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Comparative enantioselectivity in the sulphoxidation of albendazole in man, dogs and rats

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1. H.p.l.c. analyses were performed to investigate the plasma kinetics of albendazole (ABZ), the sulphoxide (SO . ABZ) and sulphone (SO_2ABZ) metabolites, as well as the chirality vs time of SO . ABZ, after oral administration to rats, dogs and man of the prochiral sulphide antiparasitic drug ABZ.

2. In all three species the initial plasma concentration ratio of the enantiomers, as soon as SO . ABZ could be detected in plasma, was that of a racemate.

3. Subsequently, the ratio (+)/(−) increased linearly with time, reaching values of 13·1 and 9·3 in man and dogs, respectively, while it decreased to 0·6 in rats.

4. The (+) enantiomer represents 80%, 70% and 41% of the area under the curve of the total SO . ABZ in man, dogs and rats, respectively.

Introduction

The rationale behind conducting animal experiments in the development of drugs destined for use in human medicine assumes a certain analogy in the metabolism of xenobiotics between man and experimental animals currently used, mainly rodents and dogs. The recent introduction of high-performance liquid chromatography on chiral stationary phases (chiral-h.p.l.c.) into routine analyses makes it possible to take into account the molecular chirality for drug evaluation. This aspect has been shown to be of great importance in pharmacology, since all steps of drug metabolism are possibly enantio-dependent (Ariens 1984, Testa 1986, Jamali *et al.* 1989, Campbell 1990).

The purpose of the present paper is to compare in man, dogs and rats the chiral behaviour of the sulphoxide metabolite (SO . ABZ) of the prochiral sulphide anthelmintic drug, albendazole (ABZ, 5-(propylthio)-1H-benzimidazol-2-yl)carbamic acid methyl ester) (see figure 1).

Materials and methods

Drug administrations and sampling

Male Sprague–Dawley rats, 140–160 g body wt, bred in specific pathogen-free conditions, were purchased from IFFA-Credo (69-l'Arbresle, France). They were dosed orally at 8 a.m. by gastric intubation with an aqueous suspension of ABZ at the dose level of 10 mg/kg body wt. Groups of six animals were killed at the following times after administration: 15 min, 30 min, 1, 2, 3, 6, 9, 12 and 18 h.

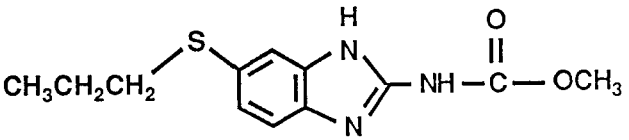

Figure 1. Molecular structure of albendazole (ABZ).

Four healthy male beagle dogs, 10–14 kg body wt, from the colony of the Veterinary School of Lyon, were dosed orally at 8 a.m. using a syringe at a dose level 10 mg/kg of ABZ (Valbazen R, 1·9% suspension). Blood samples were taken by puncture of the jugular vein 30 min, 1 h, 1 h 30 min, 2, 3, 4, 6, 8 and 12 h after dosing.

Four clinically healthy male human volunteers, 65–80 kg body wt, received a single oral administration of 10 mg ABZ/kg, as the pure substance incorporated into a yoghurt at 8 a.m., following a light continental breakfast or an overnight fast. Blood samples were taken 30 min, 1, 2, 3, 4, 6, 8, 10 and 12 h after ingestion.

In all cases, blood samples were collected into evacuated heparinized tubes and immediately centrifuged. The plasma was separated by centrifugation and stored at −18°C until analysis.

Chromatographic analysis

Pharmacokinetic profiles. Aliquots of 200 µl of plasma were introduced onto cassettes of the 'Advanced Automated Sample Processor' (AASP) Varian and subjected to chromatographic analysis on a C18 column according to the conditions previously described (Delatour *et al.* 1984). In this way the simultaneous quantification of ABZ and its metabolites sulphoxide (SO . ABZ) and sulphone (SO_2ABZ) was obtained.

Enantiometric ratio of SO . ABZ vs time. The method developed for the chromatographic resolution of the SO . ABZ metabolite has been described previously (Lienne *et al.* 1989a) and adapted to biological assays (Delatour *et al.* 1990a). In short, during preparative liquid chromatography on a C18 column of a 1–6 ml sample of plasma, depending on the SO . ABZ concentration previously determined, the fraction of interest was collected, extracted and the subsequent residue chromatographed on a chiral column of which the stationary phase was an α_1-glycoprotein immobilized on silica, 5 µm (Chiral-AGP, Interchim, Montluçon, France). The retention times (figure 2) of the enantiomers (−) and (+) of SO . ABZ were 4·6 and 9·2 min, respectively (Lienne *et al.* 1989a). An integrator provided the relative proportions (percentages) of both peaks directly.

Results

From the raw data, mean values and standard deviations were calculated as well as the areas under the curve (*AUC*) vs time. The pharmacokinetic profiles of the metabolites SO . ABZ and SO_2ABZ in man, dogs and rats are presented in figures 3–5. The enantiomeric proportions of both antipodes, regardless of the

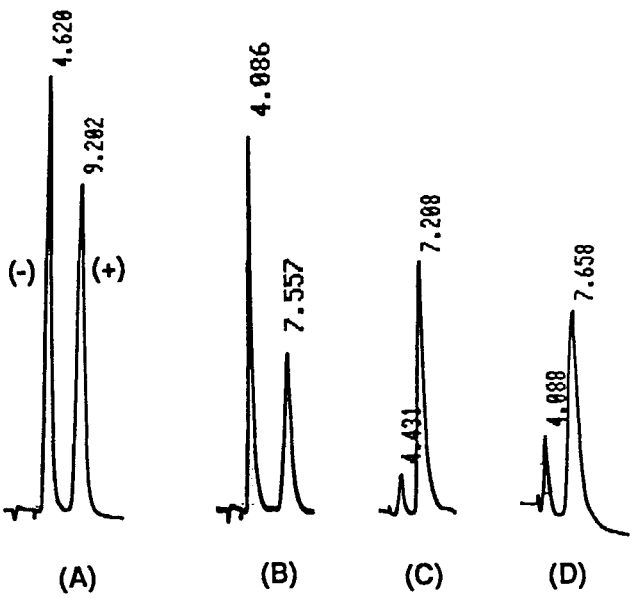

Figure 2. Chiral resolution of albendazole sulphoxide (SO . ABZ) on a α_1-glycoprotein column.
(A): calibration: 49·4(−) : 50·6(+); (B): rat 6 h: 55(−) : 45(+); (C): dog 8 h: 10(−) : 90(+); (D): man 6 h: 14(−) : 86(+).

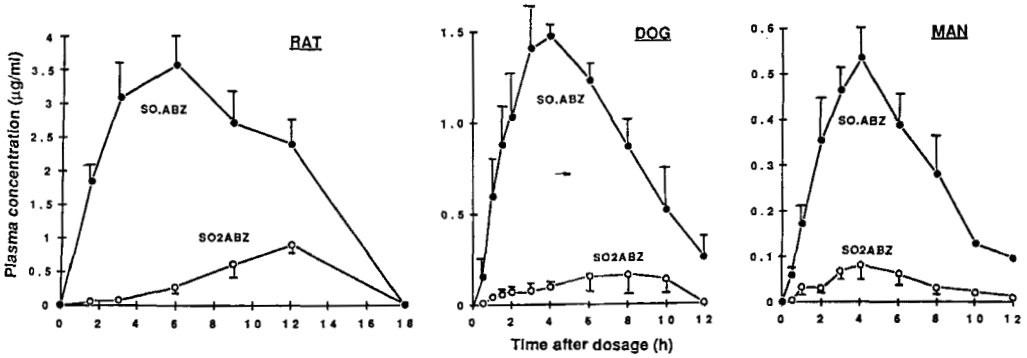

Figure 3. Pharmacokinetics of the sulphoxide and sulphone metabolites (SO . ABZ and SO_2ABZ) of albendazole in rats, dogs and man.

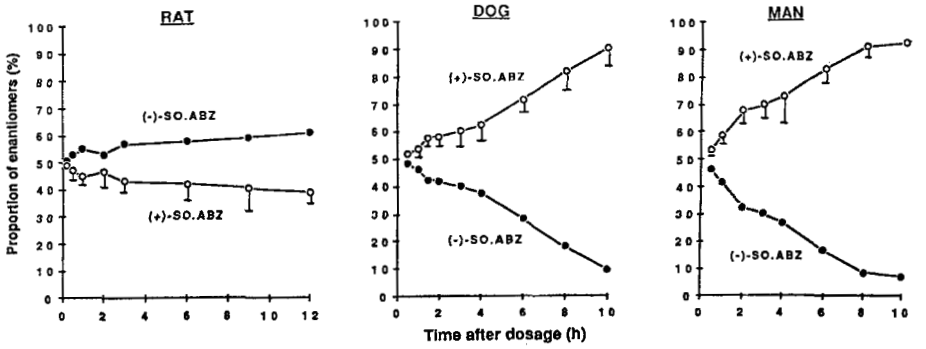

Figure 4. Evolution vs time of the percentage of the enantiomers of albendazole sulphoxide (SO . ABZ) in rats, dogs and man.

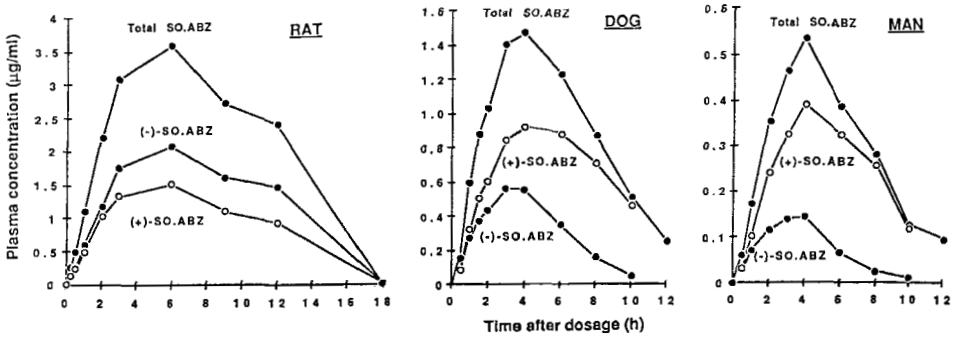

Figure 5. Plasma concentration vs time of the enantiomers of albendazole sulphoxide (SO . ABZ) in rats, dogs and man.

plasma concentration of total SO . ABZ, are presented in figure 4. The concentration (μg/ml) vs time of individual enantiomers is shown in figure 5.

Discussion

The metabolism of ABZ has been described in rats (Gyurik *et al.* 1981) and man (Penicaut *et al.* 1983), but not in dogs. In all species the main metabolites found are SO . ABZ and SO_2ABZ, while the presence of the parent compound in plasma is negligible or undetectable ($<0{\cdot}1\,\mu$g/ml). The pharmacokinetic data shown in figure 3 are consistent with those previously published concerning rats (Souhaili-el-Amri *et al.* 1988b) and man (Marriner *et al.* 1986). The bioavailability of ABZ is very different in the three species. The mean *AUC* of the active SO . ABZ metabolite is: 39·2, 10·6 and 3·5 μg h ml^{-1} in rats, dogs and man, respectively. Despite this, the overall plasma profiles seem to be rather similar (figure 3) for all three species. Particularly, the ratio of the *AUC* of SO . ABZ to the *AUC* of SO_2ABZ is 5·6 in rats, 10·0 in dogs and 7·3 in man. The proportions vs time of the antipodes of SO . ABZ (figure 4) show that at zero time (extrapolation of the curves), the plasma concentration ratio of the enantiomers is not significantly different from that of a racemate. Later, (+)SO . ABZ is always prominent in man and dogs, while (−)SO . ABZ is prominent in rats (figure 4). The plasma concentration ratio (+)/(−) increases from unity at zero time to 13·1 and 9·3 in man and dogs 10 h after administration, respectively, and decreased to 0·6 in rats 12 h after dosing. During the total time-course of the plasma kinetics (figure 5), the proportions (percentages) of the enantiomers within the area of total SO . ABZ are 20 (−) : 80 (+) in man, 30 (−) : 70 (+) in dogs and 59 (−) : 41 (+) in rats.

In the absence of complementary *in vivo* and *in vitro* investigations to date, explanations of the phenomena observed may only be hypothesized. If the plasma concentration enantiomeric ratio at zero time is the result of the reaction of sulphoxidation of ABZ, the level 50 : 50 is surprising in rats where the main enzyme responsible for this metabolic step seemed to be a microsomal FAD-containing monooxygenase (Fargetton *et al.* 1986) which is known to be product enantioselective (Ziegler 1989). Nevertheless, in rats the cytochrome P-450 is also able to produce sulphoxides *in vitro* (Waxman *et al.* 1982). With the substrate 4-tolyl ethyl sulphide, a model where the sulphur moiety is also an alkyl aryl sulphide, it has been shown that the mammalian FAD-containing oxygenase carries out sulphoxidation to yield the R-(+) enantiomer as the major product (Light *et al.* 1982) while the cytochrome P-450-dependent MFO produces selectively the S-(−) enantiomer (Waxman *et al.* 1982). Probably, at least in rats and possibly in dogs and man, but not in ruminants (Delatour *et al.* 1990b), both enzymic systems act simultaneously so that the global SO . ABZ produced appears to be a racemate at zero time.

The plasma concentration ratio (+)/(−) changes with time could be the consequence of substrate enantio-selectivity of the cytochrome P-450-dependent (Souhaili-el-Amri *et al.* 1988a) sulphonating reaction, a phenomenon already described (Auret *et al.* 1968). According to this interpretation the selective consumption of one enantiomer of SO . ABZ is responsible for the increase in the proportion of its antipode. More importantly, the discrepancy between rats on the one hand, and dogs and man on the other hand, concerning the identity of the major enantiomer in plasma was unexpected. An atypical enantiomeric ratio was also observed in rats for the sulphoxide metabolite of fenbendazole, another compound chemically related to ABZ (Lienne *et al.* 1989b).

Regardless of the kinetic and enzymic reasons for the differences between species, these results provide an example of the pertinent choice of experimental animal species when dealing with drug development studies. If, according to the general rule, the two enantiomers do not exhibit the same biological effects—information which to date is missing in the case of ABZ—pharmacological data obtained from dogs in a similar situation are certainly more pertinent than those obtained from rats. Consequently, chiral metabolic investigations should take place early in the time-course of the development of drugs destined for human therapeutics.

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