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Lack of P-glycoprotein induction by rifampicin and phenobarbital in human lymphocytes

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ABSTRACT

The efficacy of drugs acting within lymphocytes depends on their intracellular concentrations, which could be modulated by membrane efflux transporters including P-glycoprotein (P-gp), encoded by the *MDR1* gene. In particular, P-gp induction may compromise the efficacy of its substrates. Rifampicin and phenobarbital have been shown to induce P-gp in hepatic and intestinal cells through the activation of the nuclear receptors PXR and CAR. However, controversial data exist in human lymphocytes. We investigated the effect of these drugs on P-gp activity and expression in lymphocytes *in vitro* and *ex vivo*. CCRF-CEM cells and peripheral blood mononuclear cells (PBMCs) from healthy volunteers were incubated in the presence of rifampicin, phenobarbital, or without any drug. P-gp activity was measured by flow cytometry using $DiOC_6$ efflux. *MDR1*, *PXR* and *CAR* mRNA expression were measured by quantitative RT-PCR. Neither P-gp activity nor *MDR1* mRNA expression were modified by rifampicin or phenobarbital both in CCRF-CEM cells and PBMCs. Moreover, P-gp protein expression at the membrane was neither detectable nor induced. The very weak *PXR* and *CAR* mRNA expression levels in these cells could partly explain these results. Therefore, P-gp induction by rifampicin and phenobarbital may play a negligible role in drug interactions occurring within lymphocytes.

1. Introduction

P-glycoprotein (P-gp) is an ATP-dependent transmembrane efflux pump encoded by the *MDR1* (*ABCB1*) gene in humans. It actively transports various structurally unrelated drugs out of cells, including several groups of drugs whose targets are located within lymphocytes like antiretroviral agents, immunosuppressive drugs and anticancer drugs against leukaemia (Kim, 2002; Fromm, 2003; Marzolini et al., 2004). P-gp activity may thus affect the intracellular concentrations of these drugs. Many factors have been shown to influence this activity, including genetic polymorphisms, stress stimuli, sex hormones, age and the lymphocyte subset (Chaudhary et al., 1992; Giraud et al., 2009a; Hoffmeyer et al., 2000; Kim and Benet, 2004; Scotto, 2003). Furthermore, several drugs known as P-gp inducers may up-regulate P-gp expression and activity, thereby reducing the intracellular concentration and pharmacological efficacy of co-administered P-gp substrates (Ford et al., 2004; Jones et al., 2001). In particular, rifampicin, an antibiotic, and phenobarbital, an antiepileptic drug, have been shown to induce P-gp expression in the intestine and liver (Collett et al., 2004; Jigorel et al., 2006; van de Kerkhof et al., 2008; Greiner et al., 1999; Stormer et al., 2002). However, in peripheral blood lymphocytes, conflicting results exist regarding P-gp induction by rifampicin or phenobarbital (Owen et al., 2006; Becquemont et al., 2000). Moreover, the induction of *MDR1* gene expression by these drugs in hepatocytes and enterocytes has been shown to involve the activation of two nuclear receptors, the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR) (Geick et al., 2001; Owen et al., 2006; van de Kerkhof et al., 2008; Hariparsad et al., 2004; Wang and LeCluyse, 2003; Wei et al., 2000). The role of these receptors in

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MDR1 gene expression has been yet poorly characterised in human lymphocytes. In the present study, we investigated P-gp induction in human lymphocytes by rifampicin and phenobarbital *in vitro* in CCRF-CEM cells and *ex vivo* in isolated peripheral blood mononuclear cells (PBMCs). An intestinal LS174T cell line was used as a positive control for P-gp induction by these drugs. We also examined whether PXR and/or CAR may be involved in P-gp induction in lymphocytes by determining their transcript levels in these cells.

2. Materials and methods

2.1. Materials

The CCRF-CEM cell line (human T-lymphoblastoid cells) was purchased from ATCC (Molsheim, France). The HL60-DNR cell line (human myeloid leukaemia cells), overexpressing P-gp after selection by daunorubicin, and the LS174T cell line (human colon cancer cell) were kindly provided by the INSERM E0355 laboratory (Université Paris 6, Paris, France). DMEM-complete medium, RPMI 1640 medium, HEPES, sodium pyruvate and sodium bicarbonate were supplied by Invitrogen (Cergy Pontoise, France). Fetal calf serum was obtained from Bio-West (Nuaille, France) and Ficoll-Hypaque from Eurobio (Les Ulis, France). Phenobarbital was provided by Sanofi Aventis France (Paris, France). Rifampicin, 3,3′-diethyloxacarbocyanine iodide ($DiOC_6$) and cyclosporin A were purchased from Sigma–Aldrich (Saint Quentin Fallavier, France). The antibodies used were: allophycocyanine-labelled CD3 antibody (DakoCytomation, Trappes, France), phycoerythrine-cyanine 5-labelled CD19 antibody (Beckman Coulter, Villepinte, France) and phycoerythrine-labelled CD56 (Becton Dickinson, Le Pont de Claix, France), monoclonal mouse anti P-glycoprotein C219 antibody (Alexis Biochemicals, San Diego, CA), monoclonal mouse anti-β-actin antibody (Sigma–Aldrich), and rabbit peroxidase-conjugated anti-mouse secondary antibody (Abcam, Cambridge, UK). Other chemicals and reagents were purchased from Sigma–Aldrich and Invitrogen.

2.2. Subjects and isolation of PBMCs

Peripheral blood samples were obtained from eight healthy volunteers who had to receive no treatment known to influence P-gp activity. All subjects had given their written consent before inclusion. Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation on Ficoll-Hypaque gradients and incubated rapidly as described below.

2.3. Cell incubation conditions

The medium used for the CCRF-CEM and LS174T cell lines consisted of DMEM-complete medium supplemented with 10% fetal calf serum, 10 mM HEPES, 1 mM sodium pyruvate and 1.5 g/l sodium bicarbonate. PBMCs were grown in RPMI 1640 medium supplemented with 10% fetal calf serum. Aliquots of 3×10^6 cells were incubated in the presence of rifampicin (20 μM), phenobarbital (3 mM) or without any drug (control cells) and maintained at 37 °C in a 5% CO_2 humidified atmosphere. The presence of 0.1% methanol, the drug solvent, had no influence on P-gp activity. P-gp activity was quantified after incubations of 24 h, 48 h and 72 h. For mRNA expression studies, cells were incubated for 24 h and stored at −80 °C until RNA extraction.

2.4. Quantification of P-gp activity by a functional assay using $DiOC_6$

CCRF-CEM and LS174T cells were incubated in RPMI medium in the presence of $DiOC_6$ (final concentration: 25 nM) with or without cyclosporin A, a P-gp inhibitor (final concentration: 2 μM) for 30 min at 37 °C. For the *ex vivo* study, 30 μl of the suspension of incubated mononuclear cells or 30 μl of total blood were incubated for 15 min at room temperature in the presence of 30 μl of a mixture of CD3/19/56 antibodies diluted 1:7 (Giraud et al., 2009a). Then, cells were resuspended in RPMI medium and incubated in similar conditions as the cell lines. For treated cells, we checked that similar results were obtained with or without a washing out step of the drug before the addition of $DiOC_6$. Analyses were then performed with a flow cytometer (FACSCalibur, Becton Dickinson, San Jose, CA). A minimum of 3000 events were acquired in a gate selecting cells accumulating $DiOC_6$. The gated events were analysed with Cellquest software (Becton Dickinson, Franklin Lakes, NJ). Compensation was set to optimize the detection of each marker. P-gp activity was determined using physical properties for CCRF-CEM and LS174T cells and selected on the basis of antibody binding in addition to physical properties for each lymphocyte subset of PBMCs. P-gp activity was calculated as the ratio of the mean fluorescence intensity of $DiOC_6$ in the presence of cyclosporin A to that in the absence of the inhibitor.

2.5. Quantification of MDR1, PXR and CAR gene expression by quantitative RT-PCR

Total RNA was extracted from each sample using the RNeasy Mini kit according to the manufacturer's instructions (Qiagen SA, Courtaboeuf, France). Genomic DNA was eliminated by DNAse I (RNase-Free Dnase Set; Qiagen SA) treatment. The concentrations and purity of RNA samples were assessed spectrophotometrically at 260 nm using the Nanodrop® ND-1000 instrument (NanoDrop Technologies, Wilmington, DE). RNA integrity was verified by agarose gel electrophoresis. For each sample, reverse transcription (RT) was performed using 500 ng of total RNA in a final reaction mixture of 20 μl containing 500 μM of each dNTP, 10 mM dithiothreitol, 1.5 μM random hexanucleotide primers (Amersham Biosciences, Orsay, France), 20 U RNasin ribonuclease inhibitor (Promega, Charbonnières-les-Bains, France) and 100 U superscript II RNase reverse transcriptase (Invitrogen). All samples were incubated at 25 °C for 10 min, then 42 °C for 30 min and 99 °C for 5 min on a thermal cycler (PTC-100 programmable thermal controller; MJ Research Inc., Waltham, MA). cDNAs were stored at −80 °C until analyses.

The expression of *MDR1*, *PXR* and *CAR* genes, and that of the housekeeping gene encoding TATA box-binding protein (*TBP*) were analysed by quantitative polymerase chain reaction (PCR) with a Light-Cycler® instrument (Roche Diagnostics, Meylan, France) using SYBR Green fluorescence detection. The final reaction mixture consisted of 5 μl cDNA diluted 1:20, 1 μl LC-FastStart DNA Master SYBR Green kit (Roche Diagnostics), 0.5 μl of each primer (10 μM), 1.2 μl of 10 mM $MgCl_2$ and 1.8 μl nuclease-free water. Specific primers for each gene were designed using OLIGO 6.42 software (Med Probe, Oslo, Norway). For each primer pair, we performed control assays in the absence of template, for which signals were negligible. Gel electrophoresis was used to check the size of the specific amplicons of interest and the absence of other PCR products. The specificity of each reaction was also assessed by melting curve analysis. Primer sequences are shown in Table 1.

Gene expression was evaluated using the crossing-threshold value (Ct) for each sample. Expression of the target genes was normalized to that of *TBP* gene since the expression of the latter gene was not influenced by rifampicin or phenobarbital. The comparative variable ΔΔCt was calculated from the following formula: $\Delta\Delta Ct = (Ct\ target\ gene - Ct\ TBP)\ sample - (Ct\ target\ gene - Ct\ TBP)\ calibrator$. The $2^{-\Delta\Delta Ct}$ values represented the fold change in mRNA amounts in samples relative to the calibrator, arbitrarily set at 1 (Yousif et al., 2007; Giraud et al., 2009a, 2010; Dauchy et al., 2009).

Table 1
Sequences of primers used for quantitative RT-PCR.

Gene	Forward primer (5′–3′)	Reverse primers (3′–5′)	Length (bp)	GenBank accession[a]
TBP	TGCACAGGAGCCAAGAGTGAA	CACATCACAGCTCCCCACCA	132	NM_003194
MDR1	CACCCGACTTACAGATGATG	GTTGCCATTGACTGAAAGAA	81	NM_000927
PXR	CGCTTCCTGAGTCTTTTCA	CACCTGCCGATGAGTACA	115	NM_003889
CAR	GGGGTTCCAGGTAGAGTTT	GTCGGTCAGGAGAGAAGAG	122	NM_005122

[a] Gene accession number at http://www.ncbi.nlm.nih.gov.

2.6. Immunodetection of P-gp by Western blotting

CCRF-CEM cells and PBMCs were incubated in the presence of rifampicin (20 μM), phenobarbital (3 mM) or without any treatment for 48 h and lysed by incubation at 4 °C for 30 min in a buffer containing 50 mM Tris–HCl pH 7.4, 150 mM NaCl, 0.5% sodium deoxycholate, 0.5% Triton X-100 and complete™ protease inhibitor complex (Roche Diagnostics). Homogenates were centrifuged at 4 °C for 10 min at 10,000 × *g*, and supernatants were stored. The protein content was determined in each sample using the Micro BCA Protein Assay Kit (Thermo Fisher Scientific, Illkirch, France) and a bovine serum albumin calibration curve. Samples were normalized for equal amounts of protein (100 μg for CCRF-CEM cells and PBMCs – 20 μg for HL60-DNR cells). Proteins were separated using 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels and electrotransferred onto Hybond nitrocellulose membranes (Bio-Rad, Marnes la coquette, France). Non-specific binding sites were blocked overnight at 4 °C with Tris–base buffer containing 0.1% Tween 20 (TBST) and 5% non-fat milk. Membranes were probed with the C-219 antibody (diluted 1:200) for 2 h at room temperature, washed several times in TBST, incubated with rabbit peroxidase-conjugated anti-mouse secondary antibodies (1:10,000) for 30 min at room temperature and exposed to ECL™ system (GE Healthcare Europe, Orsay, France). Signals were revealed with the Bio-Rad ChemiDoc® XRS imaging device. Blots were then stripped by immersion in 0.1 M acetic acid for 2 h, and reprobed with the monoclonal mouse anti-β actin antibody (1:10,000).

2.7. Statistical analysis

Data are presented as means ± S.D. and were analysed using NCSS® Version 07.1.4 software (NCSS, Kaysville, UT). Differences in P-gp function or in *MDR1* mRNA expression following drug incubation were compared with the respective untreated control cells using Student's unpaired (cell lines) or paired (PBMCs) t-tests. All tests were two-tailed and values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Effect of rifampicin and phenobarbital on P-gp activity and MDR1 gene expression in CCRF-CEM cells

In control CCRF-CEM cells, negligible P-gp activities were observed. Indeed, the ratios of mean fluorescence intensities of $DiOC_6$ with and without cyclosporin A were very similar and close to one after 24 h, 48 h and 72 h incubations (0.98 ± 0.09, 0.99 ± 0.08 and 0.98 ± 0.06) which means that P-gp inhibition did not increase $DiOC_6$ efflux from CCRF-CEM cells (Fig. 1). After incubations with 20 μM rifampicin for 24 h, 48 h and 72 h, no induction of P-gp activity was shown either in CCRF-CEM cells or in any peripheral blood lymphocyte subset compared to control cells (Fig. 1A). Similarly, no induction of P-gp activity was observed after exposition to 3 mM phenobarbital for 24 h and 48 h. A significant but slight increase of P-gp activity by 1.13 ± 0.04 was observed in CCRF-CEM cells after 72 h incubation (Fig. 1B). Several assays were also conducted with a range of other drug concentrations (10, 30, 40 and 50 μM for rifampicin, 1, 2, 4 and 5 mM for phenobarbital). Their results confirmed the lack of P-gp induction (data not shown). Furthermore, except for 5 mM phenobarbital, no alteration of the cell viability was observed by using trypan blue staining.

A weak *MDR1* mRNA expression was detected in control CCRF-CEM cells. This expression was not modified after 24 h incubation either with rifampicin or phenobarbital (Fig. 2). Western blotting experiments did not allow the detection of P-gp expression at the protein level in CCRF-CEM cells and showed no induction of the transporter's expression by rifampicin or phenobarbital (Fig. 3).

Conversely, both P-gp activity and *MDR1* mRNA expression were induced in LS174T cells by rifampicin and phenobarbital.

3.2. Effect of rifampicin and phenobarbital on P-gp activity and MDR1 gene expression ex vivo in the different peripheral blood lymphocyte subsets

No induction of P-gp activity was observed in T-lymphocytes after 24 h, 48 h or 72 h incubation in the presence of 20 μM rifampicin or 3 mM phenobarbital (Fig. 1). Similar results were observed for B lymphocytes and NK cells. Moreover, neither rifampicin nor phenobarbital significantly increased *MDR1* gene expression after 24 h incubation of PBMCs (Fig. 2).

P-gp protein expression was neither detected nor induced by rifampicin or phenobarbital in control cells (Fig. 3).

3.3. PXR and CAR mRNA expression in CCRF-CEM cells and PBMCs

Very weak *PXR* and *CAR* mRNA expression levels were measured in seven samples of CCRF-CEM cells and six samples of PBMCs. Almost all samples exhibited a Ct value over the quantification limit of *PXR* and *CAR* mRNA expression. Most samples had even a Ct value over the detection limit (Table 2).

PXR and *CAR* transcript levels in PBMCs were compared to those measured in human hepatocytes and were shown to be at least 60- and 130-fold less abundant in PBMCs than in the liver.

Table 2
Gene expression of *PXR* and *CAR* in CCRF-CEM cells and PBMCs.

Number of samples	*PXR* gene expression		*CAR* gene expression	
	<LOD	<LOQ	<LOD	<LOQ
CCRF-CEM cells	0/7	7/7	0/7	5/7
PBMCs	4/6	6/6	1/6	6/6

The limits of quantification (LOQ) and detection (LOD) of *PXR* and *CAR* gene expression had been determined at 31 and 33 Ct, respectively.

4. Discussion

Most data reported on P-gp induction by rifampicin have been obtained in hepatic or intestinal cells (Collett et al., 2004; Jigorel et al., 2006; van de Kerkhof et al., 2008; Greiner et al., 1999). In contrast, very few studies have examined P-gp induction by this drug in lymphocytes. Moreover, many conflicting results have been

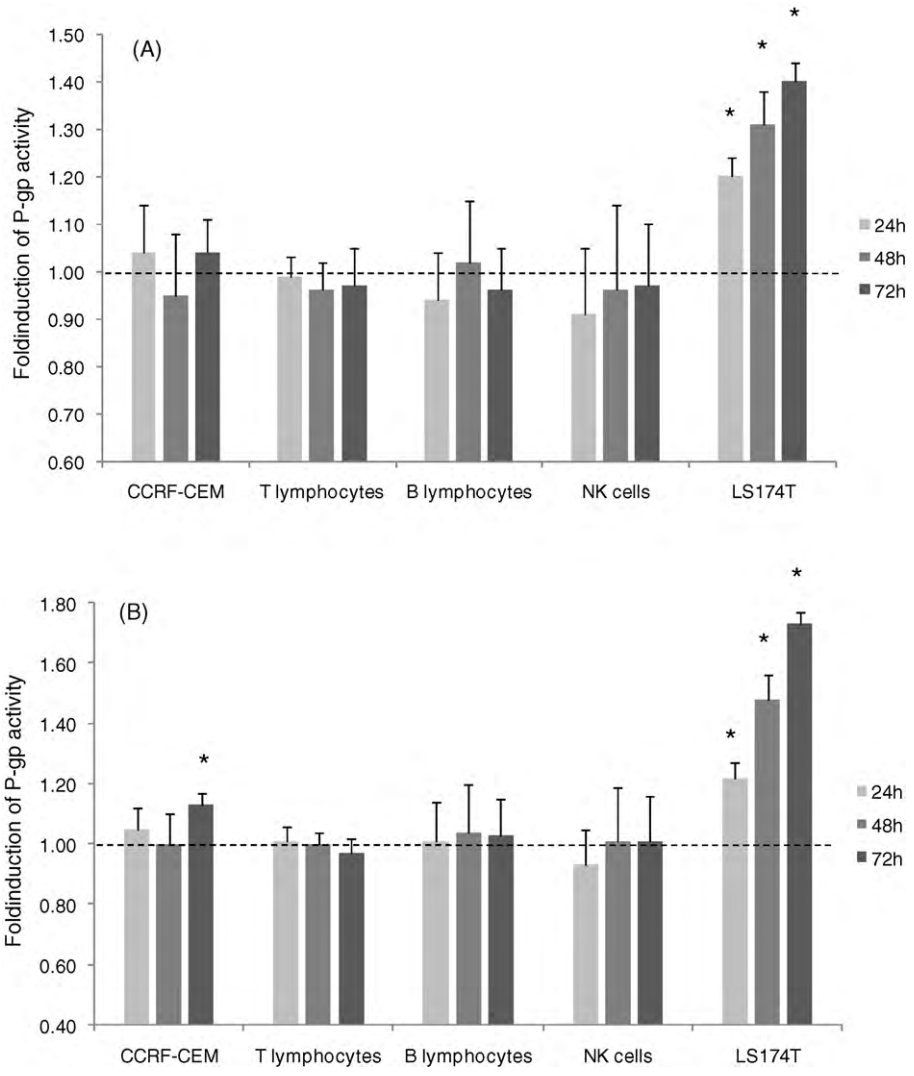

Fig. 1. Effect of rifampicin and phenobarbital on P-gp activity in CCRF-CEM cells and peripheral blood lymphocytes. CCRF-CEM cells ($n = 10$), PBMCs ($n = 8$) and LS174T cells ($n = 10$) were incubated in the presence of 20 μM rifampicin (A), 3 mM phenobarbital (B) or without any drug (control cells) for 24 h, 48 h and 72 h. LS174T cells were used as a positive control for P-gp induction. P-gp function was measured as the ratio of $DiOC_6$ mean intensity fluorescence in the presence or absence of cyclosporin A. Fold inductions are expressed as the ratio of P-glycoprotein function in treated cells compared to control cells. Results are expressed as means ± S.D. $^*P < 10^{-6}$.

reported. In agreement with ours, Becquemont et al. (2000) showed a lack of induction of P-gp activity and expression in the PBMCs of thirteen healthy volunteers who had received oral rifampicin for 4 days. Asghar et al. (2002) reported controversial results since they observed, on the one hand, a decrease of *MDR1* gene expression in the PBMCs of 42% of 50 patients who had received oral rifampicin for 1 week, but on the other hand a concomitant increase for 58% of the patients. Contrary to our results, Owen et al. (2006) reported an induction of *MDR1* gene expression in human PBMCs incubated for 6 h with rifampicin. However, this study included only four patients while ours was conducted with a higher number of healthy volunteers. Moreover, we also tested incubations for 6 h at the beginning of our work. However, we observed no *MDR1* gene induction with this incubation time, either in PBMCs or in LS174T cells, contrary to what we obtained with longer times of incubation. Concerning phenobarbital, P-gp induction by this drug has been demonstrated in hepatocytes and intestinal cells (Jigorel et al., 2006; van de Kerkhof et al., 2008; Stormer et al., 2002). However, to our knowledge, no data exist regarding P-gp induction by this drug in lymphocytes. Therefore, the present study is the first to show that phenobarbital may induce neither P-gp activity nor P-gp expression in these cells. In our opinion, the statistically significant but very weak difference in P-gp activity in CCRF-CEM cells after 72 h incubation cannot be considered biologically relevant. The lack of confirmation of this increase with the quantification of *MDR1* mRNA levels supports this judgment.

The conflicting results reported regarding P-gp induction by rifampicin in lymphocytes may be partly due to the heterogeneity between the quantification methods used (cytometric quantification of dye-efflux, immunodetection by monoclonal anti-P-gp antibodies, RT-PCR quantification of *MDR1* mRNAs) and the differences between *in vitro, ex vivo* or *in vivo* conditions. However, regarding technical conditions, the lack of induction of P-gp activity and expression that we observed may not be due to our methodology. Indeed, the techniques we used had been carefully validated at the beginning of our work. As proof, they have already been recognized as reliable and adequate in several previous publications (Giraud et al., 2009a,b; Dauchy et al., 2009; Yousif et al., 2007). One criticism could be that, like in the great majority of studies interested in transporters in lymphocytes, we used isolated PBMCs rather than isolated lymphocytes. However, monocytic cells were eliminated using their propriety of adherence to multiwell culture plates after a 2-h-preplating (Glomsda et al., 2003). Thus, since about 80% of lymphocytes are T-lymphocytes, the levels of P-gp

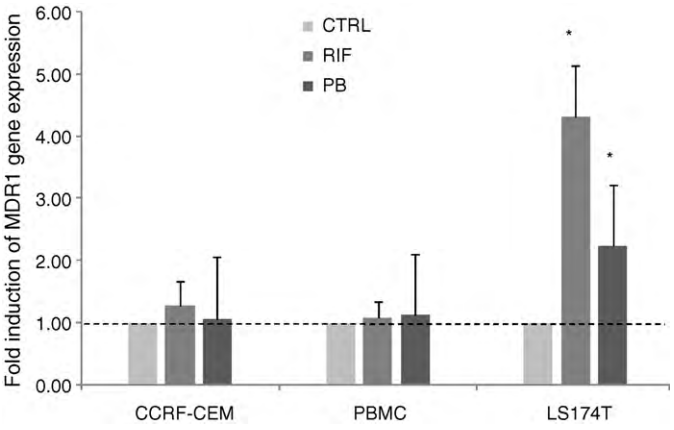

Fig. 2. Effect of rifampicin and phenobarbital on *MDR1* gene expression in CCRF-CEM cells and peripheral blood lymphocytes. CCRF-CEM cells ($n = 10$), PBMCs ($n = 8$) and LS174T cells ($n = 10$) were incubated in the presence of 20 μM rifampicin (A), 3 mM phenobarbital (B) or without any drug (control cells) for 24 h. LS174T cells were used as a positive control for the induction of *MDR1* gene expression. *MDR1* gene expression was determined using real-time quantitative RT-PCR and normalized to the *TBP* housekeeping gene expression. Fold increases in *MDR1* gene expression were calculated in treated cells relative to CCRF-CEM control cells. Results are expressed as means ± S.D.

protein and MDR1 gene expression may be considered as a good reflect of those measured in T-lymphocytes (Sleasman et al., 1997; Heldrup et al., 1992). Regarding control cells, we incubated them in parallel with drug-exposed cells at each incubation time rather than use control cells only at time 0 in order to take into account the fact that P-gp expression and activity may slightly vary in control cells depending on incubation times. The lack of P-gp induction may neither be due to our choices of drug concentrations or incubation times. The rifampicin concentration (20 μM) was chosen in the range of therapeutic concentrations and similar to those used in several other studies which have reported P-gp induction by this drug (Owen et al., 2006; Collett et al., 2004; Chandler et al., 2003; Stormer et al., 2002). The phenobarbital concentration was also in the range of those used in several studies which reported P-gp induction with no alteration of cell viability (Schuetz et al., 1996; Ohno et al., 2009; Martin et al., 2003). Common incubation times were also used (Nishimura et al., 2006; Collett et al., 2004; Stormer et al., 2002; Chandler et al., 2003; Gupta et al., 2008; van de Kerkhof et al., 2008). Moreover, concerning P-gp activity, a 96-h-incubation time was also tested but finally not used because of the observation of an associated significant decrease of cell viability. Finally, in our view, the demonstration of P-gp induction in the LS174T cell line is proof positive of the use of appropriate technical conditions in our work.

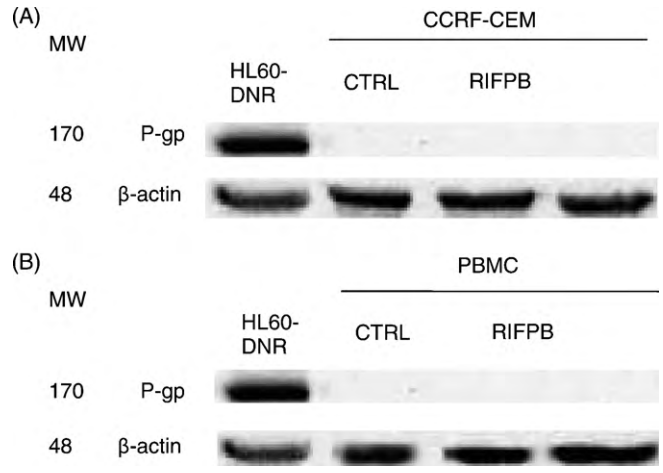

Fig. 3. Western blot analysis of P-gp protein expression in CCRF-CEM cells and PBMCs. Two samples of CCRF-CEM cells and the PBMCs of two healthy subjects were incubated in the presence of 20 μM rifampicin (RIF), 3 mM phenobarbital (PB) or without any drug (control cells; CTRL) for 48 h. HL60-DNR cells were used as a positive control for P-gp expression and β-actin as an internal control. Samples were loaded with 100 μg of proteins, except HL60-DNR cells (20 μg). (A) CCRF-CEM cells; (B) PBMCs.

Nuclear receptors present various tissue-specific expression patterns which are likely to modulate differently the regulation of *MDR1* gene expression. Differences in this regulation are in accordance with the lack of correlation already reported between P-gp expression levels in PBMCs compared to that in the intestine and liver (Albermann et al., 2005). P-gp induction by rifampicin and phenobarbital has been mainly described in hepatocytes and intestinal cells, known to express abundantly PXR and CAR nuclear receptors (Collett et al., 2004; Jigorel et al., 2006; van de Kerkhof et al., 2008; Greiner et al., 1999; Perloff et al., 2000; Dussault et al., 2001; Stormer et al., 2002; Lehmann et al., 1998). On the contrary, in the present study conducted on lymphocytes, we observed a very weak expression level of *PXR* and *CAR* genes, suggesting a very limited role of these nuclear receptors in the transcription of their target genes in these cells. This observation is in agreement with the results of Schote et al. (2007) who have quantified the expression level of twenty-four nuclear receptor genes in lymphocytes and shown that *PXR* and *CAR* genes were weakly transcribed in these cells compared to most other tissues. Several other studies have demonstrated that *PXR* and *CAR* gene expression may be very weak or even undetectable in human lymphocytes (Lehmann et al., 1998; Bertilsson et al., 1998). *PXR* gene expression was reported to be at least 250-fold lower in PBMCs than in the liver (Albermann et al., 2005; Owen et al., 2004). Furthermore, in addition to the lack of P-gp induction, the very weak *PXR* and *CAR* gene expression levels in lymphocytes may also participate to explain the very low P-gp expression in these cells. Indeed, despite the fact that, in accordance with the study of Oselin et al. (2003), we could detect *MDR1* mRNA in PBMCs and CCRF-CEM cells using RT-PCR, it must be underlined that these expression levels were low and obviously detected only because of the high sensitivity of the RT-PCR technique. In our opinion, in accordance with several other studies, P-gp protein expression could be detected neither in CCRF-CEM cells nor in PBMCs using Western blots because of the combination of two reasons (Dupuis et al., 2003; Oerlemans et al., 2006). First, the Western blot technique is far less sensitive than the RT-PCR technique. Second, several studies have already reported the lack of correlation between P-gp protein expression and *MDR1* mRNA quantification, which could be either of technical origin or due to post-transcriptional mechanisms since such mechanisms have already been reported in the literature for the *MDR1* gene (McClean et al., 1993; Yague et al., 2003; Gomez-Martinez et al., 2007; Albermann et al., 2005; Giraud et al., 2009a; Fakhoury et al., 2005).

In conclusion, the present study shows that rifampicin and phenobarbital may induce neither P-gp activity nor P-gp expression in lymphocytes, contrary to what had been observed in other cell types, in particular hepatocytes and enterocytes. This lack of induction may be partly due to different expression patterns of *PXR* and *CAR* gene expression levels between these different cell types. These results will have to be considered in studies investigating drug interactions in patients receiving multitherapies, in particular in the context of treatment for cancer or HIV infection.

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