

# Comparison of cell based and membrane based high throughput assays for the detection of drugs interacting with the BCRP transporter

Szilvia Gedev, Hristos Glavinas, Emese Kis, Ákos Pál, Dóra Méhn, Márton Jani, Berend Oosterhuis, Tünde Nagy, György Báthori, Péter Krajcsi



SOLVO Biotechnology, 62 Temesvári Krt, H-6726 Szeged, Hungary

## INTRODUCTION

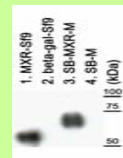
ATP Binding Cassette (ABC) transporters significantly modulate the absorption, metabolism, cellular effectivity and toxicity of pharmacological agents. The BCRP (MXR, ABC-P, ABCG2) transporter is a half transporter that is known to be responsible for certain cases of multidrug resistance. Its presence on the canalicular membrane of hepatocytes and other important lining surfaces of the human body suggest that BCRP is important in determining the pharmacokinetics of drugs and drug candidates. The aim of this study was to compare different high-throughput whole-cell based assays and membrane based assay systems such as ATPase and vesicular transport assays with respect to their capability of detecting BCRP interacting compounds.

In order to set up an ATPase assay for BCRP transporter we chose two approaches: (1) we prepared membranes from a selected mammalian cell line that express BCRP at a similar level to baculovirus infected Sf9 cells, and (2) using different inhibitors of the BCRP transporter we suppressed the baseline vanadate sensitive ATPase activity and used different transported substrate to activate the inhibited transporter. We hypothesized that both low and high permeability substrates would be detected by this membrane based system.

We also tested another membrane based assay: the vesicular transport (VT) assay. We hypothesized that it is feasible to measure the transport of low permeability compounds directly (direct VT assay) and measured the transport of <sup>3</sup>H-methotrexate and <sup>3</sup>H-estrone-3-sulfate. The vesicular transport assay can also be used in an indirect setup, detecting the modulation of the transport of a known low permeability substrate by test compounds. This setup would detect both low and high permeability interacting compounds. To test this hypothesis we measured the modulation of <sup>3</sup>H-methotrexate transport by different test compounds.

We used an ABCG2 overexpressing cell line and measured the effect of test drugs on the cellular accumulation of two BCRP substrates: Hoechst 33342 and <sup>3</sup>H-labelled prazosin. We hypothesized that since the binding site locating for substrates is located in the membrane or on the intracellular surface of the transporter these assays would not detect low permeability compounds.

## RESULTS AND DISCUSSION



The membrane preparations from baculovirus infected Sf9 cells and selected mammalian cells express BCRP at similar levels. Western blot analysis revealed that the protein expressed in mammalian cells has higher molecular mass due to the extensive glycosylation of BCRP that is not present in Sf9 cells (Ozvegy et al. 2001.)

Figure 1. BCRP in the Sf9 and mammalian membrane preparations as detected by the monoclonal antibody BXP-21 in western blot. Proteins of the membrane vesicles (the indicated amount of protein) were separated by 7.5 % SDS-PAGE and blotted on PVDF membranes.

To compare the novel human membrane preparation with the Sf9 membrane preparation containing the wild type transporter we determined the basic biochemical characteristics of the two membrane preparations (Figure 2). Both membrane preparations showed similar  $K_m$  for ATP (2.0 and 2.2 mM for MXR-Sf9 and MXR-M membranes respectively).

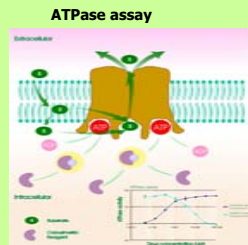
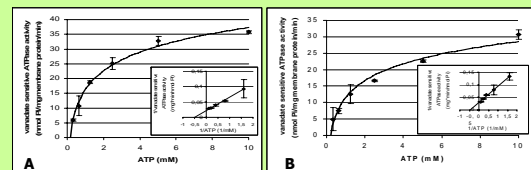


Figure 2. Vanadate sensitive ATPase activity of MXR-Sf9 (A) and MXR-M (B) preparations in the presence of different ATP concentrations. Membranes containing 20 µg of total protein were incubated at 37 °C for 40 min. Vanadate sensitive ATPase activity was determined. Insert: Lineweaver-Burk plot.

The ATPase assay can not be used with BCRP over-expressing Sf9 membrane preparations as a result of high baseline vanadate sensitive ATPase activity. This is believed to be due to an endogenous substrate of BCRP present in the Sf9 membranes. This high baseline activity cannot be further activated by transported compounds. Though mammalian membrane preparations have significant baseline vanadate sensitive ATPase activity also, they can be activated by substrates of BCRP. K0134 or K0143 can be used as an inhibitor of the baseline vanadate sensitive ATPase activity of the membrane preparations, while non-interacting compounds did not modulate it (Figure 3a and 3b).

### Insect (Sf9) vesicles Mammalian membrane vesicles

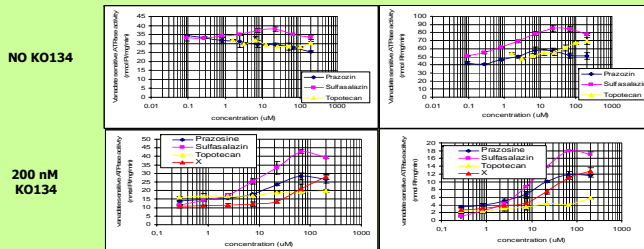
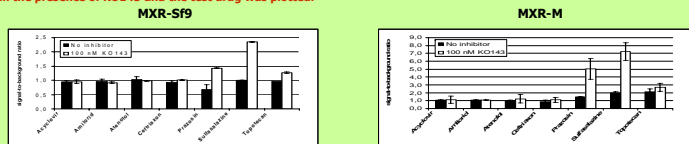


Figure 3a. Vanadate sensitive ATPase activity with and without K0134 in the presence of BCRP substrates at different concentrations. Membranes containing 20 µg of total protein were incubated at 37 °C for 40 min in the presence of different concentrations of test compounds. Vanadate sensitive ATPase activity was determined. Compound X is a drug under development that is known to interact with BCRP.

Figure 3b. MXR-Sf9 and MXR-M ATPase activity with and without K0134 (100 nM) and in the presence of different test drugs. Membranes containing 20 µg of total protein were incubated at 37 °C for 40 min in the presence of different test drugs (100 µM). Vanadate sensitive ATPase activity was determined and the ratio of baseline vanadate sensitive ATPase activity and vanadate sensitive ATPase activity in the presence of the test drug (white bars), or the ratio of K0143 inhibited vanadate sensitive ATPase activity and vanadate sensitive ATPase activity in the presence of K0143 and the test drug was plotted.



## Vesicular transport assay

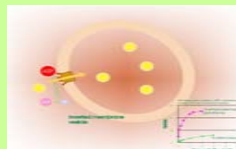
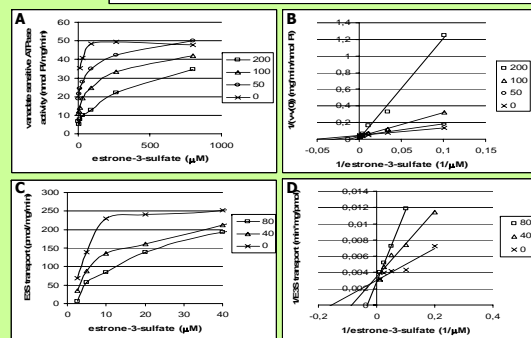
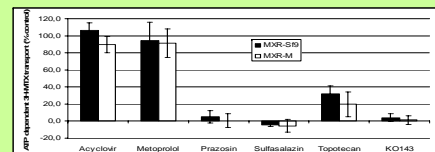


Figure 4a. Inhibition of the ATP dependent <sup>3</sup>H-methotrexate transport for Sf9 and human membrane vesicles by different substrates and non-substrates (100 µM) and the specific inhibitor K0143 (1 µM) of BCRP

Figure 4b. (A) Vanadate sensitive ATPase activity of MXR-M preparations in the presence of different concentrations of K0134 (nM, legend). (B) Lineweaver-Burk plot of the estrone-3-sulfate activated vanadate sensitive ATPase activity (v-v(0)) of MXR-M preparation in the presence of different concentrations of K0134 (nM, legend). Membranes containing 20 µg of total protein were incubated at 37 °C for 40 min. Vanadate sensitive ATPase activity was determined and v-v(0) was calculated by subtracting the vanadate sensitive ATPase activity in the presence of K0134 alone from the vanadate sensitive ATPase activity determined in the presence of both estrone-3-sulfate and the respective K0134 concentration. (C) ATP dependent <sup>3</sup>H-estrone-3-sulfate transport for MXR-M membrane vesicles at different estrone-3-sulfate concentrations in the presence of different concentrations of K0134 (nM, legend) (C) Incubation time was 1 minute. (D) Lineweaver-Burk plot of figure C.

We managed to set up the vesicular transport assay for both preparations. The indirect vesicular transport studies showed that all known substrates inhibited the transport of <sup>3</sup>H-methotrexate, while non-interacting compounds did not modulate it (Figure 4a.). The direct transport of the two low permeability compound, <sup>3</sup>H-methotrexate and <sup>3</sup>H-estrone-3-sulfate, was successful, while we did not succeed in setting up the direct transport of prazosin (data not shown). The biochemical characteristics detected in the ATPase assay and the direct vesicular transport measurements for <sup>3</sup>H-estrone-3-sulfate were in good accordance (Figure 4b.). This shows that activations detected in the ATPase assay are indicative of underlying transport processes.



## Cell-based assays



The whole-cell based assays detected the two high permeability BCRP interacting compound tested: prazosin and K0143. The other BCRP substrates assayed and did not show interaction as hypothesized (Figure 5.). This indicates that these low permeability substrates did not reach the intracellular site of interaction in these assays.

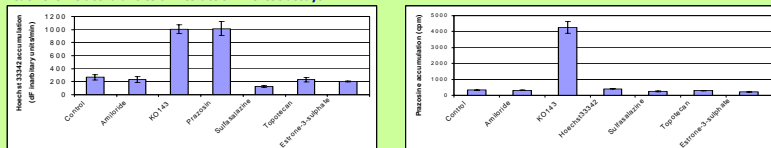


Figure 5. (A) BCRP (MXR) expressing cells were incubated in the presence of K0143 (1 µM) or test compounds (100 µM) and 50 µM Hoechst 33342 for 15 minutes. Fluorescence (355 Ex./460 Em.) was recorded every minutes and the rate of Hoechst accumulation was calculated from the slope of the fluorescence curves.

(B) BCRP (MXR) expressing cells were incubated in the presence of K0143 (1 µM) or test compounds (100 µM) and 1 µM <sup>3</sup>H labelled prazosin for 30 minutes. Cells were washed 3 times with PBS and lysed by 0.1 M NaOH. Prazosin accumulation was measured by liquid scintillation.

## CONCLUSIONS

- Both membrane preparations and cellular assays can be used with certain limitations to detect compound - BCRP transporter interactions.
- Cellular assays, ATPase assay and indirect vesicular transport assays can be used for high permeability compounds, whereas direct vesicular transport can not.
- ATPase assay, direct and indirect vesicular transport assays detect interactions of compounds with low permeability while high-throughput whole cell based assays do not.
- All in all, by choosing the adequate membrane-based assay transporter-drug interactions can be determined for the whole permeability space.

This work was also supported by Hungarian Grants GVOP-3.3.2.-2004-04-0001/3.0, GVOP-3.3.2-05/1.-2005-05-0015/3.0, GVOP-3.3.3-05/1.-2005-05-0118/3.0, GVOP-3.1.1.-2004-05-0506/3.0, GVOP-3.1.1.-2004-05-0467/3.0, GVOP-3.1.1.-2004-05-0329/3.0, GVOP-3.1.1.-2004-05-0440/3.0, GVOP-3.1.1.-2004-05-0243/3.0, NKFP-1A/06/04, NKFP-1A/04/04, NKFP-1A/05/04, RET-08/2004, RET-06/2004, Asbóth-DermaVi., Asbóth-XTTPSRT1 and FP6-NoE-005137, FP6-STREP-518246, FP6-STREP-018961, FP6- NoE-018814, EEF-Munka 00034/2000, OTKA T 043141.