Short Communication

THE ANTHELMINTHIC AGENT ALBENDAZOLE DOES NOT INTERACT WITH P-GLYCOPEPTIDE

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ABSTRACT:
Albendazole is a clinically important anthelmintic agent known to have variable and low oral bioavailability. The aim of this work was to determine whether albendazole, a CYP3A4 substrate, is also a substrate for the multidrug efflux transporter P-glycoprotein. Both in vitro and in vivo methods were used to assess the role of P-glycoprotein-mediated albendazole transport. In cultured LLC-PK1, L-MDR1, and Caco-2 cells, albendazole was found not to be a P-glycoprotein substrate; the transport across LLC-PK1 and L-MDR1 cells revealed basal to apical versus apical to basal transport to a similar extent. In addition, there was no inhibitory effect of albendazole on digoxin transport in Caco-2 cells, and P-glycoprotein inhibitors (verapamil and quinidine) did not affect transport across Caco-2 cells. The in vivo relevance of P-glycoprotein to albendazole disposition was assessed using mdrla+/− mice after intravenous administration of albendazole (15 mg/kg). A similar pattern of tissue distribution in both P-glycoprotein-deficient and wild-type mice was observed. In conclusion, albendazole is neither a substrate nor an inhibitor of P-glycoprotein. Therefore, interactions between albendazole and P-glycoprotein substrates or inhibitors are unlikely to be clinically important.

Albendazole (ABZ) is a broad-spectrum anthelmintic agent (Bennett and Guyatt, 2000; Cox, 2000) also used to treat microsporidial infections, an emerging disease of relevance, particularly among those infected with human immunodeficiency virus (Costa and Weiss, 2000). ABZ is converted in vivo into albendazole sulfoxide (ABZSO) and albendazole sulfone (ABZSO2). CYP450 and the flavin monooxygenase system have been suggested to be responsible for the sequential sulfonation of ABZ. Both systems are involved in the sulfonation (Rawden et al., 2000), whereas CYP450 is the main determinant of sulfonation (Soutaili-El Amri et al., 1998). Involvement of the two pathways in ABZ metabolism has been observed in several animal species (Galtier et al., 1986; Soutaili-El Amri et al., 1988) and among humans (Rolin et al., 1989). Moreover, intestinal metabolism and significant secretion of ABZSO into the intestinal lumen have also been demonstrated (Redondo et al., 1999). Recent data suggest CYP3A4 may be the key contributor of ABZSO formation (Rawden et al., 2000).

ABZ therapy is in hampered by its low solubility and poor absorption from the gastrointestinal tract, resulting in low bioavailability and reduced efficacy. In addition to its physicochemical properties, active transport by efflux pumps such as P-glycoprotein (P-gp), a product of the multidrug resistance gene MDRI, may have a role in ABZ disposition. P-gp is expressed in organ systems that influence drug absorption (intestine), distribution (central nervous system, leukocytes, and tests), and elimination (liver and kidney) (Cordon-Cardo et al., 1989; Tsuji et al., 1992). Moreover, P-gp is known to be coexpressed with CYP3A4, the key CYP450 involved in the metabolism of many drugs including ABZ, in organs such as the intestine and liver (Maurel, 1996). In addition, P-gp and CYP3A4 share many substrates and inhibitors (Wacher et al., 1995). Accordingly, a systematic study was undertaken to determine the extent of P-gp involvement in ABZ transport both in vitro and in vivo.

Materials and Methods

Chemicals. [3H]digoxin was obtained from PerkinElmer Life Sciences (Boston, MA) and [14C]ABZ from GlaxoSmithKline (Madrid, Spain). ABZ, ABZSO, and ABZSO2 were supplied by GlaxoSmithKline. Mebendazole, verapamil, quinidine, and probenecid were purchased from Sigma Chemical Co. (St. Louis, MO). Methanol and acetic acid were obtained from Merck (Darmstadt, Germany), acetonitrile from BDH (Poole, UK), and ethyl acetate from Sigma-Aldrich (Dorset, UK).

Transport in Cultured LLC-PK1, L-MDR1, and Caco-2 Cells. LLC-PK1 and L-MDR1 (LLC-PK1 cells transfected with MDR1 cDNA and stably expressing human MDR P-gp) were grown under identical conditions to those described by Schinkel et al. (1995). Caco-2 cells were grown under the same conditions previously described (Kim et al., 1998).

Transport experiments were carried out using the same protocol described previously (Kim et al., 1998). ABZ transport in L-MDR1 and LLC-PK1 cells was measured using five different concentrations (6.25, 12.5, 25, 50, and 100 μM) of [14C]ABZ, and four concentrations (6.25, 12.5, 25, and 50 μM) of ABZ were tested in Caco-2 cells. In addition, at the end of each experiment, filters containing the cells were washed three times with cold Dulbecco’s modified Eagle’s medium, and the radioactivity was measured. Radiolabeled digoxin was used as a control P-gp substrate.

Inhibition of P-gp-mediated transport by Caco-2 cells was determined in a...
FIG. 1. a, transepithelial transport of [3H]digoxin (5 μM) and [14C]ABZ (25 and 100 μM) in LLC-PK1 (right) and L-MDR1 (left) monolayers; b, transepithelial transport of [3H]digoxin across a Caco-2 monolayer in the absence or presence of ABZ at 0.1, 10, 100 μM.

Drugs were applied to one compartment (basal or apical), and the percentage of radioactivity appearing in the opposite compartment at defined time points was measured. Translocation from basal to apical compartments (squares, solid line); translocation from apical to basal compartments (rhombus, dotted line). Data are means ± S.D. from three or more experiments. DIG, digoxin.
similar manner after the addition of ABZ (0.1, 1, 10, 20, and 100 μM) to both the apical and basal compartments, using [3H]digoxin (5 μM) as the P-gp substrate. Complete inhibition of P-gp-mediated transport would be expected to result in the loss of dioxin’s basal-to-apical (B–A) versus apical-to-basal (A–B) transport differences. Inhibition of ABZ transport was determined in a similar manner after the addition of quinidine, verapamil (Choo et al., 2000), and probenecid (organic anion transporter inhibitor) (Payen et al., 2000) (100 μM) to both compartments and at 6.25 or 25 μM ABZ.

**Determination of Tissue Distribution in mdr1a/b(+/+) and (−/−) Mice.** Male mdr1a/b(−/−) mice (FVB/TacBR-[KO]mdr1abN7), 6 weeks of age, and genetically matched male mdr1a/b mice (FVB/MTacBR) weighing 24 to 28 g were obtained from Taconic Farms (Germantown, NY). The animals were cared for in accordance with the U.S. Public Health Service policy for the Care and Use of Laboratory Animals. The tissue distribution of [14C]ABZ was determined following i.v. injection (15 mg/kg) of an ethanol (10%)/polyethylene glycol (40%)/saline (50%) solution over 5 min into the tail vein of groups of three mice; the total volume injected was 8 μl/g. After half an hour, the animals were anesthetized using isoflurane (Isoflo; Abbott Laboratories, Abbot Park, IL), blood was removed by orbital bleeding, and the animals were sacrificed. Subsequently, tissues were harvested, weighed, and homogenized with 4% bovine serum albumin solution. Total radioactivity was determined after the addition of 75 μl of plasma or 500 μl of tissue homogenate to vials.
containing 5 ml of scintillation fluid (Scintiverse BD; Fisher Scientific Co., Fairlawn, NJ).

**HPLC Analysis of [14C]ABZ Tissue and Cell Culture Medium Levels.**
Plasma and tissue samples were obtained at half an hour following administration of [14C]ABZ, and the cell culture medium remaining at the conclusion of ABZ transport experiments in Caco-2 cells was also extracted and analyzed by HPLC (Redondo et al., 1998).

**Data Analysis.** Data are presented as the mean and standard deviation. Statistical differences were assessed by a two-sided Student’s test, with $P < 0.05$ as the limit of significance.

### Results and Discussion

It is now widely appreciated that both P-gp and CYP3A have remarkably broad substrate specificities and that considerable overlap in shared substrates exists. Given their expression in tissues of importance to drug disposition, such as the intestine, liver, and kidney, a coordinate function of P-gp and CYP3A is thought to be a major determinant in the disposition of many drugs. It should be noted, however, for some CYP3A substrates there is a lack of P-gp involvement (Kim et al., 1999). The antihelminthic agent ABZ, in addition to being a CYP3A substrate, is known to have low oral bioavailability. In addition, ABZSO is known to be actively secreted into the intestinal lumen (Redondo et al., 1999).

Accordingly, the hypothesis that P-gp may be involved in ABZ disposition would be testable in vitro and in vivo.

First, the role of P-gp in mediating the transport of ABZ was assessed in LLC-PK1 and L-MDR1 cell lines. L-MDR1 cell line only differs from the parental LLC-PK1 line by the manipulated overexpression of P-gp; thus, the potential presence of other transporters is not a confounding factor. The transepithelial transport of [3H]digoxin (5 μM) and [14C]ABZ at 25 and 100 μM in both cell lines is represented in Fig. 1a. There were no differences in ABZ transport at the tested concentrations in terms of basal to apical transport or apical to basal transport in LLC-PK1 or L-MDR1 cells. Transport of [3H]digoxin (5 μM), a prototypical P-gp substrate, was markedly polarized, extensive in the basolateral to apical direction, and markedly attenuated in the apical to basolateral direction in L-MDR1 but not in LLC-PK1 cell line. The ability of ABZ to inhibit P-gp activity was determined in Caco-2 cells at five concentrations (0.1, 1, 10, 20, and 100 μM), using digoxin as the prototypical substrate. Figure 1b shows the transepithelial transport of [3H]digoxin in the absence or presence of 0.1, 10, and 100 μM ABZ. [3H]Digoxin transport was markedly greater in the basal to apical direction than in the apical to basal direction, consistent with apical expression of P-gp in Caco-2 cells. Addition of ABZ up to 100 μM did not have any effect on digoxin transport. These data strongly suggest ABZ is not a P-gp inhibitor.

Similar to L-MDR1 cells, there was no difference at any concentration (6.25, 12.5, 25, and 50 μM) in the directional transport of ABZ, suggesting a lack of P-gp involvement in ABZ transport. From the HPLC analysis, we concluded that the amount of ABZSO formed (6.25, 12.5, and 25 μM ABZ) was about 9.5% of the ABZ remaining at the 4-h time point. In the case of 50 μM ABZ, the percentage of ABZSO formed was of 3.7%, ABZSO2 undetectable. Accordingly, formation of metabolites in Caco-2 cells seemed to be modest. The ability of verapamil, quinidine, and probenecid (100 μM) to inhibit ABZ transport was determined in Caco-2 cells using two concentrations of ABZ (6.25 and 25 μM) (Fig. 2). No significant inhibitory effects were observed.

The data obtained with the polarized cells further demonstrate that the speculated overlap between CYP3A and P-gp drug substrates is incomplete. Similarly, two other important examples are nifedipine and midazolam, which are CYP3A substrates (Rashid et al., 1995; Thummel et al., 1996), and were found not to be P-gp substrates (Kim et al., 1999). Likewise, carbamazepine, a substrate of CYP3A4 and CYP2C8, was found not to be a P-gp substrate (Owen et al., 2001). It is possible that other efflux transporters, such as canalicular multispecific organic anion transporter (cMOAT; also termed MRP2 and ABCB2), may be involved in the efflux transport of ABZ conjugates. Although the data presented in the current study do not rule out an involvement of cMOAT in terms of ABZ transport, this is less likely given the available data, which show cMOAT expression on the apical membrane of Caco-2 cells (Hirohashi et al., 2000). Indeed, we did not observe significant differences in the basal to apical versus apical to basal transport of ABZ in Caco-2 cells. Moreover, the inhibitor of anion transport (probenecid) failed to alter the directional transport of ABZ.

The lack of P-gp involvement in ABZ disposition in vivo is demonstrated by the absence of differences in tissue distribution of ABZ in mdr1a1/1b(-/-) mice compared with syngeneic animals after intravenous administration of identical doses (Table 1). Moreover, HPLC analysis of plasma and tissue samples obtained after ABZ administration demonstrated the presence of ABZSO and ABZSO2. However, there were no significant differences in the levels of these metabolites in plasma or other tissue distribution between mdr1a1/1b(-/-) mice and their wild-type counterpart (data not shown).

In conclusion, we show that ABZ, a CYP3A4 substrate with low oral bioavailability, is neither a substrate nor an inhibitor of P-gp. Accordingly, if the findings outlined in this study are extended to the in vivo situation, drug interactions with ABZ and known P-gp substrates or inhibitors are unlikely to occur.

### Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>mdr1a1/1b(+/+)</th>
<th>mdr1a1/1b(-/-)</th>
<th>(-/-)/(+/+)</th>
<th>mdr1a1/1b(+/+)</th>
<th>mdr1a1/1b(-/-)</th>
<th>(-/-)/(+/+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>4.95 ± 0.46</td>
<td>5.28 ± 1.03</td>
<td>1.07</td>
<td>0.41 ± 0.11</td>
<td>0.52 ± 0.05</td>
<td>1.28</td>
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<tr>
<td>Brain</td>
<td>2.01 ± 0.51</td>
<td>2.73 ± 0.29</td>
<td>1.36</td>
<td>0.82 ± 0.11</td>
<td>0.96 ± 0.05</td>
<td>1.05</td>
</tr>
<tr>
<td>Heart</td>
<td>4.10 ± 0.92</td>
<td>4.55 ± 0.62</td>
<td>1.11</td>
<td>1.39</td>
<td>1.04 ± 0.05</td>
<td>1.33</td>
</tr>
<tr>
<td>Lungs</td>
<td>5.12 ± 0.34</td>
<td>7.11 ± 0.22</td>
<td>1.20</td>
<td>4.23 ± 0.98</td>
<td>4.91 ± 0.80</td>
<td>1.16</td>
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<tr>
<td>Liver</td>
<td>21.20 ± 6.38</td>
<td>25.51 ± 0.83</td>
<td>1.20</td>
<td>1.23 ± 0.10</td>
<td>2.11 ± 1.28</td>
<td>1.71</td>
</tr>
<tr>
<td>Spleen</td>
<td>6.12 ± 0.94</td>
<td>10.50 ± 4.57</td>
<td>1.71</td>
<td>1.40 ± 0.16</td>
<td>1.49 ± 0.22</td>
<td>0.93</td>
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<tr>
<td>Kidney</td>
<td>7.89 ± 0.11</td>
<td>7.78 ± 0.36</td>
<td>0.99</td>
<td>1.91 ± 0.73</td>
<td>2.23 ± 0.45</td>
<td>1.16</td>
</tr>
<tr>
<td>Colon</td>
<td>9.31 ± 2.83</td>
<td>12.03 ± 4.71</td>
<td>1.29</td>
<td>0.98</td>
<td>1.08 ± 0.18</td>
<td>0.94</td>
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<tr>
<td>Testis</td>
<td>5.30 ± 0.63</td>
<td>5.27 ± 0.10</td>
<td>1.00</td>
<td>0.48 ± 0.06</td>
<td>0.45 ± 0.06</td>
<td>0.94</td>
</tr>
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