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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 IC_{50} values in the 1–1.5 μM range. This interaction may have implications in human ABCG2-mediated drug–drug interactions of ivermectin.

Keywords: BCRP; macrocyclic lactones; ADME; toxicity; ABC transporter

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 mice[2] 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 ABCB1[6] and multiple members of the ABCC subfamily[7] 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 mice[11–13] and in humans.[14,15] Interaction of ivermectin with ABCG2/Abcg2 seems to display species specificity as it was shown to inhibit bovine ABCG2[16] but not the mouse ortholog.[17] No interaction was seen *in vivo* in Abcg2 knockout versus wild-type 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 Fülöp (University of Szeged, Hungary). All other chemicals were from Sigma (Hungary, Budapest).

Cell Lines

PLB985-BCRP[19] and parental cells were kindly provided by Dr. Katalin Német (National Blood

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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% CO_2, 37°C).

Vesicular Transport Assay

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

For K_i 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_i was derived from the x-axis coordinates of the intersections of fitted linears.

Hoechst Assay

The Hoechst assay was performed as described earlier.[19] 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.[20] 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 IC_{50} 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[21] ivermectin inhibited E3S transport with an IC_{50} of 1.5 μM (Fig. 1A). The drug inhibited ABCG2-mediated efflux of the Hoechst dye with a similar potency (IC_{50} of 1 μM, Fig. 1B). The good correlation observed between IC_{50} values measured in membrane as well as cellular systems (1.5 μM vs. 1 μM, respectively) indicates that ivermectin has a reasonable membrane permeability.[20] 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.[7]

The inhibitory potency of ivermectin is within the range of other known potent ABCG2 inhibitors

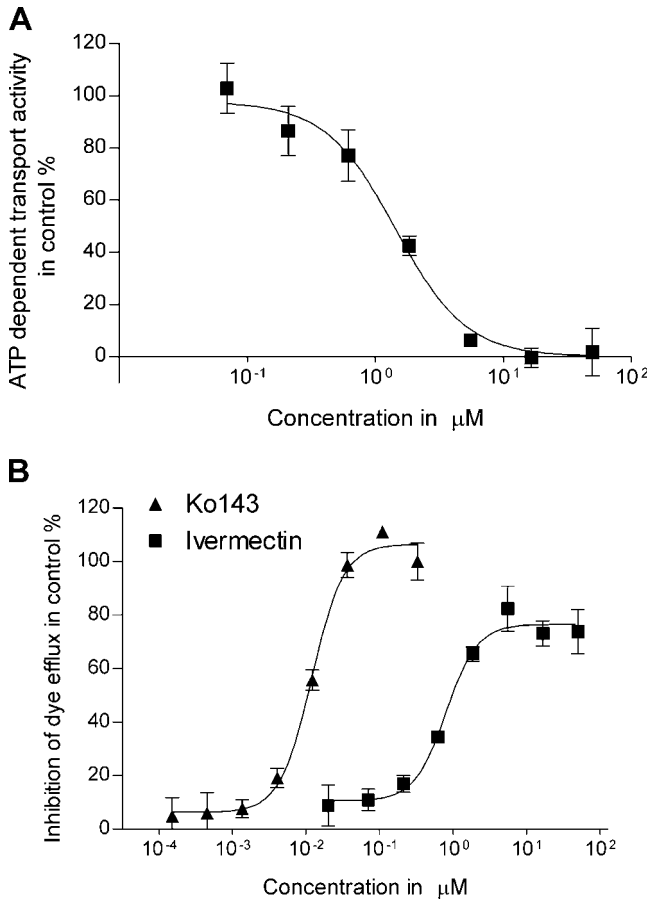

Figure 1. Ivermectin inhibits ABCG2 function in membrane vesicles and in cells. Ivermectin was incubated at final concentrations indicated with membrane vesicles (A) or cells (B) overexpressing human ABCG2. Transport of 3H-E3S (A) or Hoechst33342 (B) was measured and inhibition of transport is shown. Data points represent arithmetic means with standard deviations of three replicates.

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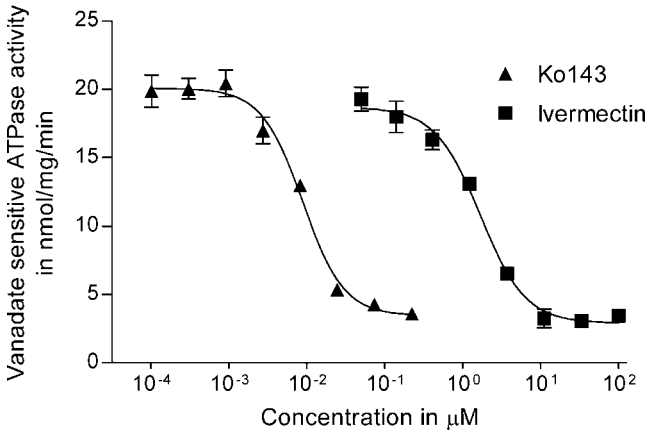

Figure 2. Effect of ivermectin on ABCG2 ATPase. Membrane vesicles (20 μg/well) were incubated with indicated concentrations of ivermectin for 40 min at 32°C. The sodium orthovanadate sensitive activity was plotted. Data points represent arithmetic means with standard deviations of three replicates.

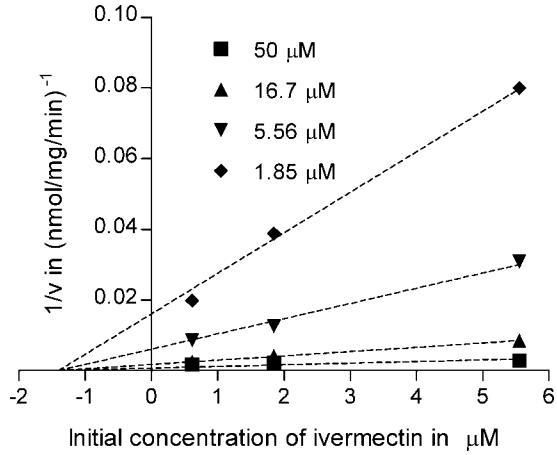

Figure 3. Inhibition of BCRP-mediated E3S transport by ivermectin in Dixon's representation. Linear portion of ivermectin series is shown. E3S concentrations were 50, 16.7, 5.56, and 1.85 μM. The intersections yield a K_i of 1.4 μM.

(Tab. 1). Ivermectin seems particularly potent in cellular assays.

In sum, ivermectin interacts with human ABCG2 with an affinity (Tab. 1; K_i of 1.4 μM, determined in vesicular transport assay, Fig. 3) close to the affinity (0.44 μM) 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 a ABCB1/Abcb1−/− 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 nM[9] that is about 10% of the IC_{50} 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.

Table 1. Comparison of Observed IC_{50} Values of ABCG2 Inhibitors in *In Vitro* Assays

	Vesicular Transport	ATPase Inhibition	Hoechst Efflux Inhibition
Ivermectin	1.5	1.68	1.0
Ko134	0.069	0.011	0.22
GF120918	0.046	0.11	None detected
Sulfasalazine	0.21	None detected	None detected
Novobiocin	0.11	22	19

Values are given in μM.

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