

## INHIBITION OF P-GP/ATPASE ACTIVITY OF RAT SMALL INTESTINE MEMBRANE VESICLES BY THE NOVEL, DIHYDROPYRIDINE MDR REVERTER DP7

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Permeability glycoprotein (P-gp) is an ATP-dependent active transporter that confers multidrug resistance (MDR) to cancer cells against many unrelated drugs. The energetic coupling of ATP hydrolysis with drugs transport allows to investigate P-gp function by assaying its ATPase activity.

We have developed a test system for P-gp/ATPase activity with use of rat intestine membrane vesicles and analysed the effects of novel 3,5-dibenzoyl-1,4-dihydropyridines proven to inhibit P-gp of mouse lymphoma MDR-1 transfected cell line (1). Vesicles were prepared by following the method described by Kessler (2). ATPase activity was measured spectrophotometrically at 37°C using an ATP-regenerating system (pyruvate kinase and phosphoenolpyruvate) coupled to lactate dehydrogenase and NADH, by monitoring NADH absorbance decay with time at 340 nm (3). Membrane ATPases not related to drug transport, i.e. mitochondrial ATPases, Na<sup>+</sup>, K<sup>+</sup> ATPases and Ca-ATPases, were inhibited by the addition of 10 mM sodium azide, 0.5 mM ouabain and 1 mM EGTA, respectively. Residual ATPase activity was fully ascribable to P-gp owing to its complete inhibition by low μM concentrations of Na-orthovanadate. This basal ATPase activity could be stimulated by drugs known as transport substrates, such as verapamil.

NADH absorbance decay with time curve followed a polynomial equation. ATPase activity was calculated by the curve tangent at 1300 sec.

Verapamil gave a typical bell shaped concentration-activation curve with 30 μM maximum effective concentration. DP7 was tested at various concentrations (0.025-10 μM) either on basal or 30 μM verapamil-stimulated ATPase activity. Neither pyruvate kinase nor lactate dehydrogenase were inhibited by DP7 which, however, inhibited concentration-dependently both P-gp ATPase activities, with IC<sub>50</sub> values of about 1 μM.

Data showed that the inhibition mechanism of DP7 was competitive in nature, with a K<sub>i</sub> of 5.3 μM.

(Supported by a grant from Ministero degli Affari Esteri, Roma)

### References:

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