ABSTRACT

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Title of Doctoral Thesis **Biotransformation and transport of xenobiotics in helminths**

Infectious diseases caused by parasitic helminths are a major problem threatening health of domestic and wild animals and affecting agricultural industry worldwide. Treatment of helminthoses is based on administration of anthelmintic drugs, with benzimidazoles being the most important group. Unfortunately, the irrational use of similar anthelmintics has led to the development of drug resistance in helminths, thus causing a serious problem in the veterinary practice. Possible mechanisms of drug resistance development include changes of pharmacokinetic processes (changes in drug transport or increased drug deactivation), which are based on an increased activity of biotransformation enzymes and transporters in helminths. Understanding the mechanisms of drug resistance and defence strategies of parasites against drugs can prolong the efficacy of current anthelmintics and help to find new strategies for the control of helminthoses. Although drug metabolizing enzymes and transporters of helminths serve as an efficient defence against the negative action of xenobiotics, they have been relatively little investigated so far. Therefore, the aim of my doctoral thesis was to study the defence strategies of helminths, namely biotransformation enzymes, metabolic faith and transport of selected anthelmintic drugs, in the representatives of the main groups of parasitic helminths. From the class of flukes, we tested Dicrocoelium dendriticum. Haemonchus contortus was studied as a representative of group Nematoda and Hymenolepis diminuta served as a model organism of tapeworms.

To achieve our goals, we performed in vitro (subcellular fractions of homogenate from parasites' bodies) and ex vivo (living parasites cultivated with medium) experiments. Activities of reduction enzymes, which play important role in parasites, were assayed towards model substrates. Anthelmintic drugs metabolized via carbonyl reduction in all examined species are represented by mebendazole (MBZ) and flubendazole (FLU). Although drug oxidation enzymes have not been considered to be important in helminths, obtained results in our experiments have confirmed the existence of an oxidative drug metabolism in these organisms. From the anthelmintics tested, albendazole (ABZ) undergoes sulphoxidation in D. dendriticum and H. contortus, but not in H. diminuta. Some helminths' oxidation enzymes, which play the significant role in defence against oxidative stress induced by redox-cycling drugs or host immune system, may contribute to the biotransformation of the drugs. From these enzymes, superoxide dismutase, catalase and peroxidase showed activity in all parasites. The ability to form methyl derivatives (D. dendriticum, H. diminuta) and glucose conjugates (H. contortus) have brought the first evidence that even helminths conjugate drugs with endogenous substrate in order to deactivate them. Comparison of sensitive and resistant isolates of H. contortus revealed higher formation of FLU metabolites in resistant individuals. It suggests that higher activity of biotransformation enzymes can contribute to the drug resistance development in this helminth species. Transport studies have confirmed a key role of passive diffusion in the transport of drugs in helminths. In the case of D. dendriticum the results indicated also the participation of active transport of ABZ and its sulphoxide.

The obtained results expand the knowledge of defence strategies in helminths and prove that all tested helminths are able to effectively transform the structure of the drugs and other xenobiotics. By this way, parasites may be protected against toxic effects of drugs and it can contribute to the drug resistance in parasites. Therefore, the knowledge of drug biotransformation and transport in parasitic helminths may improve the pharmacotherapy of helminthoses in target species.