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Research Report

The transport of nifurtimox, an anti-trypanosomal drug, in an *in vitro* model of the human blood–brain barrier: Evidence for involvement of breast cancer resistance protein

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ABSTRACT

Human African trypanosomiasis (HAT) is a parasitic disease affecting sub-Saharan Africa. The parasites are able to traverse the blood–brain barrier (BBB), which marks stage 2 (S2) of the disease. Delivery of anti-parasitic drugs across the BBB is key to treating S2 effectively and the difficulty in achieving this goal is likely to be a reason why some drugs require highly intensive treatment regimes to be effective. This study aimed to investigate not only the drug transport mechanisms utilised by nifurtimox at the BBB, but also the impact of nifurtimox–eflornithine combination therapy (NECT) and other anti-HAT drug combination therapies (CTs) on radiolabelled-nifurtimox delivery in an *in vitro* model of drug accumulation and the human BBB, the hCMEC/D3 cell line. We found that nifurtimox appeared to use several membrane transporters, in particular breast-cancer resistance protein (BCRP), to exit the BBB cells. The addition of eflornithine caused no change in the accumulation of nifurtimox, nor did the addition of clinically relevant doses of the other anti-HAT drugs suramin, nifurtimox or melarsoprol, but a significant increase was observed with the addition of pentamidine. The results provide evidence that anti-HAT drugs are interacting with membrane transporters at the human BBB and suggest that combination with known transport inhibitors could potentially improve their efficacy.

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Abbreviations: BBB, blood–brain barrier; BCRP, breast cancer resistance protein; CT, combination therapy; HAT, human African trypanosomiasis; NECT, nifurtimox–eflornithine combination therapy; S1, stage 1 of human African trypanosomiasis; S2, stage 2 of human African trypanosomiasis

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1. Introduction

Human African Trypanosomiasis (HAT) is caused by *Trypanosoma brucei gambiense* (*T. b. gambiense*) and *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*), two species of parasitic protozoans belonging to the genus *Trypanosoma*. The trypanosomes are spread by the biting Tsetse fly which acts as an intermediate host. The disease, if left untreated, then manifests as two distinct stages. The first stage (S1) is generally asymptomatic and characterized by presence of the parasites in the blood and lymphatic systems of the human host. The second stage (S2) is characterized by parasites in the brain and cerebrospinal fluid (CSF) and can occur months (*T. b. rhodesiense*) or years (*T. b. gambiense*) after the initial infection. In S2, a variety of central nervous system (CNS) disorders become apparent including insomnia and changes in sleeping cycle which give the disease the name 'sleeping sickness' (for a recent review of HAT's effects on the CNS see Kristensson et al., 2010). If HAT remains untreated it is fatal, thus anti-parasitic chemotherapy is crucial. Fortunately, several drugs are available to treat the disease but have different efficacies depending on the disease stage and pathogen being targeted. The drugs also pose several other problems; they can be expensive, require intensive administration programmes which are unrealistic in a resource poor setting and some are toxic to patients. Treatment of S2 requires that the drug crosses the blood–brain barrier (BBB); the highly specialised microvasculature that separates the cerebral tissue from the blood circulation (Abbott et al., 2006). S1 acting drugs are pentamidine and suramin which are effective against *T. b. gambiense* and *T. b. rhodesiense*, respectively (Brun et al., 2010; Sanderson et al., 2007; Sands et al., 1985). S2 drugs are melarsoprol, eflornithine and nifurtimox. Several recent reviews discuss the S2 acting drugs in further detail (Brun et al., 2010; Lutje et al., 2010).

Our research group has investigated the ability of suramin, pentamidine, eflornithine and nifurtimox to cross the BBB using an *in situ* brain/choroid plexus perfusion technique in anaesthetised mice (Jeganathan et al., 2011; Sanderson et al., 2007, 2008, 2009). Our latest study focused on nifurtimox, an anti-parasitic nitrofuran that was originally used to treat Chagas disease; a closely related condition to HAT caused by *Trypanosoma cruzi* (Gonnert and Bock, 1972; Haberkorn and Gonnert, 1972), but has since been used in compassionate treatment for HAT when other methods have failed (Moens et al., 1984; Van Nieuwenhove, 1992). Nifurtimox is now used against S2 in combination with eflornithine (Checchi et al., 2007). Nifurtimox is cheap, orally active and effective against *T. b. gambiense* and, to a lesser extent, *T. b. rhodesiense* (Bouteille et al., 2003; Haberkorn, 1979; Lutje et al., 2010). Importantly, our group have shown that nifurtimox is able to cross the murine BBB *in situ*, but undergoes an efflux removal process from the brain via an unidentified process, in which the adenosine triphosphate (ATP) binding cassette (ABC) transporter P-glycoprotein, (P-gp) is not involved (Jeganathan et al., 2011). The identify of this efflux mechanism is of special interest with the fact that nifurtimox–eflornithine combination therapy (NECT) is now becoming the first course of treatment against S2 HAT (Yun et al., 2010), having been shown to both improve efficacy and reduce harmful side effects (Priotto et al., 2007, 2009). The precise mechanisms behind the success of this particular combination therapy (CT) have yet to be fully revealed, however, it is possible CT could improve delivery to the brain. Our group have shown that nifurtimox delivery to the mouse brain is improved with the addition of the S1 acting drug pentamidine (Jeganathan et al., 2011), which we have previously identified as being a substrate for cellular transport mechanisms at the BBB, including P-gp (Sanderson et al., 2009). These findings highlight not only the need to elucidate the transport mechanisms utilized by nifurtimox at the BBB, but also the effect of CT on its delivery.

Our earlier work has focused on *in vivo* murine models of the BBB, however, in order to translate the research to the human situation this present study uses a human *in vitro* BBB model, the hCMEC/D3 cell line. The hCMEC/D3 cell line is the most promising immortalized human BBB cell line available today, exhibiting many of the characteristics that are essential for a good predictive BBB *in vitro* model (Poller et al., 2008; Weksler et al., 2005). These include expression of tight junction proteins, polarized expression of multiple ABC/SLC transporters and restrictive permeability (Dauchy et al., 2009; Tai et al., 2009b). The following study is the first to investigate nifurtimox transport interactions in a human model of the BBB.

2. Results

2.1. *hCMEC/D3 — expression of endothelial cell marker von Willebrand factor*

We confirmed the endothelial cell phenotype by staining monolayers of cells grown on collagen-coated coverslips for vascular endothelial marker, von Willebrand factor (vWF) (Fig. 1).

2.2. *Influence of self-inhibition on [^{3}H]nifurtimox accumulation*

By varying the concentrations of unlabelled nifurtimox in accumulation buffer alongside [^{3}H]nifurtimox and [^{14}C]sucrose, we were able to assess any roles played by major BBB transport proteins in the transport and subsequent accumulation of [^{3}H]nifurtimox and [^{14}C]sucrose, compared to appropriate controls. Accumulation of [^{3}H]nifurtimox was not significantly affected by the addition of unlabelled nifurtimox at a clinically relevant dose of 6 μM or an increased dose of 12 μM (Fig. 2). The addition of 60 μM and 150 μM unlabelled nifurtimox, however, caused significant increases in [^{3}H]nifurtimox accumulation at all time points ($p<0.001$) compared to DMSO [^{3}H]nifurtimox controls.

2.3. *Roles of P-gp and BCRP in [^{3}H]nifurtimox accumulation*

To assess any roles played by major BBB transport proteins in the transport and subsequent accumulation of [^{3}H]nifurtimox and [^{14}C]sucrose, a variety of drugs were used individually in the accumulation buffer alongside [^{3}H]nifurtimox and [^{14}C]sucrose and compared to appropriate controls. The influences of P-gp and BCRP in the transport of [^{3}H]nifurtimox, were tested