THE INHIBITORY POTENTIAL OF TB DRUGS ON ATP-BINDING CASSETTE TRANSPORTERS MRP1-5, P-GP, BCRP AND BSEP

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ABC transporters

- ABC transporters actively transport substrates across the cell membrane with the use of ATP hydrolysis
- Located at pharmacological barriers and important in ADME of drugs
- Transporter interactions have a major impact on later phases of drug development
- *In vitro* high-throughput: “fail fast - fail cheap”
Aim

To study the inhibitory potential of a range of anti-TB drugs on the ABC transporters MRP1-5, BCRP, P-gp and BSEP in vitro, to obtain insight in the role of efflux transporters in drug-drug interactions.
Vesicle assay

✓ Vesicles: cell membrane preparations containing transporter(s)

✓ Functional expression in vesicles:

human ABCB1: MDR1 / Pgp
human ABCB11: BSEP
human ABCC1: MRP1
human ABCC2: MRP2
human ABCC3: MRP3
human ABCC4: MRP4
human ABCC5 MRP5
human ABCG2: BCRP
Transporters in different organs

KIDNEY

- OAT1
- OAT2
- OAT3
- OCT2
- SLCO4C1
- MRP2
- MRP4
- P-gp *
- BCRP *
- MATE1
- MATE2k
- URAT1 *
- PEPT1
- PEPT2

LIVER

- MRP1
- MRP3
- MRP4
- MRP5
- MRP6
- OAT1A2
- OATP1B1
- OATP1B3
- OATP2B1
- NTCP
- OAT1
- OCT1
- OCT3

BLOOD-BRAIN-BARRIER

- BCRP *
- P-gp *
- MRP2
- MRP4
- MRP5
- MRP1
- MRP3
- MRP4
- MRP5
- MRP6
- BSEP
- MDR3
- MATE1
- ABCG5
- ABCG8

INTESTINE

- MRP1
- MRP3
- MRP2
- BCRP *
- P-gp *
- OCT1
- OATP2B1

Legend:

- Green: ATP mediated transporter functional in vesicles
- Red: ATP mediated transporter non-functional
- Yellow: Non ATP mediated transporter functional in cell line
- Purple: Non ATP mediated transporter non-functional

# Also functional in vesicles
* Also functional in cell line
I. First line oral agents
- Rifampicin
- Isoniazid
- Pyrazinamide
- Ethambutol
- Linezolid

II. Injectable agents
- Amikacin

III. Fluoroquinolones
- Moxifloxacin

IV. Oral bacteriostatic second line agents
- Cycloserine
- Ethionamide
- PAS

V. Agents with unclear role
- Amoxicillin
- Clofazimine
- Linezolid

Agents experimental in TB treatment (EPI)
- Thioridazine
- Timcodar
- SQ109
Vesicle assay (II)

Assay ingredients:

✓ Vesicles
✓ Model substrate (RA label)
✓ Potential drug inhibitors (200 µM)
✓ ATP
Inhibitory effects TB-drugs – high-throughput

200 µM solutions -> derive concentration-effect curves
Inhibitory effects TB-drugs – high-throughput

RIF, CLF and EPIs are most important significant inhibitors
IC50 – heat map

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Radboudumc
IC50 – P-gp

- 1 µM = 0.7 mg/L
- 2 µM = 0.8 mg/L
- 11 µM = 3.5 mg/L
- 23 µM = 9.2 mg/L
- 29 µM = 24 mg/L

- Curve shift right -> higher IC50
- Free concentrations: possible relevant/irrelevant
Conclusion

1. Supposedly mycobacterial EPIs (timcodar, SQ109 and thioridazine) inhibit a range of human ABC transporters at possibly clinical relevant concentrations.

2. Clofazimine shows potent *in vitro* transporter inhibition. In clinical setting DDI are relatively unknown.

3. Rifampicin shows inhibition at probably clinical irrelevant free concentrations.
Conclusion (II)

✓ Vesicle assay can be used to screen for transport inhibition in an early stage of drug development, to predict DDI expectancy:

1. IC50 values represent free concentrations at target site
   -> no *in vivo* protein binding and drug accumulation

2. No effect on expression levels
   -> rifampicin induces expression *in vivo*

3. Mechanism of inhibition unknown (competitive vs direct inhibition)
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