THE INTERACTION BETWEEN ANTI-TUBERCULOSIS DRUGS AND P-GLYCOPROTEIN

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Resistance to anti-tuberculosis drugs is increasing and several mechanisms may account for this (Bambeke et al 2000). Active drug transporters such as P-glycoprotein (P-gp) have been shown to influence the cellular accumulation of antiretrovirals and anticancer drugs (Jones et al 2001, Germann 1996). Since mycobacterium tuberculosis is also an intracellular pathogen, we sought to investigate if P-gp could influence the cellular accumulation of the anti-tuberculosis drugs (rifampicin, isoniazid, pyrazinamide and ethambutol) in vitro.

Flow cytometry and immunoblotting techniques were used to determine the expression of P-gp on CEM (parental) and CEMVBL100 (P-gp overexpressing) T-lymphoblastoid cell lines. The cytotoxicity of the drugs in each cell line was measured by the MTT formazan formation assay. Cells (2 x 10^5 cells/ml) were incubated (37°C, 5% CO2) in the absence or presence of the drugs (rifampicin, 25 – 450 µM; isoniazid, 1 – 100 mM; pyrazinamide, 1 – 40 mM and ethambutol 0.5 – 50 mM) in RPMI supplemented with 10 % FCS. Cytotoxicity was expressed as the concentration of drug giving 50% cell death (EC50). For drugs showing differential toxicity between the cell lines, the study was repeated in the presence and absence of 100 nM Tariquidar (XR9576, a potent P-gp inhibitor). The cellular accumulation ratio of [14C]-isoniazid (10 µM) and [3H]-rifampicin (10 µM) was also determined in each cell line. All experiments were repeated 4 times and analysed using the Mann-Whitney statistical test.

Flow cytometry and western blot confirmed the overexpression of P-gp in CEMVBL100 compared to the parental CEM cell line. There were significant differences in the EC50 values between CEM and CEMVBL100 for rifampicin (107 ± 3 µM, 155 ± 3 µM, p < 0.05) and ethambutol (1.95 ± 0.77 mM, 3.37 ± 0.53 mM respectively, p < 0.05). 100 nM tariquidar significantly decreased the EC50 of rifampicin in CEMVBL100 (149 ± 12 µM to 102 ± 5 µM, p < 0.05) and for ethambutol (15.05 ± 1.41 mM to 9.54 ± 1.04 mM, p < 0.05). No differential cytotoxicity was observed for isoniazid and pyrazinamide in the cell lines. There was a significant decrease in the accumulation ratio of [3H]-rifampicin in the CEMVBL100 (2.19 ± 0.07) compared to the CEM (5.55 ± 0.59, p < 0.05). However there was no significant difference in the accumulation ratio of [14C]-isoniazid in the cell lines.

These findings confirm that rifampicin is a substrate for P-gp. Furthermore there is evidence that ethambutol may also be a substrate for the transporter. However the lack of cytotoxicity of isoniazid and pyrazinamide between the two cell lines suggests that they may not be P-gp substrates.

