FLUORESCENT COMPOUNDS AS FUNCTIONAL INDICATORS OF THE EFFLUX TRANSPORTERS P-GLYCOPROTEIN AND MRP1 IN BEWO CELLS

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Rhodamine, fluorescein and the acetoxy methyl (AM) derivative (calceinAM) of the fluorescent indicator calcein were used to evaluate the functional role of the efflux transport system in BeWo cells. Uptake studies with P-glycoprotein or MRP1 inhibitors show significant enhancement of accumulation of calcein. CyclosporinA, verapamil, indomethacin, probenecid, sodium orthovanadate and vinblastine affected the uptake, efflux and transport of calcein. The transport was polarized with greater permeability from the apical to the basolateral direction. The inhibition of MRP1 and Pgp resulted in a decrease of apical to basolateral transport. However, none were able to decrease transport from basolateral to the apical side significantly. The efflux of calcein was decreased by all the compounds tested, indicating an inhibition of MRP1. Since both calcein and calceinAM are extruded by MRP1, whereas only calceinAM is a substrate of P-glycoprotein, the effect of modulators on the transport of calceinAM is indicative of the functional activity of both P-glycoprotein and MRP1. Fluorescein uptake was only affected by MRP1 inhibitors. P-glycoprotein inhibitors verapamil and C219 had no effect on the uptake of fluorescein, suggesting that it is an effective indicator of MRP1 functionality. However the transport of fluorescein did not show polarization. This may be due to fluorescein showing permeability (~ $1x10^{-5}$ cms^{-1}) ten times greater than that of calcein. Studies with rhodamine (substrate of P-glycoprotein) did not reveal the expected functional activity in the presence of the same modulators. Therefore, rhodamine could not be used as a functional indicator of P-glycoprotein activity in BeWo cells due to the presence of other transporters which interfere with rhodamine uptake and transport.