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Induction of cytochrome P450 enzymes by albendazole treatment in the rat

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Abstract

The anthelmintic drug albendazole (ABZ), methyl(5-(propylthio)-1H-benzimidazol-2-yl)carbamate, is a benzimidazole highly efficient in the treatment of neurocysticercosis. The effects of ABZ treatment (i.p. and p.o. administration) on the expression of several cytochrome P450 (CYP) enzymes were evaluated in rat liver in order to characterize the spectrum of altered CYP enzymes involved in the metabolism of environmental mutagens and carcinogens, after drug intake. Intraperitoneal administration of ABZ (50 mg/kg body weight/day/three days in corn oil) to rats, caused an induction of hepatic activities of CYP1A1-associated ethoxyresorufin O-deethylase (EROD) 65 fold, CYP1A2-associated methoxyresorufin O-demethylase (MROD) 6 fold, CYP2B1-associated penthoxyresorufin O-dealkylase (PROD) 4 fold, CYP2B2-associated benzyloxyresorufin O-dealkylase (BROD) 14 fold, as well as a partial reduction of CYP2E1-associated 4-nitrophenol hydroxylase (4-NPH) activity. CYP3A-associated erythromycin *N*-demethylase (END) activity was not modified under the same treatment conditions. Western blot analysis was conducted to explore if the increased catalytic activity was a result of an increased protein content; only CYP1A1/2 showed a visible increase in protein concentration after ABZ inoculation, therefore, the increased PROD and BROD activities could be attributed to the induction of CYP1A1/2. Results with the two main metabolites of ABZ (15 mg/kg body weight/day/three days, i.p.) indicated that ABZ sulfoxide (ABZSO) but not ABZ sulfone ($ABZSO_2$) displayed the same pattern of CYP induction than ABZ. Oral administration of ABZ at the human therapeutic dose of 20 mg/kg body weight/day/three days, produced an increase in CYP1A1/2 protein content 24 h after the first intake. The protein level remained high during the treatment, and up to 72 h after the last administration; basal protein levels were almost recovered 48 h later.

Keywords: Cytochrome P450; Anthelmintic; Neurocysticercosis; Albendazole

1. Introduction

Albendazole (ABZ) is a broad spectrum anthelmintic drug widely used in human and veterinary medicine (Theodorides et al., 1976). It shows high efficacy in the treatment of human hydatidosis (Saimot et al., 1983) and neurocysticercosis (Sotelo, 1997). The bioconversion steps of this drug by rat liver microsomes involve an initial S-oxidation by flavin containing monooxigenases (FMO) and cytochrome P450 (CYP) 3A, yielding ABZ sulfoxide (ABZSO) (Moroni et al., 1995), and a second S-oxidation mainly by CYP1A1 producing ABZ sulfone ($ABZSO_2$) (Souhaili-El Amri et al., 1988b). The anthelmintic activity and embryotoxicity of ABZ in rats is due to ABZSO; on the contrary, $ABZSO_2$ is neither active nor toxic (Delatour et al., 1984). Previous studies on the CYP induction properties of ABZ revealed that administration of this drug by oral route at a dose of 10 mg/kg/10 days, increased the catalytic activity and protein content of CYP1A1 (Souhaili-El Amri et al., 1988a), but results for additional CYP enzymes were inconclusive since, appropriate CYP antibodies or substrates for determining associated activities were not available.

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It is now recognized that the ability of a chemical to induce certain CYP isoforms is likely to be a critical factor in determining its toxic and carcinogenic potential (Mori et al., 1993; Rice et al., 1994). Some authors have suggested that the induction of lung CYP1A1 in smokers is associated with a high risk of lung cancer (Anttila et al., 1991; Bartsch et al., 1992). On the other hand, an inverse relationship has been demonstrated between breast aryl hydrocarbon hydroxylase activity (CYP1A1) and survival rate in human breast cancer (Pyykko et al., 1989). Besides chronic adverse effects, acute toxic manifestations have also been reported. There are several circumstances in which a drug combination is associated with increased risk of toxic reactions. For example, the antituberculosis drug isoniazid has been identified as an inducer of CYP2E1 (Ryan et al., 1986), one of the enzymes that catalyzes the formation of the putative hepatotoxic metabolite of acetaminophen (Raucy et al., 1989). Patients under isoniazid treatment presented severe hepatotoxicity when ingesting a dose of acetaminophen not normally associated with toxicity (Crippin, 1993).

Our goal in the present study was to explore the capacity of ABZ to modulate the expression of some CYP family enzymes. For this purpose, rats were intraperitoneally injected with 1/100 LD_{50} of ABZ, ABZSO, or $ABZSO_2$, and hepatic CYP1A1/2, CYP2B1/2, CYP2E1 and CYP3A expressions were assessed by the evaluation of CYP-associated enzymatic activities (Burke et al., 1985, 1994) and western blot analysis. In order to explore ABZ inducing properties at a therapeutic dose as well as the CYP induction kinetics, rats were treated with a human oral therapeutic dose of ABZ, and hepatic CYP1A1/2 modification was followed by immunoblot analysis at 24, 48, 72, 120 and 168 h after the beginning of treatment.

2. Materials and methods

2.1. Chemicals

ABZ, pentoxyresorufin, benzyloxyresorufin, methoxyresorufin, β-NADPH and diaminobenzidine were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). ABZSO and $ABZSO_2$ were kindly donated by SmithKline Beecham (Worthing, UK). Ethoxyresorufin and resorufin were obtained from Molecular Probes (Eugene, Oregon, USA). 4-Nitrophenol and 4-nitrocatechol were purchased from Aldrich Chemical Company, Inc. (Milwaukee, Wisconsin, USA). All electrophoresis reagents were purchased from Bio-Rad Laboratories (Hercules, California, USA).

2.2. Antibodies

Goat anti-rat polyclonal antibodies against CYP1A1/2, CYP2B1/2, CYP2E1 and CYP3A2, were obtained from Daiichi Pure Chemicals Co. (Tokyo, Japan). Anti CYP1A1/2 recognizes both rat CYP1A1 and CYP1A2; anti CYP2B1/2 recognize rat CYP2B1 and rat CYP2B2; anti CYP2E1 recognize rat CYP2E1 with a minor cross reactivity with rat CYP2C11 and rat CYP2C13; and finally, anti CYP3A2 also recognizes rat CYP3A1 on inmunoblots (Gentest Corporation, 1999). Rabbit anti-goat IgG was obtained from Sigma Chemical Co. (St. Louis, Missouri, USA).

2.3. Animal treatment

Male wistar rats (200–250 g) were housed in polypropylene cages and subjected to a 12-h light–dark cycle in an animal care facility. Animals were allowed unrestricted access to laboratory rodent chow and distilled water.

In a first experiment, four groups of animals were used (four animals/group). One group was treated with ABZ (50 mg/kg/day/three days, i.p.). A second group was injected with ABZSO, and a third one with $ABZSO_2$, both at 15 mg/kg/day/three days, i.p. A control group was dosed with corn oil. All animals were euthanized 24 h after the last dose and their livers were aseptically recovered and pooled by groups for processing and analysis.

In a second experiment, five experimental groups (each with its corresponding control) were used (three animals/group). Rats were administered ABZ (20 mg/kg/day) suspended in corn oil, by gastric intubation. Control animals received the vehicle only. The first group received a one day treatment and was euthanized 24 h later. The second group was treated with the same dose for two consecutive days and euthanized 24 h after the last dose. Three consecutive doses were given to the third group and animals were euthanized 24 h after the last administration. The fourth and fifth groups were also treated for three consecutive days but the animals were euthanized 72 and 120 h after the last dose, respectively. Livers were immediately and aseptically removed. Animals from each group were analyzed individually.

2.4. Microsomes preparation

Liver S9 fractions were prepared according to the procedure described by Maron and Ames (1983). Briefly, livers were minced in small pieces, homogenized in 150 mM KCl (3 ml/g wet liver), and centrifuged at 9 000 g for 10 min. The supernatant (S9) was subsequently centrifuged at 100 000 g for 60 min. The pellet was resuspended to its initial volume in a solution

containing 67.5 mM K_2HPO_4 and 32.5 mM KH_2PO_4 (pH 7.4) and recentrifuged at 100 000 g for 60 min. Microsomal fractions were finally resuspended in potassium phosphate buffer with 1 mM EDTA, 0.1 mM DTT, and 20% glycerol, in a volume of 1.5 ml/g of starting wet tissue. Samples were then stored at −70°C until analysis. All solutions and glassware were kept at 4°C. Protein concentrations were determined by the Bradford method (Bradford, 1976) using Bio-Rad (Hercules, California, USA) reagent.

2.5. Alkoxyresorufin O-dealkylases

Microsomal AROD activities were measured spectrofluorometrically by monitoring the formation of resorufin according to Burke's method (Burke et al., 1985) with some modifications. Excitation and emission wavelengths were set at 520 and 585 nm, respectively. 50 mM tris-HCl-25 mM $MgCl_2$, pH 7.6 buffer was used. Buffer (1.91–1.94 ml), substrate (10–40 μl) and microsomal sample (10–40 μl) were placed in appropriate amounts in a fluorimeter cuvette, and incubated at 37°C for 3 min. The reaction was started by the addition of 500 μM NADPH (20 μl of a 50 mM solution in buffer). With a total 2 ml volume of reaction, the cuvette was then placed in the fluorimeter and the reaction followed for 3 min, recording every 15 s the fluorescence readings. Substrates were dissolved in dimethylsufoxide as follows: Ethoxyresorufin, 50 μM; methoxyresorufin, 0.5 mM; pentoxyresorufin, 1.0 mM; and benzyloxyresorufin, 1.0 mM. Catalytic activities were calculated from a standard curve of resorufin (2.5–50 pmol/ml).

2.6. 4-Nitrophenol hydroxylase

Hydroxylation of 4-nitrophenol to 4-nitrocatechol was determined by a modification of the method described by Koop (1986). 4-Nitrophenol (0.2 mM) was dissolved in a 50 mM Tris–HCl-25 mM $MgCl_2$ (pH 7.4) buffer. 930 μl of this solution and 50 μl of microsomal sample were incubated at 37°C for 5 min. The reaction was started by adding 20 μl of 50 mM NADPH and incubated for another 10 min. Reaction mixtures were stopped by the addition of 0.5 ml of 0.6 N perchloric acid followed by centrifugation. 4-Nitrocatechol formation was then spectrophotometrically determined in 1 ml of supernatant plus 0.1 ml of NaOH (10 N), at 510 nm. A standard curve with 4-nitrocatechol (5–50 nmol/ml) was used to calculate microsomal activity.

2.7. Erythromycin N-demethylase

Erythromycin *N*-demethylation was assessed spectrophotometrically according to Alexidis et al. (1996) measuring the production of formaldehyde. The incubation mixture (1 ml final volume) contained: erythromycin 10 mM, 1 mg microsomal protein and buffer (10 mM $MgCl_2$, 150 mM KCl, 50 mM Tris–HCl). The mixture was pre-incubated three min at 37°C and the reaction was started by adding NADPH 10 mM and incubated for additional 30 min. Reaction mixtures were stopped by the addition of 0.5 ml 12.5% TCA followed by centrifugation. 1 ml of clear supernatant was mixed with 1 ml Nash reagent, heated at 50°C for 30 min and measured spectrophotometrically at 412 nm. A standard formaldehyde solution (100 nmol/ml) was used for the calibration curve.

2.8. Gel electrophoresis and immunoblot analysis

Polyacrylamide gel electrophoresis was carried out at room temperature in the presence of SDS using the discontinuous buffer system described by Laemmli (1970). 5 μg of microsomal protein were loaded per well. The slab separating gel ($7 \times 8 \times 0.075$ cm^3) contained 10% acrylamide and 0.3% bis-acrylamide. The electrophoresis was run under constant voltage (100 V). After electrophoresis, proteins were transferred to an 8×6 cm^2 nitrocellulose membrane following the method of Towbin et al. (1979). The membrane was then treated with 5% skim milk in phosphate-buffered saline (PBS) for 1 h at room temperature, incubated with anti-CYP antibody in a 0.5% skim milk in PBS solution (1:750) for 1 h, washed three times with PBS-0.05% Tween 20, incubated for an additional hour with the secondary antibody in 0.5% skim milk in PBS solution (1:2000) and washed again. Immunocomplexes were detected using 3′, 3-diaminobenzidine and hydrogen peroxide. Relative increases over controls for each CYP isoform were determined by densitometry of the spots by means of a microcomputer program (RFLP Scan 3.0, Scanalytics Inc., Fairfax, VA, USA). CYP1A1 and CYP1A2 as well as CYP2B1 and CYP2B2 bands were considered in conjunction to calculate the relative increase described in the results as CYP1A1/2 and CYP2B1/2 respectively. CYP2E1 and CYP3A2 specific bands were considered alone for calculations.

2.9. Statistics

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

3. Results

3.1. Enzymatic activities

Enzymatic activities of liver microsomes from ABZ

Table 1
Effect of ABZ and ABZ metabolites treatment on AROD[a], 4-NPH[b] and END[c] activities in rat hepatic microsomes (values represent means ± SD for at least three samples)

Enzymatic activity (associated CYP)	Control	ABZ (ABZ/Control)	ABZSO (ABZSO/Control)	$ABZSO_2$ ($ABZSO_2$/Control)
EROD activity (1A1)	91.94 ± 12.98	5971.95 ± 347.31** (65.0)	1848.94 ± 86.06** (20.1)	62.32 ± 8.11 (0.7)
MROD activity (1A2)	49.26 ± 2.78	301.60 ± 57.08** (6.0)	293.74 ± 43.11* (6.0)	78.40 ± 4.38* (1.6)
PROD activity (2B1)	10.64 ± 0.20	44.12 ± 2.94** (4.1)	22.84 ± 2.19* (2.1)	14.48 ± 0.48* (1.4)
BROD activity (2B2)	36.25 ± 2.77	508.92 ± 73.71* (14.0)	198.17 ± 7.30* (5.5)	28.47 ± 4.95 (0.8)
4-NPH activity (2E1)	1.26 ± 0.05	0.76 ± 0.14* (0.6)	1.06 ± 0.08* (0.8)	1.11 ± 0.19 (0.9)
END activity (3A2)	16.05 ± 1.53	15.4 ± 1.25 (1.0)	16.8 ± 1.39 (1.0)	16.0 ± 0.23 (1.0)

[a] Expressed in pmol of resorufin/mg of protein × min.
[b] Expressed in nmol of 4-nitrocatechol/mg of protein × min.
[c] Expressed in nmol of formaldehyde/mg of protein × 30 min.
** $P < 0.001$.
* $P < 0.05$.

treated rats are shown in Table 1. Ethoxyresorufin O-deethylase (EROD; associated to CYP1A1) and methoxyresorufin O-demethylase (MROD; associated to CYP1A2) activities were increased 65.0 and 6.0 times, respectively, by i.p. treatment with ABZ. Additionally, hepatic penthoxyresorufin O-dealkylase (PROD; associated to CYP2B1 activity) and benzyloxyresorufin O-dealkylase (BROD; associated to CYP2B2 activity) showed 4.0- and 14.0-fold increases respectively. 4-NPH activity decreased by 40% following ABZ treatment.

i.p. Intraperitoneal injection of ABZSO lead to a substantial increase in EROD and MROD activities (20.1- and 6.0-fold, respectively). PROD and BROD activities were also affected, showing an increase over controls of 2.1 and 5.5 times, respectively. A decrease in 4-NPH was produced by ABZSO. In contrast, $ABZSO_2$ did not substantially modify AROD activities as shown in Table 1.

3.2. Western-blot analysis

Following i.p. treatment with ABZ or ABZSO, a 4.2- and a 4.3-fold increase respectively of CYP1A1/2 content in liver microsomes over controls was observed, while CYP2B1/2, CYP2E1 and CYP3A levels remained unaffected (Fig. 1). No alteration of any CYP was noted after $ABZSO_2$ treatment.

Oral administration of ABZ at a human therapeutic dose of 20 mg/kg/day/three days also caused an increase in the hepatic concentration of CYP1A1 (Fig. 2) A 2.2-fold increase over the control value was noted 24 h after the first administration of the drug and it was 2.5 times higher after the second day of treatment; it reached a maximum of 2.7-fold 24 h after the last administration. Induction was maintained 72 h after the last dose (2.4-fold increase) and decreased towards normal protein levels 48 h later (1.6-fold over controls). No variations in CYP2B1/2, CYP2E1 and CYP3A protein content were detected after oral administration of ABZ (data not shown).

4. Discussion

In this study, the treatment of rats with ABZ caused a substantial increase in catalytic activity associated to CYP1A subfamily and, in a minor extent, to CYP2B subfamily, as well as a discrete reduction in 4-NPH activity (Table 1). No changes in the CYP3A-associated END activity were found in liver microsomes from ABZ, ABZSO, or $ABZSO_2$ treated animals. In comparison with other potent inducers of CYP1A (Lewis, 1996), the inducing potential (fold increase/dose) of

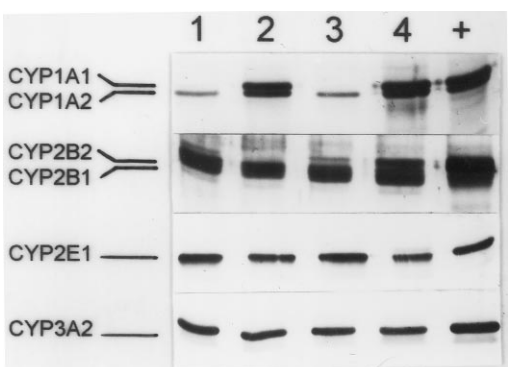

Fig. 1. Western blots detecting different CYP proteins in hepatic microsomes from rats treated with: 1, corn oil; 2, ABZSO; 3, $ABZSO_2$; and 4, ABZ. Pools were made from each experimental group. + lane contains microsome standards from rats administered with inducers for each CYP: 1A1/2, 3-methylcholanthrene; 2B1/2 and 3A, phenobarbital; 2E1, acetone.

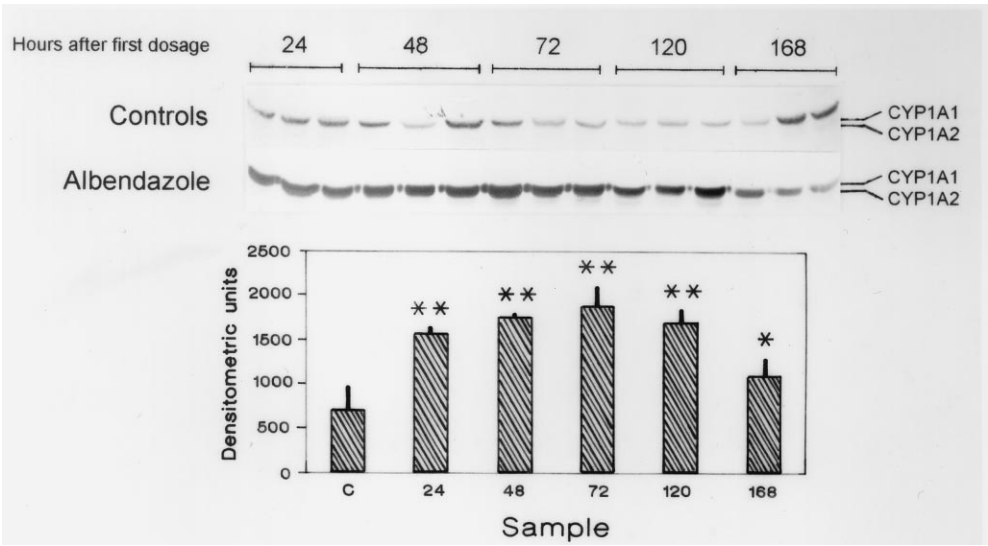

Fig. 2. CYP1A1/2 immunodetection in microsomes from rats receiving corn oil (controls) or ABZ (20mg/kg/day), and sacrificed at different times after the first administration; 24 h after first dosage rats received a single dose of 20 mg/kg, 48 h group received two doses and 72 h animals received three doses. 120 and 168 h groups were euthanized one and two days after receiving three consecutive doses. Each lane contains microsomes from a single rat. The bottom panel is a graphic presentation of the relative increase of CYP1A1/2 levels determined by densitometry of the nitrocellulose blots. Each value represents the mean densitometric unit $\pm$S.D. from three animals for each sample, and from 15 animals for control (c). $^{**}P < 0.001$; $^{*}P < 0.01$.

ABZ (300) is above that exhibited by potent inducers like 2-naphpthylamine (75), acridine orange (33) and 4-aminobiphenyl (25).

In the case of CYP1A1/2, the pattern of modulation of CYP immunoreactive proteins by ABZ paralleled that of EROD and MROD related activities in that both parameters showed an induction over controls. CYP3A immunoreactive protein and EDM activity remained unaffected and a marginal reduction of CYP2E1 protein and 4-NPH activity was noted in microsomes from ABZ treated rats (Table 1, Fig. 1).

Increases in PROD and BROD activities after ABZ and ABZSO treatment (Table 1) were not followed by increases in protein contents detected by immunoblot (Fig. 1). These results could be attributed to the high induction of CYP1A since both members of this subfamily proved to have PROD and BROD activities (Burke et al., 1994). We cannot rule out the possibility that other CYP forms not included in this study, like CYP2C6, were responsible for the PROD activity seen in hepatic microsomes form ABZ treated animals.

The pattern of CYP modulation observed after ABZ intake could help to explain some data from ABZ pharmacokinetics and metabolism reported previously. The biotransformation of ABZSO into $ABZSO_2$ by rat liver microsomes induced with 3-MC, ABZ, Aroclor or isosafrole is biphasic (Souhaili-El Amri et al., 1988b), indicating the involvement of two different enzyme systems. The high affinity component only requires 1.97 μg/ml of ABZSO to reach half its metabolic capacity and the plasma concentration was estimated to be 3 μg/ml (Souhaili-El Amri et al., 1988a), suggesting a major participation of this component, namely CYP1A1, in the conversion of sulfoxide to the sulfone metabolite. The CYP1A1 inducibility observed after ABZ intake (Table 1, Figs. 1 and 2) predicts that plasma levels of the sulfoxide derivative will be reduced and those of the sulfone metabolite will be augmented after repeated doses of ABZ. This is the case in the rat in which plasma concentration of ABZSO was reduced in 51.8% and that of $ABZSO_2$ increased to 92% after 10 days of ABZ treatment (Souhaili-El Amri et al., 1988a). Although no such study has been done in humans, a reduction of the mean ABZ sulfoxide plasma concentration was seen in two different populations under ABZ treatment when comparing a single dose scheme vs. an eight days dosage (Jung et al., 1990, 1992). Taken into account that ABZSO is the pharmacological active molecule, repeated doses of ABZ could influence its therapeutic effectiveness.

Data from the pharmacokinetic interaction between ABZ and dexamethasone in humans and rat, suggest the involvement of CYP3A in the sulfoxidation of ABZ (Moroni et al., 1995; Jung et al., 1990). In the present study, no CYP3A induction was found after ABZ treatment, which agrees with the result reported by Souhaili-El Amri et al. (1988a,b) who found no stimulation of the in vivo or in vitro sulphoxidation pathway after drug administration.

Considering the high levels of CYP1A activity reached after ABZ treatment, interactions of clinical relevance with other pharmaceutical drugs could be predicted. Imipramine (antidepressant), flutamide (antiandrogenic) and clozapine (antipsychotic), are metab-

olized by CYP1A2 to more toxic metabolites (Yang et al., 1999; Amitai and Frischer, 1994; Shet et al., 1997; Fau et al., 1994; Tugnait et al., 1999; Tschen et al., 1999) and the widely used drug theophyllin is also metabolized by CYP1A2. Concomitant administration of these drugs with ABZ should be avoided.

On the other hand, there are several evidences indicating that CYP1A1 and CYP1A2 proteins are involved in the activation of a wide range of pro-carcinogens and mutagens, such as benzo[a]dimethylbenzanthracene, β-naphthylamine, aflatoxin B_1, 4-aminobiphenyl, benzidine, and heterocyclic amines produced during the pyrolisis of aminoacids (Gonzalez and Gelboin, 1994; Schut and Snyderwine, 1999); therefore, increases in CYP1A will give rise to higher levels of genotoxic reactive intermediates resulting from the exposure to these genotoxic agents (Nebert et al., 1990).

Results reported here confirm and extend previous data on the inducing properties of the widely used anthelmintic drug ABZ. Until experimental data on the possible genotoxic and/or drug–drug interactions resulting from the concomitant intake of ABZ and other xenobiotics are obtained, individuals under therapeutic regimen with this drug should avoid the exposure to other drugs and environmental mutagens/carcinogens that are putative substrates for the CYP1A subfamily. According to the kinetics of the induction observed in our experiments, the levels of CYP1A1/2 enzymes return nearly to normal, 120 h after the last dose (Fig. 2), diminishing the probable risk associated to drug–drug interactions or increased electrophilic metabolites resulting from exposure to environmental mutagens/carcinogens.

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