A fast and simple method for measuring P-glycoprotein (Pgp) inhibition

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The Fluorosome®-trans-pgp fluorescence intensity assay specifically measures the inhibition of P-glycoprotein multidrug transporter

BMG LABTECH’s microplate reader with onboard injectors used to set up fluorescence kinetic measurements

Materials & Methods

The Pgp is a 170 kDa intrinsic membrane protein that effluxes a wide range of drugs from the cell. Pgp is also an outwardly directed flippase for fluorescent phospholipid and glycosphingolipid derivatives, which suggests that it may also translocate drug molecules from the inner to the outer membrane leaflet. The membrane lipid bilayer plays an important role in Pgp function and may regulate both binding and transport of drugs.

Isolation and Purification of Pgp

Purified Pgp (>90%) is isolated from Pgp-over-expressing CH®B30 cells by detergent (CHAPS) extraction followed by affinity chromatography on Con A-Sepharose. Micellar Pgp is reconstituted into lipid bilayers by mixing in solution with CHAPS-solubilized micellar phospholipid, and the detergent is removed by gel exclusion chromatography. The resulting reconstituted Pgp is aliquoted and stored frozen at -70°C.

Manufacture of Fluorosome-trans-pgp

Frozen reconstituted Pgp is thawed and mixed in buffer with fluorophore (BSA-fluorescein) then converted into Fluorosome-trans-pgp by extrusion. The resulting unilamellar vesicles are separated from unencapsulated fluorophore by gel exclusion chromatography. The fluorosome-trans-pgp is then subjected to Quality Control Criteria; size, fluorescence, passive permeability, and ATP-stimulated transport of a control Pgp substrate.

The Fluorosome-trans-pgp assay procedure.

1. 98 μl of Fluorosome-trans-pgp in buffer containing 3 μM of the substrate S-HR was added to each well of a 96-well half-well plate.
2. A 2 μl aliquot of a DMSO solution of test compound was added. Each aliquot contained test compound at one of 7 concentrations to give a predetermined concentration range.
3. The plate was placed in the microplate reader, and the fluorescence was monitored at 485/520 for 60 seconds as follows:
   a. a fluorescence baseline was established during the first 18 seconds;
   b. at 18 seconds, 5 μl of a solution of ATP in buffer was injected into each well (final concentration 2 mM), and the fluorescence was monitored for 42 seconds;
   c. The slope from 30 to 60 seconds was immediately calculated by BMG LABTECH’s MARS data analysis software.

Introduction

P-glycoprotein (Pgp; ABCB1), a member of the ATP-binding cassette (ABC) superfamily, exports structurally diverse hydrophobic compounds from the cell driven by ATP hydrolysis. Pgp expression has been linked to the efflux of chemotherapeutic drugs in human cancers leading to multidrug resistance. Pgp activity can also result in low oral absorption and poor brain penetration. Interaction of drugs with the Pgp may also cause an increase in toxicity of co-administered compounds.

Interaction of drugs with active transporters such as Pgp is of increasing interest to the pharmaceutical industry based, in large part, on new draft FDA guidelines requiring knowledge of whether a drug candidate is a substrate and/or inhibitor of Pgp.

The Fluorosome Company’s Fluorosome®-trans-ppg assay, together with BMG LABTECH’s microplate readers, provide a rapid, sensitive and specific assay, together with BMG LABTECH’s microplate reader with onboard injectors used to set up fluorescence kinetic measurements.

Assay Principle

Fig. 1: Principle of the Fluorosome®-trans-pgp Assay.
A typical picture of fluorescence signals measured over time is given in Figure 2.

**Results & Discussion**

The slope for each well (Fig. 2) was plotted against concentration of test compound, and IC$_{50}$ values were calculated by using a robust fit for a single order decay (Fig. 3 and 4).

**Conclusion**

We report a novel assay specific for measuring inhibition of the P-glycoprotein multidrug transporter (Pgp; ABCB1) and an ideal instrument for its use.

- **Simple assay procedure:** No sterile conditions, non-compound specific, no radiolabel or LC-mass spectroscopy
- **Specificity:** Fluorosome-trans-pgp contains only the P-glycoprotein transporter
- **Low Sample Requirement:** A 100 μl volume of Fluorosome-trans-pgp Reagent per assay requires 1 nanomole drug
- **Speed:** 1 minute per inhibition assay - e.g. an 8 point IC$_{50}$ determination + 2 references takes only 10 minutes
- **Popular format:** 96 well half-well or 384 well microplates - one well per inhibition assay

Correlation of Fluorosome-trans-pgp IC$_{50}$ Data and Cell Monolayer IC$_{50}$ Data

IC$_{50}$ values are given as μM concentrations. Bidirectional transport inhibition studies were conducted on the same set of compounds as were measured with the Fluorosome-trans-pgp assay, examples of which are shown in Fig. 5. Bidirectional transport inhibition studies employed LLC-MDR1 cell monolayers and [3H] digoxin [0.1 μM] as the substrate.

Results using the Fluorosome-trans-pgp assay are in excellent agreement with those obtained by cell culture methodology.