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Host ABC transporter proteins may influence the efficacy of ivermectin and possibly have broader implications for the development of resistance in parasitic nematodes

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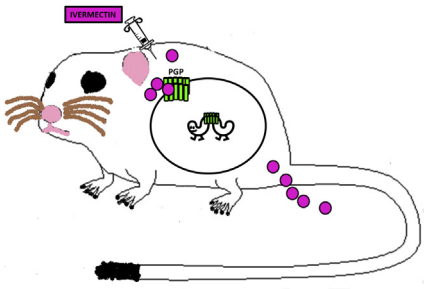
HIGHLIGHTS

- ABC transporters function to extrude compounds from cells and are expressed by host and parasite.
- *Brugia malayi* macrofilariae were not affected by ivermectin.
- The absence of macrofilaricidal effects was associated with low amounts of ivermectin in worms.
- The expression of ABC proteins was higher in worms treated *in vitro* and in treated gerbils.
- The results suggest that host ABC transporters may influence the efficacy of ivermectin.

GRAPHICAL ABSTRACT



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ABSTRACT

ABC transporter proteins function to extrude compounds from the cell. These proteins present an obstacle for treatment and for overcoming drug resistance as they are expressed by both host and parasite, and function similarly. The contribution of host ABC proteins to drug efficacy was examined using ivermectin and a *Brugia malayi* model system. Parallel *in vitro* and *in vivo* experiments were conducted using equal concentrations of ivermectin. The motilities and fecundity of *B. malayi* exposed to ivermectin *in vitro* were significantly lower than those treated *in vivo*. The higher motilities were correlated with low concentrations of ivermectin in worms extracted from treated hosts. The expression of ABC proteins was significantly higher in worms treated *in vitro* compared to those treated *in vivo* as well as in gerbils treated with ivermectin than in non-treated controls. The results suggest that host ABC transporters may influence the efficacy of ivermectin.

1. Introduction

Filarioid diseases in humans are caused by infection with the parasitic worms *Onchocerca volvulus*, *Brugia malayi*, *Brugia pahangi*, *Brugia timori* and *Wuchereria bancrofti*; over one billion people are currently at risk of acquiring these infections (WHO, 2013). Ivermectin is the only macrocyclic lactone that is currently approved

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for use in humans to treat these infections (Omura and Crump, 2004). The African Program for Onchocerciasis Control (APOC), and the former Onchocerciasis Control Program (OCP), recommends a single annual dose of 150 μg/kg of ivermectin to reduce microfilariae in the skin; repeated annual doses can suppress production of new microfilaria for several months (Gardon et al., 2002). The strategies for the Global Program to Eliminate Lymphatic Filariasis (GPELF) are similar to APOC, with mass drug administration of either a single-dose of albendazole in combination with ivermectin, in *O. volvulus* endemic areas, or a single-dose of albendazole in combination with diethylcarbamazine, in areas where *O. volvulus* is not endemic (Gyapong et al., 2005). Both APOC and GPELF utilize ivermectin for reduction of morbidity and interruption of transmission. As these control methods for parasitic nematodes rely on drugs, this in combination with their misuse and overuse, has resulted in drug resistance in a number of target species including *O. volvulus* (Awadzi et al., 2004a, 2004b; Osei-Atweneboana et al., 2007, 2011). Since ivermectin has systemic actions and must cross the tissues of the host organism before reaching the target parasites located *in refugia* (e.g., *B. malayi* in lymph nodes and *O. volvulus* in nodules), the presence of drug transporters in the host that efflux ivermectin will influence drug bioavailability and potentially drug efficacy and selection of resistant worms.

The four main mechanisms described for drug resistance are drug inactivation or modification, alteration of target site, alteration of a metabolic pathway and reduced drug accumulation by decreasing drug permeability and/or increasing active efflux of drugs across the cell surface (Li and Nikadio, 2009). The principal mechanism described for multidrug resistance (MDR) is active efflux by membrane transporters with broad substrate specificity. The transporters with established roles in MDR are members of the Drug Metabolite Transporter (DMT) superfamily of proteins which includes the ABC (ATP binding cassette; ABC transporters) Systems (Higgins, 2007).

ABC transporters are drug efflux pumps that function as a natural defense mechanism and influence the bioavailability and disposition of drugs through active extrusion of the compound from the cell, thereby contributing to MDR. Classic MDR is the consequence of the over-expression of P-glycoprotein (PGP) and the Multidrug Resistance associated Protein (MRP) (Higgins, 2007). By extruding the chemotherapeutic agent from the cell, PGP and MRP lower the effective drug concentration (Binkhathlan and Lavasanifar, 2013). In mammals, these transporters are expressed in a variety of tissues as well as the blood–brain barrier and provide a very efficient barrier of protection for the organism because they limit the entrance of many drugs, however as a consequence they also restrict drug efficacy (Sarkadi et al., 2006). Studies using mammals have shown that the principal physiological role of PGP is to protect organisms from toxic substances. Evidence for this function includes the identification of PGP expression at sites that are involved in drug excretion and from studies involving mice that were deficient in the gene coding for PGP. These mice showed altered sensitivity to compounds transported by PGP and were extremely sensitive to ivermectin compared to normal mice (Schinkel et al., 1994). When the function of PGP is inhibited by substrates that are competitive inhibitors (e.g., verapamil), drug sensitivity of MDR organisms is restored. This has been shown for *Haemonchus contortus* resistant to ivermectin (Molento and Prichard, 1999; Lifschitz et al., 2010; Bartley et al., 2012).

The primary mechanism of action of ivermectin results in paralysis and death of nematodes through activation of glutamate-gated chloride channels (GluCls) in muscle and nerve cells (Cully et al., 1994; Dent et al., 1997; Hibbs and Gouaux, 2011; Morenoa et al., 2010) and through effects on gamma-aminobutyric acid (GABA) receptors (Feng et al., 2002). A number of studies on nematodes have suggested efflux of ivermectin by PGP as a protective or resistance conferring mechanism (Ardelli and Prichard, 2013; Ardelli and Prichard, 2008; Dicker et al., 2011; James and Davey, 2009; Lespine et al., 2011; Tompkins et al., 2010; Williamson et al., 2011). In *B. malayi*, *in vitro* sensitivity to ivermectin was potentiated when it was co-administered with a number of PGP inhibitors, particularly verapamil (Tompkins et al., 2011).

It is clear that functional ABC transporters, particularly PGP, are critical in protecting the host from ivermectin toxicity (Schinkel et al., 1994). Furthermore, these transporters are involved in drug resistance in both humans (Sharom, 2014) and nematodes (Sangster et al., 2002). Since these transporters are an obstacle to cancer therapy because they confer MDR, it is likely that they will influence drug efficacy and possibly contribute to drug resistance in parasitic infections, particularly in the case of ivermectin as it is transported by mammalian PGP. Using a *B. malayi*-ivermectin-gerbil model, the purpose of this study was to investigate the contribution of host ABC transporters to drug efficacy.

2. Materials and methods

2.1. In vivo experimental design

Twelve male gerbils (60 g) were procured from Charles River Laboratories (Wilmington, VA, USA). Upon arrival at the at the National Institutes of Health/National Institute of Allergy and Infectious Disease (NIH/NIAID) Filariasis Research Reagent Resource Center (FR3 - College of Veterinary Medicine, University of Georgia, USA), the gerbils were weighed, assigned randomly to four groups ($n = 3$) and then a physical exam was performed. The gerbils were housed socially, in suspended polysulfone cages (Ancare, Bellmore, NY) on an aluminium rack (Research Equipment Corporation). Cages were bedded with corncob bedding (The Anderson's, Maumee, OH) and gerbils were provided nestlets (Ancare, Bellmore, NY) as environmental enrichment. Food (Lab Diet 5053, irradiated, Lab Diet, St. Louis, MO) and filtered municipal water in water bottles were provided *ad libitum*. The room temperature range was 65–75 °F and the light cycle was 12:12. All research procedures were approved by the University of Georgia's Institutional Animal Care and Use Committee (Registration number A2010 07-124).

The four groups were treated as follows: non-infected and non-treated (Group A); infected but not treated (Group B); not infected and treated (Group C); and infected and treated (Group D). After one week of acclimation, gerbils in Groups B and D were inoculated subcutaneously with 100 L3s of *B. malayi*. After the *B. malayi* had sexually matured, 200 μg/kg of ivermectin were administered subcutaneously in a volume of 100 μL to gerbils in Groups C and D. One week post-treatment, gerbils were euthanized and necropsied. During necropsy, the abdominal, thoracic cavities and lymphatic vessels were examined for adult *B. malayi*. Adult worms were removed and shipped immediately to Brandon University. Tissues (blood, brain, spleen, liver, intestines) were extracted from gerbils, immediately frozen and shipped to Brandon. Upon arrival in Brandon, adult worms were placed immediately in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin and allowed to acclimatize for 24 h before their motility was determined in a blind experiment. The number of microfilariae released by females into the culture medium was determined as described (Tompkins et al., 2010). Following measurements of motility (three independent trials), worms were immediately frozen for use in determining the amount of ivermectin in tissues and the gene expression profiles of *Brugia* ABC transporters. The gerbil tissues were used to determine the amount of ivermectin and the