All chemicals were obtained from Sigma. After 3 days of treatment, the medium was removed and fresh medium was added to LS180 cells purchased from ATCC (Manassas, VA, USA). Briefly, the cells were seeded at a density of 1.5 × 10^5 cells/ml in 48-well tissue culture plates with 500 μl medium for Pgp and CYP 3A4 induction, assays of Rh123 uptake and Pgp CYPA4 activity. Cells were incubated in a humidified incubator (37°C, 5% CO2) and treated with rifampicin, ritonavir or vinblastine for 3 days with daily changes of medium. The vehicle control cells were treated with 0.1% DMSO for 3 days, also with daily medium changes.

**Evaluation of Pgp and CYP3A4 mRNA expression (qPCR):** RNA was isolated from cells using RNeasy Mini Kit (Qiagen, Cat# 74104) and treated with DNase (RNase-free DNase set, Qiagen, Cat# 79654), followed by the protocol supplied by the manufacturer. The cDNA synthesis was performed using Postscript First Strand cDNA Synthesis Kit (NEB, Cat# E6050L). cPCR samples were analyzed using 10 ng of cDNA sample per reaction and Pgp- and CYP3A4-specific primers and probes with the Light Cycler 480 Instrument and the Light Cycler 480 Software (version 1.5). The sequences of primers and probes of qPCR were designed using Universal Probe Library (UPL, Roche, Basel, Switzerland). Relative gene expressions of Pgp and CYP3A4 were normalized by the housekeeping gene -actin.

**Evaluation of Pgp and CYP3A4 protein expression (Western blot):** Cell protein was isolated with RIPA Lysis Buffer (Santa Cruz BioTech, Cat# sc-24948) following the manufacturer’s protocol. The concentration of protein was measured using the Pierce BCA protein assay kit (Thermo Scientific, Cat# 23225). 45 μg of protein was separated on a 4-20% SD-polyacrylamide gel (Pierce) and transferred to PVDF membrane (Millipore, Cat# IPVH08132). Pgp and CYP3A4 protein was detected using the monoclonal antibody for Pgp (C219, Abcam, Cat # ab3364) and polyclonal antibody for CYP3A4 (Millipore, Cat# AB1254). Rh123 uptake assay: After 3 days of treatment, the medium was removed and fresh medium without Pgp inducers added. Cells were incubated for 1 hour prior to initiating the Rh123 uptake assay. The cellular fluorescence of Rh123, normalized to the protein concentration, was used as an inverse measure of the relative cellular Pgp activity i.e., lower cellular Rh123 fluorescence reflects higher Pgp activity.

**CYP3A4-mediated biotransformation activity in vitro:** Formation of 6 β-Hydroxytestosterone from testosterone (10 μM, 1 mM Na pyruvate, 1% (v/v) MEM non-essential amino acids, 100 μl/ml penicillin and 100 μg/ml streptomycin. Four cell batches were cultured for this study.

**Pgp induction:** LS180 cells were seeded at 1.5 × 10^5/ml in 48-well tissue culture plates with 500 μl medium for Pgp and CYP 3A4 induction, assays of Rh123 uptake and Pgp CYPA4 activity. Induction of Pgp and CYP3A4 functional activity in LS180 cells: Rh123 uptake was measured after 3 days of treatment with the three Pgp inducers or 0.1 % DMSO as a control. Reduced Rh123 uptake correlates with higher Pgp activity. As shown in Table 2, Rh123 uptake was 22% and 38% lower in cells treated with rifampicin and ritonavir respectively, compared with vehicle-treated cells. CYP3A4 enzyme activity in cells treated by ritonavir and vinblastine was not significantly increased. Only rifampicin treatment produced higher CYP3A4 activity (P<0.01, Table 3).

**RESULTS**

Pgp and CYP3A4 mRNA and protein induction: The qPCR and WB showed that expression of Pgp in untreated LS180 cells was low (Table 1, Figure 1). These results are similar to previously reported results. Expression of Pgp and CYP3A4 mRNA in LS180 cells were increased 4- to 10-fold and 2- to 31-fold, respectively, by rifampicin, ritonavir and vinblastine. Increased Pgp protein expression was also induced by all three agents. The order of Pgp induction based on the level of expression of the corresponding mRNA and protein was: rifampicin > ritonavir > vinblastine. However, there is no pronounced effect of the three Pgp inducers on expression of CYP3A4 protein in these cells. Based on the results of qPCR and WB, induction of Pgp protein did not parallel the dose at low concentrations of vinblastine. However, at higher concentrations of M, Figure 2 and 3). However, significantly higher induction was seen at a higher concentration (55 μM).

**CONCLUSION**

1. In the present study, we demonstrated the applicability of the LS180 cell line as a suitable in vitro model for investigating Pgp induction. Even though LS180 cell monolayers do not have sufficient integrity to characterize transcellular drug transport, they may be useful for investigating the intestinal regulatory mechanism responsible for Pgp induction by drugs.

2. Rh123 uptake into LS-180 cells can be used to evaluate the functional activity of Pgp. Our results indicate that LS180 cells respond to rifampicin or ritonavir treatment by decreasing Rh123 uptake, which is indicative of an increase in Pgp activity. This change in functional activity of Pgp occurred in parallel with increased expression of Pgp mRNA and protein.

3. Our findings suggest that induction of Pgp mRNA parallels induction of CYP3A4 mRNA in LS180 cells and may share a common mechanism, most likely mediated by PXR.

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