ABSTRACT: Ivermectin is an antiparasitic drug frequently administered to humans. It has a limited brain exposure that is attributed to the efflux activity of ABCB1/Abcb1. ABCG2/Abcg2 is also a major transporter present in most pharmacologically important barriers. However, interaction of ivermectin with Abcg2 shows species specificity and in many studies was confounded by the masking effect of ABCB1/Abcb1. In this study using cellular and membrane assays we show that ivermectin displays a high-affinity interaction with human ABCG2 with IC50 values in the 1–1.5 μM range. This interaction may have implications in human ABCG2-mediated drug–drug interactions of ivermectin.

INTRODUCTION

Ivermectin has long been considered a model substrate of ABCB1 (P-glycoprotein, MDR1). Ivermectin brain exposure increased 70-fold in CF-1 mice deficient in Abcb1a,1 87-fold in Abcb1a knockout mice2 leading to a 100-fold increase in neurotoxicity.2 Abcb1 is a crucial determinant of ivermectin pharmacokinetics inasmuch as several coadministered Abcb1-interacting drugs have been shown to significantly alter pharmacokinetics of ivermectin across a board of different species (reviewed in Refs. 3,4).

Ivermectin is used in veterinary medicine to treat gastrointestinal infections. It has also been approved for human use. It is mostly used in tropical countries for ochorcerciasis but it is also used in most of the occidental countries to treat strongyloidiasis and scabies. Although it has been used to treat humans for more than 20 years, very little pharmacokinetics data was published. Nevertheless, it is known that ivermectin is metabolized essentially through CYP3A4 in the liver.5 The interaction with human ABC transporters such as ABCB16 and multiple members of the ABCC subfamily7 has also been described and may contribute to the disposition of the drug in humans as it has been previously shown in mice.1,2,8 A clinical drug–drug interaction with azithromycin yielding an estimated 1.37-fold increase in ivermectin bioavailability was also published.9 As azithromycin, an ABCB1 substrate does not interact with CYP3A4; it was suggested that this drug–drug interaction was ABCB1-mediated.9

ABCG2/Abcg2 (BCRP, MXR) is a broad substrate specificity transporter expressed in multiple pharmaceutically and physiologically important barriers (reviewed in Ref.10). It affects pharmacokinetics of drugs in mice11–13 and in humans.14,15 Interaction of ivermectin with ABCG2/Abcg2 seems to display species specificity as it was shown to inhibit bovine ABCG216 but not the mouse ortholog.17 No interaction was seen in vivo in Abcg2 knockout versus wildtype mice comparison either.18 Albeit, the in vivo data were generated on an Abcb1a,b background.

In this study utilizing multiple in vitro methods we are showing that ivermectin interacts with human ABCG2 with high affinity.

MATERIALS AND METHODS

Chemicals

Ko134 and Ko143 was from Solvo Biotechnology (Budaörs, Hungary). GF120918 was a kind gift from Prof. Ferenc Fülop (University of Szeged, Hungary). All other chemicals were from Sigma (Hungary, Budapest).

Cell Lines

PLB985-BCRP19 and parental cells were kindly provided by Dr. Katalin Német (National Blood
Transfusion Service, Hungary). Cells were maintained in Gibco's Advanced RPMI 1640 from Csertex (Hungary, Budapest). All media were supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 μg/mL penicillin–streptomycin and were grown under standard conditions (5% CO₂, 37°C).

**Vesicular Transport Assay**

The vesicular transport assay was performed using the PREDIVEZ Kit (Solvo Biotechnology) for human ABCG2 (SB-ABCG2-HAM-PREDIVEZ™-VT kit) using estrone-3-sulfate (E3S) as probe substrate according to the manufacturer’s recommendations.

For $K_d$ determination ivermectin was set to final concentrations 50, 16.7, 5.56, 1.85, 0.617, 0.206, and 0.0686 μM, and its effect measured on E3S transport at 50, 16.7, 5.56 and 1.86 μM concentrations. Data were plotted in Dixon’s representation and $K_d$ was derived from the x-axis coordinates of the intersections of fitted lines.

**Hoechst Assay**

The Hoechst assay was performed as described earlier. Accumulation of Hoechst 33342 dye in PLB985-BCRP cells was measured in a fluorometer (Fluoroskan Ascent Type 374) at 350 nm (excitation) and 460 nm (emission). The fluorescence intensities were recorded for 15 min. The positive control measurements to determine 100% inhibition were carried out in the presence of 300 nM Ko134.

**ATPase Activity**

ATPase activity was measured as described previously. The PREDEASY ATPase kit for ABCG2-HAM was from Solvo Biotechnology and was used according to the manufacturer’s instructions.

**Data Analysis**

Experiments were carried out at least twice with data points measured with three parallels. In the case of ATPase and Hoechst assays relative inhibition was plotted with the 100% reference provided by 300 nM Ko134. For vesicular transport studies relative activity was plotted with vehicle control as 100% reference.

Sigmoidal dose–response curves were fitted onto effect versus log concentration plots with GraphPad PRISM 4.0 (GraphPad Software, Inc., La Jolla, CA) and IC₅₀ values derived from best-fit parameters.

**RESULTS AND DISCUSSION**

To test interaction of ivermectin with human ABCG2 inhibition experiments were carried out. In mammalian membrane vesicles specifically overexpressing human ABCG2 ivermectin inhibited E3S transport with an IC₅₀ of 1.5 μM (Fig. 1A). The drug inhibited ABCG2-mediated efflux of the Hoechst dye with a similar potency (IC₅₀ of 1 μM, Fig. 1B). The good correlation observed between IC₅₀ values measured in membrane as well as cellular systems (1.5 μM vs. 1 μM, respectively) indicates that ivermectin has a reasonable membrane permeability. To get a preliminary indication on the nature of interaction, ivermectin was tested in an ATPase assay using membranes overexpressing the human protein. Interestingly, ivermectin inhibited the basal vanadate sensitive activity of ABCG2 with the same efficacy as Ko134, the reference inhibitor (Fig. 2). The lack of stimulation of the basal ABCG2 activity, however, does not necessarily indicate lack of transport as ivermectin inhibited the basal ATPase activity of ABCB1 in a similar fashion.

The inhibitory potency of ivermectin is within the range of other known potent ABCG2 inhibitors.
Ivermectin seems particularly potent in cellular assays. In sum, ivermectin interacts with human ABCG2 with an affinity (Tab. 1; $K_i$ of 1.4 $\mu$M, determined in vesicular transport assay, Fig. 3) close to the affinity (0.44 $\mu$M) observed for ABCB1.22 To elucidate the mechanism of the interaction transport experiments need to be performed in a relevant barrier model. In vivo investigations have to be carried out using ABCB1/Abcb1 and ABCG2/Abcg2 specific inhibitors or ABCG2/Abcg2 knockout animals generated on an ABCB1/Abcb1/C0/C0 background in a species where ivermectin interaction with ABCG2/Abcg2 can be demonstrated in vitro. Alternatively, ABCG2 and ABCB1 functions can specifically be knocked down using RNAi technology employing adenovirus or adeno-associated virus vectors.

In some humans ivermectin plasma levels may reach 100 nM9 that is about 10% of the IC50 values measured in vitro (Tab. 1). Albeit this is the total plasma concentration of ivermectin it was shown that total plasma concentration of a drug yields better in vitro–in vivo correlations than using the unbound plasma concentration when calculations are based on total concentration of the drug in the in vitro assay.23,24 Therefore, ivermectin is a potential perpetrator in drug–drug interactions in countries where it is licensed for human use. Nevertheless, clinical effect of this interaction on common substrates of ABCB1 and ABCG2 may be limited due to the masking effect of ABCB1.

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**REFERENCES**


**Table 1.** Comparison of Observed IC50 Values of ABCG2 Inhibitors in In Vitro Assays

<table>
<thead>
<tr>
<th></th>
<th>Vesicular Transport Inhibition</th>
<th>ATPase Inhibition</th>
<th>Hoechst Efflux Inhibition</th>
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<tbody>
<tr>
<td>Ivermectin</td>
<td>1.5</td>
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<td>1.0</td>
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<tr>
<td>Ko143</td>
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<td>None detected</td>
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<tr>
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<td>19</td>
</tr>
</tbody>
</table>

Values are given in $\mu$M.


