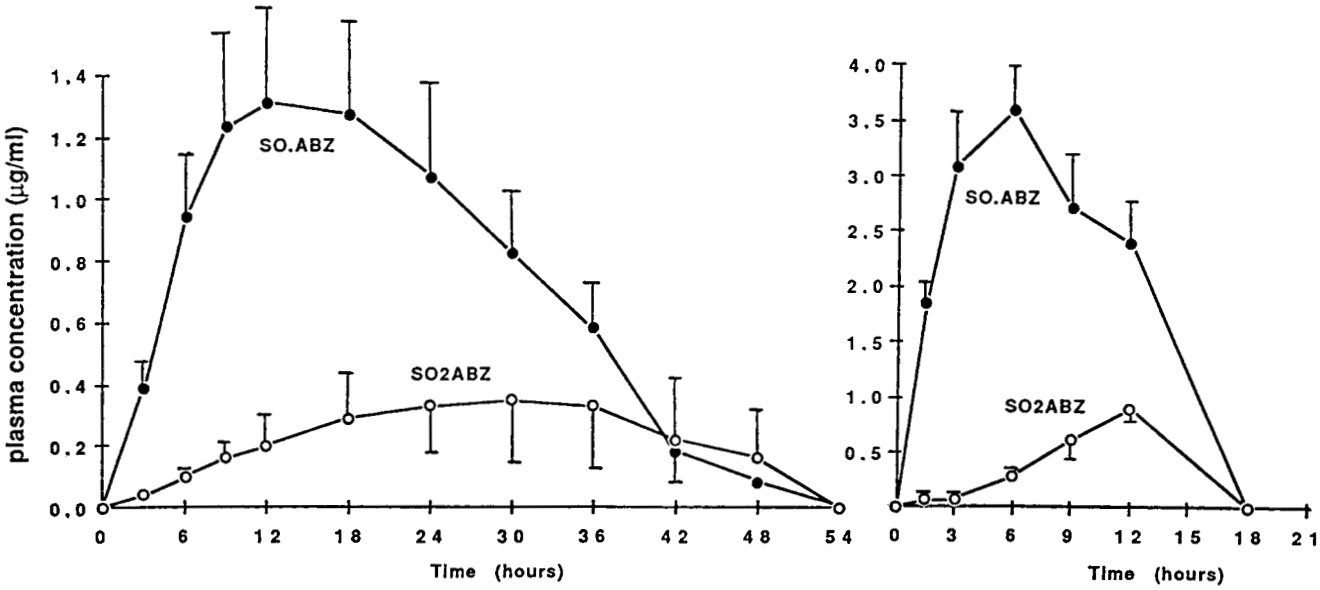

Fig. 4. Pharmacokinetics of ABZ (metabolites SO.ABZ and SO_2ABZ) after an oral administration to sheep (left) and rats (right).

the enantiomeric proportions (Fig. 5), and the evolution vs. time of the plasma concentrations (µg/ml) of individual enantiomers (Fig. 6). Additional details will be provided later elsewhere.[5]

In rat and sheep plasmas, unmodified ABZ has never been detected (<0.01 µg/ml) while both metabolites of S-oxidation have been consistently present for at least 18 h in rats and 48 h in sheep. The ratio of the area under the curve vs. time (AUC) of SO.ABZ/SO_2ABZ is 5.6 in rats and 2.9 in sheep. These figures are consistent with those previously reported.[12,18]

The enantiomers (−) and (+) of SO.ABZ are detected in plasma of both species, but their proportions are different from one animal species to another. Enantiomer (−)SO.ABZ is dominant in rats, while (+)SO.ABZ is in sheep. In addition, the plasma concentration ratio (+)/(−) is not a constant but linearly moves vs. time. In rats, it decreases from 1.0 to 0.6 between 15 min and 12 h postdose, while in sheep it increases from 3.3 to 23.4 between 3 and 36 h postdose. It is very difficult to measure accurately the plasma concentration enantiomeric ratio earlier than 15 min in rats and 3 h in sheep because of the very low plasma concentrations at short times after dosage. For this reason, the enantiomeric ratio at T_0 is not accessible experimentally, but only by extrapolation of the curves. In these conditions, the ratio (+)/(−) at T_0, which is not significantly different to a racemate in rats, seems to be about 75/25 = 3 in sheep. Finally, during the total course of the kinetics, enantiomers (−) and (+)

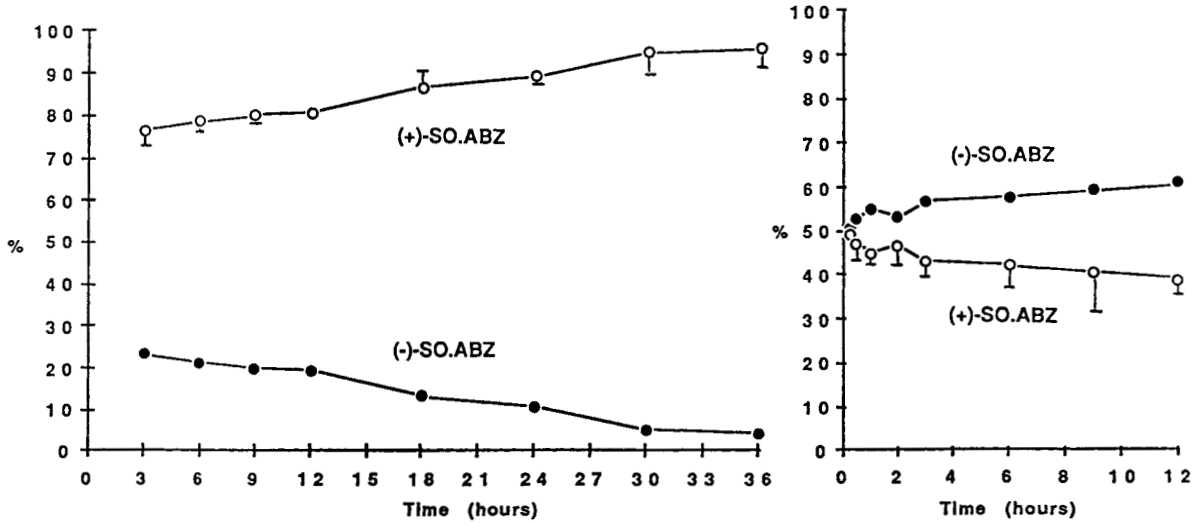

Fig. 5. Percentage vs. time of the enantiomers of metabolite SO.ABZ after an oral administration of ABZ to sheep (left) and rats (right).

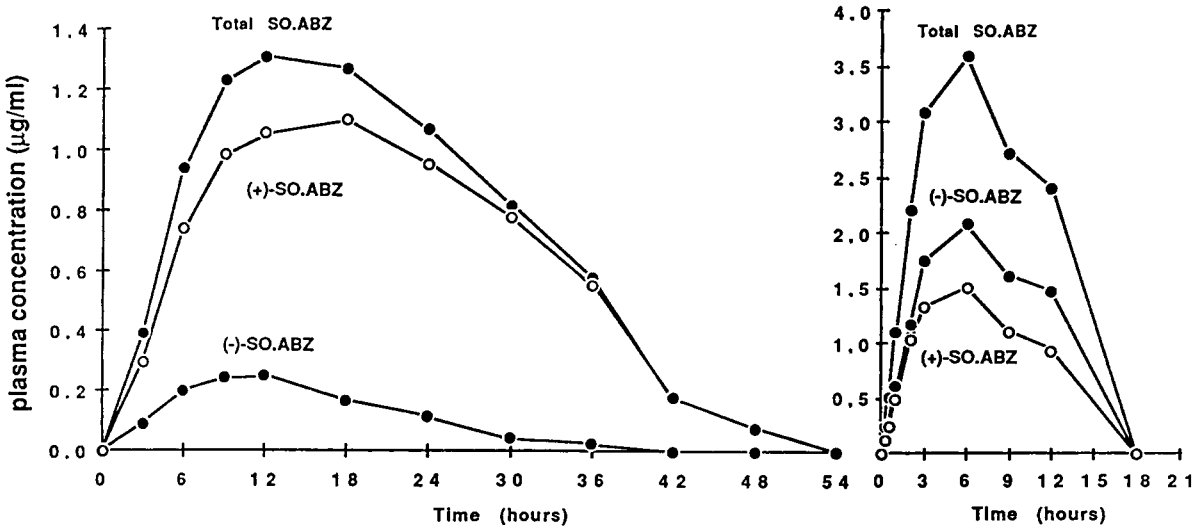

Fig. 6. Plasma concentration (µg/ml) vs. time of the enantiomers of the metabolite SO.ABZ after an oral administration of ABZ to sheep (left) and rats (right).

correspond to 59 and 41% in rats, and 14 and 86% in sheep, of the area of total SO.ABZ, respectively. The latter ratio is not greatly different from the one previously observed in goats.[8]

These significant discrepancies in the enantiomeric behaviour of SO.ABZ between rats and sheep are still unclear. If the (+)/(−) ratio at T_0 results directly from hepatic enzymatic sulfoxidation, the interspecies difference was unexpected since the main enzyme responsible for this first metabolic step is flavin dependent in both rats[9] and sheep.[10] Nevertheless, it has been shown with the substrate 4-tolyl ethyl sulfide, a chemical structure closely related to ABZ, that the in vitro sulfoxidation by rat cytochrome *P*-450 leads preferentially to the enantiomer (−)S,[21] while the FAD-containing monooxygenase produces selectively the (+)R enantiomer.[16] Probably, as opposed to sheep, both enzymic systems act equivalently in rats so that an apparent racemate is produced. In the same vein, the change vs. time of the level of (+)/(−) in an opposite trend in rats vs. sheep, could be explained theoretically by a number of parameters; let us mention the possible substrate enantioselectivity of the cytochrome responsible for the sulfonation of SO.ABZ, a phenomenon already described[4] and/or the possible enantiomeric difference in plasma protein binding.

Additional investigations are required, involving in vitro studies and the use of enzymatic inducers and inhibitors, to understand the facts observed. Finally, which enantiomer is selectively or exclusively responsible for the biological activity is yet unknown. The present example suggests once again that, in the course of drug development, interspecies similarities occurring in classical pharmacokinetic profiles do not imply identity in chiral behaviour. Consequently, chiral experimental studies in pharmacology would often be demanding work, but necessary for a better understanding of the metabolism–activity relationship.

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