A novel in vitro transporter assay in ABCB4 - overexpressing cells

C. Temesszentandrási-Ambrus[I], G. Nagy[I], A. Bui[I], **Z. Gáborik**[I]

[I]Solvo Biotechnology, Charles River Laboratories Hungary, Budapest, Hungary

Multidrug resistance protein 3 (MDR3, ABCB4) expression is almost exclusively limited to the canalicular membrane of hepatocytes, where it transports phosphatidylcholine into the bile, playing an important role in bile formation by protecting the biliary tree from the detergent activity of bile salts and maintaining cholesterol solubility. ABCB4 polymorphisms and deficiencies in humans are associated with hepatobiliary disorders ranging from progressive familial intrahepatic cholestasis type 3 (PFIC3) to ABCB4-related cholestatic liver disorders of varying manifestation and severity. It has been suggested that its inhibition by drugs may lead to cholestasis and drug-induced liver injury (DILI), although there are only a few identified substrates and inhibitors of ABCB4. Since ABCB4 shares up to 76% identity and 86% similarity in its amino acid sequence, as well as substrates and inhibitors with ABCB1, we developed an ABCB4-expressing Abcb1-knock out MDCKII cell line for transcellular transport assays using digoxin as a substrate. This in vitro system facilitates the screening of ABCB4-specific drug substrates and inhibitors with no confounding ABCB1 activity present. Abcb1KO-MDCKII-ABCB4 cells provide a reproducible, conclusive, and straightforward assay to study drug interactions. Screening a set of drugs with different DILI outcomes have shown that this assay is suitable to test ABCB4 inhibitory potency. Our results are consistent with prior findings concerning hepatotoxicity causality, and provide a new tool to identify drugs as potential ABCB4 inhibitors and substrates.