ABSTRACT

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Title of diploma thesis: Effect of colchicine on P-glycoprotein expression and activity in caco-2 cells

The caco-2 cell line is one of the most widely used human cell culture models. These cells derived from human colorectal adenocarcinoma and have been accepted as a reliable *in vitro* model for intestinal drug excretion mediated by P-glycoprotein studies. P-glycoprotein is an ATP-dependent efflux pump encoded by the MDR1 gene in humans, which is highly expressed in several cancer cells conferring a multidrug resistance phenotype.

P-glycoprotein is inducible by many drugs including dexamethasone, rifampicin, the herbal antidepressant St. John's wort (hyperforin and hypericin) and chemotherapeutic agents, namely doxorubicin, daunorubicin and vinblastine. The sensibility of P-glycoprotein from caco-2 cells to different inducing compounds is yet to be clearly established. Colchicine is a toxic natural product and secondary metabolite, originally extracted from plants of the genus Colchicum – Colchicum autumnale. This compound is used as an anticancer drug and was already reported as a P-glycoprotein inducer. Thus, the main objective of the present work was to evaluate the potential changes in P-glycoprotein expression and activity, when caco-2 cells are exposed to colchicine.

Caco-2 cells were exposed to a range of colchicine concentrations (0,1 μM – 100 μM), for a maximum period of 96 hours. Colchicine cytotoxicity was evaluated at different time points by the MTT assay. P-glycoprotein expression and transport activity were evaluated by flow cytometry, using a fluorescein isothiocyanate conjugated antibody (CD 243) and the P-glycoprotein fluorescent subtract rhodamine 123, respectively. The obtained results suggest that colchicine is cytotoxic for all the tested concentrations when caco-2 cells are exposed for more than 24 hours. For that reason, caco-2 P-glycoprotein expression and transport activity were evaluated only after 24 hours incubation with colchicine. Exposure of these cells to colchicine for 24 hours resulted in a small but significant increase in P-glycoprotein expression levels, although no significant changes were observed in P-glycoprotein transport activity. The observed results were important to characterize these cells in order to study the induction mechanism to protect cells from toxic compounds, including therapeutic drugs.