"Inside-Out" Membrane Vesicles: an in vitro Model to Study Transporter-mediated Drug Interactions that can Lead to Liver Toxicity

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Abstract
Multidrug resistance proteins (MRPs), MRP2 and the bile salt export pump (BSEP) in particular, are two major ABC efflux transporters that contribute to drug-induced liver toxicity. MRP2 is essential for hepatoprotective elimination of many drugs, drug conjugates, and endogenous compounds, such as bilirubin glucuronides. MRP2 dysfunction caused by inherited MRP2 deficiency or drug-mediated inhibition results in hyperbilirubinemia. BSEP is the rate-limiting step of bile salt transport across hepatocyte membranes. Disruption of BSEP in hepatocytes leads to accumulation of cytosolic bile salts in the Liver, resulting in liver toxicity (e.g., cholestasis). In light of the evidence showing the importance of MRP2 and BSEP in drug-induced liver toxicity, the International Transporter Consortium (ITC) recommends evaluating the potential interaction of drugs (new molecular entities) with MRP2 and BSEP. In addition, other ABC transporters, i.e., BCRP localized on the canalicular membrane of hepatocytes are the target of drug-drug interaction (DDI). The study focused on the use of "inside-out" membrane vesicles, which are prepared from insect cells (Sf9) infected with baculovirus expressing human MRP2, BSEP or BCRP (LCMA), to access drug interactions for these transporters in vitro. The data demonstrate that in the presence of ATP, MRP2 vesicles showed significant uptake (>20-fold) for taurocholate (TCA) and estradiol-17p-glucuronide, known MRP2 substrates, compared to control vesicles (vesicles assayed with the non-hydrolyzable ATP analog AMP). Taurocholate, a BSEP probe substrate, showed more than 20-fold uptake activity in BSEP vesicles, compared to the control. MRP2-mediated uptake of the probe sulforaphane glucuronide was inhibited by 5.1 µM MK571 (a protein kinase C activator) and 10 µM Ko143 (a bile acid transport inhibitor). BSEP-mediated taurocholate uptake was shown to be inhibited by sodium taurocholate (NaTCA), 3-aminobenzamide, trinitrobenzenesulfonic acid (TNBSA), etc. BSEP-mediated estradiol-17p-glucuronide was inhibited by BCRP modulators (sulfasalazine, estrone-3-sulfate, novobiocin, FTC, and Ko143). The data indicate that "inside-out" membrane vesicles are a quick and useful tool to screen MRP2, BSEP, and BCRP inhibitors that can potentially cause liver toxicity or DDIs in vivo.

Introduction
The ATP binding cassette (ABC) family is a multi-transmembrane protein that plays a vital role in drug absorption, distribution, elimination, and toxicity. Inverted plasma membrane vesicles prepared from transporter over-expressed cells are increasingly used to study ABC efflux transporters.1 The advantage of this model versus whole cell model is that the test articles do not need to be preloaded into the cells. The vesicle models work for both permeable and poorly permeable drugs. In this study, we present the application of inside-out vesicles in characterizing ABC transporter substrate specificity and drug inhibitory effect on transporter-mediated drug excretion.

Materials and Methods

Materials
Corning® Gentest™ Human MRP2 (Cat. No. 451458), Human BSEP (Cat. No. 453502) and Human BCRP (Cat. No. 451302) vesicles; Corning Gentest MRP/BCRP Vesicle Assay Kit (Cat. No. 450500); and BSEP Vesicle Assay Kit (Cat. No. 450510); 10 mM Buffer for MRP/BCRP Vesicle Assay (Cat. No. 450600) and 10 mM Wash Buffer for BSEP Vesicle Assay (Cat. No. 450601).

Transport Uptake Assay

The uptake was carried out using a Millipore filtration system (Gelman vacuum manifold). A 48 pmol reaction mixture containing 50 µL GSH (used only for MRP2) and probe substrates or test compounds in uptake buffer was pre-incubated at 37°C for 5 minutes. For inhibitory studies, test inhibitor was pre-incubated in uptake buffer with 50 µg vesicles and substrates for 5 min. The uptake was initiated by addition of 5 mM ATP or AMP, followed by incubation at 37°C for 15 minutes. The assay was terminated by rapid filtration, followed by washing with cold washing buffer. After filtration, the assay was ready for analysis by scintillation counter or LC-MS/MS following the manufacturer’s protocol.

Data Analysis

ATP-dependent uptake activity was reported as uptake activity in the presence of ATP subtracting uptake activity in the presence of AMP. The percentage of remaining activity was reported as the uptake activity in the presence of inhibitor divided by the uptake activity in the absence of inhibitor. SignalP® software was used to analyze the kinetics data. IC50 was calculated by linear interpolation using the following equation:

\[ IC50 = \frac{Vmax \times IC50 \times V_{PPE}}{(V_{max} - V_{PPE}) \times IC50 + V_{PPE}} \]

where Vmax is the maximum uptake activity in the absence of AMP. IC50 was calculated using SignalP® and shown as Intersection of IC50s in the graph.

Conclusions
Corning Life Sciences offers a broad portfolio of ABC transporter vesicle products that feature robust performance with a high signal-to-noise-ratio. The IC50 of selected probe substrates and IC50 values of selected transporter modulators were determined using the Corning® Gentest™ ABC Transporter Vesicles and Assay Kit. The data were comparable with literature reports. Methods were developed and validated for vesicle uptake assay for radiolabeled or non-radiolabeled chemicals using the rapid filtration system and the LC-MS/MS detection approach. Inside-out recombinant membrane vesicles are robust and predictive tools to screen MRP2, BSEP, and BCRP inhibitors that can potentially cause liver toxicity or DDI in vivo.