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RESEARCH PAPER

Effects of budesonide on P-glycoprotein expression in intestinal cell lines

A Maier[1], C Zimmermann[1], C Beglinger[2], J Drewe[1] and H Gutmann[1]

[1]*Department of Clinical Pharmacology and Toxicology, University Hospital Basel, Basel, Switzerland and* [2]*Department of Internal Medicine, Division of Gastroenterology, University Hospital of Basel, Basel, Switzerland*

Background and purpose: P-glycoprotein (P-gp) is an important efflux transporter that supports the barrier function of the gut against invading antigens and against administered drugs. Since glucocorticoids, such as budesonide, are frequently used during inflammatory bowel disease we investigated how budesonide influences P-gp expression in different intestinal cell lines.
Experimental approach: LS180 and Caco-2 cells were incubated with budesonide and changes in P-gp expression were determined on mRNA, protein and functional level. The mRNA expression levels of glucocorticoid receptor (*GR*) and pregnane X receptor (*PXR*) were determined in these cell lines. PXR receptor was transiently transfected into Caco-2 cells.
Key results: Budesonide showed an induction of P-gp in LS180 cells and a down-regulation in Caco-2 cells. Expression levels of nuclear receptors revealed high expression of *PXR* only in LS180 cells and exclusive expression of *GR* in Caco-2 cells. Mifepristone, an anti-glucocorticoid, could not reverse the down-regulation of P-gp by budesonide in Caco-2 cells. In *PXR*-transfected Caco-2 cells the budesonide-mediated down-regulation of P-gp was abolished. Furthermore the expression of cytochrome P450 3A4 (*CYP3A4*), another PXR target gene, was induced in *PXR*-transfected Caco-2 cells after budesonide treatment.
Conclusions and Implications: Budesonide has the potential to influence *MDR1* expression *in vitro*. In LS180 cells, the induction of *MDR1* by budesonide probably is mediated via PXR. The mechanism of the down-regulation in Caco-2 cells still remains unclear, but GR does not seem to be involved. Further studies are required to evaluate how budesonide alters P-gp expression *in vivo*.
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Keywords: *P*-glycoprotein; *MDR1*; *ABCB1*; budesonide; Caco-2, LS180; intestine; PXR; glucocorticoid receptor

Abbreviations: AP-1, activator protein-1; CYP3A4, cytochrome P450 3A4; DMSO, dimethylsulphoxide; GR, glucocorticoid receptor; MDR, multidrug resistance; NF-κB, nuclear factor-κB; *P*-gp, *P*-glycoprotein; PXR, pregnane X receptor; R123, rhodamine 123

Introduction

Glucocorticoids are an important therapeutic option in treating inflammatory disorders like asthma, rheumatoid arthritis or inflammatory bowel disease (IBD). Budesonide is a newer synthetic glucocorticoid that is increasingly used in IBD patients (Kane *et al*., 2002). If applied in a controlled release formulation, budesonide is, after oral administration, topically released in the distal small intestine and colon, the predominant sites of inflammation (Klotz and Schwab, 2005). There, it is well absorbed, but it is extensively pre-systemically metabolized (Schwab and Klotz, 2001). Consequently, upon oral administration budesonide exerts strong anti-inflammatory effects in intestinal tissue with minimal systemic side effects (Hofer, 2003).

The molecular mechanism of glucocorticoid action involves intracellular binding to the glucocorticoid receptor (GR), followed by a translocation of activated GR to the nucleus. There, it stimulates or inhibits gene expression by binding to glucocorticoid response elements on DNA (Wright *et al*., 1993). Moreover, ligand-bound GR can repress a number of pro-inflammatory genes by physically associating with transcription factors via direct protein–protein interactions. Examples are the repression of nuclear factor-κB (NF-κB) or activator protein-1 (AP-1) (Smoak and Cidlowski, 2004).

Glucocorticoids such as dexamethasone are further known to activate the pregnane X receptor (PXR) (Kliewer *et al*., 1998). PXR belongs to the group of orphan nuclear receptors that function as heterodimers with the retinoic X receptor (Chawla *et al*., 2001). They are important regulators of

Correspondence: Professor J Drewe, Department of Clinical Pharmacology and Toxicology, University Hospital Basel, Hebelstr. 2, CH-4031 Basel, Switzerland.
E-mail: juergen.drewe@unibas.ch
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xenobiotic metabolism and upon activation they can induce the expression of metabolizing enzymes (e.g. cytochrome P450 3A4 (CYP3A4)) and drug transporters such as *P*-glycoprotein (*P*-gp) (Bertilsson *et al*., 1998; Geick *et al*., 2001).

P-gp is the gene product of the multidrug resistance gene 1 (*MDR1*, gene symbol: *ABCB1*). It is an adenosine triphosphate-dependent drug efflux pump with wide substrate specificity (Juliano and Ling, 1976). It has barrier function in tissues such as kidney, blood brain barrier and intestine (Cordon-Cardo *et al*., 1990). In the intestine, *P*-gp is localized in the apical membrane of epithelial cells and is continuously expressed along the intestinal tract (Thiebaut *et al*., 1987; Zimmermann *et al*., 2005). Therefore, *P*-gp can influence significantly the bioavailability of many drugs, including glucocorticoids, as it has been shown previously that budesonide, dexamethasone and prednisone are substrates of this transporter (Ueda *et al*., 1992; Fromm, 2003; Dilger *et al*., 2004). Besides the induction through PXR, the expression level of *MDR1* is dependent on a complex transcriptional regulation with a redundancy of signalling pathways (Labialle *et al*., 2002).

Several publications demonstrated that dexamethasone induces *MDR1* expression in the intestine of rats (Lin *et al*., 1999; Yumoto *et al*., 2001; Perloff *et al*., 2004). So far, there is no explanation of how glucocorticoids, in particular budesonide, influence *MDR1* expression in human intestinal cells. Here, we have investigated how the glucocorticoid budesonide influences the expression of *MDR1* in different human intestinal cell lines. We chose the Caco-2 cell line, as it shows transporter expressions comparable to those in the human jejunum (Taipalensuu *et al*., 2001). Furthermore, these cells are an established model for small intestinal transport (Hidalgo *et al*., 1989). Secondly, we used LS180 cells, which are a suitable model for intestinal gene induction studies (Bhat *et al*., 1995; Thummel *et al*., 2001; Zhou *et al*., 2004). Their expression level of efflux transporters is similar to human colonic tissue (Pfrunder *et al*., 2003). The findings of this study could be relevant in evaluating the involvement of *P*-gp in glucocorticoid action and glucocorticoid side effects.

Methods

Cell culture

The LS180 (used between passage 40 and 50) and Caco-2 cell lines (used between passage 54 and 70) were purchased from American Type Culture Collection (Manassas, NE, USA). LS180 and Caco-2 cells were cultured in Dulbecco's modified Eagle's medium with Glutamax-I, supplemented with 10% ($v\,v^{-1}$) fetal bovine serum, 1% non-essential amino acids, 1% sodium pyruvate, 50 $\mu g\,ml^{-1}$ gentamycin (Invitrogen AG, Basel, Switzerland). Cells were seeded into 12-well plastic culture dishes (3.8 cm^2 per well, BD Falcon AG, Allschwil, Switzerland) and were maintained in a humidified 37°C incubator with a 5% carbon dioxide in air atmosphere. After Caco-2 cells had reached confluence, or near confluence for LS180, they were treated with the substances as indicated or with vehicle alone. Medium was changed every 24 h. Toxicity was tested in advance for all applied substances using sulforhodamine B staining (Sigma-Aldrich, St Louis, MO, USA) (Skehan *et al*., 1990).

Real-time RT-PCR

After the indicated incubation procedures LS180 and Caco-2 cells were disintegrated by adding lysis buffer RLT (Qiagen, Hilden, Germany) and homogenized by using QIAshredder columns (Qiagen). Total RNA was extracted from cell lysates using the RNeasy Mini Kit (Qiagen). RNA was quantified with a Nanodrop Spectrophotometer (Witeg AG, Littau-Luzern CH). The purity of the RNA preparations was high as demonstrated by the 260 nm over 280 nm ratio (range 1.8–2.1). After DNase I digestion (Gibco, Life Technologies, Basel, Switzerland) 0.75 μg of total RNA was reversed transcribed by Superscript II (Gibco) according to the manufacturer's protocol using random hexamers as primers (Applied Biosystems, Rotkreuz, Switzerland).

TaqMan analysis was carried out on a 7900HT Sequence Detection System (Applied Biosystems). Polymerase chain reaction (PCR) conditions were 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Each TaqMan reaction contained 10 ng of cDNA in a total volume of 10 μl. qPCR Mastermix Plus from Eurogentec (Seraing, Belgium) was used. Primers and probes were used at concentrations of 900 and 225 nM, respectively. They were synthesized by Invitrogen (Basel, Switzerland) and by Eurogentec (Seraing, Belgium), respectively. Primers and probes for MDR1 and PXR were designed according to the guidelines of Applied Biosystems with help of the Primer Express 2.0 software. Sequences for glucocorticoid receptor-α (GR-α) and GR-β were adopted from a previous paper (Pedersen and Vedeckis, 2003). Corresponding sequences of primers and probes for TaqMan analysis are shown in Table 1. All samples were run in triplicates and not reverse-transcribed RNA served as a negative control. For the quantification, the expression of the genes of interest was normalized to glyceraldehyde-3-phosphate dehydrogenase mRNA expression.

Table 1 Primers and probes for TaqMan analysis

Gene	*Probe*
MDR1	5′-AAGCTGTCAAGGAAGCCAATGCCTATGACTT-3′
GR-α	5′-TTTCAACCACTTCATGCATAGAAT-3′
GR-β	5′-CATAACATTTTCATGCATAGAATCCAAGAGTTTTGTCA-3′
PXR	5′-AGCCCTTGCATCCTTCACATGTCATGA-3′
CYP3A4	5′-TTCTCCTGGCTGTCAGCCTGGTGC-3′
Gene	*Forward primer*
MDR1	5′-CTGTATTGTTTGCCACCACGA-3′
GR-α	5′-GGCAGCGGTTTTATCAACTGA-3′
GR-β	5′-AACTGGCAGCGGTTTTATCAA-3′
PXR	5′-GGCCACTGGCTATCACTTCAA-3′
CYP3A4	5′-TCTCATCCCAGACTTGGCCA-3′
Gene	*Reverse primer*
MDR1	5′-AGGGTGTCAAATTTATGAGGCAGT-3′
GR-α	5′-AATGTTTGGAAGCAATAGTTAAGGAGA-3′
GR-β	5′-TGTGAGATGTGCTTTCTGGTTTTAA-3′
PXR	5′-GTTTCATGGCCCTCCTGAAA-3′
CYP3A4	5′-CATGTGAATGGGTTCCATATAGATAGA-3′

Table 2 Primers for cDNA standards

Gene	*Forward primer*	*Reverse primer*
MDR1	5′-ACAGTCCAGCTGATGCAGAGG-3′	5′-CCTTATCCAGAGCCACCTGAAC-3′
GR-α	5′-TACCCTGCATGTACGACCAA-3′	5′-TTTTGGTATCTGATTGGTGATGA-3′
GR-β	5′-TACCCTGCATGTACGACCAA-3′	5′-TTGTCGATGAGCATCAGTTG-3′
PXR	5′-GCAGTCCAAGAGGCCCAGAA-3′	5′-CGTCGGACATGATCATCTCCTTC-3′
CYP3A4	5′-TAGTGATGGCTCTCATCCCAGA-3′	5′-TGAAGGTTGGAGACAGCAATGA-3′

For absolute quantification, we used external standard curves. Standards were gene-specific cDNA fragments that cover the TaqMan primer/probe area and they were generated by PCR. Sequences of the corresponding primers are shown in Table 2. The PCR products were purified by running a 1.5% agarose gel and a subsequent gel extraction (gel extraction kit, Qiagen). The standards were quantified using the PicoGreen reagent (Molecular Probes, Eugene, OR, USA) and were checked by sequencing (Microsynth GmbH, Balgach, Switzerland).

Western blot analysis

LS180 and Caco-2 cells were incubated for 72 h with 25 μM budesonide or with vehicle (0.25%. dimethylsulphoxide (DMSO)) as a negative control. LS180 cells additionally were incubated with 10 μM rifampicin. Then proteins were extracted with protein extraction buffer (20 mM Tris-HCl, 1% Igepal CA-630, 0.5 mM sodium orthovanadate), including 1 mM of the protease inhibitor phenylmethylsulfonyl fluoride (Sigma-Aldrich, St Louis, MO, USA) and protease inhibitor cocktail tablet, Complete Mini (Roche Diagnostics, Germany). The quantification of the protein content was performed with the bicinchoninic acid protein assay kit (Pierce Chemical, Rockford, IL, USA). Protein concentration was determined by measuring the absorbance at 562 nm with Spectra MAX 250 Microplate Spectrophotometer (Molecular Devices Corporation, California, USA).

For immunoblotting, 50 μg of total protein extract was mixed with Laemmli sample buffer (Bio Rad Laboratories, Reinach, Switzerland) and transferred to the polyacrylamide gel. Gel electrophoresis was performed with a Mini Protean 3 Electrophoresis Cell (Bio Rad) applying 80 V for 15 min for the stacking gel (4% acrylamide) and 120 V for 1 h for the separating gel (10% acrylamide). After electrophoresis, proteins were blotted to the nitrocellulose membrane (250 mA for 2.5 h) using a Mini Trans-Blot Cell (Bio Rad). Protein transfer was verified by Ponceau S staining. The membrane was blocked overnight at 4°C with phosphate-buffered saline (PBS) containing 5% milk powder and 0.05% Tween 20. After washing three times for 15 min (0.05% Tween in PBS), the membrane was incubated for 2 h at room temperature with the primary, mouse anti-human antibody C219 against *P*-gp, 0.1 mg ml^{-1} (Alexis Corporation, Lausen, Switzerland) diluted 1:100 in PBS containing 0.05% Tween and 1% milk powder. As loading control, β-actin mouse monoclonal antibody (abcam, Cambridge, UK) was used with the dilution of 1:1000. After the first incubation, the membrane was washed three times for 15 min and then incubated with the secondary, horseradish peroxidase-conjugated, rabbit anti-mouse immunoglobulin-G (Amersham, Buckinghamshire, UK) diluted 1:1000. Secondary antibody incubation was performed for 1 h at room temperature. Membranes were washed, and *P*-gp and β-actin detection was performed with the enhanced chemiluminescence system (ECL-Detection-Kit, Amersham). The molecular weight was identified by using Precision Plus Protein TM Standard Dual Color (Bio Rad).

Rhodamine 123 accumulation assay

LS180 and Caco-2 cells were incubated for 72 h either with medium containing 20 μM budesonide or vehicle only (0.2% DMSO). LS180 cells were additionally incubated with 10 μM rifampicin. Medium was changed every day. Following drug treatment, cells were washed with Hanks'-buffered saline solution (HBSS) (supplemented with 1 mM pyruvate throughout) and incubated for 30 min at 37°C with HBSS containing additionally 100 μM verapamil and 0.5 μM rhodamine 123 (R123) (Molecular Probes, Eugene, OR, USA). In this step, cells are loaded with R123, whereas *P*-gp function is blocked with verapamil. R123 uptake was stopped by transferring the cells to ice. They were washed three times with ice-cold HBSS in the presence or absence of 100 μM verapamil. R123 efflux was started by incubating the cells with or without 100 μM verapamil in HBSS at 37°C. After 60 min, cells were washed at 4°C and lysed in 400 μl 5% Triton X-100. Samples of the homogenized cell lysates (200 μl) were analysed with a HTS 7000 Plus Bio Assay Reader (Perkin Elmer Ltd, Buckinghamshire, UK) with 485 nm excitation and 535 nm emission filters. The ratio of intracellular R123 fluorescence in the absence and presence of verapamil is indicative of the activity of *P*-gp. This approach has been reported previously for the measurement of *P*-gp activity in LS180 cells (Collett *et al*., 2004).

mRNA decay measurement

The measurement of mRNA stability was performed in Caco-2 cells using actinomycin D as an inhibitor of transcription. Cells were preincubated for 1 h with budesonide 25 μM or vehicle. Actinomycin D (5 μg ml^{-1}) was added and total RNA was extracted after different time points. The expression level of *MDR1* mRNA after 0, 4, 8, 24 and 48 h was determined by real-time reverse transcription (RT)-PCR.

Transfections

The Full ORF Expression vector of *PXR* (IOH34726-pT-Rex-DEST30) was purchased from the Deutsches Ressourcenzentrum für Genomforschung GmbH. The empty vector was

obtained by cutting the *PXR* plasmid with the restriction enzymes *Eco*RV and *Mlu*I followed by purification on a 0.5% agarose gel. The cut ends were blunt-ended with T4 DNA polymerase and self-circulated with T4 DNA ligase. Transfection was performed with Lipofectamine 2000 (Invitrogen) during 48 h following the manufacturer's protocol using 1.6 μg DNA and 4 μl Lipofectamine 2000. As negative control, the empty vector was used. After transfection Caco-2 cell were incubated with budesonide 25 μM and rifampicin 10 μM or vehicle only. Transfection was verified by measuring *PXR* mRNA expression using TaqMan analysis.

Statistics

Treatment groups were compared to control group by analysis of variance. If this analysis revealed significant differences and more than one treatment group was included in the analysis, pairwise comparisons of treatment groups with the control group was performed subsequently using Dunnett's two-sided multi-comparison test or multiple unpaired two-sided *t*-test with Bonferoni's correction, as appropriate, to account for the multiplicity of testing. All tests were performed using the SPSS for Windows software (version 14.0). The level of significance was $P<0.05$.

Materials

Budesonide (Sigma-Aldrich, St Louis, MO, USA), rifampicin (Fluka Chemie, Buchs SG, Switzerland) and actinomycin D (Sigma-Aldrich) were dissolved in DMSO. Mifepristone (Sigma-Aldrich) and R123 (Molecular Probes, Eugene, OR, USA) were dissolved in ethanol.

Results

MDR1 mRNA expression

The basal *MDR1* mRNA expression level was higher in Caco-2 cells compared to LS180 cells. The effect of budesonide on *MDR1* mRNA expression was investigated in these two different intestinal cell lines (LS180 and Caco-2). Cells were incubated for 48 h with increasing budesonide concentrations and mRNA expression was analysed using real-time RT-PCR. In LS180 cells, we observed a dose-dependent induction of *MDR1* mRNA expression by budesonide (Figure 1a). Rifampicin as a positive control also showed an induction. In Caco-2 cells, *MDR1* mRNA expression decreased in a dose-dependent manner after budesonide treatment (Figure 1b).

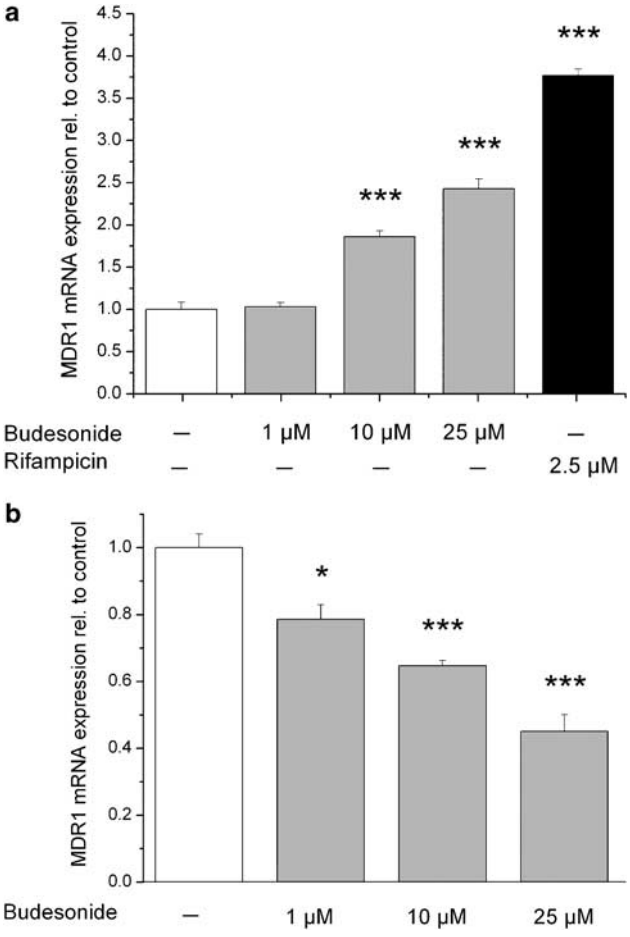

Figure 1 Effect of budesonide on *MDR1* mRNA expression in LS180 (**a**) and Caco-2 (**b**) cell lines. Cells were incubated for 48 h and mRNA expression was determined by quantitative real-time PCR. Rifampicin was used as a positive control for *MDR1* induction in LS180 cells. Results are normalized to control cells and expressed as mean $\pm$ s.e.m. ($n=4$). *$P<0.05$, ***$P<0.001$ vs control cells.

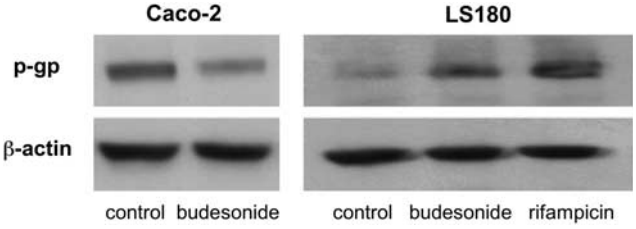

Figure 2 Effect of budesonide on *P*-gp protein expression. LS180 and Caco-2 cells were incubated for 72 h with 25 μM budesonide or vehicle. Rifampicin was used as a positive control for *MDR1* induction in LS180 cells. *P*-gp protein and β-actin were determined by Western blot analysis. β-Actin was used as loading control.

MDR1 protein expression

The observed changes in *MDR1* mRNA expression in LS180 and Caco-2 cells were confirmed on protein level by Western blot analysis (Figure 2). In three independent assays, cells were incubated for 72 h with 25 μM budesonide and 10 μM rifampicin. Compared to control cells, *MDR1* protein levels increased in LS180 cells and decreased in Caco-2 cells after budesonide treatment.

Measurement of P*-gp activity*

R123 is a fluorescent *P*-gp substrate that can be used for the determination of *P*-gp activity (Figure 3). After passive diffusion into the cells, it is actively transported out of the cell by *P*-gp. Measurement of cellular R123 accumulation revealed that the observed changes of *MDR1* expression were reflected by changes in *P*-gp function. In LS180 cells, where *MDR1* is induced by budesonide, relative R123 uptake was

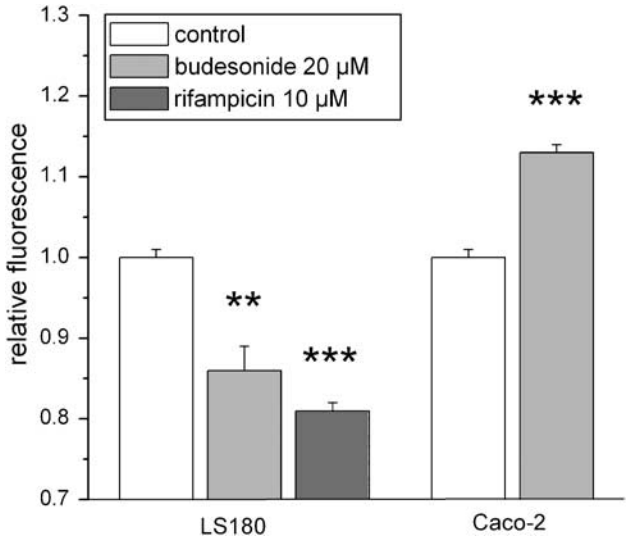

Figure 3 *P*-gp activity was assessed using R123 accumulation in LS180 and Caco-2 cells. Cells were pretreated with 20 μM budesonide or vehicle for 72 h. Rifampicin was used as a positive control for *P*-gp induction. Data represent R123 fluorescence normalized to verapamil-treated cells. A decrease in intracellular fluorescence is indicative of an increase in *P*-gp activity and *vice versa*. Results are expressed as mean $\pm$ s.e.m. ($n = 3$–4). $^{**}P < 0.01$, $^{***}P < 0.001$ vs control cells.

significantly lower compared to control cells. Similar effects were observed for rifampicin, a positive control for *MDR1* induction in LS180 cells. In Caco-2 cells, the decreased *MDR1* expression led to a significant accumulation of intracellular R123.

Expression of the nuclear receptors PXR and GR in intestinal cell lines

To elucidate the differential effects of budesonide on the expression of *MDR1* in the cell lines studied, we determined whether this could be attributed to differential expression levels of nuclear receptors (Figure 4). Glucocorticoids exert their anti-inflammatory effects through the GR-α. However, the PXR can also be activated by glucocorticoids like dexamethasone. Real-time PCR analysis ($n = 4$ for both cell lines) revealed low expression levels of *PXR* in Caco-2 cells and high expression in LS180 cells. *GR-α*, on the other hand, was exclusively expressed in Caco-2 cells. As the GR-β apparently exhibits inhibitory effects on the GR-α, we analysed also the expression rate of this receptor. However, no *GR-β* mRNA was detectable in either cell line. There was no effect of budesonide treatment on these receptors. In addition, basal *MDR1* mRNA expression was not correlated to PXR expression level in both investigated cell lines.

Effect of mifepristone on MDR1 mRNA expression in Caco-2 cells

To determine whether the GR plays a role in the budesonide-induced downregulation of *MDR1* in Caco-2 cells, mifepristone (RU486) a known anti-glucocorticoid was used. Mifepristone could not reverse the effect of budesonide when co-applied; when the drug was given alone, it induced a similar downregulation of *MDR1* (Figure 5).

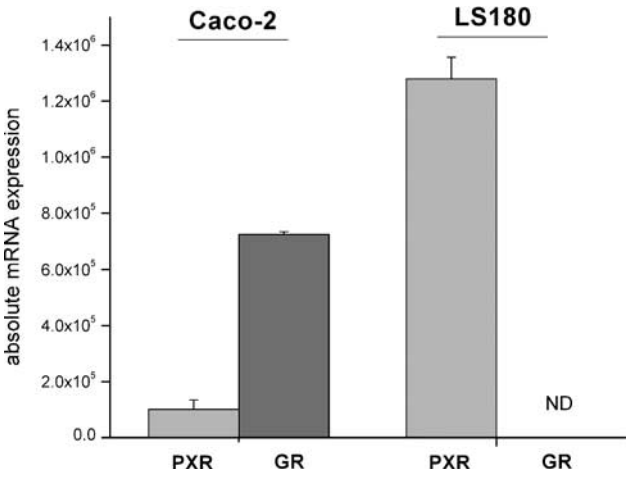

Figure 4 Expression of *PXR* and *GR-α* mRNA in the investigated intestinal cell lines Caco-2 and LS180. Expression was determined by quantitative real-time PCR. Results are expressed as mean $\pm$ s.e.m. ($n = 4$). ND = not detectable.

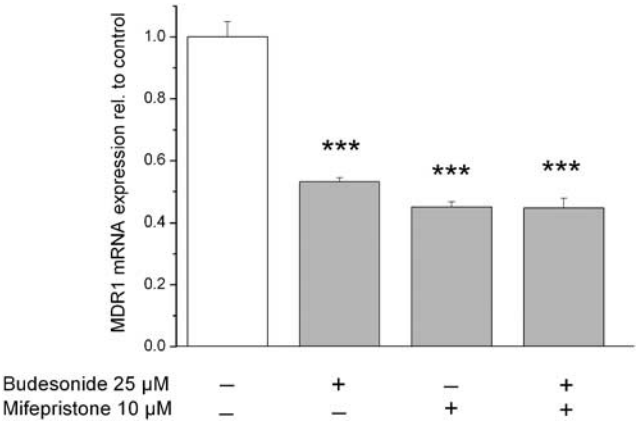

Figure 5 Effect of the anti-glucocorticoid mifepristone on *MDR1* mRNA expression. Cells were incubated for 48 h and mRNA expression was determined by quantitative real-time PCR. Results are normalized to control cells and expressed as mean $\pm$ s.e.m. ($n = 4$). $^{***}P < 0.001$ vs control cells.

MDR1 mRNA stability

We have shown that budesonide decreases the expression of *MDR1* in Caco-2 cells on the mRNA level. However, beside a transcriptional regulation, this result could also reflect a decrease in mRNA stability. Therefore, we determined the decay of *MDR1* mRNA after the addition of the transcription inhibitor actinomycin D in Caco-2 cells (Figure 6). The stability of *MDR1* mRNA was not different between control cells and cells treated with budesonide. For both treatments, the half-life of *MDR1* mRNA in Caco-2 cells appeared to be about 20 h.

Transfection of PXR into Caco-2 cells

To investigate whether PXR is involved in the regulation of *MDR1* by budesonide, *PXR* was transiently transfected into Caco-2 cells. Caco-2 cells express only low levels of endogenous *PXR* and known PXR activators such as rifampicin are not able to induce PXR target genes in Caco-

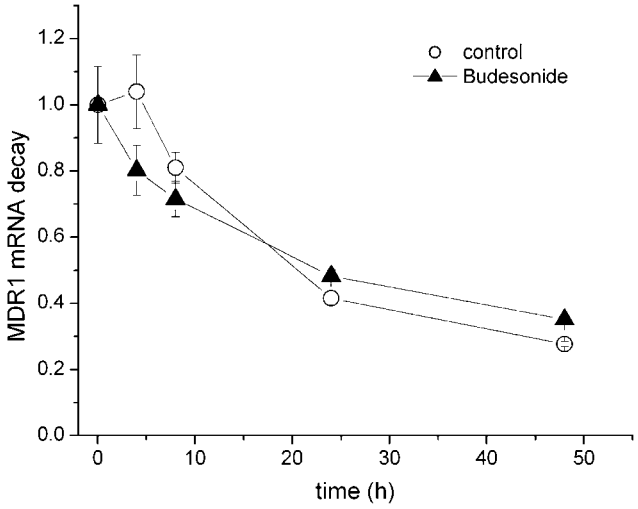

Figure 6 Effect of budesonide on *MDR1* mRNA stability in Caco-2 cells. Cells were treated with 25 μM budesonide or vehicle, whereas transcription was inhibited with actinomycin D (5 μg ml^{-1}). mRNA expression was determined by quantitative real-time PCR after 0, 4, 8, 24 and 48 h incubation. Each data value is expressed relative to the expression at 0 h. Data represent mean $\pm$ s.e.m. ($n=3$).

2 cells (data not shown and Pfrunder *et al*., 2003). In a control experiment, cells transfected with the empty vector showed the expected downregulation of *MDR1* mRNA after budesonide treatment and no changes after rifampicin treatment compared to control cells. On the other hand, *PXR* transfection could reverse the downregulation of *MDR1* mRNA after budesonide treatment. Rifampicin, the positive control for PXR activation, induced *MDR1* mRNA in PXR-transfected Caco-2 cells (Figure 7a).

CYP3A4, another target gene of PXR, is only slightly expressed in Caco-2 cells. Nevertheless, in parallel to *MDR1*, we could see a significant induction of *CYP3A4* in PXR-transfected Caco-2 cells after treatment with budesonide and rifampicin (Figure 7b).

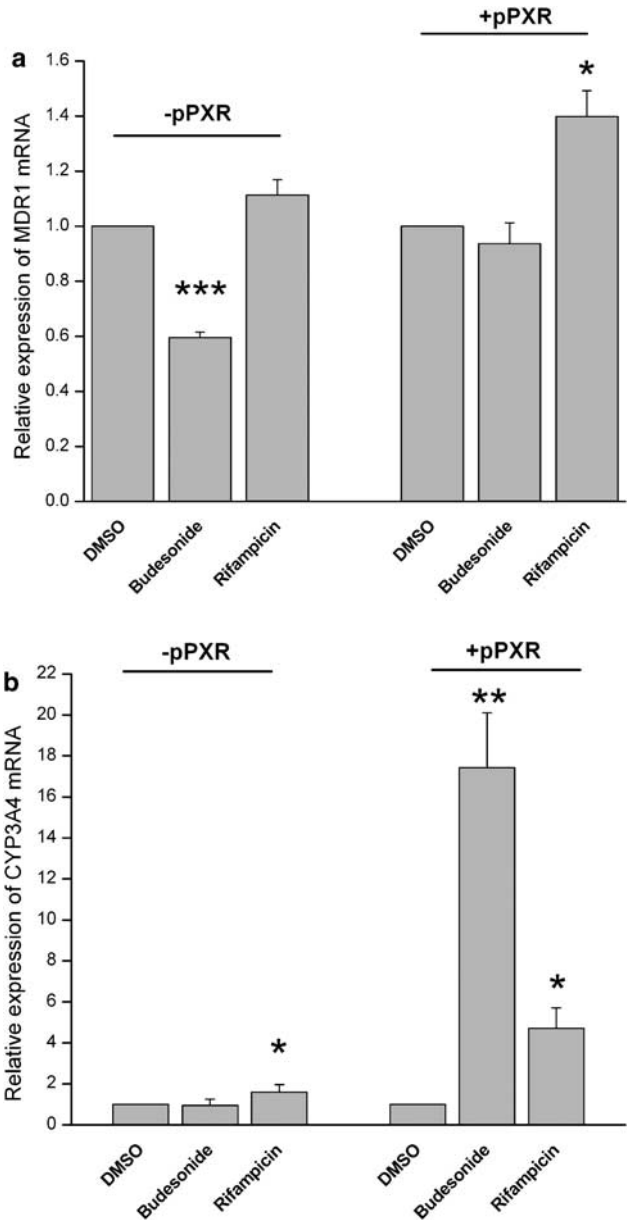

Figure 7 Expression of *MDR1* (**a**) and *CYP3A4* (**b**) in Caco-2 cells transfected with the empty vector (−pPXR) and PXR vector (+pPXR) after treatment with budesonide, rifampicin or vehicle only. In two independent experiments, cells were transfected during 48 h, followed by 48 h drug treatment. mRNA expression was determined by quantitative real-time PCR. Results are normalized to the respective control cells and expressed as mean $\pm$ s.e.m. ($n=5$–6). *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs control cells.

Discussion

At present, there are no data on the intestinal regulation of *MDR1* by budesonide. Given that this glucocorticoid is often used in the treatment of IBD, we investigated its effects on *MDR1* expression in two different, frequently used intestinal cell lines. Our results indicate that the regulation of this efflux transporter by budesonide is complex. *MDR1* expression was induced in LS180 cells; in contrast, it was downregulated in Caco-2 cells.

An altered intestinal expression of *P*-gp can have important clinical implications. On the one hand, an induction of *MDR1* can lead to an increased efflux of *P*-gp substrates out of the enterocytes (Westphal *et al*., 2000). Ineffective therapy or even therapy resistance could be the outcome. On the other hand, a decreased expression can impair the barrier function of the intestinal epithelial cells. In this respect, it was shown that mice deficient for *MDR1a* developed an inflammation of the large intestine similar to IBD (Panwala *et al*., 1998). The inflammation was dependent on the presence of intestinal bacteria, suggesting a function of *P*-gp to protect the body from toxins produced by intestinal bacteria. This hypothesis is in concordance with data from patients with ulcerative colitis, where the expression of *MDR1* and *PXR* was significantly reduced in mucosal biopsy specimens from non-affected regions of the colon and terminal ileum (Langmann *et al*., 2004). Here, we have shown that, in Caco-2 cells, *MDR1* expression was repressed by budesonide in a dose-dependent manner. An enhanced mRNA decay is not responsible for the observed *MDR1*

downregulation (as shown in the experiments with actinomycin D), although increased *MDR1* mRNA decay with dexamethasone has been reported in primary rat hepatocytes (Schuetz *et al*., 1995).

In contrast to LS180 cells, the *GR* was highly expressed in Caco-2 cells. This led us to assume that the GR could mediate the observed downregulation of *MDR1* in this cell line. The downregulation by budesonide occurred, however, in the range of micromolar concentrations only, despite the fact that the GR receptor has a high affinity for glucocorticoids. Furthermore, the addition of mifepristone (RU486), an established antagonist at the GR, did not abolish this effect. In fact, mifepristone repressed *MDR1* expression in the same way as budesonide. Therefore, the involvement of the GR can most likely be ruled out, but the mechanism behind the repression of *MDR1* in Caco-2 cells remains still unclear. However, a not yet identified nonspecific effect appears to be most likely.

In contrast, when we used LS180 cells as an intestinal model, incubations with budesonide showed an increase in *MDR1* expression. We assumed that budesonide might be able to activate PXR, leading to an induction of *MDR1* and other PXR target genes.

An nonspecific induction of *MDR1* through toxic drug effects could be ruled out by performing cytotoxicity assays before the incubation procedures. In our experiments, the induction occurred with budesonide concentrations starting from 10 μM. This is in concordance with the fact that PXR is a receptor with only low affinity for glucocorticoids, as the affinity of corticosterone for PXR was reported to be in the range of 10–30 μM (Sheppard, 2002). The applied budesonide concentrations up to 25 μM are high, but they could represent relevant local concentrations in the gut lumen after oral administration or when the drug is applied rectally as an enema. The induction of *MDR1* in the intestine through glucocorticoids has been shown previously for dexamethasone in the rat (Lin *et al*., 1999; Yumoto *et al*., 2001; Perloff *et al*., 2004). The mechanism of this upregulation by glucocorticoids seems to involve PXR. Pascussi *et al*. (2001) demonstrated that dexamethasone activates the nuclear receptor PXR. Furthermore, it has been shown that PXR mediates *MDR1* induction (Geick *et al*., 2001).

From our experiments, there are several lines of evidence to support the suggestion that budesonide induced *MDR1* expression through activation of PXR. Firstly, *PXR* expression was high in LS180 cells, where we observed an induction of *MDR1*. Secondly, rifampicin, a known activator of PXR (Geick *et al*., 2001), induced *MDR1* in parallel with budesonide in LS180 cells. Finally, the PXR target genes *MDR1* and *CYP3A4* were induced in *PXR*-transfected Caco-2 cells compared to cells transfected with the empty vector.

In *PXR*-transfected Caco-2 cells, the induction of *MDR1* apparently resulted in a reversal of the previously observed downregulation. For *CYP3A4*, on the other hand, a clear induction could be seen. Surprisingly, this induction of *CYP3A4* was even more prominent with budesonide compared to rifampicin.

PXR is known to be expressed in the human intestine. Therefore, budesonide treatment might lead to increased intestinal levels of *P*-gp and CYP3A4. Thus, potential drug–drug interactions have to be taken into account, when substrates of *P*-gp or CYP3A4 are co-administered. On the other hand, the intestinal expression level of *PXR* itself is a factor that can vary in diseases, such as ulcerative colitis, where *PXR* has been shown to be downregulated (Langmann *et al*., 2004). Consequently, results from *in vivo* studies in healthy subjects cannot directly be applied to the situation in patients with IBD.

In conclusion, we have shown in this study that budesonide has the potential to influence the expression of intestinal *MDR1 in vitro*. The investigated intestinal cell lines showed opposing regulatory effects of this transporter. Further studies have to be carried out to evaluate the impact of glucocorticoid treatment on intestinal *P*-gp expression *in vivo*.

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Conflict of interest

The authors state no conflict of interest.

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