

BCRP VESICULAR TRANSPORT ASSAY

Background

ATP Binding Cassette (ABC) transporters are increasingly recognized as important determinants of drug absorption, distribution, metabolism, and excretion (ADME) and also play an important role in drug-drug interactions (DDI). Among the ABC transporters identified to date, P-glycoprotein (P-gp, also known as MDR1, ABCB1) has the broadest substrate specificity and is therefore extensively studied for its DDI potential. However, recent evidence points to a comparable importance for Breast Cancer Resistance Protein (BCRP, also known as ABCG2) in terms of expression level, activity and broad substrate specificity. In many “barrier” tissues (eg. brain endothelial cells), BCRP and P-gp appear to play a synergistic role in the protection of the tissue by extruding drugs and toxins. The recent ITC white paper¹ has recommended screening for BCRP in addition to P-gp substrate/inhibition potential that is already recommended by the European Medical Agency guidelines and will be included as a part of a future US Food and Drug Administration draft guidance document.

Assay Outline

In vitro systems for evaluating the BCRP efflux transporter activity of test compounds include cell lines and membrane vesicles. Membrane vesicles prepared from cell systems over-expressing transporter proteins have the distinct advantage of direct accessibility of test compounds to the transporter¹. Test compounds may be screened for substrate and/or inhibitor potential using a vesicular transport assay.

Substrate Potential:

- One or two concentrations of test compound(s) in triplicate are incubated with BCRP vesicles in the presence of either ATP or AMP
- As a positive control, BCRP vesicles are also incubated with [³H]-methotrexate in the presence of ATP or AMP
- Following incubation, the vesicles are separated by trapping on a glass fibre filter plate.
- Test compounds are quantified using validated LC/MS-MS methods and [³H]-methotrexate is quantified by liquid scintillation counting.
- A two-fold difference in measured uptake velocities of test compound between ATP and AMP would suggest that the test compound is a substrate of BCRP

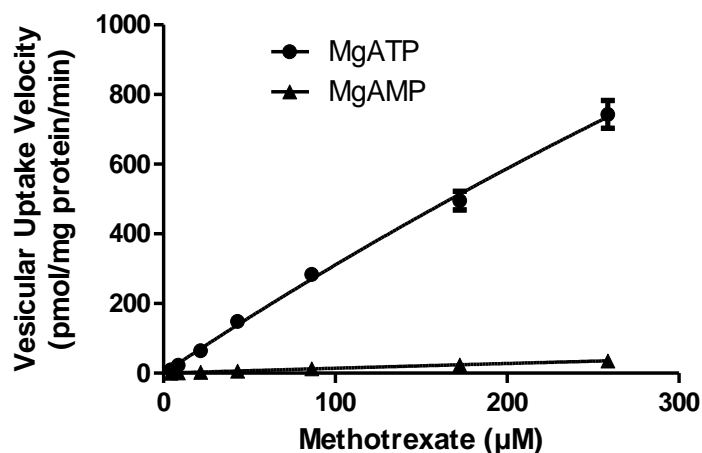
Inhibition Potential:

- Six non-zero concentrations of test compound(s) in triplicate are incubated with BCRP vesicles along with the prototypical substrate, [³H]-methotrexate, in the presence of ATP.
- As a positive control, BCRP vesicles are also incubated with Sulfasalazine.
- Following incubation, the vesicles are separated by trapping on a glass fibre filter plate.
- [³H]-methotrexate is quantified by liquid scintillation counting.
- % inhibition of [³H]-methotrexate transport will be plotted against test compound concentration and IC₅₀ (the concentration which inhibits transport activity by 50%) will be calculated for the test compound.

Vesicle	Prototypical Substrate	Prototypical Inhibitor
BCRP	Methotrexate	Sulfasalazine

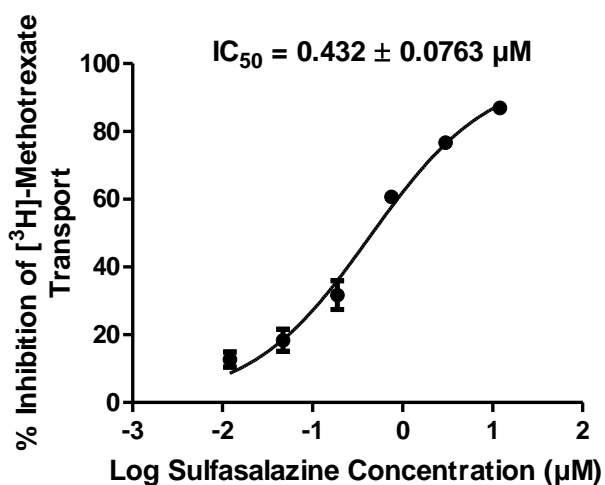
96-well format assay, high quality vesicle preparations, and LC-MS/MS quantification of test compounds ensures a high quality data output with a rapid turnaround time and reduced costs.

Experimental Results



Substrate Potential:

Vesicles (Genomembrane, Japan), were incubated with [³H]-methotrexate at seven non-zero concentrations (0-250 µM) in the presence of either ATP or AMP. The ~20 fold difference in methotrexate uptake velocity between ATP and AMP demonstrates that methotrexate is an excellent substrate of BCRP in the vesicular uptake assay. The data (mean ± S.D) are depicted graphically in the figure.



Inhibitor Potential:

Vesicles (Genomembrane, Japan), were incubated with 6 concentrations of the inhibitor, sulfasalazine (0-12 µM), along with the prototypical substrate, [³H]-methotrexate, in the presence of ATP. Sulfasalazine greatly inhibited BCRP-mediated methotrexate transport with an IC₅₀ of 0.432 ± 0.0763 µM demonstrating the viability of the vesicular uptake model for performing inhibition studies. The data (mean ± S.D) are depicted graphically in the figure.

NoAb's vesicular assay for BCRP transport allows the evaluation of the substrate and inhibitor potential of a compound, which helps to identify and avoid potential adverse drug interactions. NoAb also offers a complementary MDR1 over-expressing MDCK cell line assay for evaluation of P-gp substrate and inhibitor potential. Other Efflux Transporter assays are in development. In addition, NoAb also offers Caco-2 bi-directional permeability assay for assessing both permeability and P-gp substrate/inhibitor potential of compounds. All of these services are examples of NoAb's commitment to providing the best drug discovery tools for our clients, helping to shape drug discovery.

[1] ITC White Paper (2010): Membrane Transporters in Drug Development. Nature Rev Drug Disc 19(3):215-36.

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