



Inhibitory effect of albendazole and its metabolites on cytochromes P450 activities in rat and mouflon *in vitro*

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Abstract:

Cytochromes P450 (CYP) belong to the most important biotransformation enzymes, therefore, their inhibition may lead to serious pharmacological and toxicological consequences. Albendazole (ABZ) is a benzimidazole anthelmintic widely used in human and veterinary medicine. The effects of ABZ on CYP were investigated on the rat (*Rattus norvegicus*) and mouflon (*Ovis musimon*) hepatic microsomes. Besides ABZ, its two main metabolites (albendazole sulfoxide, ABZSO, and albendazole sulfone, ABZSOO) were tested to clarify which compound is responsible for the inhibitory effect. After preincubation of microsomes with the benzimidazoles (1, 5 and 25 μM), CYP activities, ethoxyresorufin *O*-deethylase (EROD) and benzyloxyresorufin *O*-dearylase activities were measured. The results showed that both ABZ and ABZSO, but not ABZSOO, exhibited significant potency to inhibit CYP activities measured in both tested species. Since ABZ as well as ABZSO are known inducers of EROD activity, our results clearly demonstrate that the drug can act as inducer and also as inhibitor of the same enzyme. In *in vitro* studies the CYP inhibition may mask the CYP induction. The extent of inhibition observed in mouflon was significantly higher than in rat. This finding emphasizes the importance of performance of inhibition studies in target animal species. Possible consequences of CYP inhibition should be taken into account during the anthelmintic therapy of mouflons with ABZ.

Key words:

albendazole, benzimidazole anthelmintic, enzyme inhibition, rat, mouflon

Abbreviations: ABZ – albendazole, ABZSO – albendazole sulfoxide, ABZSOO – albendazole sulfone, BROD – benzyloxyresorufin *O*-dearylase, CYP – cytochrome P450, EROD – ethoxyresorufin *O*-deethylase,

CYP, i.e. a decrease in enzyme activity, caused by interaction of the xenobiotic with an enzyme itself or by interference with the synthesis of CYP enzyme, represents one of the important consequences of exposure of organism to xenobiotics. As CYPs participate in metabolism of many drugs, their inhibition may essentially alter biotransformation processes and may have an impact on pharmacological or toxicological consequences of drug administration and exposure to environmental contaminants. Therefore, the ability and potency of drugs to inhibit the biotransformation

Introduction

Cytochromes P450 (CYP) belong to the most important biotransformation enzymes. The inhibition of

of exogenous or endogenous compounds requires careful attention [6, 17, 23]. In human pharmacology and toxicology, these problems have already been thoroughly studied but the interest of veterinary pharmacologists has been only sporadic [17, 24].

Albendazole (ABZ) is an important representative of benzimidazole anthelmintics that is adopted worldwide in human and veterinary medicine [8]. It is indicated in the cases of treatment and prevention of endoparasitological diseases. Recently, ABZ has been described to modulate activity of CYPs in rat and human [3, 19, 22]. In all mentioned papers, the ABZ potency to induce CYPs has been reported. The results of our previous studies [4, 5] have shown significant induction of ethoxyresorufin *O*-deethylase (EROD) and benzyloxyresorufin *O*-dearylase (BROD) activities caused by ABZ and its two metabolites in the rat hepatocytes. However, the non-linear dependence of the induced CYP activities on concentration of ABZ or albendazole sulfoxide (ABZSO) has been found [4, 5]. To explain the relative decrease in the extent of enzyme induction in hepatocytes treated with higher concentrations of inducers [4, 5] several hypotheses have been proposed. One of them has discussed possi-

ble inhibitory potency of ABZ or its metabolites on the tested CYPs [4]. This our hypothesis has been partially supported by study describing inhibitory effects of antifungal drugs with imidazole structure on CYP3A4 [2]. Thus, the study to evaluate the inhibitory effects of ABZ on CYPs in rat has been initiated.

In addition to rats, also mouflons were included in the study, because they represent one of the target animal species for ABZ treatment. Mouflon, originally a wild animal species (game species) is often bred in farms nowadays. Mouflons are sensitive to various endoparasitoses (muelleriosis, trichostrongylosis, dicrocoeliosis, nematodiosis, fasciolosis), hence, the use of ABZ for their treatment is common.

This study was designed to evaluate and compare the effects of ABZ on CYP activities (EROD and BROD) in the rat (laboratory animal) and mouflon (target species) *in vitro*. Parent drug (ABZ) and its two main metabolites, ABZSO and albendazole sulfone (ABZSOO), were tested to clarify which compound is responsible for the inhibitory effect (Fig. 1).

Materials and Methods

Chemicals

ABZ was purchased from Sigma-Aldrich (Prague, Czech Republic). ABZSO and ABZSOO were provided by Jang & Company (Glasgow, UK). Ethoxyresorufin and bovine serum albumin (BSA) were obtained from Fluka (Prague, Czech Republic). Benzyloxyresorufin, resorufin, Tris, coenzyme NADPH and bicinchoninic acid (BCA), protein assay reagent were obtained from Sigma-Aldrich (Prague, Czech Republic). All other chemicals were of HPLC or analytical grade.

Animals

Six young adult intact males, sexually mature were used for experiments. All animals used were healthy and have not been subjected to any pharmacological treatments. Mouflons (*Ovis musimon*) were breeding in a game enclosure under the usual conditions in Czech Republic. Six male Wistar rats (*Rattus norvegicus* var. *alba*) were maintained under constant conditions of heating and lighting and were fed with stan-

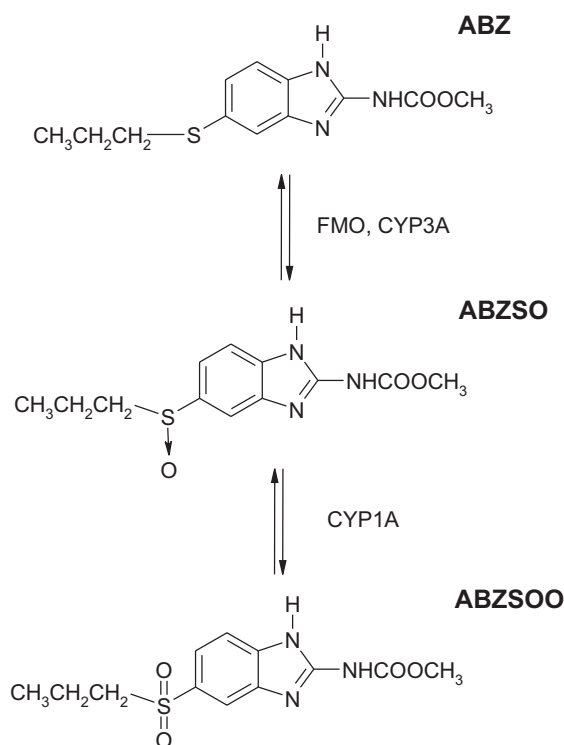


Fig. 1. Structure and the main metabolic pathway of ABZ

dard pelletes ad libitum. Rats (300–330 g) were sacrificed by decapitation under ether anesthesia. Mouflons, weighing approximately 25 kg, were shot. The liver (without any macroscopic alterations) was removed immediately after death of animal.

Preparation of hepatic microsomes

The *lobus intermedialis* (mouflon) or whole liver (rat) were cut into small pieces, and stored frozen in liquid nitrogen till preparation of subcellular fractions. The samples of microsomes for our experiments were prepared from mixture of livers of six rats or mouflons. Liver samples were homogenized at the w/v ratio of 1:6 in 0.1 M sodium phosphate buffer, pH 7.4, using the Potter-Elvehjem homogenizer and sonication was performed with Sonopuls (Bandeline, Germany). The microsomal fractions were isolated by fractional ultracentrifugation of the liver homogenate in the same buffer. A re-washing step (followed by the second ultracentrifugation) was added at the end of the microsomes preparation procedure. Microsomes were finally resuspended in a buffer containing 20% glycerol (v/v) and were stored at -80°C until required for assay. Cellular protein was assayed according to BCA method using BSA as a standard [21].

Enzyme assays

The measurements of EROD and BROD activities were performed as described by Burke et al. [7] with small modifications. Briefly, the reaction mixture contained 920 μl of 0.1 M Tris-HCl buffer (pH 7.4), 50 μl of MgCl_2 (0.1 M), 10 μl of hepatic microsomes (protein content 5–8 mg/ml), 5 μl of ethoxyresorufin (stock solution in dimethylsulfoxide, DMSO, final concentrations in reaction mixture were 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 μM) or benzyloxyresorufin (stock solution in DMSO, final concentrations in reaction mixture were 0.1, 0.25, 0.5, 0.75, 1.0, 2.5 μM), 10 μl of NADPH (0.4 mg of NADPH in 0.1 M Tris buffer, pH 7.4) and 5 μl of benzimidazole (ABZ, ABZSO or ABZSOO stock solution in DMSO) at three different concentrations (final concentrations in reaction mixture were 1, 5 or 25 μM). Concentration of DMSO in reaction mixture did not exceeded 0.1%. At this concentration, DMSO alone had no effect on EROD or BROD activity. The benzimidazole was incubated with microsomes in Tris-HCl buffer containing MgCl_2 at 37°C for 30 s, after that ethoxyresorufin or

benzyloxyresorufin were added and the reaction was initiated by NADPH. A kinetic measurement of fluorescence was performed at $\lambda_{\text{ex}} = 535\text{nm}$ and $\lambda_{\text{em}} = 585\text{ nm}$ and it was read for 60 s. Then, standard amount of resorufin, 5 pmol/reaction mixture (5 μl of stock solution in DMSO) was added. The linear fluorescence response was detected at resorufin concentration range 0–100 nM (calibration curve). The whole experiment lasted 90 s. No interference of DMSO, ABZ, ABZSO and ABZSOO was observed at excitation/emission wavelengths used for resorufin detection. All incubations were performed in triplicates.

Kinetic analysis and analysis of inhibition type and extent

Michaelis-Menten parameters, i.e. apparent maximum velocity (V_{max}) and apparent Michaelis constant (K_{m}) were estimated by non-linear regression analysis using GraphPad Prism 3.0 kinetic software. The type of inhibition was determined graphically using Lineweaver-Burk plot of reciprocals of reaction velocity ($1/v$) vs. reciprocals of substrate concentration ($1/s$). Inhibitor dissociation constants (K_{i} and αK_{i} values, respectively) were obtained graphically from Dixon plots. The concentration of inhibitor causing 50% reduction of activity, compared to the appropriate control value (IC_{50} value), was determined graphically from dose-response plot of enzyme fractional activity (v_i/v_0) vs. inhibitor concentration (only for two substrate concentrations: 0.25 and 2.5 μM) [9, 20].

To describe affinity of CYP for the tested inhibitors, the inhibition dissociation constants (K_{i} and αK_{i} , respectively) were determined. The factor α reflects the effect of an inhibitor on the affinity of the enzyme for its substrate, and the effect of the substrate on the affinity of the enzyme for the inhibitor. Mixed inhibition cannot be described by a single inhibitor dissociation constant K_{i} as free enzyme and the enzyme-substrate complex have different affinities for the inhibitors. Rather, in these cases both K_{i} and αK_{i} have to be determined to fully characterize the observed inhibition pattern [20].

ABZ biotransformation assay

The short-term incubations of the rat and mouflon hepatic microsomes with ABZ were performed to study the metabolism of the drug during the inhibition ex-

Tab. 1. Michaelis-Menten parameters (apparent maximum velocity, V_{max} [pmol/min/mg]; apparent Michaelis constant, K_m [μ M]) of EROD and BROD measured in rat hepatic microsomes in the presence of 1, 5 and 25 μ M ABZ or 1, 5 and 25 μ M ABZSO

Inhibitor	Activity	Kinetic parameter	Concentration of inhibitor [μ M]			
			0	1	5	25
ABZ	EROD	V_{max}	163.8 \pm 14.0	137.8 \pm 1.9*	129.3 \pm 3.2*	106.6 \pm 7.2*
		K_m	1.43 \pm 0.31	1.10 \pm 0.04	1.12 \pm 0.07	1.07 \pm 0.20
	BROD	V_{max}	289.8 \pm 13.3	290.2 \pm 9.8	299.0 \pm 11.9	232.9 \pm 5.9*
		K_m	0.89 \pm 0.09	0.99 \pm 0.07	1.25 \pm 0.10*	1.49 \pm 0.07*
ABZSO	EROD	V_{max}	188.2 \pm 19.9	199.1 \pm 16.4	197.9 \pm 9.6	195.1 \pm 9.6
		K_m	1.00 \pm 0.30	1.58 \pm 0.32	1.94 \pm 0.22*	2.22 \pm 0.24*
	BROD	V_{max}	309.9 \pm 13.4	288.5 \pm 18.8	253.9 \pm 16.8*	242.1 \pm 3.8*
		K_m	0.82 \pm 0.08	0.81 \pm 0.13	0.71 \pm 0.12	0.85 \pm 0.03

Each value represents the mean \pm SD of three independent experiments ($n = 3$). The microsomes were obtained from livers of six rats. * Significantly different ($p < 0.05$) as compared to the control

Tab. 2. Inhibitor dissociation constants (K_i and αK_i) and the concentration of inhibitor causing 50% reduction of activity, relative to the appropriate control value (IC_{50}) of EROD and BROD activities measured in rat and mouflon hepatic microsomes in the presence of variable concentrations of ABZ or ABZSO (1, 5 and 25 μ M)

Inhibitor	Activity	Rat hepatic microsomes				Mouflon hepatic microsomes			
		K_i [μ M]	αK_i [μ M]	IC_{50} [μ M]		K_i [μ M]	αK_i [μ M]	IC_{50} [μ M]	
				0.25 μ M substrate	2.5 μ M substrate			0.25 μ M substrate	2.5 μ M substrate
ABZ	EROD	18	63	25	>25	10		1	5–25
	BROD	41	93	≥ 25	>25	19	23	5–25	5–25
ABZSO	EROD	13–17		5	>25	10–13		1–5	>25
	BROD	118		>25	>25	48		≥ 25	≥ 25

All incubations were performed in triplicate ($n = 3$). The microsomes were obtained from livers of six rats or mouflons

periment. The reaction mixture included 225 μ l of sodium phosphate buffer (0.1 M, pH 7.4) containing ABZ (its final concentrations in the whole reaction mixture were 1, 5 or 25 μ M), 25 μ l of hepatic microsomes (protein content was 6 mg/ml) and 50 μ l of NADPH (0.25 mg NADPH in 0.1 M sodium phosphate buffer, pH7.4). All incubations were performed at 37°C, the times of incubation were 0, 30, 60 and 90 s. Addition of 180 μ l of acetonitrile stopped the reactions. Then, the samples were centrifuged and the supernatants were stored at 20°C until analysis.

HPLC analysis

HPLC was carried out using a Spectra Series P200 gradient pump, a HP 1100 Series autosampler, a HP 1100 Series thermostated column compartment, Phil-

ips fluorescence detector ($\lambda_{ex} = 290$ nm, $\lambda_{em} = 320$ nm) fitted with a Discovery C18 (5 μ m, 150 mm 4.6 mm) reverse-phase column (Supelco, Bellefonte, USA) protected with Discovery C18 precolumn (20 mm 4 mm). The solvent system was acetonitrile : 0.1 M ammonium acetate pH 4.7, at a proportion 28:72 (v/v) with flow rate of 1.0 ml/min. All measurements were carried out at 25°C. Data from chromatographic runs were processed using the Chromatography Station for Windows CSW32. The concentrations of two main ABZ metabolites (ABZSO and ABZSOO) were assessed using the analytical standards.

Statistical analysis

In this study, the one-way ANOVA was performed for comparison of the effects of three benzimidazoles on

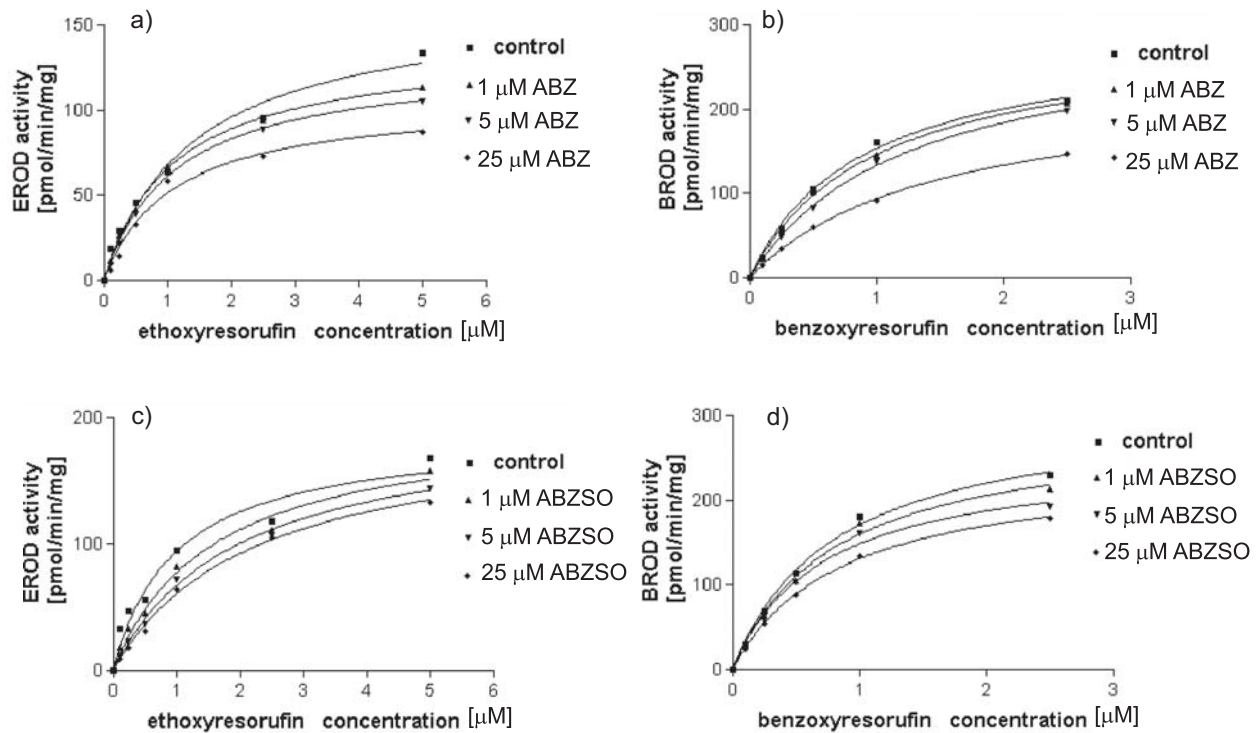


Fig. 2. Plots of enzyme activity vs. substrate concentration. a) EROD and b) BROD activities measured in rat hepatic microsomes in the presence of 1, 5 and 25 μM ABZ; c) EROD and d) BROD activities measured in rat hepatic microsomes in the presence of 1, 5 and 25 μM ABZSO. All incubations were performed in triplicate ($n = 3$); data are expressed as the mean of the measured enzyme activities [pmol/min/mg]. The microsomes were obtained from livers of six rats

two CYP activities in two different species. In addition, Student's *t*-test was used to assess whether the mean of variable differs between two groups compared.

Results

Effects of ABZ and ABZSO on EROD and BROD activities in rat microsomes

The significant inhibition of EROD was observed after incubation of rat microsomes with ABZ or ABZSO (Fig. 2). The extent of inhibition depended on ethoxyresorufin and inhibitor concentrations. The highest decrease in EROD activities (by 70%, $p < 0.001$) was provoked by 25 μM ABZ or ABZSO at 0.1 μM ethoxyresorufin. In case of BROD activities, the significant inhibition ($p < 0.01$) was observed only after incubations with 25 μM ABZ at all benzyloxyresorufin con-

centration (approximately 40% below control values) (Fig. 2). Also ABZSO decreased BROD activities only slightly (5 μM ABZSO caused inhibition by 10%, $p < 0.05$, and 25 μM ABZSO by 25% below control activity, $p < 0.01$) and no dependence of extent of inhibition on benzyloxyresorufin concentration was found (Fig. 2). Kinetic and inhibition parameters (K_m , V_{max} , K_i and IC_{50}) of the mentioned reactions are presented in Tables 1 and 2.

Effects of ABZ and ABZSO on EROD and BROD activities in mouflon

Both ABZ and ABZSO provoked strong inhibition of EROD activities in mouflon microsomes (Fig. 3). At 0.1 μM concentration of ethoxyresorufin, 25, 5 and 1 μM ABZ caused EROD inhibition by 85% ($p < 0.001$), 75% ($p < 0.001$) and 40% ($p < 0.01$), respectively. 25 μM ABZSO also led to the strongest inhibitory effect (80% below the control, $p < 0.001$) at the 0.1 μM concentration of ethoxyresorufin. The degree of inhibition was dependent on the ethoxyresorufin concen-

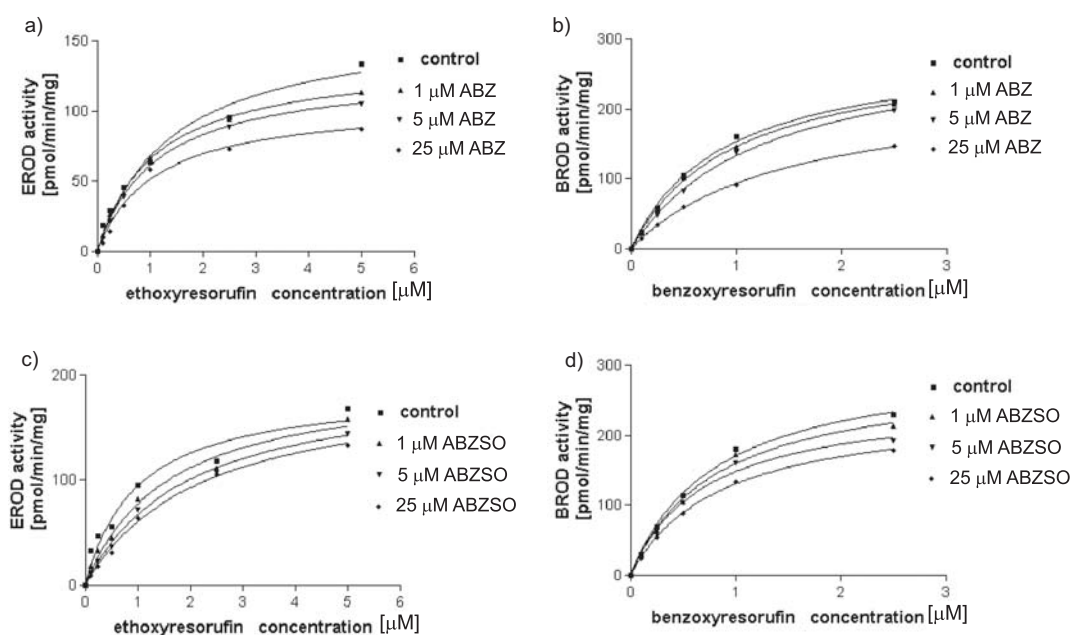


Fig. 3. Plots of enzyme activity vs. substrate concentration. a) EROD and b) BROD activities measured in mouflon hepatic microsomes in the presence of 1, 5 and 25 μM ABZ; c) EROD and d) BROD activities measured in mouflon hepatic microsomes in the presence of 1, 5 and 25 μM ABZSO. All incubations were performed in triplicate ($n = 3$); data are expressed as the mean of the measured enzyme activities [pmol/min/mg]. The microsomes were obtained from livers of six mouflons

Tab. 3. Michaelis-Menten parameters (apparent maximum velocity, V_{max} [pmol/min/mg]; Michaelis constant, K_m [μM]) of EROD and BROD activities measured in mouflon hepatic microsomes in the presence of variable concentrations of albendazole (ABZ) or albendazole-sulfoxide (ABZSO), respectively (1, 5 and 25 μM)

Inhibitor	Activity	Kinetic parameter	Concentration of inhibitor [μM]			
			0	1	5	25
ABZ	EROD	V_{max}	235.3 ± 7.6	233.4 ± 12.5	221.3 ± 16.2	208.7 ± 18.7
		K_m	0.75 ± 0.05	$1.55 \pm 0.15^*$	$2.60 \pm 0.30^*$	$3.89 \pm 0.50^*$
	BROD	V_{max}	19.6 ± 2.7	16.9 ± 2.9	17.2 ± 1.5	$9.3 \pm 2.0^*$
		K_m	0.67 ± 0.23	0.73 ± 0.31	1.12 ± 0.20	0.82 ± 0.41
ABZSO	EROD	V_{max}	78.2 ± 2.5	78.1 ± 1.5	79.3 ± 2.0	77.5 ± 6.9
		K_m	0.26 ± 0.03	$0.59 \pm 0.03^*$	$1.08 \pm 0.06^*$	$1.47 \pm 0.25^*$
	BROD	V_{max}	25.4 ± 2.3	$18.3 \pm 1.9^*$	$15.4 \pm 1.6^*$	$13.4 \pm 1.6^*$
		K_m	0.73 ± 0.16	0.64 ± 0.16	0.59 ± 0.16	0.60 ± 0.19

Each value represents the mean \pm SD of three independent experiments ($n = 3$). The microsomes were obtained from livers of six mouflons. * Significantly different ($p < 0.05$) as compared to the control

tration. BROD activities in mouflon microsomes were also inhibited by ABZ as well as ABZSO, but to a lower extent than EROD (maximally by 60% or 40%, respectively, $p < 0.01$). Kinetic and inhibition parameters (K_m , V_{max} , K_i and IC_{50}) of the mentioned reactions are presented in Tables 2 and 3.

Effects of ABZSO on EROD and BROD on rat and mouflon microsomes

In contrast to ABZ and ABZSO, no inhibitory potency on EROD or BROD activities measured was observed after the incubations of rat as well as mou-

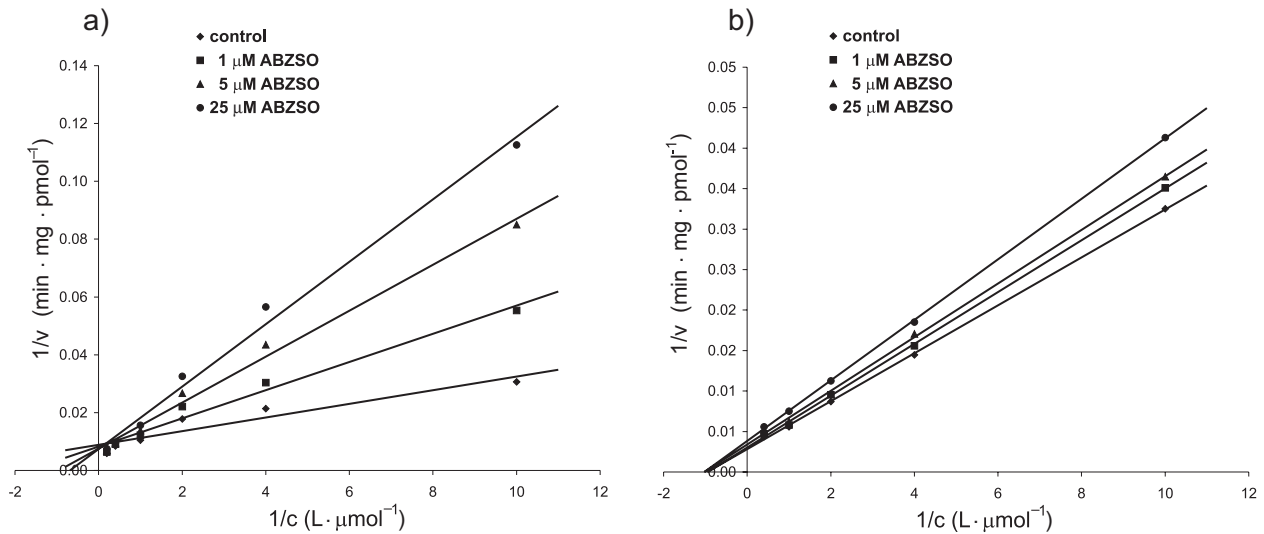


Fig. 4. Lineweaver-Burk plots of reciprocals of reaction velocity ($1/v$) vs. reciprocals of substrate concentration ($1/c$). a) EROD and b) BROD activities measured in rat hepatic microsomes in the presence of 1, 5 and 25 μM ABZSO. All incubations were performed in triplicate ($n = 3$). The microsomes were obtained from livers of six rats

flon hepatic microsomes with ABZSOO, even at the highest tested concentration of the drug (25 μM) (data not shown).

Inter-species differences in EROD and BROD inhibition by ABZ and ABZSO

ABZ and ABZSO caused a stronger inhibition of EROD and BROD activities in mouflon than in rat microsomes. ANOVA indicated the significant inter-species difference ($p < 0.001$) in the effects of ABZ and ABZSO on BROD activities. In case of EROD activities, the significant inter-species difference ($p < 0.001$) in inhibition potency of ABZ was proved using ANOVA except 0.1 μM ethoxyresorufin. The inhibitory effects of ABZSO were also significantly different in rat compared to mouflon; the level of significance of this inter-species difference was $p < 0.01$ at 0.1 and 0.25 μM ethoxyresorufin and $p < 0.001$ at 0.5, 1.0 and 2.5 μM ethoxyresorufin.

Metabolism of ABZ during the inhibition experiments

Metabolic transformation of ABZ into metabolites during the inhibition experiments (90 s) was also

studied. In mouflon hepatic microsomes, the time-dependent formation of ABZSO from ABZ was detected. At the concentrations of ABZ of 1.0 and 5.0 μM , almost 50% of ABZ was biotransformed into ABZSO during the short-term incubation. At the highest concentration of ABZ (25 μM), the concentration of ABZSO was only slightly higher than at 5.0 μM ABZ (the reaction was already saturated with the substrate). In comparison to mouflon, only slight metabolism of ABZ into ABZSO (not more than 5%) during the short-term incubation was found in rat hepatic microsomes. No ABZSOO was detected after 1.5-min incubation of ABZ with microsomes of both species.

Discussion

In our study, the measurements of EROD and BROD activities were performed to evaluate the inhibitory effects of ABZ on CYP. EROD activity is specific for CYP1A in rat [7]. In other species, EROD activities were also related to CYP1A [17]. In rat, BROD activity is mainly catalyzed by CYP2B and partly by

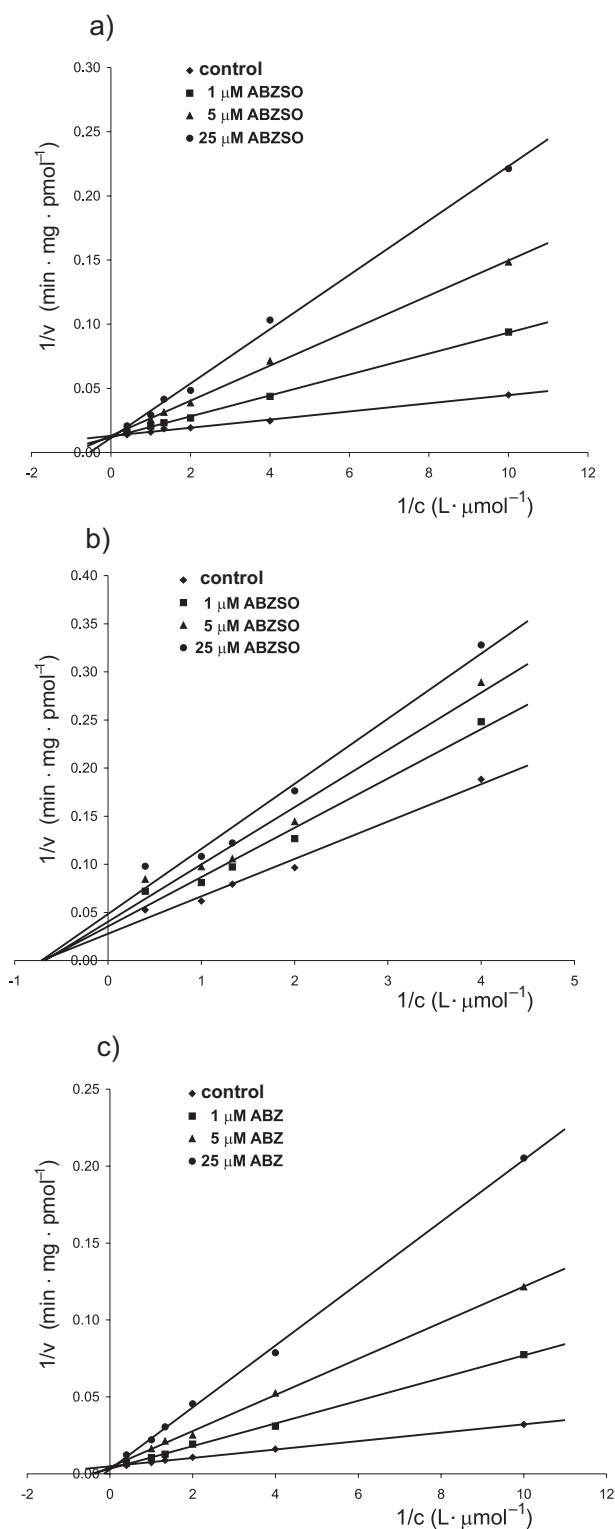


Fig. 5. Lineweaver-Burk plots of reciprocals of reaction velocity ($1/v$) vs. reciprocals of substrate concentration ($1/c$). a) EROD and b) BROD activities measured in mouflon hepatic microsomes in the presence of 1, 5 and 25 μM ABZSO and c) EROD activities measured in mouflon hepatic microsomes in the presence of 1, 5 and 25 μM ABZ. All incubations were performed in triplicate ($n = 3$). The microsomes were obtained from livers of six mouflons

CYP3A [18]. On the other hand, BROD activity in other species (e.g. human, pig) was mainly attributed to CYP3A [1, 7, 12, 15].

Besides ABZ, also its two main metabolites, ABZSO and ABZSOO, were tested in our project with the aim to find out, which compound is responsible for the inhibitory effects of the drug on CYPs. It is well known that in rat as well as in sheep [14, 16] ABZ is rapidly metabolized through two-step S-oxidation yielding first ABZSO followed by ABZSOO. While ABZSO has anthelmintic activity, ABZSOO is pharmacologically inactive [10, 11, 13]. Flavine-containing monooxygenases (FMO) and CYP3A participate in the first metabolic step [16], whereas the formation of the sulfone is mainly catalyzed by CYP1A subfamily [22].

The results of our preliminary experiments showed that ABZSOO (even at 25 μM concentration) did not cause any inhibition of the measured activities. Hence, the detailed inhibition study aimed to find the extent, type and kinetic parameters of inhibition was performed only with ABZ and ABZSO.

The competitive type of inhibition of EROD activities by ABZSO was found in both tested species as deduced from K_m and V_{max} values, and from Lineweaver-Burk plot (Figs. 4a and 5a). This is in agreement with fact that ABZSO is metabolized by CYP1A [22]. In mouflon, competitive inhibition was also proven for the inhibition of EROD activities by ABZ (Fig. 5c) and this was a surprising result, as participation of CYP1A in ABZSO formation has not been reported. Probably ABZSO, which is formed in relatively high amount in incubation mixture during the experiments, is responsible for the inhibitory effect of the drug. Nevertheless, it has to be also taken into account that CYP1A may contribute on the conversion of ABZ to ABZSO in mouflon.

In both species, ABZSO was found to affect BROD activities as noncompetitive inhibitor. This was indicated by K_m and V_{max} values as well as Lineweaver-Burk plot (Figs. 4b and 5b). In the case of rat microsomes, the kinetic constants (K_m and V_{max}) showed the tendency of competition (dependence of the effect of ABZ on benzyloxyresorufin concentration), but only at the concentrations of ABZ of 1 and 5 μM . In mouflon hepatic microsomes, even no sign of competition was observed. The influence of ABZSO included in the incubation mixture and/or the participation of several CYP on BROD activity may explain our results. Some kind of mixed type inhibition was suggested also for the inhibition of EROD activities in the rat hepatic microsomes by ABZ.

If we compare K_i and αK_i values determined for the inhibitions, CYP1A seems to have higher affinity for ABZ and ABZSO than CYP of BROD activities. Moreover, better binding to ABZSO compared to ABZ should be expected for CYP1A.

Although the most of inhibitory effects found in our study should not have serious (even any) clinical consequences as plasma concentrations of benzimidazoles are considerably lower than the determined K_i (αK_i) and IC_{50} values, inhibition of CYP1A activities in mouflon by ABZ and ABZSO may be considered as therapeutically significant. This inhibition should not have any negative effects on the anthelmintic activity of ABZ, it could be rather positive, as CYP1A plays an important role in deactivation of anthelmintically active ABZSO. However, influence of ABZ on metabolism of co-administrated drugs, which are biotransformed by CYP1A (e.g. fenbendazole, thiabendazole, caffeine, estradiol), cannot be excluded. In respect of this fact, co-administration of ABZ with drugs mentioned above should be avoided in mouflon breeding.

In our previous study [5], ABZ has been described to induce EROD and BROD activity in rat. The presented study showed that this drug had also inhibitory potency on the mentioned CYP activities. Taken together, one compound can act as an inducer as well as an inhibitor of the same enzyme. Thus, inducing effect of a drug may be masked by its inhibitory potency, especially in *in vitro* experiments.

In conclusion, our results confirmed significant inhibitory effect of ABZ on EROD and BROD activity. ABZ and its metabolite ABZSO are both responsible for the inhibitory potency of the drug. The extent of EROD and BROD inhibition was much higher in mouflon than in rat. Hence, our findings clearly demonstrate inter-species differences in response to inhibitors and, thus, the importance of performance of inhibition studies in target animal species. Possible consequences of CYP inhibition should be taken into account in the studies of CYP induction by ABZ using *in vitro* models and also during the anthelmintic therapy of mouflons with this drug.

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