

Characterization of Substrates and Inhibitors of P-glycoprotein

Purpose

P-glycoprotein (Pgp), also known as MDR1, is a member of the ABC transporter superfamily and functions as an ATP dependent drug efflux pump. Pgp is able to transport xenobiotics of various structures out of cells. Since intestinal expression of Pgp may affect the oral absorption of drugs, Pgp plays an important role in multi-drug resistance.

Compounds that interact with Pgp can be characterized as substrates or inhibitors of Pgp. Pgp substrates typically stimulate the ATP consumption, whereas inhibitors reduce Pgp-ATPase activity that was actively stimulated by a known substrate, such as verapamil.

Assay protocol

A recombinant human Pgp membrane fraction was incubated with a non-limiting concentration of ATP. The reaction was stopped by the addition of an ATP detection reagent. Compounds were tested in presence and absence of Na_3VO_4 , a highly specific inhibitor of Pgp to correct the resulting ATP consumption for unspecific background activity. Inhibitors of Pgp-ATPase were identified in the presence of 200 μM verapamil.

- The specific Pgp substrate verapamil displayed an EC_{50} value of 10.8 μM .
- Mean ATPase activity stimulated by 200 μM verapamil ranged from 93.2 ± 12.6 pmol $\text{ATP}_{\text{consumed}}/\mu\text{g}$ Pgp/minute.
- Cyclosporine A was used as a reference compound for an inhibitor of 200 μM verapamil-induced ATP consumption and displayed an IC_{50} value of 7.3 μM (Figure 2).

Results

A setup of drugs was tested for their ability to stimulate ATP consumption (Figure 1).

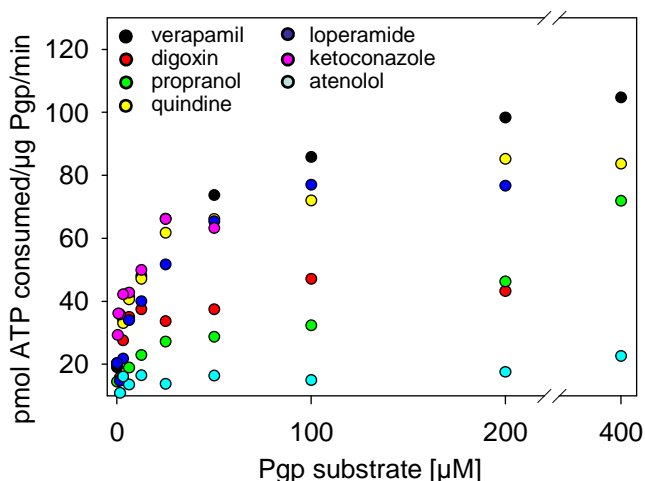


Figure 1: Stimulation of Pgp-ATPase activity by different test compounds

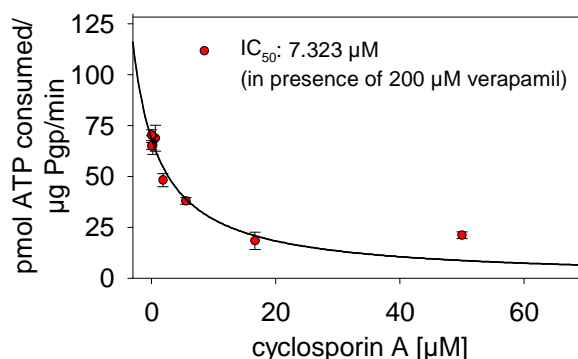


Figure 2: Inhibition of Pgp-ATPase activity by Cyclosporin A

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