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DRUG DISCOVERY AND RESISTANCE

Inhibitory potential of tuberculosis drugs on ATP-binding cassette drug transporters

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SUMMARY

Background: Multiple-drug therapy for tuberculosis (TB) and TB-associated co-morbidity increase the likelihood of drug–drug interactions (DDIs). Inhibition of membrane transporters is an important mechanism underlying DDIs. In this study, we assessed the *in vitro* inhibitory potential of currently used first and second-line TB drugs and of proposed mycobacterial efflux pump inhibitors (EPIs) on the major ABC transporters relevant to drug transport, namely P-gp, BCRP, BSEP and MRP1-5.
Methods: Membrane vesicles isolated from transporter-overexpressing HEK293 cells were used to study the inhibitory action of TB drugs and EPIs on the transport of model substrates [^{3}H]-NMQ (P-gp); [^{3}H]-E_1S (BCRP); [^{3}H]-TCA (BSEP); [^{3}H]-E217βG (MRP1, 3 and 4) and [^{3}H]-MTX (MRP2 and 5).
Results: A strong inhibition (IC_{50} value <15 μM) was observed for clofazimine (P-gp, BCRP and MRP1), thioridazine (BCRP), timcodar (P-gp, BSEP and MRP1) and SQ109 (P-gp and BCRP). Rifampicin inhibited all transporters, but less potently.
Conclusions: Co-administration of clofazimine, thioridazine, timcodar, SQ109 and possibly rifampicin with drugs that are substrates for the inhibited transporters may lead to DDIs. The mycobacterial EPIs potently inhibited a wider range of human ABC transporters than previously reported. These vesicular transport data are especially valuable considering the current emphasis on development of TB drug regimens.

1. Introduction

Tuberculosis (TB) remains a prevalent global health problem. In 2013, an estimated 9.0 million people developed active TB and 1.5 million died from the disease [1]. Treatment of TB requires long and intensive combination drug therapy. The first-line regimen for drug-sensitive TB contains four drugs, namely rifampicin, isoniazid, pyrazinamide and ethambutol [2] (Table 1). For multiple drug resistant TB (MDR TB), a range of second-line TB drugs are in use (Table 1), which are administered for longer periods, generally have less potency and are associated with more toxicity [3]. In recent years, novel TB drugs have been developed (bedaquiline and delamanid) and available TB drugs are being repurposed or optimized as first-line TB drugs (e.g. high dose rifamycins, moxifloxacin and clofazimine). In addition, various new compounds are still being evaluated for treatment of both drug-sensitive and resistant TB [3,4]. Overall, in TB drug development the focus is on development of regimens rather than just single new TB drugs, and in this context, drug-drug interactions (DDIs) between available and (potential) new TB drugs need to be assessed.

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Table 1
Overview of first-line and second-line drugs and EPIs.[*,†]

Group	Drugs
Group I: first-line oral agents	rifampicin, rifabutin, isoniazid, pyrazinamide and ethambutol
Group II: injectable agents	amikacin, kanamycin, capreomycin and streptomycin
Group III: fluoroquinolones	levofloxacin, moxifloxacin and ofloxacin
Group IV: oral bacteriostatic second-line agents	para-aminosalicylic acid (PAS), cycloserine, terizidone, ethionamide and protionamide
Group V: agents with unclear role in treatment of DR-TB	clofazimine, linezolid, amoxicillin/clavulanate, thioacetazone, imipenem/cilastatin, high-dose isoniazid and clarithromycin
Other: Adjuvant drugs proposed to be mycobacterial efflux pump inhibitors (EPIs) [15,19,20]	e.g. SQ109, thioridazine, timcodar and verapamil

[*] Adapted from Table 7.1 of the WHO guideline: Treatment of Tuberculosis: Guidelines for National Programmes (2009) [2].
[†] The underlined TB drugs were evaluated in this study.

Because TB is increasingly associated with co-morbidities, drug therapy is becoming even more complex. An estimated 1.1 million (13%) of the people who developed TB in 2013 were HIV-positive [1]. In addition, the relative burden of type 2 diabetes mellitus (DM) to the TB epidemic is increasing, as the incidence of DM is rapidly rising in countries where TB is endemic and DM is a prominent risk factor for active TB [5]. The concurrent use of antimycobacterial, antiretroviral, and antidiabetic drugs further increases the likelihood of DDIs to occur in this patient population [6].

Inhibition of membrane transporter activity is one of the mechanisms known to result in relevant DDIs. In general, drugs need to cross multiple cellular barriers in order to reach their sites of action. Uptake and efflux via membrane transporters play a vital role in the passage across these barriers and, therefore, in the absorption, distribution and excretion of drugs. Efflux transporters also protect sanctuary sites such as brain and placenta against xenobiotics and increase their elimination via liver and kidney. Many transporters of the ATP binding cassette (ABC) transporter protein family are involved in the cellular efflux of drugs [7]. ABC proteins are primary active transporters that mediate the translocation of substrates across the membrane at the expense of ATP hydrolysis. Interaction of a drug with such a transporter could alter the systemic or local disposition of other compounds that are substrates of the same transporter [8]. Consequently, drug concentrations of the other substrate drug can be increased or decreased and the safety and efficacy of this drug may be at risk [8,9]. As an example for an interaction affecting systemic exposure, the antimycobacterial drug clarithromycin (classified as a group V anti-TB drug) has demonstrated to increase the oral bioavailability and reduce the non-glomerular renal clearance of digoxin, a P-gp substrate, probably by inhibiting intestinal and renal P-glycoprotein [10]. This inhibitory effect has also been demonstrated in an *in vitro* model [11] and may form a reasonable explanation for the reports of clarithromycin induced digoxin intoxication [12].

Considering the likelihood of DDIs to occur in TB treatment, we aimed to clarify the *in vitro* interaction potential of available TB drugs with the major ABC transporters relevant to drug transport, namely P-gp, BCRP, BSEP and MRP1-5 [13]. For this purpose, we used membrane vesicles isolated from transporter-overexpressing HEK293 cells. In drug development, inside-out membrane vesicles are recognized as an appropriate *in vitro* model to assess whether a drug is an *in vivo* transporter substrate or inhibitor [13]. This model has as a major advantage that drugs can be directly applied to the cytoplasmic compartment and influx, rather than efflux, is measured. In the field of TB treatment, vesicular transport data are especially valuable, because of the limited knowledge on interactions of TB drugs with ABC transporters and the current emphasis on development of TB drug regimens. Next to first and second-line TB drugs, we assessed the inhibitory potential of three so-called mycobacterial efflux pump inhibitors (EPIs) [14], i.e. thioridazine, timcodar and SQ109 (Table 1). EPIs are proposed to work as co-adjuvant drugs to increase intramycobacterial concentrations of TB drugs, which may even reverse *Mycobacterium tuberculosis* efflux pump induced resistance [14–20].

2. Materials and methods

2.1. Chemicals

The following TB drugs were purchased from Sigma–Aldrich (Zwijndrecht, the Netherlands): rifampicin, isoniazid, pyrazinamide, ethambutol, amikacin, moxifloxacin hydrochloride, cycloserin, ethionamide, 4-aminosalicylic acid (PAS), amoxicillin, clofazimine, linezolid and thioridazine hydrochloride. SQ109 was kindly provided by Sequella Inc. (Rockville, MD, USA) and timcodar by Vertex Pharmaceuticals Inc. (Boston, MA, USA), respectively. P-gp substrate [^{3}H]-N-methyl quinidine ([^{3}H]-NMQ) and unlabeled NMQ were obtained from Solvo Biotechnology (Szeged, Hungary). Substrates [^{3}H]-estrone sulfate ([^{3}H]-E_1S), [^{3}H]-taurocholic acid ([^{3}H]-TCA) and [^{3}H]-estradiol 17β-glucuronide ([^{3}H]-$E_2$17βG) were purchased from Perkin Elmer Life Sciences (Waltham, MA, USA). [^{3}H]-methotrexate ([^{3}H]-MTX) was purchased from Moravek Biochemicals (Brea, CA, USA). Adenosine 5'-triphosphate disodium salt (ATP) (from bacterial source) was purchased from Sigma–Aldrich (Zwijndrecht, the Netherlands).

2.2. Membrane vesicles preparation

Membrane vesicles were obtained from PharmTox (Nijmegen, the Netherlands, http://www.pharmtox.nl). They were prepared from Human Embryonic Kidney (HEK) 293 cells overexpressing P-gp, BCRP, BSEP, MRP1-5 or the cytosolic enhanced Yellow Fluorescent Protein (eYFP) [21]. In short, human P-gp, BCRP, BSEP and MRP1-5 were cloned behind a CMV promotor into a Baculovirus. HEK293 cells were transduced with these viruses and harvested by centrifugation. The 100,000 g membrane fraction was homogenized in ice-cold TS buffer (10 mM Tris-HEPES and 250 mM sucrose, pH 7.4) and high shear passage through a 100 μm opening was used to prepare membrane vesicles. Protein concentration of these vesicles was determined using the Bio-Rad protein assay (Bio-Rad laboratories Inc, Veenendaal, the Netherlands). Vesicles were snap-frozen in liquid nitrogen and stored at −80 °C. Functional activity of the investigated transporters with respect to their model substrates is presented in Supplementary Table 1.

2.3. Screening for ABC transporter activity inhibition

A rapid filtration technique was applied to study the inhibitory action of 200 μM TB drugs and EPIs on the transport of model

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