Novel Molecular Insights into the Inhibitory Potential of Antifungal Drugs on ATP-Binding Cassette Transporters MRP1 – 5, BCRP, P-gp and BSEP

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Introduction

- Understanding drug disposition of systemically administered antifungal drugs is pivotal for the appreciation of antifungal efficacy in animals and humans.
- Although drug-efflux pumps play a key role in drug disposition, little is known on the role of efflux transporters in drug-drug interactions with antifungal drugs.

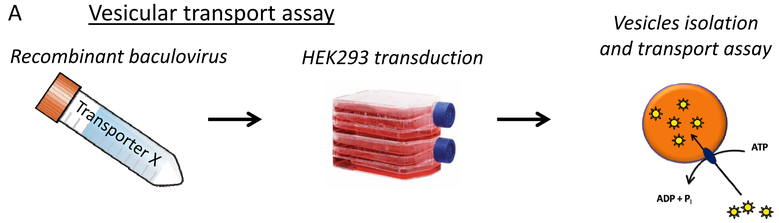
Objective

Exploration of the inhibitory potential of selected antifungal drugs on the ATP-binding cassette (ABC) transporter family MRP1-5, BCRP, P-gp and BSEP in vitro.

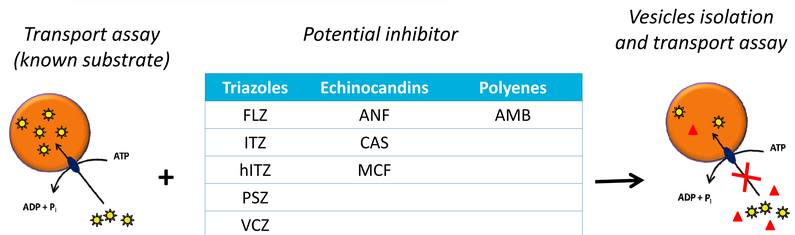
Methods

- A vesicle system was used to determine the inhibitory potential of selected antifungal drugs on predefined substrates of the ATP-binding cassette (ABC) transporter family MRP1-5, BCRP, P-gp and BSEP.
- Fluconazole (FLZ), posaconazole (PSZ), isavuconazole (ISAV), voriconazole (VCZ), itraconazole (ITZ), hydroxy-itraconazole (hITZ), anidulafungin (ANF), caspofungin (CAS), micafungin (MCF) and amphotericin B deoxycholate (AMB) were used in this study.
- After transduction of HEK293 cells with recombinant baculoviruses containing MRP1-5, P-gp, BCRP and BSEP genes, membrane vesicles were isolated and tested for overexpression and functionality (Western blot, data not shown) (A).
- The vesicular transport assay was used to study the inhibitory action of antifungal drugs on the transport of model substrates (i.e. [3H]-E1S, [3H]-E217βG, [3H]-MTX, [3H]-NMQ and [3H]-TCA) by the efflux transporter (B).
- Antifungal drugs capable of inhibiting the transport of model substrates via the studied efflux pumps for >50% in our screening were selected for concentration-dependent inhibition.
- The log(inhibitor) vs. response curve was plotted and the Hill-equation with variable slope was fitted to the data using Graphpad Prism version 5.03.

A Vesicular transport assay



B Inhibitory potential of antifungal drugs



Triazoles	Echinocandins	Polyenes
FLZ	ANF	AMB
ITZ	CAS	
hITZ	MCF	
PSZ		
VCZ		
ISAV		

Results

- FLZ and VCZ did not inhibit ATP-dependent transport >50% for all studied efflux transporters (Fig.1).
- P-gp, BCRP and BSEP were all inhibited by the triazoles ITZ, hITZ, PSZ and ISAV (Fig.1).
- ANF and ITZ were incapable to fully inhibit transporters, probably due to poor solubility (ANF soluble up to 10 µM and ITZ up to 2,5 µM).
- MCF was unique in inhibiting all studied efflux transporters (Fig.1).
- Only BCRP was inhibited by AMB, (Fig.1, see table 1 for IC50 values).

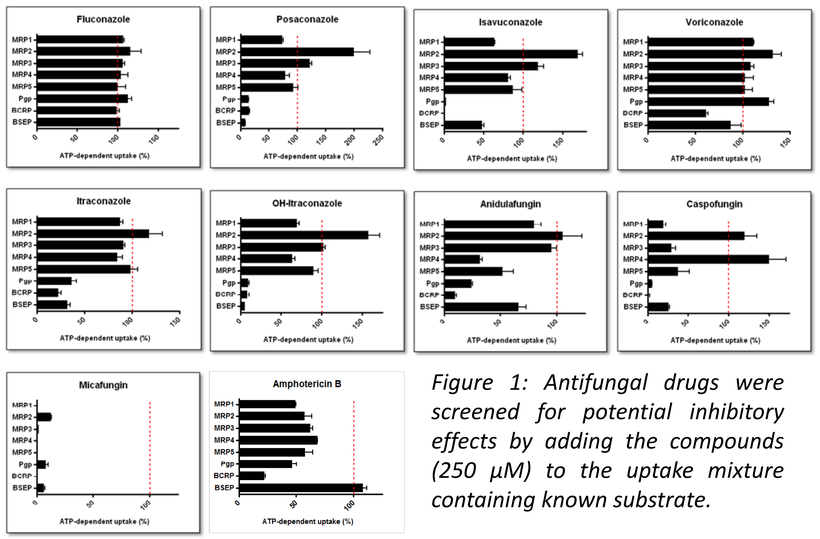
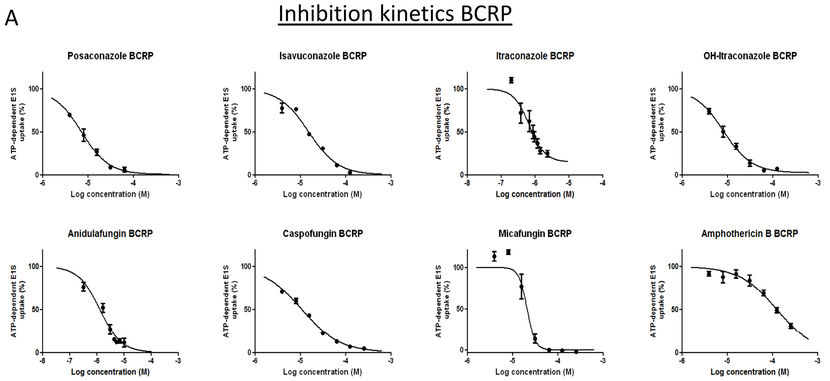


Figure 1: Antifungal drugs were screened for potential inhibitory effects by adding the compounds (250 µM) to the uptake mixture containing known substrate.

A Inhibition kinetics BCRP



B Inhibition kinetics MRP1-5

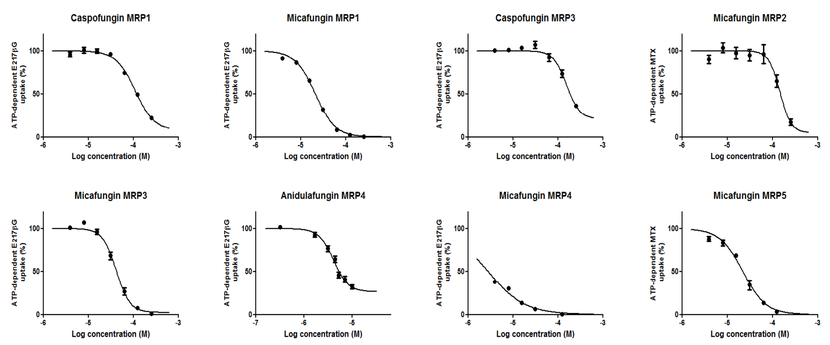


Figure 2: IC_{50} curves of BCRP (A) and MRP1-5 (B) with antifungals showing >50% inhibition during screen (concentration-dependent inhibition transport studies).

Overview IC_{50} values (µM)

	FLZ	ITZ	hITZ	PSZ	VCZ	ISAV	ANF	CAS	MCF	AMB
MRP1	.	.	.	.	.	.	.	112	21	.
MRP2	.	.	.	.	.	.	.	.	148	.
MRP3	.	.	.	.	.	.	.	158	42	.
MRP4	.	.	.	.	.	.	4	.	3	.
MRP5	.	.	.	.	.	.	.	>200	22	.
BCRP	.	0.4	8	7	.	15	1	11	21	127
P-gp	.	2	9	9	.	9	4	34	45	.
BSEP	.	4	17	34	.	94	.	182	85	.

Conclusions

- Although echinocandins (ANF, CAS and MCF) have a low potential for clinically relevant drug-drug interactions, they demonstrated in vitro inhibitory activity against efflux transporters.
- Despite the wide range and variability of drug-drug interactions with azole antifungals, they exhibit limited inhibition of efflux transporters.
- Interestingly, FLZ and VCZ did not exert any inhibition on the efflux transporters studied. Similarly, ITZ/hITZ and PSZ both inhibited the same efflux transporters.

This study was supported by a research grant by Merck, Sharpe & Dohme Corp. in 2013

24th European Congress of Clinical Microbiology and Infectious Diseases
Barcelona, Spain; 10 – 13 May 2014

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