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, albendazole sulfoxide (ABZSO) and oxfendazole (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 (ABC1), 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 MDRCKII-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 (Elhima 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 (Lanusse 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 (Lanusse and Prichard, 1993; Lanusse 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., 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/ABC1), 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). This work was supported by Grant NKI 2000-2271 of the Dutch Cancer Society.

ABBREVIATIONS: ABZ, albendazole; ABC, ATP-binding cassette; ABZSO, albendazole sulfoxide; FBZ, fenbendazole; HPLC, high performance liquid chromatography; Ko143, 3-(6-isobutyl-9-methoxy-1,4-dioxo-1,2,3,4,6,7,12,12a-octahydropyrazino[1',2':1,6]pyrido[3,4-b]indol-3-yl)-propionic acid tert-butyl ester; MF, median of fluorescence; OXF, oxfendazole; \( P_{app} \), apparent permeability coefficient; PhiP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; BCRP, breast cancer resistance protein; P-gp, P-glycoprotein.
A concentration of 292 nm.

For ABZSO and OXF, 0.8 ml/min for ABZ, and 1.5 ml/min for FBZ. UV OXF, 39:61 for ABZ, and 50:50 for FBZ. The flow rate was set to 1 ml/min ammonium acetate (0.025 M, pH 6.6) at a proportion of 67:33 for ABZSO and OXF or 5 μM Ko143 to both the apical and basal compartments, using [14C]PhIP as BCRP/Bcrp1 substrate (van Herwaarden et al., 2003; Pavek et al., 2005). At the end of the experiment, filters were excised and measured for radioactivity.

Accumulation Assays. In vitro accumulation assays were carried out as described previously (Pavek et al., 2005). Mitoxantrone (5 μM) was used as fluorescent substrate and OXF was used as inhibitor at different concentrations. Relative cellular accumulation of mitoxantrone was determined by flow cytometry using a FACSCalibur cytometer in at least 5000 cells from histogram plots using the median of fluorescence (MF). Inhibitory potencies of compounds were calculated as described (Pavek et al., 2005) in MDCKII-BCRP or MDCKII-Bcrp1 cells according to the following equation: inhibitory potency (IP) = (MF with OXF − MF without inhibitor)/(MF with Ko143 − MF without inhibitor) × 100%.

HPLC Analysis. HPLC analysis of the samples from the transport assay was carried 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, 39:61 for ABZ, and 50:50 for FBZ. The flow rate was set to 1 ml/min for ABZSO and OXF, 0.8 ml/min for ABZ, and 1.5 ml/min for FBZ. UV absorbance was measured at 292 nm.

Results and Discussion

To investigate whether methylcarbamate benzimidazoles are efficiently transported by apical ABC transporters, we determined the involvement of P-gp, MRP2, and Bcrp1 in ABZ, FBZ, ABZSO, and OXF transport in vitro, using the polarized canine kidney cell line MDCKII and its subclones transduced with human MDR1 and MRP2, and murine Bcrp1 cDNAs. In addition, human BCRP-transduced MDCKII subclones were tested for the possible role of human BCRP in the in vitro transport of ABZSO and OXF. The parental and transduced cell lines were grown to confluent polarized monolayers on porous membrane filters, and vectorial transport of the tested compound (10 μM) across the monolayers was determined.

For ABZSO and OXF, apically directed translocation was highly increased and basolaterally directed translocation drastically decreased (Fig. 1, A, B, E, and F) in the Bcrp1-transduced compared with the MDCKII parental cell line. When the selective Bcrp1 inhibitor Ko143 was used (Allen et al., 2002), this Bcrp1-mediated transport was completely inhibited (Fig. 1, C and G), resulting in a vectorial translocation pattern equal to that of the MDCKII parental cell line. When the human BCRP-transduced cell line was used, the difference in the directional transport was lower than in the case of the murine Bcrp1-transduced cell line (Fig. 1, D and H) for both ABZSO and OXF. In contrast, in the MDR1- and MRP2-transduced MDCKII cell lines, the vectorial translocation was similar to that in the MDCKII parental cell line (Fig. 2, A and B). No significant vectorial transport was observed for ABZ and FBZ in MDCKII and its subclones transduced with murine Bcrp1 and human MDR1 and MRP2 cDNAs (Fig. 2, C and D).

Together, these results show highly efficient transport of ABZSO and OXF by murine Bcrp1, moderate transport by human BCRP, and no transport by MDR1 or MRP2. ABZ and FBZ are not Bcrp1, MRP2, or P-gp substrates in vitro. This is in agreement with the lack of P-gp involvement in ABZ transepithelial transport using LLC-PK1 cells transfected with MDR1 cDNA obtained by Merino et al. (2002).

It is interesting to note that the simple sulfoxidation of ABZ and FBZ converts non-Bcrp1 substrates into well transported Bcrp1 substrates. This is in agreement with the involvement of P-gp in ABZ transepithelial transport using LLC-PK1 cells transfected with MDR1 cDNA obtained by Merino et al. (2002).

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), 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 methyccarbamate benzimidazoles and known BCRP/Bcrp1 substrates in therapeutic-target species (humans, livestock).

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References

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