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The inability of tapeworm *Hymenolepis diminuta* and fluke *Dicrocoelium dendriticum* to metabolize praziquantel

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ABSTRACT

Biotransformation enzymes can, to a certain extent, protect parasitic worms against the toxic effects of anthelmintics and can contribute to drug-resistance development. The objective of our work was (1) to find and identify phase I and II metabolites of the anthelmintic praziquantel (PZQ) formed by the lancet fluke (*Dicrocoelium dendriticum*) and the rat tapeworm (*Hymenolepis diminuta*) and (2) to compare PZQ metabolites in helminths with PZQ biotransformation in rat as host species. Ultra high performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS) was used for this purpose. During *in vitro* incubations, mitochondria-like and microsomes-like fractions (prepared from homogenates of adult worms or from rat liver homogenate) were incubated with 10 and 100 μM PZQ. Liquid/liquid extraction was used for samples during *in vitro* experiments. In the *ex vivo* study, living *D. dendriticum* and *H. diminuta* adults were incubated in RPMI-1640 medium in the presence of 50 nM or 100 nM PZQ for 24 h. After incubation, the worms were removed from the medium and homogenized. Homogenates of worms, medium from the incubation of worms or rat hepatocytes and rat urine (collected during 24 h after oral PZQ administration) were separately extracted using solid-phase extraction.

The results showed that both *D. dendriticum* and *H. diminuta* enzymatic systems are not able to metabolize PZQ. On the other hand, thirty one different phase I and four phase II PZQ metabolites were detected in rat samples using UHPLC/MS/MS analyses. These results show that our experimental helminths, as the members of tapeworm and fluke groups of parasites, are not able to deactivate PZQ, and that the biotransformation enzymes of the studied helminths do not contribute to PZQ-resistance.

1. Introduction

Praziquantel (PZQ) is a pyrazinoisoquinoline drug used in both veterinary and human medicine as the drug of choice against many parasitic diseases caused by cestodes and trematodes. PZQ is the primary treatment for human schistosomiasis caused by genus *Schistosoma*, for which it is usually effective in a single dose (Cioli and Pica-Mattoccia, 2003). This anthelmintic has no activity against nematodes, probably due to differences in the metabolism of purines (Jeziorski and Greenberg, 2006; Angelucci et al., 2007).

The exact mechanism of action of PZQ remains still unclear, although there are some theories attempting to explain it. A rapid influx of calcium ion (Ca^{2+}) and other morphological changes of the tegument in schistosomes were observed. In accordance with this, β subunits of voltage-gated Ca^{2+} channels, located in parasites, have been identified as the molecular target of PZQ (Doenhoff et al., 2008). Another hypothesis puts forth that PZQ causes calcium influx by the blocking of adenosine receptors

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(Angelucci et al., 2007). Either way, PZQ evidently causes quick and total paralysis in parasites.

In the last decade, several cases indicating a lower efficiency of PZQ against *Schistosoma mansoni* have been noticed and in some case reports PZQ totally failed to cure schistosomiasis. Determinations of possible resistance mechanisms remain speculative (Doenhoff et al., 2008; Barakat and Morshedy, 2010). Generally, drug-resistance may be caused by many mechanisms, e.g. changes in the target structure, in drug biotransformation or in drug transport (uptake/efflux) (Ouellette, 2001).

The metabolism of PZQ has been studied quite extensively in mammals, in which PZQ mono- and di-hydroxy derivatives, glucuronides and sulfates have been observed (Meier and Blaschke, 2000; Giorgi et al., 2001). In helminths, the biotransformation of PZQ has not yet been studied, although biotransformation can lead to a modification of efficiency or even the inactivation of drugs. To fill this gap, two helminths species, the lancet fluke (*Dicrocoelium dendriticum*) from flukes, and the rat tapeworm (*Hymenolepis diminuta*) from tapeworms were used for the study of PZQ biotransformation. These species were chosen as representatives of two classes of flatworms in which PZQ is often used for the treatment of parasitoses (Marangi et al., 2003; Rana et al., 2007; Patamia et al., 2010). Efficiency of PZQ in sheep infected by *D. dendriticum* was about 90% at a dose of 50 mg/kg (Wolff et al., 1984). *D. dendriticum* is a parasite found in wild and domestic ruminants (sheep, goat, cattle), and occasionally in rabbits, pigs, dogs, horses and humans (Otranto and Traversa, 2002). Diagnostic methods are based mostly on coprological methods and the post-mortem examination of liver and bile. Newer techniques are based on immunodiagnostic methods such as ELISA tests (Broglia et al., 2009).

H. diminuta has one intermediate host – beetles of genus *Tribolium*, *Tenebrio* or others. The adult worms live in the small intestine of final hosts, rats and other rodents (Arai, 1980). Because of the easy maintenance of the *H. diminuta* cycle in the laboratory for many years, this helminth is often used as a model for other cestodes.

The present study was designed to determine if *D. dendriticum* and *H. diminuta* are able to metabolize PZQ. The metabolites of phase I or phase II of PZQ biotransformation formed by *D. dendriticum* and *H. diminuta* were searched for *in vitro* as well as *ex vivo*. PZQ biotransformation was also tested in rats with the goal of comparing PZQ metabolites formed in helminths with those formed in their hosts.

2. Materials and methods

2.1. Chemicals

Praziquantel was purchased from Sigma–Aldrich (Prague, Czech Republic). Liquid sterile-filtered RPMI-1640 medium (Roswell Park Memorial Institute medium), HAM F12 medium, Williams' E medium, fetal calf serum and all other chemicals (LC–MS, HPLC or analytical grade) were obtained from Sigma–Aldrich (Prague, Czech Republic).

2.2. Laboratory animals

Female Wistar rats (16–18 weeks) were obtained from BioTest (Konárovice, Czech Republic). They were kept on standard rat chow with free access to tap water, in animal quarters under a 12-h light–dark cycle. The rats were cared for and used in accordance with the *Guide for the care and use of laboratory animals* (Protection of Animals from Cruelty Act. No. 246/92, Czech Republic).

2.3. Isolation of hepatocytes for in vitro experiment

The hepatocytes were obtained from livers of 2 female rats by two-step collagenase method (Berry et al., 1991), and the isolated hepatocytes were mixed together. Three million of viable (65%) cells in 3 ml of culture medium ISOM (1:1 mixture of Ham F12 and Williams' E) were placed into 60-mm plastic dishes pre-coated with collagen. The foetal calf serum was added in culture medium (5%) to favour the cells attachment during first four hours and after that medium without serum was changed. The cultures were maintained without substrates 4 h at 37 °C in a humid atmosphere of air and 5% CO_2.

2.4. Collection of parasite material

D. dendriticum adults were isolated from naturally infected mouflon ewes (*Ovis musimon*, $n = 5$, age 5–7 years) bred in the Vlkov game preserve (Czech Republic). The infected mouflon ewes were culled according to Czech slaughtering rules for farm animals (stunning, exsanguination). After removal of the liver from the abdominal cavity (up to 5 min), the liver was coated with a polyethylene sac, immersed in warm saline solution (0.9% NaCl in water, 38 °C) and transported to the laboratory (up to 60 min). There the liver tissue was cut and repeatedly washed with a saline solution (38 °C) with the aim of collecting the fluke adults. In the following experiments, the mixture of flukes isolated from individual mouflons was used. Freshly isolated living flukes were washed three times with a 0.1 M phosphate buffered saline (pH 7.4, 38 °C) containing 60 μg ml^{-1} penicillin and 100 μg ml^{-1} streptomycin.

H. diminuta adults were isolated from artificially infected male Wistar rats (20–25 weeks). Rats were infected by cysticercoids of *H. diminuta* previously isolated from beetles *Tenebrio molitor*. Beetles were infected by eggs of this parasite two weeks before dissection. After two months post infection, rats were decapitated and small intestines were removed. Tapeworms were flushed out from small intestines by 0.1 M phosphate buffer (pH 7.4, 38 °C) to Petri dish. All tapeworms were rinsed three times with a 0.1 M phosphate buffered saline (pH 7.4, 38 °C) containing 60 μg ml^{-1} penicillin and 100 μg ml^{-1} streptomycin.

The isolated parasites were either used immediately for *ex vivo* experiments or frozen at −80 °C for preparation of subcellular fractions for *in vitro* studies.

2.5. Preparation of subcellular fractions

The frozen flukes or tapeworms were thawed at room temperature and homogenized in 0.1 M sodium phosphate buffer (pH 7.4) at the ratio of 1:6 (w/v), using a Potter–Elvehjem homogeniser and sonication with Sonopuls (Bandelin, Germany). The subcellular fractions were prepared by fractional ultracentrifugation of the homogenate with the same buffer. The 20,000 × *g* sediment (60 min, 4 °C) corresponds to the mitochondrial fraction (MIT) in vertebral tissues fractionation. The sediment was then resuspended in the buffer and rewashed by repeating centrifugation at 20,000 × *g* (60 min, 4 °C). Cytosol-like (CYT) and microsome-like (MIC) fractions correspond to the supernatant and sediment of the centrifugation at 105,000 × *g* (60 min, 4 °C). A re-washing step in the same buffer (followed by the second ultracentrifugation) was included at the end of the preparation procedure for the microsomes. The mitochondria and microsome-like fractions were finally resuspended in 0.1 M sodium phosphate buffer (pH 7.4) containing 20% glycerol (v/v). The subcellular fractions from rat liver were prepared by the same procedure. All subcellular fractions were stored at −80 °C.

2.6. In vivo experiment

For *in vivo* studies urine of adult female rats after oral administration of PZQ suspension (100 mg per kg of body weight) was collected during 24 h post administration. All samples were stored at −80 °C before extraction and analysis.

2.7. Ex vivo experiment

Freshly isolated *D. dendriticum* (50 adults for each sample) and *H. diminuta* (2 adults for each sample) were cultivated in plastic flasks in 5 ml or in 10 ml, respectively, of RPMI-1640 medium (containing 60 μg ml^{-1} penicillin and 100 μg ml^{-1} streptomycin) under humid atmosphere of air and 5% CO_2 at 38 °C. PZQ was predissolved in dimethyl sulphoxide (DMSO). The final concentration of DMSO in medium was only 0.1% (v/v) to prevent its harmful impact on living parasites. Final concentrations of PZQ used for incubations of *D. dendriticum* were 25 nM, 50 nM, and 1 μM. Final concentrations of PZQ used for incubation of *H. diminuta* were 0.1 μM, 1 μM, and 10 μM.

The parasites were incubated with PZQ for 2, 4, 8, and 24 h. After the incubation, medium was taken up, placed into plastic tubes, frozen and stored at −80 °C. The parasites were repeatedly washed up, transferred into plastic tubes, frozen and stored at −80 °C. In blank samples, either medium containing the drugs but not the parasites was incubated or the parasites were incubated in a drug-free medium. Prior to the analysis, parasite bodies were homogenized in redistilled water at the ratio of 1:3 (w/v) using Sonopuls (Bandelin, Germany). Medium and the parasite homogenate were then extracted using solid-phase extraction.

2.8. Incubation of hepatocytes primary culture with PZQ

After attachment of rat hepatocytes, the ISOM medium was replaced with fresh serum-free medium with 10 μM PZQ, pre-dissolved in DMSO. The concentration of DMSO in medium was 0.1% (v/v). The hepatocytes were incubated with drugs for 2 h or 24 h at 38 °C in a humid atmosphere of air and 5% CO_2. Aliquots of medium (0.5 ml) were collected and stored frozen at −24 °C prior to their extraction.

2.9. Incubation of subcellular fractions with PZQ in vitro

The subcellular fractions from parasites (MIC and MIT together) and rats (MIC) were incubated with substrate PZQ and coenzyme NADPH. The total volume of the reaction mixture (0.3 ml) contained 100 μl of fractions (approximately 0.4 mg of proteins), anthelmintic drugs (100 μM) pre-dissolved in DMSO, 1 mM NADPH and 0.1 M sodium phosphate buffer (pH 7.4). The blank samples contained either 100 μl of 0.1 M sodium phosphate buffer (pH 7.4) instead of fractions, or the fractions were incubated without drugs. The concentration of solvents in all reaction mixtures and blank samples was 1% (v/v). All incubations were carried out at 37 °C for 30 min under aerobic conditions.

2.10. Solid-phase extraction and sample preparation

Samples from *in vivo* and *ex vivo* experiments were extracted using solid-phase extraction method. Two ml of the medium, parasite homogenate or 4 ml of urine were centrifuged at 3000 × *g* for 5 min. The supernatant was loaded onto a Waters Oasis HLB extraction cartridge (1cc, 30 mg, 30 μm particles; Waters) previously conditioned by washing with 1 ml methanol and 1 ml redistilled water. In the next step, the cartridge was washed with 1 ml 2.5% (v/v) methanol. Compounds of interest were eluted with 1.5 ml of methanol.

The eluates were evaporated to dryness using vacuum concentrator (Eppendorf 5310, Hamburg, Germany) and stored at −80 °C until LC/MS analysis.

2.11. Liquid/liquid extraction

Liquid/liquid extraction was used only for samples of microsomal and mitochondria fractions incubated with PZQ. At the end of the incubation, 30 μl of ammonium solution (concentrated, 25% (v/v)) and 700 μl of ethyl acetate were added. After shaking (3 min, vortex) and centrifugation (3 min, 5000 × *g*) of the mixture, the supernatants were removed and subsequently evaporated to dryness using vacuum concentrator. Samples were stored at −80 °C until LC/MS analysis.

2.12. Ultra high performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS)

Particular samples were quantitatively dissolved in 200 μl mixture of acetonitrile/water (1:1, v/v).

UHPLC/MS/MS chromatograms of samples were measured in both positive and negative polarity modes using electrospray ionization (ESI) and hybrid quadrupole-time-of-flight mass analyzer (micrOTOF-Q, Bruker Daltonics, Germany). The atmospheric pressure chemical ionization (APCI) was also used for preliminary experiments. UHPLC was performed on an Agilent 1290 Infinity Liquid Chromatograph (Agilent Technology, Waldbronn, Germany) using Kinetex C18 column 150 mm × 2.1 mm, 1.7 μm (Phenomenex, Torrance, CA, USA), temperature 25 °C, flow rate 0.3 ml/min and the injection volume 0.3 μl. The mobile phase consisted of acetonitrile (A) and 5 mM ammonium acetate buffer with pH 6.5 (B). The linear gradient was as follows: 0 min – 15% A, 10 min – 55% A, 15 min – 95% A; and finally washing and reconditioning of the column. The quadrupole-time-of-flight mass spectrometer was used with the following setting of tuning parameters: capillary voltage 4.5 kV, drying temperature 220 °C, the flow rate and pressure of nitrogen were 8 l/min and 1.3 bar, respectively. The external calibration was performed with sodium formate clusters before individual measurements. ESI mass spectra were recorded in the range of m/z 50–1000 both in positive- and negative-ion modes. The isolation width $\Delta m/z$ 4 and the collision energy 20 eV using argon as the collision gas were used for MS/MS experiments.

3. Results and discussion

In the present project, the biotransformation of PZQ was studied in two helminths species, fluke *D. dendriticum* and tapeworm *H. diminuta*, as well as in rats as host species. All *in vitro* and *ex vivo* methods applied for this purpose had been used successfully in our previous studies leading to the identification of several new metabolites of benzimidazole anthelmintics in helminths (Cvilink et al., 2008, 2009).

The analytical characterization of PZQ and its metabolites in biological samples were performed using UHPLC/ESI-MS/MS. First, a full scan and a tandem mass spectra of standard PZQ were measured and interpreted in the positive-ion and negative-ion mode of ESI and APCI. No significant differences among the obtained ESI and APCI mass spectra were found. Due to the expectation of phase II metabolite formation, the ESI was further applied for the subsequent measurement. Both ESI polarity modes were used to obtain individual $[M+H]^+$ and $[M-H]^-$ ions in the full scan spectra and the structural information in tandem mass spectra. Emphasis was given on the precise calibration of mass scale to achieve a high mass accuracy typically better than 3 ppm and reproduced results. This allowed the determination of the exact molecular weight of observed ions as well as provided information about the elemental composition of present metabolites. The combination of different UHPLC/MS/MS scans, such as reconstructed ion current chromatograms, constant neutral loss chromatograms and exact mass filtration, was used for the unambiguous detection and identification of individual metabolites. Moreover, the comparison of chromatograms of PZQ biotransformation samples with the placebo experiments together with the prediction of metabolites and subsequent target screening was performed using advanced software tools. First, the Metabolite Predict algorithm was applied for the prediction of possible PZQ metabolites in three generations, including all metabolic rules. Then, the list of proposed metabolites, including their elemental composition with exact m/z values of their $[M+H]^+$ and $[M-H]^-$ ions, was downloaded into the Metabolite Detect Software. The difference chromatogram, which provides information about the metabolites presence, was generated as a subtraction of the placebo sample from the biotransformation sample (e.g. the difference chromatogram for sample of rat microsomes is shown in Fig. 1).

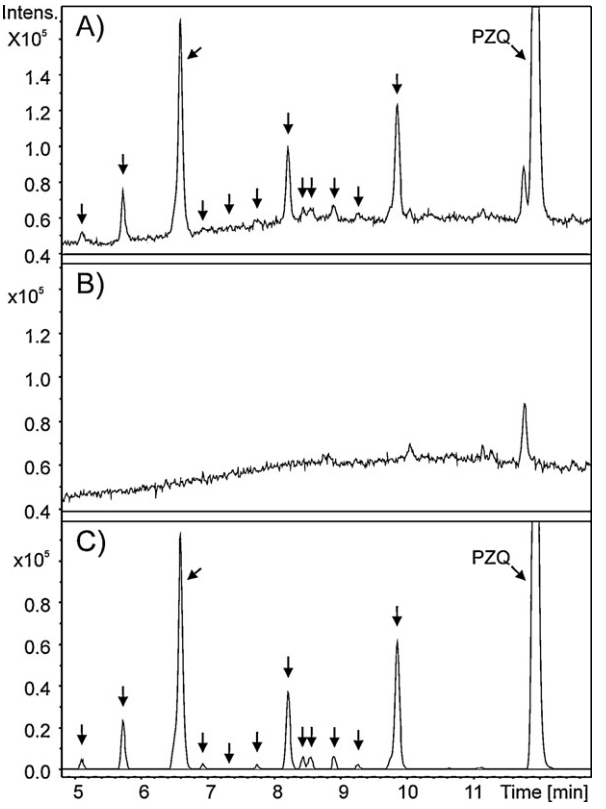

Fig. 1. Subtraction of placebo samples from PZQ biotransformation samples using Metabolite Detect Software: (A) total ion current chromatogram of rat microsomes extract; (B) total ion current chromatogram of particular placebo sample; (C) difference chromatogram.

First, phase I metabolites of PZQ in *D. dendriticum* and *H. diminuta* were searched for using PZQ incubation of the worms' subcellular fractions. The mixture of microsomal and mitochondrial fractions from *D. dendriticum* and *H. diminuta* was incubated with 10 and 100 μM PZQ. In all samples only protonated molecule of PZQ with m/z 313 at the elution time of 12.0 min was observed and no phase I metabolites produced by *D. dendriticum* or *H. diminuta* were detected.

Consequently, *ex vivo* incubations of PZQ in various concentrations were done with living helminths previously isolated from their hosts. These experiments were focused on both the formation of phase I and II metabolites of PZQ. Lower concentrations of PZQ than that had been used in

Table 1
Metabolites of praziquantel in rat detected by UHPLC/MS/MS.

t_R (min)	Theoretical m/z of $[M+H]^+$	Metabolic reaction (elemental composition change)	Mass accuracy (ppm) of experimental m/z of $[M+H]^+$		
			Microsomes	Hepatocytes	Urine
3.2	505.2180	+O, $+C_6H_8O_6$	n.d.	−0.1	−1.9
3.3	361.1757	+3O	n.d.	n.d.	−3.3
3.3	345.1809	+2O	n.d.	n.d.	2.9
3.4	505.2180	+O, $+C_6H_8O_6$	n.d.	−1.6	0.0
3.5	361.1757	+3O	n.d.	−0.3	−2.2
3.6	345.1809	+2O	n.d.	−0.9	1.7
3.7	505.2180	+O, $+C_6H_8O_6$	n.d.	0.0	−2.5
3.7	521.2129	+2O, $+C_6H_8O_6$	n.d.	n.d.	−3.1
3.7	343.1652	+2O, −2H	n.d.	1.1	−1.5
3.8	345.1809	+2O	n.d.	−5.5	−0.6
3.9	343.1652	+2O, −2H	n.d.	n.d.	−4.9
4.0	345.1809	+2O	n.d.	−1.4	2.9
4.2	345.1809	+2O	n.d.	1.4	0.3
4.2	343.1652	+2O, −2H	n.d.	5.5	−0.3
4.3	343.1652	+2O, −2H	n.d.	−0.6	−0.8
4.6	345.1809	+2O	n.d.	n.d.	−0.3
4.6	345.1809	+2O	n.d.	−3.4	−0.3
4.7	345.1809	+2O	n.d.	−3.7	−0.8
4.9	345.1809	+2O	n.d.	−2.8	−0.6
5.1	345.1809	+2O	−4.6	−4.6	−1.7
5.2	343.1652	+2O, −2H	n.d.	2.9	−2.9
5.3	343.1652	+2O, −2H	n.d.	2.9	−2.3
5.8	329.1860	+O	3.9	1.8	4.3
6.6	329.1860	+O	−0.3	0.9	−0.3
7.0	327.1703	+O, −2H	4.8	4.6	0.6
7.3	327.1703	+O, −2H	2.1	3.0	−3.1
7.8	329.1860	+O	2.4	n.d.	n.d.
7.9	327.1703	+O, −2H	n.d.	n.d.	1.2
8.2	329.1860	+O	3.9	n.d.	n.d.
8.4	329.1860	+O	3.9	n.d.	n.d.
8.5	329.1860	+O	4.5	n.d.	n.d.
8.9	327.1703	+O, −2H	n.d.	n.d.	1.5
8.9	329.1860	+O	2.4	n.d.	n.d.
9.3	329.1860	+O	0.0	n.d.	n.d.
9.9	329.1860	+O	3.6	n.d.	n.d.
12.0	313.1910	Parent drug (PZQ)	3.5	n.d.	3.2

t_R, retention time; metabolic reaction, the description of metabolite formation; mass accuracy, particular mass accuracies of experimental m/z of $[M+H]^+$; n.d., not determined.

the *in vitro* procedures were employed due to toxic effect of PZQ on worms. At PZQ concentration in a medium of up to 50 nM and 100 nM for *D. dendriticum* and *H. diminuta*, respectively, movement by the helminths was visible for several hours. But at higher PZQ concentrations (1 μM and 10 μM), the quick convulsion of worms was observed. In this case it was not possible to determine if helminths were dead or alive during the experiment. After 24-h

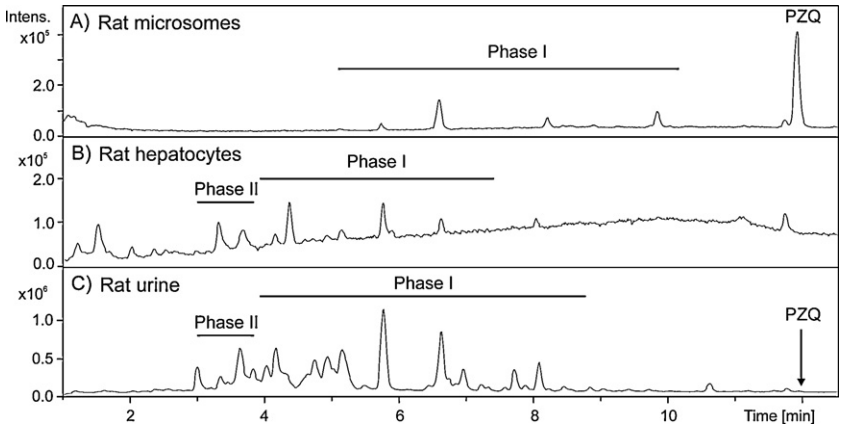

Fig. 2. UHPLC/ESI-MS total ion current chromatograms with labeled retention regions for phase I and II metabolites of PZQ in the rat: (A) rat microsomes; (B) rat hepatocytes; (C) rat urine.

incubations, medium and worm homogenates were analyzed by UHPLC/MS/MS technique. In all samples, only PZQ was detected regardless of the PZQ concentration used. No phase I or phase II metabolites of PZQ produced by *D. dendriticum* or *H. diminuta ex vivo* were found.

On the other hand, when the biotransformation of benzimidazole anthelmintics was studied in *D. dendriticum*, several metabolites were found and identified *in vitro* as well as *ex vivo* (Cvilink et al., 2009). *D. dendriticum* was able to reduce the carbonyl group of flubendazole and mebendazole, to oxidize sulfur in the albendazole and to form methylated derivatives of flubendazole and mebendazole as phase II metabolites (Cvilink et al., 2009). In *H. diminuta*, the biotransformation of anthelmintic drugs has not been described yet, but in another tapeworm, *Moniezia* spp., the biotransformation of flubendazole and albendazole was found. Albendazole undergoes oxidative metabolism to its sulphoxide and the carbonyl group of flubendazole is reduced in *Moniezia* spp. (Solana et al., 2001; Moreno et al., 2004). The finding of no PZQ metabolites in *D. dendriticum* and *H. diminuta* indicates that these helminths are not able to deactivate PZQ through biotransformation. This result was slightly surprising.

With the aim of checking the accuracy of our extraction and analytical methodology, biotransformation of PZQ was also tested in rat *in vitro*, at subcellular and cellular levels, and *in vivo*. Rat liver microsomal fractions were incubated with 100 μM PZQ, and primary cultures of rat isolated hepatocytes were incubated with 10 μM PZQ. Obtained samples were analyzed using UHPLC/MS/MS. Particular total ion current chromatograms are shown in Fig. 2, PZQ with *m*/*z* 313 being found only in rat microsomes (elution time 12.0 min). In samples obtained from hepatocytes, PZQ was fully metabolized to phase I and II metabolites. The hydroxylation or epoxidation (+O), hydroxylation combined with alcohol dehydrogenation (+O, −2H) from phase I PZQ biotransformation and glucuronidation ($+C_6H_8O_6$) from phase II biotransformation were observed in rat microsomes and hepatocytes (Table 1).

For *in vivo* study of PZQ biotransformation in rat, adult female rats were treated orally with PZQ (100 mg per kg of body mass) and urine was subsequently collected for 24 h. PZQ together with its 28 metabolites were found in rat urine. Similar biotransformation pathways as in case of rat hepatocytes were detected *in vivo* in rats. Results are shown in Fig. 2 and Table 1.

In total, 31 different phase I and 4 phase II metabolites were detected in all rat samples (microsomes, hepatocytes, urine) during 12 min of UHPLC/MS/MS analyses. These results clearly demonstrate the accuracy of all methods used in this study. Moreover, the high sensitivity of analyses led to the identification of some metabolites that had as of yet not been reported. In humans, hydroxyderivatives, glucuronides and sulfate of PZQ have been found (Meier and Blaschke, 2000). In mice, hydroxylation represents the main metabolic pathway for PZQ (Ali et al., 1990). Cytochrome P450 enzymes are known to catalyze the phase I metabolism of PZQ in mammals (Li et al., 2003; Godawska-Matysik and Kieć-Kononowicz, 2006). In rats, mono- and di-hydroxy derivatives along with glucuronide conjugates of PZQ have been reported (Meier and Blaschke, 2001; Giorgi et al., 2001). In our experiments, 12 different metabolites of PZQ produced by rat microsomes, 24 PZQ metabolites produced by rat hepatocytes and 28 metabolites in rat urine were detected. No analytical methods used in previous PZQ biotransformation studies mentioned above were sensitive enough to identify such a profusion of different PZQ metabolites.

4. Conclusions

The present study was designed to advance our knowledge about metabolism of PZQ in two helminth species. Despite the highly sensitive UHPLC/MS/MS analyses, no PZQ metabolite formed in *D. dendriticum* and *H. diminuta* was detected. On the other hand, this technique allowed us to find and identify 35 different PZQ metabolites formed in rats. Some of these metabolites had not been described yet. The finding of no PZQ metabolite in *D. dendriticum* and *H. diminuta* indicates that these members of flukes and tapeworms are not able to deactivate PZQ through biotransformation. Therefore, biotransformation does not contribute to the development of PZQ-resistance. On the other hand, due to the lack of data from other trematodes and cestodes this conclusion could not be generalized to all species of these classes.

Conflict of interest

There is no conflict of interest.

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