QUANTITATIVE PROTEIN ABUNDANCE MEASUREMENTS

TNO innovation for life

Membrane embedded transporter proteins play an important role in the absorption, distribution and excretion of many drugs. A reliable prediction of human pharmaco-kinetics of (new) drugs based on PBPK modeling is highly dependent on accurate determination of the absolute protein expression levels of these transmembrane transporter proteins.

The importance of membrane bound drug transporters in drug absorption, distribution, metabolism, elimination, and as such toxicity and efficacy has generally been established and recognized. Making use of recent developments we are now able to accurately measure the concentration of membrane transporters within the plasma membrane using lipid chromatography linked to tandem mass spectrometry (LC-MS/MS). So far, this has been a missing link in extrapolating from in vitro studies to the in vivo situation.

YOUR ADVANTAGE

- Delivery of appropriate physiological scaling factors to extrapolate in vitro data to whole organ or whole body PK.
- Accurate comparison of differential transfected cell lines by determining the protein expression levels within the plasma membrane (e.g. comparison of cells expressing a wild-type or mutant variant of a transporter protein).
- Method also applicable for highly sensitive detection of SNPs, post-translational modifications and/or phosphorylation of proteins.

SAMPLE PREPERATION AND LC-MS/MS ANALYSIS

For each specific transporter protein we select a proteotypic tryptic peptide, based on selection criteria as described by Kamiie et al.[1] Plasma membranes are isolated using an in house optimized sucrose gradient protocol. Extracted samples are spiked with known amounts of labeled peptides (used as internal references), followed by trypsin digestion and analyzed by LC-MS/MS in the selected reaction monitoring mode (SRM).

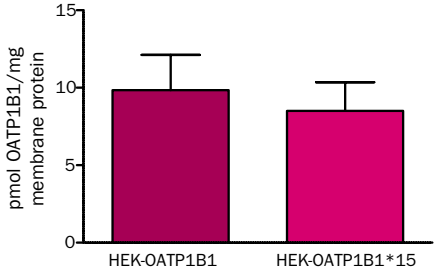

*Figure 1. Absolute plasma membrane protein expression levels of OATP1B1 in HEK-OATP1B1 and HEK-OATP1B1*15 cells as measured by LC-MS/MS (n=3; mean ± SD).[2]*

FEATURES

Absolute expression levels can be determined in different organs (e.g. liver, kidney, intestine, brain) and different cell lines (hepatocytes, transfected cells). Two examples are presented in Figure 1 and 2.

Every specific membrane-embedded protein that is not listed in the table below can be monitored upon request.

Membrane-bound receptor proteins can potentially also be measured using the same methodology.

The sensitivity of the LC-MS/MS methodology is at the low pmol/mg protein level. The required sample amount is 100-500 mg tissue or 0.3-1 · 10^8 cells.

COMBINED SERVICES

LC-MS/MS services for protein quantification can be combined with other expertise, including:

- Cell-based transporter assays
- In vivo animal studies (KO and transgenic models)
- In vivo PK-PD studies, e.g. using animal models for a specific disease
- In silico PBPK modeling to predict drug behavior based on cell or animal studies

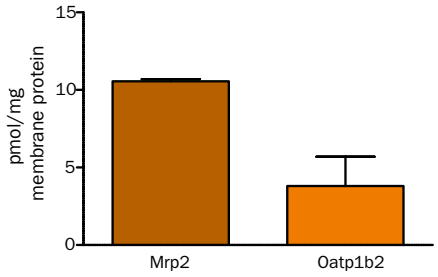

Figure 2. Absolute plasma membrane protein expression levels of Mrp2 and Oatp1b2 in rat (Wistar) livers as measured by LC-MS/MS (n=3; mean ± SD).

THE FOLLOWING PROTEINS CAN CURRENTLY BE QUANTIFIED:

MDR1, BCRP, MRP2, BSEP	(human, rat, mouse)
OATP1B1, OATP1B3, OATP2B1	(human)
Oatp1a4, Oatp1b2, Oatp2b1	(rat)

QUANTIFICATION OF THE FOLLOWING PROTEINS IS UNDER DEVELOPMENT:

MRP1, MRP3, NTCP, ASBT	(human, pig)
OCT1, OCT3, OCTN2	(human, pig)
OATP1C1, OATP3A1, OATP4A1, OATP4C1	(human, pig)
GLUT1, MCT1, MCT5, PEPT1	(human, pig)

Quantification of additional proteins can be performed upon request

REFERENCES

[1] *Kamiie J, Ohtsuki S, Iwase R, Ohmine K, Katsukura Y, Yanai K, Sekine Y, Uchida Y, Ito S, Terasaki T (2008). Pharm.Res. 25: 1469-1483.*

[2] *Van de Steeg E, Greupink R, Schreurs M, Nooijen IHG, Verhoeckx KCM, Hanemaaijer R, Ripken D, Monshouwer M, DeGroot J, Verwei M, Russel FGM, Huisman MT, Wortelboer HM (2012) Drug-drug interactions between rosuvastatin and oral antidiabetic drugs occurring at the level of OATP1B1 (submitted).*

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