Short Communication

TRANSPORT OF ANTHELMINTIC BENZIMIDAZOLE DRUGS BY BREAST CANCER RESISTANCE PROTEIN (BCRP/ABCG2)

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ABSTRACT:
Methylcarbamate benzimidazoles [albendazole (ABZ), fenbendazole (FBZ), and their respective sulfoxide derivatives, albenzazole sulfoxide (ABZSO) and oxendazole (OXF)] are therapeutically important anthelmintic agents with low bioavailability. We studied their in vitro interaction with the apical ATP-binding cassette (ABC) drug efflux transporters, breast cancer resistance protein (BCRP/ABCG2), P-glycoprotein (ABCBI), and MRP2 (ABCC2) using MDCKII cells transduced with human MDR1, MRP2, and BCRP, and murine Bcrp1 cDNAs. These ABC drug efflux transporters extrude a wide range of xenotoxins from cells in intestine, liver, and other organs, thus affecting the bioavailability of many compounds. In transport experiments, ABZSO and OXF were transported efficiently by murine Bcrp1 and moderately by human BCRP, but not by MDR1 or MRP2. ABZ and FBZ were not found to be Bcrp1, MRP2, or P-glycoprotein substrates in vitro. OXF was found to be a good BCRP/Bcrp1 inhibitor, with somewhat higher potency in the MDCKII-BCRP cell line. The latter results were confirmed by flow cytometry experiments demonstrating inhibition by OXF of murine Bcrp1- and human BCRP-mediated mitoxantrone transport. Further studies of interactions between OXF and known BCRP/BCrp1 substrates will be of interest. The use of efficacious BCRP/Bcrp1 inhibitors might increase the extent and duration of systemic exposure to ABZSO and OXF, with possible therapeutically beneficial effects in extra-intestinal infections.

Albendazole (ABZ), fenbendazole (FBZ), and their sulfoxide derivatives (albendazole sulfoxide/ricobendazole, ABZSO; and fenbendazole-sulfoxide/oxfendazole, OXF) are methylcarbamate benzimidazoles with a broad-spectrum anthelmintic activity, widely used in human and veterinary medicine. They are used against several systemic parasitoses including nematodes, hydatidosis, taeniasis, and others (Campbell, 1990). They are also active against various protozoa (Katiyar et al., 1994) and Gram-positive bacteria (Elma et al., 1981). ABZ is used to treat microsporidial and cryptosporidial infections, which can cause lethal diarrhea in patients treated with immunosuppressive drugs or those infected with HIV (Zulu et al., 2002; Didier et al., 2004). In addition, ABZ has shown antitumor activity (Morris et al., 2001; Pourgholami et al., 2004). Both sulfoxide derivatives (ABZSO and OXF) are the main active metabolites found in plasma after oral administration of the parent compounds, ABZ and FBZ (Lanuse and Prichard, 1993), but they are also available as commercial formulations themselves. Especially FBZ, but also ABZ, and their sulfoxides are poorly absorbed from the gastrointestinal tract (Lanuse and Prichard, 1993; Lanuse et al., 1995; Capece et al., 2000). For treatment of intestinal luminal parasites, this is ideal: intestinal concentration of the drug remains high, and there is little risk of systemic toxicity. However, for systemic treatments elsewhere in the body, substantial (systemic) uptake of the drugs is needed, and low benzimidazole bioavailability is a disadvantage. ABZ and FBZ are known to cross plasma membranes by passive diffusion due to their lipophilicity (Mottier et al., 2003), but the existence of additional (uptake) transport mechanisms cannot be excluded.

Low water solubility of benzimidazoles has been considered a limiting factor in their low bioavailability (Capece et al., 2000), but for many drugs, it has been shown that their oral availability is also reduced by ATP-binding cassette (ABC) drug efflux transporters present in the apical membrane of intestinal epithelia (among other epithelia) (Zhu, 1999; Jonker et al., 2000). Significant direct secretion of ABZSO into the intestinal lumen has been demonstrated (Redondo et al., 1999; Merino et al., 2003). The understanding of the mechanism involved in the low availability of these compounds might lead to the design of new strategies to modify this pharmacokinetic property when desired and, thus, enhance therapeutic efficacy in systemic treatments.

In this paper, we studied the in vitro transport of some therapeutically relevant methylcarbamate benzimidazoles by apical ABC transporters [breast cancer resistance protein (BCRP/ABCG2), P-glycoprotein (P-gp/ABCB1), MRP2/ABCC2] using MDCKII cells transduced with human MDR1, MRP2, and BCRP, and murine Bcrp1 cDNAs.

Materials and Methods

Chemicals. ABZ and MBZ were obtained from Sigma-Aldrich (St. Louis, MO). ABZSO was kindly supplied by GlaxoSmithKline (Madrid, Spain). FBZ and OXF were supplied by Rhône Mérieux (Lyon, France). PhiP and PhiM were synthesized in-house.

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ABBREVIATIONS: ABZ, albendazole; ABC, ATP-binding cassette; ABZSO, albenzazole sulfoxide; FBZ, fenbendazole; HPLC, high performance liquid chromatography; Ko143, 3-(6-isobutyl-9-methoxy-1,4-dioxo-1,2,3,4,6,7,12a-octahydropyrazino[1,2-b:1,6]pyrido[3,4-b]indol-3-yl)-propionic acid tert-butyl ester; MF, median of fluorescence; OXF, oxendazole; Papp, apparent permeability coefficient; PhiP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; BCRP, breast cancer resistance protein; P-gp, P-glycoprotein.
Absorbance was measured at 292 nm. For ABZSO and OXF, 0.8 ml/min for ABZ, and 1.5 ml/min for FBZ. UV separation was performed on a reversed-phase column (Nucleosil 120 C18, 10 μm; 250 mm × 4 mm). The composition of the mobile phase was acetonitrile/ammonium acetate (0.025 M, pH 6.6) at a proportion of 67:33 for ABZSO and OXF. In contrast, in the MDR1- and MRP2-transduced MDCKII cell line. When the human BCRP-transduced cell line was used, the transport was completely inhibited (Fig. 1, C and G), resulting in a difference in the directional transport was lower than in the case of the MDCKII parental cell line. When the selective Bcrp1 inhibitor Ko143 (C and G) was present as indicated. Results are the means; error bars indicate the standard deviations (n = 3). Closed circles, translocation from the basolateral to the apical compartment; open circles, translocation from the apical to the basolateral compartment. The apparent permeability coefficient (P_app) corresponding to 10% of transport in 1 h was 12.3 × 10⁻⁶ cm/s.

**Results and Discussion**

To further characterize the interactions of the methylcarbamate benzimidazoles with Bcrp1/BCRP, the ability of these compounds...
to modulate mitoxantrone accumulation in murine Bcrp1- and BCRP-expressing cell lines was tested in flow cytometry experiments. BCRP inhibition increases the accumulation of mitoxantrone in Bcrp1- and BCRP-transduced cells and thus increases the MF. OXF had the strongest inhibitory potency in both cell lines (75.2 ± 2.5% in MDCKII-Bcrp1 and 112.7 ± 1.2% in MDCKII-BCRP at a concentration of 375 μM; Fig. 3A), whereas ABZSO only reached an inhibitory potency of 6.9 ± 0.4% in MDCKII-Bcrp1 and 28.9 ± 2.6% in MDCKII-BCRP at a concentration of 375 μM (not shown). ABZ and FBZ had an inhibitory potency of less than 15% at 50 μM (limit of solubility) in both cell lines (not shown).

Since OXF was the most potent inhibitor of Bcrp1, its inhibitory potential on transepithelial transport of the dietary carcinogen PhIP was tested. PhIP is an excellent BCRP/Bcrp1 substrate (van Herwaarden et al., 2003; Pavek et al., 2005). OXF (at 325 μM) completely reversed transepithelial transport of PhIP across MDCKII-BCRP cells, like the potent Bcrp1/BCRP inhibitor Ko143 (at 5 μM; Fig. 3A), whereas ABZSO only reached an inhibitory potency of 6.9 ± 0.4% in MDCKII-Bcrp1 and 28.9 ± 2.6% in MDCKII-BCRP at a concentration of 375 μM (not shown). ABZ and FBZ had an inhibitory potency of less than 15% at 50 μM (limit of solubility) in both cell lines (not shown).

The duration of exposure of the parasite to the benzimidazole drug is a key determinant of efficacy. Consequently, strategies have been developed both experimentally and commercially to extend this exposure period through the development of sustained delivery systems and by modifying the metabolism of the drug in the host species (McKellar and Jackson, 2004). In this study, we demonstrated that ABZSO and OXF are both substrates of BCRP and Bcrp1 in vitro. It may thus be that the clinical use of efficacious BCRP/Bcrp1 inhibitors might improve their oral bioavailability and, hence, also their systemic target exposure, by reducing their intestinal elimination and
their hepatobiliary secretion, since Bcrp1 is also expressed in the biliary canalicular membrane of hepatocytes and mediates the hepatobiliary excretion of its substrates (Jonker et al., 2000; van Herwaarden et al., 2003). It will therefore be of interest to further study the possible in vivo effect of BCRP in the pharmacokinetics of these benzimidazole drugs and potential drug interactions with these methylcarbamate benzimidazoles and known BCRP/Bcrp1 substrates in therapeutic-target species (humans, livestock).

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References


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