Application Forum

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

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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 leading to multidrug resistance, low oral absorption, and poor brain penetration. Interaction of drugs with Pgp is of interest based 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*-Pgp assay, together with BMG LABTECH's FLUOstar Omega, provides a rapid, sensitive, and specific Pgp liposome assay (Figure 1) to measure the ability of a compound to compete with a Pgp substrate for transport.

The Fluorosome-*trans*-Pgp assay

Ninety-eight microliters of Fluorosome-*trans*-Pgp in buffer containing 3 μ M substrate S-HR was added to each well of a 96-well half-well plate. A two-microliter aliquot of a DMSO solution of test compound was added. Each aliquot contained test compound at one of seven concentrations to give, after dilution into the well, the desired concentration of compound. Blank and reference wells (no compound) had 2 μ L DMSO added to them. The plate was placed in the FLUOstar Omega multidetection plate reader, and fluorescence intensity was monitored at ex 485 nm/em 520 nm for 60 seconds: a baseline was established during the first 18 s; then 5 μ L of ATP was injected into each well and the fluorescence was monitored for 42 s. The slope from 30 to 60 s was immediately calculated by BMG LABTECH's MARS data analysis software.

Results & discussion

The slope was plotted against the concentration of test compound, and IC_{50} values were calculated by a fit for a single-order decay (Figure 2). We report a novel assay specific for measuring inhibition of the P-glycoprotein multidrug transporter (Pgp; ABCB1) and an ideal instrument for its use. 1) Simple assay procedure: No sterile conditions, non-compound

Figure 1. Principle of the Fluorosome®-*trans*-pgp Assay.

Figure 2. Single-order decay fit with nicardipine as test compound.

specific, no radiolabel or LC-mass spectroscopy 2) Specificity: Fluorosome®-*trans*-Pgp contains only the Pgp transporter 3) Low sample requirement 4) Speed: 1 min per inhibition assay; an 8-point IC_{50} determination plus 2 references takes 10 min.

References

The Fluorosome Company: www.fluorosome.com
The Microplate Reader Company: www.bmglabtech.com

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