cyprotex AN EVOTEC COMPANY

In vitro **ADME & PK**

Human MRP Efflux Transporter Substrate Identification (MRP2, MRP3, MRP4) for Screening or Regulatory Reporting Purposes

Background Information

'Membrane transporters can have clinically relevant effects on the pharmacokinetics and pharmacodynamics of a drug in various organs and tissues by controlling its absorption, distribution and elimination.'

[2]Draft FDA Guidance for Industry - In Vitro Metabolism- and Transporter-Mediated Drug-Drug Interaction Studies, October 2017

- MRP2 (multidrug resistance associated protein 2; ABCC2), MRP3 (ABCC3) and MRP4 (ABCC4) are ATP binding cassette (ABC) efflux transporters which are located on the brush border membrane of enterocytes (MRP2), the canalicular membrane (MRP2) or sinusoidal membrane (MRP3, MRP4) of hepatocytes, the brush border membrane of renal proximal tubule epithelial cells (MRP2, MRP4) and at the blood-brain barrier (MRP4)[1].
- Consequently, these efflux transporters influence the absorption, distribution, metabolism and excretion of drugs/and or metabolites within the body.
- The International Transporter Consortium (ITC)[1] indicate that because MRP2, MRP3 and MRP4 are important determinants of hepatobiliary disposition of polar drug metabolites, for example glucuronide conjugates, then being a substrate of these transporters may contribute to the overall victim and perpetrator DDI potential of the parent drug. Furthermore, the draft FDA guidances[2,3] indicate that the DDI potential of metabolites versus the major drug transporters, and other emerging transporters such as MRPs when appropriate, be assessed on a case by case basis.
- Cyprotex's MRP efflux transporter substrate identification assay determines if your compound is a substrate of these key hepatobiliary transporters.

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 (MRP2, MRP3 or MRP4) incubated in the presence of ATP and AMP (absence of ATP)

Test Article Concentrations
Screening study:

- Single concentration (typically 1 µM), single timepoint for 7 compounds
- Two concentrations (typically 1 and 10 µM), single time point for 3 compounds
- Two concentrations (typically 1 and 10 µM), two time points for a single compound

Regulatory study:

- Typically 1, 10, 50 and 100 µM (depending on customer requirements) plus inhibition at two substrate concentrations (two time points)

Time Points
Typically, 10 min or 10 and 20 min (depending on customer requirements)

Analysis Method
MicroBeta® scintillation counter (radiolabelled substrates)
LC-MS/MS analysis (non-radiolabelled substrates)

Data Delivery
Cellular uptake and fold accumulation
Written report available on request

Figure 1

Uptake of [3H]-estradiol 17ß-glucuronide (30 µM) in MRP2 membrane vesicles with ATP or AMP in the presence and absence of the inhibitor MK-571 (300 µM).

To confirm ATP-dependent transporter involvement in the uptake of estradiol 17ß-glucuronide in the MRP2 membrane vesicles, the inhibitor MK-571 was included in the incubations. This reduced the uptake to similar levels (< 2 fold) as observed in the plus AMP background condition.

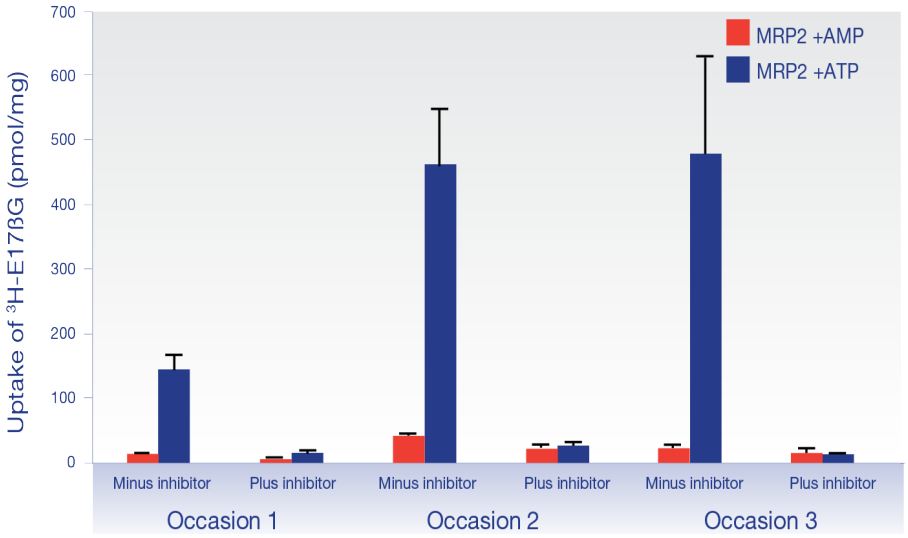


Figure 2

Uptake of [3H]-estradiol 17ß-glucuronide (1 µM) in MRP3 membrane vesicles with ATP or AMP in the presence and absence of the inhibitor terfenadine (300 µM).

To confirm ATP-dependent transporter involvement in the uptake of estradiol 17ß-glucuronide in the MRP3 membrane vesicles, the inhibitor terfenadine was included in the incubations. This reduced the uptake to similar levels (< 2 fold) as observed in the plus AMP background condition.

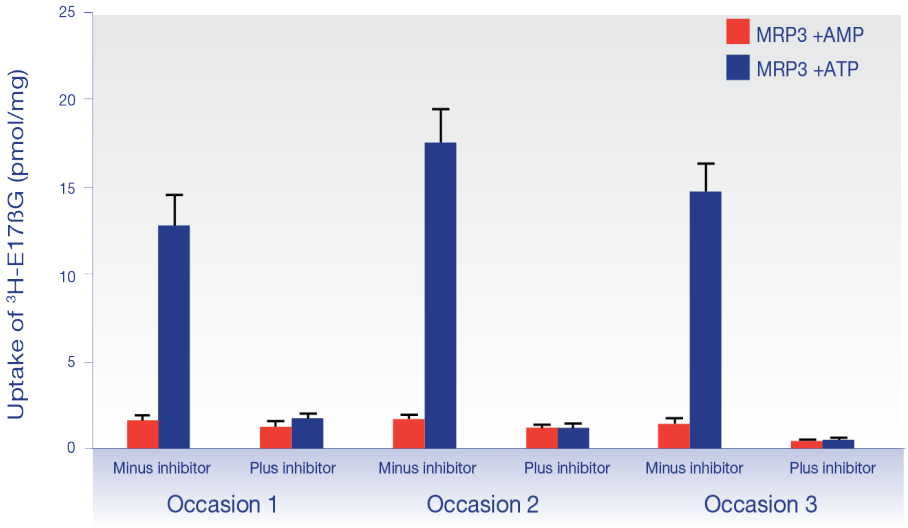
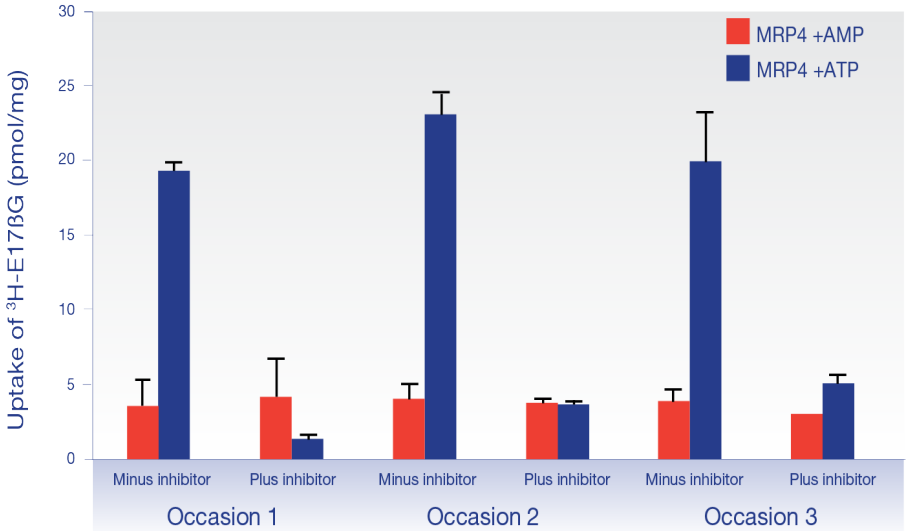


Figure 3

Uptake of [3H]-estradiol 17ß-glucuronide (1 µM) in MRP4 membrane vesicles with ATP or AMP in the presence and absence of the inhibitor MK-571 (10 µM).

To confirm ATP-dependent transporter involvement in the uptake of estradiol 17ß-glucuronide in the MRP4 membrane vesicles, the inhibitor MK-571 was included in the incubations. This reduced the uptake to similar levels (< 2 fold) as observed in the plus AMP background condition.



References

[1] Zamek-Gliszczynski MJ *et al*., (2018) Transporters in Drug Development: 2018 ITC Recommendations for Transporters of Emerging Clinical Importance. *Clin Pharmacol Ther* **104(5)**: 890-899.

[2] Draft FDA Guidance for Industry – Drug Interaction Studies – Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations, February 2012

[3] Draft FDA Guidance for Industry – In Vitro Metabolism- and Transporter- Mediated Drug-Drug Interaction Studies, October 2017