

In vitro ADME & PK

BSEP, MRP2, MRP3 and MRP4 Inhibition

Background Information



'Proactive evaluation and understanding of BSEP inhibition is recommended in drug discovery and development to aid internal decision making on DILI risk..'

³Kenna JG *et al.*, (2018) *Clin Pharmacol Ther* **104**(5): 916-932.

- BSEP (bile salt export pump; ABCB11) is an ATP binding cassette (ABC) efflux transporter located on the canalicular membrane of hepatocytes, and is the major transporter for the secretion of bile acids from hepatocytes into bile in humans¹.
- Because of the link between BSEP inhibition and initiation of cholestatic DILI, the European Medicines Agency Guideline on the Investigation of Drug Interactions (2012) recommends *in vitro* screening of BSEP inhibition².
- MRP2 (multidrug resistance associated protein 2; ABCC2), MRP3 (ABCC3) and MRP4 (ABCC4) are ATP binding cassette (ABC) efflux transporters located on the canalicular membrane (MRP2) or sinusoidal membrane (MRP3, MRP4) of hepatocytes^{3,4}.
- MRP3 and MRP4 efflux transporters are upregulated under cholestatic conditions suggesting they provide a protective role against bile acid-mediated hepatotoxicity by alleviating increases in intracellular bile acid concentrations, which may occur as a result of impaired biliary excretion due to inhibition of BSEP^{3,4,5}. Understanding whether a compound is able to inhibit MRP transporters may therefore provide useful additional information towards helping evaluate the risk of DILI.
- Cyprotex offer BSEP, MRP2, MRP3 and MRP4 inhibition assays which investigate inhibition of the uptake of prototypical probe substrates (taurocholic acid for BSEP and estradiol 17 β -D-glucuronide for MRPs) into inside-out membrane vesicles overexpressing the human ABC-transporter of interest.

Related Services

P-gp
BCRP
Human SLC Transporters

Protocol

Test System

Sf9 insect cell-derived or mammalian (HEK293) cell-derived inside-out membrane vesicles overexpressing a single transporter (BSEP, MRP2, MRP3 or MRP4) incubated in the presence of ATP and AMP (absence of ATP).

Probe Substrate

[³H]-Taurocholic acid
[³H]-Estradiol 17 β -glucuronide

Test Article Concentrations

6 concentrations plus 0 μ M (triplicate wells) (final test article concentrations dependent on customer requirements)

Time Points

Dependent on transporter

Analysis Method

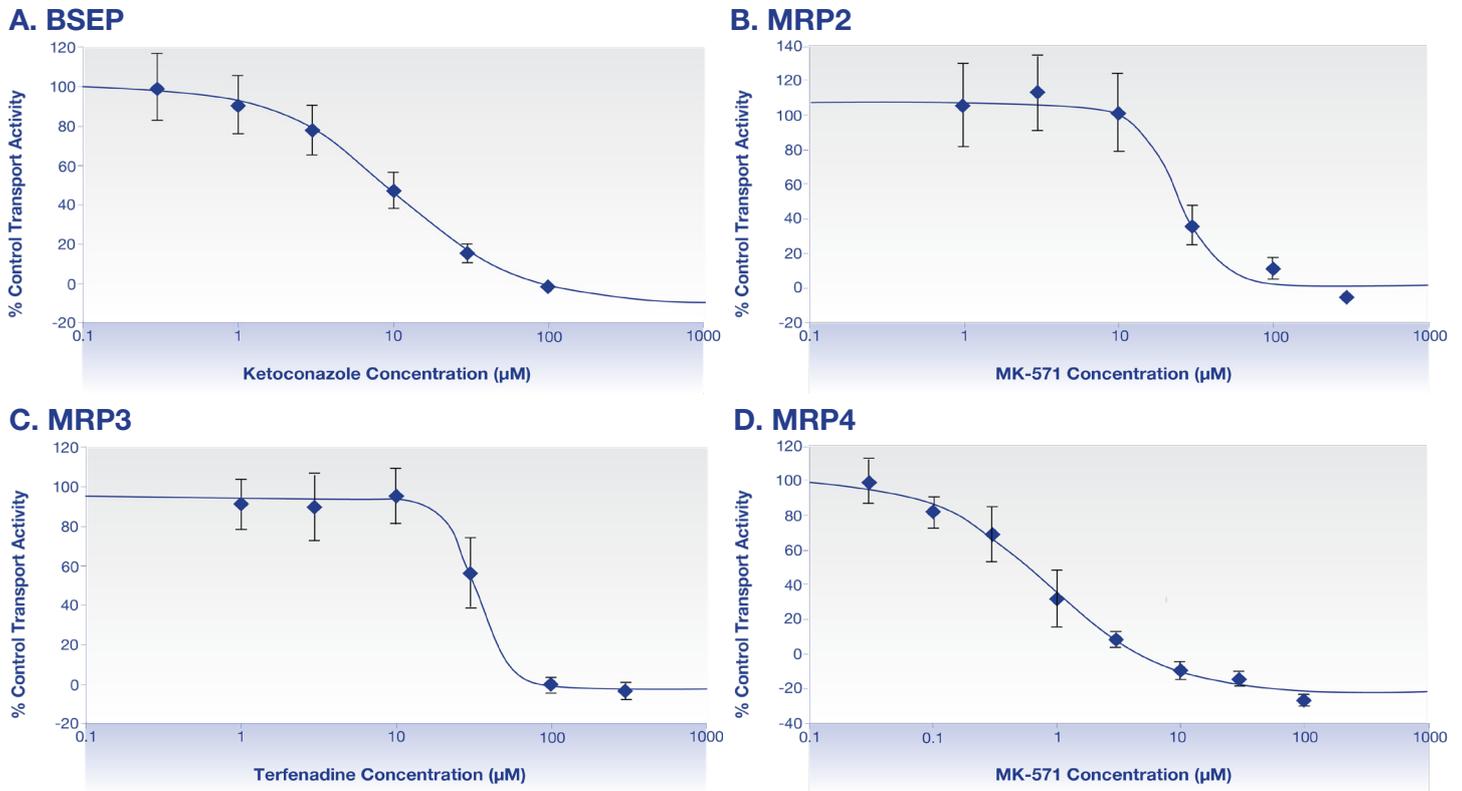
Radiochemical detection using scintillation counting

Data Delivery

IC₅₀
Written report available on request

Figure 1

BSEP-mediated taurocholic acid (A) and MRP-mediated estradiol 17 β -D-glucuronide (B-D) transport in the presence of a range of concentrations of inhibitor expressed as a percentage of vehicle control (mean \pm standard deviation; n=3-9 wells, triplicate incubations performed on 3 separate occasions).

**Table 1**

Inhibition of human BSEP- and MRP-mediated transport of the prototypical substrates, taurocholic acid and estradiol 17 β -glucuronide, respectively.

Transporter	Substrate	Inhibitor	IC ₅₀ \pm Standard Deviation (μM)
BSEP	Taurocholic acid	Ketoconazole	8.78 \pm 1.25
MRP2	Estradiol 17 β -glucuronide	MK-571	22.6 \pm 6.38
MRP3	Estradiol 17 β -glucuronide	Terfenadine	33.5 \pm 6.77
		Fidaxomicin	1.06 \pm 0.117
MRP4	Estradiol 17 β -glucuronide	MK-571	0.555 \pm 0.238
		Indomethacin	3.79 \pm 0.342
		Ibuprofen	42.0 \pm 23.7

The incubation conditions for each of the species have been fully characterised for the chosen substrates based on time linearity and uptake kinetics (V_{max} and K_m).

The chosen substrate concentration is much lower than the determined K_m , and as such IC₅₀ equates to K_i (assuming competitive inhibition).

References

- Wang L *et al.*, (2002) The role of bile salt export pump mutations in progressive familial intrahepatic cholestasis type II. *J Clin Invest* **110**(7): 965-972.
- The European Medicines Agency (EMA) Guideline on the Investigation of Drug Interactions (Adopted 2012)
- Kenna JG *et al.*, (2018) Can bile salt export pump inhibition testing in drug discovery and development reduce liver injury risk? An International Transporter Consortium perspective. *Clin Pharmacol Ther* **104**(5): 916-932.
- Zamek-Gliszczyński MJ *et al.*, (2018) Transporters in drug development: 2018 ITC recommendations for transporters of emerging clinical importance. *Clin Pharmacol Ther* **104**(5): 890-899
- Morgan RE *et al.*, (2013) A multifactorial approach to hepatobiliary transporter assessment enables improved therapeutic compound development. *Toxicol Sci* **136**: 216-241