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Inducing Effect of Albendazole on Rat Liver Drug-Metabolizing Enzymes and Metabolite Pharmacokinetics

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Inducing Effect of Albendazole on Rat Liver Drug-Metabolizing Enzymes and Metabolite Pharmacokinetics. SOUHAILI-EL AMRI, H., FARGETTON, X., BENOIT, E., TOTIS, M., AND BATT, A. M. (1988). *Toxicol. Appl. Pharmacol.* **92**, 141–149. Albendazole (ABZ), methyl (5-(propylthio)-1H-benzimidazol-2-yl)carbamate, is a broad spectrum anthelmintic drug. *S*-oxidation to the sulfoxide (SO-ABZ) and the sulfone (SO_2-ABZ) are the first steps of its bioconversion. SO-ABZ is pharmacologically active and embryotoxic in rats. In the present study, rat liver microsomal drug-metabolizing enzymes were assayed after 10 days oral administration with 40 μmol ABZ/kg per day. The activities of 4-nitroanisole *O*-demethylase, benzo[*a*]pyrene hydroxylase, 7-ethoxycoumarin *O*-deethylase, and 7-ethoxyresorufin *O*-deethylase increased 6-, 7-, 8-, and 30-fold, respectively. By immunoblotting an increase in cytochrome *P*-448 was observed. UDP-glucuronosyltransferase (GT) type 1 activities (1-naphthol, 7-hydroxycoumarin, 4-nitrophenol, and 4-methylumbelliferone) were significantly higher than in control microsomes (3- to 4-fold), while GT type 2 activities and bilirubin-GT remained unchanged. Microsomal epoxide hydrolase (benzo[*a*]pyrene oxide) increased 2-fold. Microsomal γ-glutamyltransferase activity was unchanged. The *in vivo* SO-ABZ plasma level was decreased when the SO_2-ABZ plasma level was increased. *In vitro* sulfoxidation and sulfonation were, however, unchanged. Although a range of imidazole derivatives, including benzimidazole itself, were commonly reported as inhibitors of monooxygenase activities, ABZ behaved as an inducer of cytochrome *P*-448, GT1, and epoxide hydrolase. © 1988 Academic Press, Inc.

Albendazole (ABZ), methyl (5-(propylthio)-1H-benzimidazol-2-yl)carbamate (Zentel, Valbazen), is a broad-spectrum anthelmintic, widely used in human and veterinary medicine (Saimot *et al.*, 1983; Theodorides *et al.*, 1976). *S*-oxidations of the drug to albendazole sulfoxide (SO-ABZ) and albendazole sulfone (SO_2-ABZ) are the first steps of bioconversion (Gyurik *et al.*, 1981). These oxidations are of a primary importance in the expression of anthelmintic activity and toxicity of ABZ, since it has been shown that in the plasma of man (Penicaut *et al.*, 1983; Marriner *et al.*, 1986), rats (Delatour *et al.*, 1984), cattle (Prichard *et al.*, 1985), and sheep (Marriner and Bogan, 1980) the parent compound remains undetectable (<0.01 μg/ml). SO-ABZ is pharmacologically active and embryotoxic in rats in contrast with SO_2-ABZ, which is neither active nor toxic (Delatour *et al.*, 1981). Previous studies have demonstrated that ABZ was converted to SO-ABZ and SO_2-ABZ by rat liver microsomes (Fargetton *et al.*, 1986).

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Imidazoles and benzimidazoles are able to interact with liver drug-metabolizing enzymes. Inhibiting effects have been shown with cimetidine (Bast *et al.*, 1984; Rendic *et al.*, 1984), omeprazole (Jensen and Gugler, 1986), etomidate (Horai *et al.*, 1985), ketoconazole, and their derivatives (Van den Bossche *et al.*, 1985; Sheets *et al.*, 1986; Kedderis and Rickert, 1985) and some benzimidazoles (Holder *et al.*, 1976). On the other hand imidazole itself is an active inducer of alcohol-oxidizing cytochrome *P*-450 (*P*-450 Alc or *P*-450-30) in rabbits (Koop *et al.*, 1985). Inducing effects due to miconazole, ketoconazole, itraconazole (Laurijsen *et al.*, 1985), and benzimidazole (Holder *et al.*, 1976) have been also demonstrated. Moreover, a novel imidazole SC-37211 (Searle Research Department) was recently reported to induce UDP-glucuronosyltransferase activity toward 4-nitrophenol (GT1) (Comer *et al.*, 1985). Imidazole derivatives also enhance styrene oxide hydrolase (James and Sloan, 1985).

The aim of the present work was to study the possible inhibiting and inducing activities of the benzimidazole drug, albendazole, on the liver drug-metabolizing enzymes. We focused our attention on cytochrome *P*-450-dependent monooxygenase activities, microsomal epoxide hydrolase, UDP-glucuronosyltransferases (GT1, GT2, bilirubin, and testosterone) and γ-glutamyltransferase activities. Finally, we studied the *in vivo* changes in plasma concentrations of albendazole and its metabolites after repeated administration of the drug in rats.

MATERIALS AND METHODS

Chemicals. ABZ, SO-ABZ, and SO_2-ABZ were supplied by Smith Kline and French Laboratories (Paoli Pike, West Chester, PA). Other chemicals and biochemicals were obtained from commercial sources.

Animals. Male Sprague–Dawley rats (180–200 g) were purchased from Iffa-Credo (l'Arbresle, France).

Treatment of animals. The animals were randomly separated into two groups, treated and control, respectively, housed in cages of three animals each so as to compare pairs of cages. Each treated rat received a daily (at 8:00 AM) oral dose of 10.6 mg (4×10^{-5} mol/kg) ABZ/kg by gastric intubation of aqueous suspensions for 10 days. The control group was dosed by the vehicle only. This drug dosage had previously been demonstrated to be embryotoxic in rats.

Kinetic studies. On the 11th day at 8:00 AM, 24 rats of each group which had been fasted overnight received a single oral dose of 10.6 mg ABZ/kg. The animals were killed by decapitation (three animals at each time) at 0, 0.75, 1.5, 3, 6, 9, 12, and 18 hr postdose. Blood was collected in EDTA and immediately centrifuged; plasma was separated and stored frozen at −20°C. ABZ, SO-ABZ, and SO_2-ABZ were extracted from plasma and determined by HPLC according to methods previously described (Delatour *et al.*, 1984).

Preparation of microsomes. On the 11th day at 8:00 AM, six rats of each group which had been fasted overnight were killed by decapitation. The livers were immediately removed and perfused with 0.9% NaCl containing 10 mM K-phosphate buffer, pH 7.4. The excised liver was minced and homogenized in a threefold volume of a 0.25 mM sucrose containing 50 mM K-phosphate buffer, 1 mM EDTA, pH 7.4. The homogenate was successively centrifuged at 800*g* for 10 min, at 13,500*g* for 20 min, and at 105,000*g* for 60 min to isolate the microsomal fraction. The microsomal pellet was washed with 10 mM $Na_2P_2O_7$, pH 7.4 to eliminate the hemoglobin. The microsomes were then suspended in 100 mM potassium phosphate, 1 mM EDTA, 1 mM dithiothreitol, 20% glycerol, pH 7.4, and stored at −80°C.

Assays. The protein content of the microsomal suspension was determined using the method of Lowry *et al.* (1951) with bovine serum albumin as a standard. Cytochrome *P*-450 and cytochrome b_5 were measured according to Omura and Sato (1964).

The sulfoxidation of ABZ by liver microsomes was assessed by the amount of SO-ABZ produced. In a final volume of 1 ml of phosphate buffer (100 mM, pH 7.4), a typical reaction mixture contained 2 mg of microsomal protein, 0.5 mM NAD, 0.5 mM NADP, 3.2 mM glucose 6-phosphate, 1 U glucose-6-phosphate dehydrogenase, and ABZ (200 μM) as the substrate. ABZ was dissolved in 5 μl of dimethyl sulfoxide. Incubations were carried out in glass vials in an oscillating water bath at 37°C for 2 min. Vials without the NADPH regenerating system were used as the control. ABZ and SO-ABZ were extracted from incubation mixtures, identified (retention time, uv spectrum) by comparison with pure synthetic compounds, and determined by HPLC according to methods previously described (Delatour *et al.*, 1984). The sulfonation of SO-ABZ by liver microsomes was assessed by the amount of SO_2-ABZ formed in the incubation mixture, identical to that which was used for the

sulfoxidation assay but with SO-ABZ (200 μM) as the substrate and an incubation time of 1 hr.

N-demethylation of aminopyrine, ethylmorphine, or benzphetamine was carried out by the method of Yang and Strickhart (1974), and *O*-demethylation of 4-nitroanisole was carried out by the method of Netter and Seidel (1964). The activities of benzo[*a*]pyrene hydroxylase, 7-ethoxycoumarin deethylase, 7-ethoxyresorufin deethylase, and aniline hydroxylase were determined according to Nebert and Gelboin (1968), Ullrich and Weber (1972), Rifkind and Muschick (1983), and Mazel (1972), respectively. NADPH cytochrome *c* reductase was determined as described by Strobel and Dignam (1978).

UDP-glucuronosyltransferase activities were determined according to Mulder and Van Doorn's method (1975), as modified by Colin-Neiger *et al.* (1984) with the substrates 1-naphthol, 7-hydroxycoumarin, 4-nitrophenol, 4-methylumbelliferone, *cis*-myrtanol, nopol, menthol, and testosterone. Bilirubin-UDP-glucuronosyltransferase was measured by the method of Heirwegh *et al.* (1972). Epoxide hydrolase was estimated with benzo[*a*]pyrene oxide by the method of Dansette *et al.* (1979), and γ-glutamyltransferase was estimated by the method of Szasz (1969).

Statistics. All direct comparisons to a single control group were conducted with the unpaired Student *t* test.

Purification of cytochrome P-450. The liver microsomes obtained from phenobarbital (80 mg/kg, ip, in saline for 5 days)- and 3-methylcholanthrene (80 mg/kg, ip, in corn oil, once, 5 days before termination)-treated rats were used for the purification of cytochromes *P*-450 (PB-B or *P*-450 b) and *P*-448 (M C2 or *P*-450 c), respectively, according to the procedures of Guengerich *et al.* (1982) and of Wolf and Oesch (1983).

Preparation of antisera. Antiserum to the purified enzyme was raised in rabbits. The animals were inoculated subcutaneously along the flank at several sites with 150 μg of purified cytochrome *P*-450 isoenzyme. The immunogen was diluted in 0.9% NaCl to 0.5 ml and mixed with 0.5 ml of Freund's complete adjuvant. Similar booster injections, but using Freund's incomplete adjuvant, were given at 3-week intervals. Each rabbit was bled from the ear vein 8 days after the second booster challenge. The anti-cytochrome *P*-450 and anti-cytochrome *P*-448 IgG were purified using a protein A-Sepharose CL 4B column (Pharmacia), after 50% ammonium sulfate precipitation of antiserum proteins.

Specificity of antisera. Antisera were examined for reactivity toward purified cytochrome *P*-450 isoenzymes, as well as toward other microsomal proteins, by double-immunodiffusion analysis according to the method of Ouchterlony.

Electrophoretic separation of proteins. Discontinuous sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed according to Laemmli (1970). The stacking and running gels were 5 and 10% acrylamide, respectively. Electrophoresis was performed at room temperature for 2 hr under a constant current of 25 mA.

Electroblotting. "Western blots" were performed with a trans-blot system (Bio-Rad laboratories) to transfer proteins to nitrocellulose sheets (Schleicher & Schuell, FRG) for probing with antibodies (Burnette, 1981).

RESULTS

Cytochrome P-450-Dependent Monooxygenases (Table 1)

Following treatment of rats with albendazole, total liver microsomal cytochrome *P*-450 increased approximatively 2-fold. The activities of 4-nitroanisole *O*-demethylase, 7-ethoxycoumarin *O*-deethylase, benzo[*a*]-pyrene hydroxylase, and 7-ethoxyresorufin *O*-deethylase increased 6-, 7-, 8-, and 30-fold, respectively, in microsomes prepared from ABZ-treated rats (ABZ-microsomes) relative to the values in microsomes prepared from untreated rats (UT-microsomes). In contrast the activities of aminopyrine and ethylmorphine dealkylases were only slightly enhanced, while neither benzphetamine *N*-demethylase nor aniline hydroxylase was increased significantly.

Immunoblotting

Polyclonal antibodies raised to cytochrome *P*-448 (M C1 or P-450 c) cross-reacted with liver microsomes from ABZ-pretreated rats in a manner similar to 3-MC-treated rat liver microsomes. Polyclonal antibodies raised to cytochrome *P*-450 (PB-B or P-450 b) reacted with liver microsomes of ABZ-treated rats in a pattern comparable to that of control liver microsomes (Fig. 1).

UDP-Glucuronosyltransferase Activities

The data are presented in Table 2. UDP-glucuronosyltransferase type 1 activities, naphthol, 7-hydroxycoumarin, 4-nitrophe-

TABLE 1

EFFECT OF ALBENDAZOLE TREATMENT ON CYTOCHROME *P*-450 CONTENT AND ON CYTOCHROME *P*-450-DEPENDENT MONOOXYGENASE ACTIVITIES OF RAT LIVER MICROSOMES

	UT-microsomes	ABZ-microsomes
Cytochrome *P*-450[a]	0.57 (0.07)	1.22 (0.10)**
Aminopyrine *N*-demethylase[b]	3.76 (0.76)	5.28 (0.72)*
Ethylmorphine *N*-demethylase[b]	3.46 (0.71)	5.46 (1.58)*
Benzphetamine *N*-demethylase[b]	3.88 (0.58)	4.90 (0.95)
7-Ethoxycoumarin *O*-deethylase[b]	0.37 (0.04)	2.55 (0.47)**
7-Ethoxyresorufin *O*-deethylase[b]	0.028 (0.005)	0.869 (0.124)**
4-Nitroanisole *O*-demethylase[b]	0.92 (0.05)	6.24 (0.83)**
Benzo[*a*]pyrene hydroxylase[b]	0.03 (0.11)	2.46 (0.45)**
Aniline hydroxylase[b]	0.36 (0.04)	0.32 (0.8)

Note. The results are expressed as means (standard deviation) of six separate determinations performed with microsomes prepared from six individual rats. UT, Untreated rat microsomes. ABZ, ABZ-treated microsomes.

[a] Expressed as nmol/mg microsomal protein.

[b] Expressed as nmol/min/mg microsomal protein.

* $p < 0.05$.

** $p < 0.001$.

nol, and 4-methylumbelliferone, were significantly higher in ABZ-microsomes than in control microsomes, while UDP-glucuronosyltransferase type 2 activities, nopol, menthol, *cis*-myrtanol, and bilirubin-UDP-glucuronosyltransferase, remained unchanged. The UDP-glucuronosyl testosterone activity was not significantly enhanced in ABZ-microsomes.

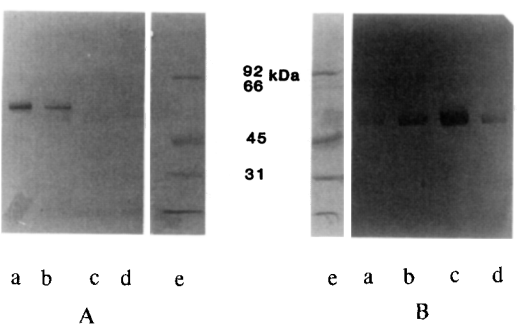

FIG. 1. Cytochrome *P*-448 (MC 2 or *P*-450 c) induction in microsomes from albendazole-treated rats. (A) Anti-cytochrome *P*-448 (MC 2 or *P*-450 c) polyclonal antibodies reacted with blots. (B) Anti-cytochrome *P*-450 (PB B or *P*-450 B) polyclonal antibodies reacted with blots. a, 3-Methylcholanthrene-treated rat liver microsomes; b, albendazole-treated rat liver microsomes; c, phenobarbital-treated rat liver microsomes; d, control rat liver microsomes; e, molecular-weight standards.

TABLE 2

EFFECT OF ALBENDAZOLE TREATMENT ON UDP-GLUCURONOSYLTRANSFERASE ACTIVITIES OF RAT LIVER MICROSOMES

Substrate	UT-microsomes	ABZ-microsomes
Naphthol	48.4 (6.3)	161.8 (33.9)*
Hydroxycoumarin	65.0 (6.0)	265.7 (46.8)*
4-Nitrophenol	17.6 (1.6)	68.3 (8.5)*
4-Methylumbelliferone	45.6 (4.2)	167.2 (20.7)*
Nopol	4.6 (0.7)	4.4 (1.4)
Menthol	10.3 (0.7)	12.0 (1.7)
cis-Myrtanol	10.7 (0.9)	11.7 (3.3)
Bilirubin	0.81 (0.07)	0.94 (0.16)
Testosterone	7.3 (0.4)	9.4 (1.2)

Note. Results are expressed as nmol/min/mg microsomal protein. Values are the mean (standard deviation) of six separate determinations performed with microsomes prepared from six individual rats. UT, Untreated rat microsomes. ABZ, ABZ-treated microsomes.

* $p < 0.001$.

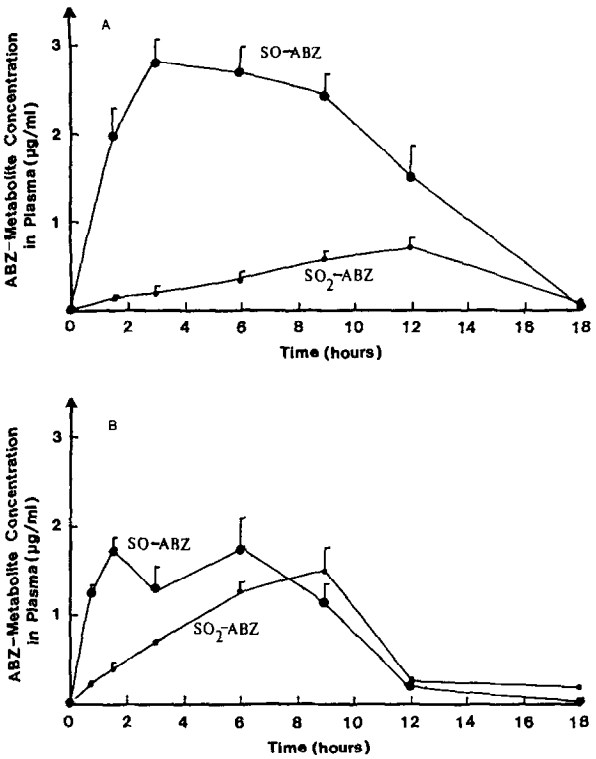

FIG. 2. Concentrations in plasma of albendazole sulfoxide (SO-ABZ) and albendazole sulfone (SO_2-ABZ) after administration of albendazole. (A) One dose (10.6 mg/kg). (B) Ten days treatment with 10.6 mg/kg each day.

Epoxide Hydrolase and γ-Glutamyltransferase

The epoxide hydrolase activity increased significantly from 11.44 (2.45) in UT-microsomes to 21.02 (4.52) nmol/mg protein^{-1} min^{-1} in ABZ-microsomes. γ-Glutamyltransferase activity in microsomal membranes remained unchanged.

Metabolization of Albendazole

As shown in Fig. 2A, after repeated administrations of ABZ, the plasma concentration of SO-ABZ showed a marked depression. The area under the SO-ABZ plasma concentration vs time curve (AUC) decreased from 31.035 μg/hr ml^{-1} (control) to 14.955 μg/hr ml^{-1} (treated), corresponding to a 51.8% reduction. In the same time the plasma concentration of SO_2-ABZ increased (Fig. 2B). The SO_2-ABZ AUC increased by 92% (6.496 μg/hr ml^{-1} for the control and 12.499 μg/hr ml^{-1} for the treated rats). In contrast, ABZ-microsomes and UT-microsomes, studied at pH 7.4, did not exhibit significant differences from control with respect to ABZ sulfoxidation and SO-ABZ sulfonation (Table 3). ABZ itself was sulfoxidized but not sulfonated.

DISCUSSION

Although it has been reported that imidazole derivatives, such as benzimidazole, inhibit the activity of hepatic mixed-function oxidases (Holder *et al.*, 1976; Testa and Jen-

TABLE 3

EFFECT OF ALBENDAZOLE TREATMENT ON ABZ-SULFOXIDASE AND SO-ABZ-SULFONASE ACTIVITIES OF RAT LIVER MICROSOMES

	UT-microsomes	ABZ-microsomes
ABZ-sulfoxidase (nmol/min/mg protein)	0.66 (0.36)	0.78 (0.21)
SO-ABZ-sulfonase (pmol/hr/mg protein)	252 (53)	329 (50)

Note. Values are the means (standard deviations) of six separate determinations performed with microsomes prepared from six individual rats. UT, Untreated rat microsomes. ABZ, ABZ-treated rat microsomes.

ner, 1981; Jensen and Gugler, 1986), we found no evidence for an inhibitory effect of ABZ in any of the enzyme systems tested. This lack of inhibitory activity may be related to the fact that ABZ is a substituted benzimidazole derivative. In fact 2-substituted benzimidazole derivatives are weak inhibitors (Dickins *et al.*, 1975). Wilkinson *et al.* (1972) have proposed that at least one of the imidazole nitrogens must be free in order for the molecule to have an inhibitory effect.

Our results show that ABZ is an inducer of cytochrome *P*-448. The activity of 7-ethoxyresorufin *O*-deethylase was increased 30-fold after pretreatment with ABZ. UDP-glucuronosyltransferase type 1 and epoxide hydrolase were also increased. Such a pattern of enzyme induction was also observed by us, in one sample of human liver, obtained by resection from a patient treated with ABZ. These observations are in agreement with reports showing that different imidazole or benzimidazole derivatives induce 7-ethoxycoumarin deethylase activity (Reinke *et al.*, 1985), 7-ethoxyresorufin deethylase (Müller-Enoch, 1986), epoxide hydrolase (James *et al.*, 1985), or UDP-glucuronosyltransferase type 1 (Laurijsen *et al.*, 1985; Comer *et al.*, 1985). Such a pattern of enzyme induction may be related to the fact that benzimidazole derivatives are stabilized in a planar configuration by the two double bonds of the imidazole ring. Planar compounds induce the metabolism, via cytochrome *P*-448 and UDP-glucuronosyltransferase type 1, of planar substrates (Bock *et al.*, 1980; Okulicz-Kozaryn *et al.*, 1981; Parke, 1986).

ABZ did not induce aniline hydroxylase activity. A similar result has been reported by Reinke *et al.* (1985), for imidazole in rats, although imidazole induces cytochrome *P*-450–ethanol in rabbits (Koop *et al.*, 1985).

In vivo ABZ stimulates its own metabolism via the sulfonation pathway but not via the sulfoxidation pathway. This latter observation (the lack of stimulation of sulfoxidation) can be explained on the basis that sulfoxidation activity depends upon flavine monooxygenase, which is not inducible (Ziegler, 1980). *In vitro* we did not observe a significant increase in SO-ABZ sulfonase activity. The lack of correlation between the *in vivo* and *in vitro* results may be explained by the relatively weak SO-ABZ sulfonase activity *in vitro*. Whereas the enzymes for sulfoxidation are now better known (Mittchell and Waring, 1986; Damani, 1986), the enzyme involved in ABZ sulfonation is not yet identified. The protein responsible for this reaction has been recently isolated in our laboratory and is presently being characterized.

A final question concerns the potential toxicological consequences of induction of cytochrome *P*-448 and UDP-glucuronosyltransferase type 1 by ABZ. Although it has been suggested that metabolism of drugs by the cytochrome *P*-448 pathways may produce toxic metabolites (carcinogens or hepatotoxics) (Parke and Ioannides, 1984; Ioannides *et al.*, 1983, 1984), several substances, such as β-naphthoflavone, which induce cytochrome *P*-448 are not carcinogenic. Similar arguments have been reported concerning the possible toxicological significance of induction of GT1 (Bock *et al.*, 1982) and epoxide hydrolase (Dent and Graichen, 1982) activi-

ties. An unequivocal reply to this question requires further investigation. Although some benzimidazole derivatives have been reported to be mutagenic/carcinogenic, their effect was borderline and required high doses (Delatour and Parish, 1986).

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