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

Background Information

- 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).

- Consequently, these efflux transporters influence the absorption, distribution, metabolism and excretion of drugs and/or metabolites within the body.

- The International Transporter Consortium (ITC) 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 guidance indicates 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.

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

Related Services

- P-gp
- BCRP
- Human SLC Transporters

To find out more contact enquiries@cyprotex.com
To confirm ATP-dependent transporter involvement in the uptake of estradiol 17β-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.

References

