

Christian Zimmermann · Heike Gutmann
Juergen Drewe

Thalidomide does not interact with P-glycoprotein

Received: 24 May 2005 / Accepted: 25 July 2005 / Published online: 1 September 2005
© Springer-Verlag 2005

Abstract *Background:* There is growing clinical interest in thalidomide for the treatment of various disorders due to its anti-inflammatory, immunomodulatory, and anti-angiogenic properties. In numerous clinical trials thalidomide is used as an adjunct to standard therapy. Therefore, clinicians should be aware of all possible drug–drug interactions that might occur with this drug. P-glycoprotein (P-gp), a drug efflux transporter that is expressed in many tissues, is the cause of several drug–drug interactions. P-gp induction or inhibition can lead to ineffective therapy or side-effects. In this study, we investigated thalidomide's potential to cause drug–drug interactions on the level of P-gp. *Methods:* LS180 cells were incubated with thalidomide for 72 h in order to determine P-gp induction using real-time RT-PCR. A human leukaemia cell line over-expressing MDR1 (CCRF-CEM/MDR1) was used to measure uptake of rhodamine 123, a P-gp substrate, in the presence of thalidomide. Dose-dependent and bi-directional transport of thalidomide through Caco-2 cell monolayers was performed to assess site-directed permeability. Transport rates were determined using HPLC including chiral separation of the thalidomide enantiomers. *Results:* Thalidomide did not induce P-gp expression in LS180 cells. The uptake of rhodamine 123 in CCRF cells over-expressing MDR1 was not influenced by co-incubation with thalidomide. The transport through Caco-2 monolayers was linear and the permeability was similar for both directions. No differences between the thalidomide enantiomers were observed. *Conclusions:* Our

study indicates that thalidomide is neither a substrate, nor an inhibitor or an inducer of P-gp. Therefore, P-gp-related drug–drug interactions with thalidomide are not likely.

Keywords Thalidomide · P-glycoprotein · Enantiomers · Intestinal transport

Introduction

Thalidomide was widely used in Europe as a sedative-hypnotic drug (Contergan) since 1956. In the early 1960s it was withdrawn from the market due to its obvious teratogenic effects. Later, in 1965, it was reported to show remarkable efficacy against erythema nodosum leprosum [1]. The recent renewed interest in thalidomide is based on its potential to treat inflammatory and autoimmune disorders and to its anti-angiogenic activity [2]. Anti-inflammatory and immunomodulatory effects of thalidomide have been explained by a degradation of mRNA encoding tumour necrosis factor- α (TNF- α) in monocytes [3]. Furthermore, thalidomide seems to inhibit the production of the cancer-associated growth factor interleukin-6 [4, 5] and the production of several other cytokines [6]. Clinical applications of thalidomide include aphthous ulcers, Behçet's syndrome, rheumatoid arthritis, graft-versus-host disease, and inflammatory bowel disease. In addition, malignant diseases such as haematological cancers, prostate cancer, and renal-cell carcinoma have been treated with thalidomide [7, 8].

Thalidomide (α -phthalimidoglutarimide) is a neutral racemic compound derived from glutamic acid. It is applied in equimolar amounts of (+)-(R)- and (–)-(S)-enantiomers that rapidly interconvert at physiological pH [9]. The parent compound undergoes spontaneous hydrolysis in aqueous solution at pH 7, leading to twelve hydrolysis products [10]. This non-enzymatic hydrolysis is the main breakdown mechanism of thalidomide in the body, whereas hepatic metabolism seems to play a minor role [11]. Therefore, only low concentrations of

C. Zimmermann · J. Drewe (✉)
Department of Clinical Pharmacology and Toxicology
Petersgraben, University Hospital Basel
and Department of Pharmaceutical Sciences,
Petersgraben 4, CH-4031 Basel, Switzerland
E-mail: juergen.drewe@unibas.ch
Tel.: (business)- +41-612653848
Fax: +41-612658581

H. Gutmann
Department of Pharmaceutical Sciences,
University of Basel, Basel, Switzerland

5-hydroxythalidomide were detectable in a human study [12]. Additionally, it was shown in vitro that thalidomide is a poor substrate for cytochrome P-450 isoenzymes and that it does not inhibit the metabolism of CYP-specific substrates [13]. These results suggest that thalidomide is not involved in clinically important drug–drug interactions caused by an inhibition of cytochrome P-450 metabolism.

However, interactions may also occur at the level of drug transporters such as P-gp, the gene product of MDR1 (ABCB1). P-gp is an efflux pump, located on the apical membrane of cells. It actively extrudes a variety of substances and presumably functions as a biological barrier against xenobiotics and pathogens [14]. It is expressed in many tissues such as intestine, kidney, liver, and blood brain barrier [15, 16]. Due to its broad substrate specificity it limits the absorption of numerous unrelated substances such as HIV drugs, anticancer drugs, and endogenous compounds [17–19]. Therefore, competition for substrate binding, inhibition or up-regulation of this transporter can lead to significant changes in the pharmacokinetics of P-gp substrates. Many cases of drug–drug interactions on the basis of these mechanisms have already been reported [20].

Due to its anti-angiogenic activity, thalidomide is being tested in patients who suffer from various cancers [21]. In tumour cells, P-gp is often over-expressed, making them resistant to P-gp substrates [22]. Whether thalidomide is also a substrate of this transporter could therefore be of interest. Moreover, many drugs used in cancer therapy such as actinomycin C, etoposide, teniposide, paclitaxel, docetaxel, and topotecan, as well as glucocorticoids and morphine are P-gp substrates. The co-administration of a potential inhibitor or inducer of P-gp could influence the therapy due to the described mechanisms.

Since thalidomide is mostly used as an adjuvant therapy, clinicians should be aware of all possible drug–drug interactions. So far, there is nothing known about the affinity of thalidomide to transporter proteins such as P-gp. In this study, experiments were performed to determine the effect of thalidomide on the function and expression of this clinically important drug efflux transporter. This knowledge can help to assess the risk of P-gp-related drug–drug interactions during therapies where thalidomide is applied. Moreover, it might further elucidate the process of thalidomide absorption.

Materials and methods

Determination of MDR1 mRNA induction with quantitative real-time RT-PCR

(+/-)-Thalidomide and rifampicin (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) were dissolved in dimethyl sulphoxide (DMSO). LS180 cell line (used

between passage 40 and 45) was purchased from ATCC (Manassas, USA). Cells were cultured in Dulbecco's MEM with Glutamax-I, supplemented with 10% (v/v) foetal calf serum, 1% non-essential amino acids, 1% sodium pyruvate, and 50 µg/ml gentamycin. Cultures were maintained in a humidified 37°C incubator with a 5% carbon dioxide in air atmosphere. The cells were seeded into six well plastic culture dishes (9.2 cm²/well) and after they had reached confluence they were treated with 10 and 100 µM thalidomide and 10 µM rifampicin for 72 h.

The stability of thalidomide in our cell culture media at 37°C was determined over a period of up to 4 hours. The concentration decreased to 74, 53, and 18% after 1, 2, and 4 h, respectively (data not shown). Consequently, in the incubation experiments we applied relatively high thalidomide concentrations (10 and 100 µM) and the medium was changed twice a day. Although we could not exclude significant degradation, this approach reflects the clinical situation of oral thalidomide administration, which is given two times a day.

At the end of the culture period, total RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA was quantified with a GeneQuant photometer (Pharmacia, Uppsala, Sweden). The purity of the RNA preparations was high as demonstrated by the 260/280 nm ratio (range 1.8–2.0). After DNaseI digestion (Gibco, Life Technologies, Basel Switzerland) 1 µg of total RNA was reverse-transcribed by Superscript (Gibco, Life Technologies, Basel, Switzerland) according to the manufacturer's protocol using random hexamers as primers.

TaqMan analysis was carried out on a Gene Amp 5700 Sequence Detector (Applied Biosystems). PCR conditions were 10 min, 95°C followed by 40 cycles of 15 s, 95°C and 1 min, 60°C. Each TaqMan reaction contained 25 ng of cDNA in a total volume of 25 µL using TaqMan Universal PCR Mastermix (Applied Biosystems). The concentrations of primers and probes were 900 nM and 225 nM, respectively. They were designed according to the guidelines of Applied Biosystems with the help of the Primer Express 2.0 software (MDR1 forward primer: 5'-CTGTATGTTTGCCACCACGA-3'; MDR1 probe: 5'-AAGCTGTCAAGGAAGCCAATGCCTATGACTT-3'; MDR1 reverse primer: 5'-AGGGTGTCAAATTTATGAGGCAGT-3'; GAPDH forward primer 5'-GGTGAAGGTCGGAGTCAACG-3'; GAPDH probe 5'-CGCCTGGTCACCAGGGCTG C-3'; GAPDH reverse primer 5'-ACCATGTAGTTGAGGTCAA TGAAGG-3'). Primers were synthesised by Invitrogen (Basel, Switzerland), probes by Eurogentec (Seraing, Belgium). For absolute quantification, we used external standard curves. Standards were gene-specific cDNA fragments that cover the TaqMan primer/probe area. All samples were run in triplicates and RNA that was not reverse-transcribed served as a negative control. Results are expressed as ratios of MDR1 to GAPDH expression.

Rhodamine 123 uptake in CCRF-CEM/MDR1 cells

CCRF-CEM/MDR1 cells (human leukaemic T-lymphocytes over-expressing MDR1) were a gift from Altana Pharma Ltd. (Konstanz, Germany). The cells were grown in suspension and were cultured in RPMI 1640 medium with Glutamax-I, supplemented with 10% FCS and 50 µg/mL gentamycin. Cells were continuously cultured in the presence of 1 µg/mL vincristine under 5% CO₂/95% air atmosphere at 37°C. One day before the experiment, cells were grown in vincristine-free medium.

Two million CCRF-CEM/MDR1 cells per millilitre were pre-incubated at 37°C for 10 min in the presence of 10, 100, and 300 µM thalidomide or 100 µM verapamil (both Sigma-Aldrich Chemie, Schnelldorf, Germany), followed by an incubation with additional 5 µM rhodamine 123 (Molecular Probes, Eugene, OR). After 15 min, rhodamine 123 uptake was stopped by transferring samples to ice. Cells were washed three times in the presence of the previously applied drug at 4°C and lysed in 1% Triton X-100. Aliquots were transferred into Optiplate-96 plates (Packard, Zürich, Switzerland) and fluorescence of the lysate was analysed with a HTS 7000 Plus Bio Assay Reader (Perkin Elmer Ltd., Buckinghamshire, UK) with 485 nm excitation and 535 nm emission filters. Prior to the assay, possible quenching of rhodamine 123 due to thalidomide or verapamil was checked for all concentrations used in the uptake experiment.

Transport of (±)-thalidomide across Caco-2 cell monolayers

Caco-2 cells (used between passage 54 and 59) were purchased from ATCC (Manassas, USA). They were cultured in Dulbecco's MEM with Glutamax-I, supplemented with 10% foetal calf serum, 1% non-essential amino acids, 1% sodium pyruvate, and 50 µg/mL gentamycin. Cells were seeded with a density of 6×10^4 cells/cm² onto Snapwell polycarbonate membrane filters (12 mm diameter, 0.4 µm pore size; Costar, Cambridge, MA) and were cultured in standard six-well cluster plates in a humidified 37°C incubator with a 5% CO₂ in air atmosphere. The membranes with the cell monolayer were placed into a side-by-side diffusion chamber (Costar) 16 days after seeding. The experiments were performed at 37°C with sample volumes of 4 mL per compartment. Cells were washed twice and equilibrated for 10 min with pre-heated transport buffer [Hank's balanced salt solution (HBSS) + 1 mM pyruvate].

Bi-directional transport was initiated by adding transport buffer containing 100 µM thalidomide to the donor chamber (apical or basolateral side of the cell monolayer). The transport buffer was removed from the acceptor compartment after 5, 10, 15, and 30 min and replaced with fresh buffer. For the investigation of dose-

dependent transport 10, 50, 100, and 250 µM thalidomide were added to the apical compartment. After 10 minutes the buffer in the basolateral compartment was removed for analysis.

According to Eriksson et al. [23] the drawn samples were instantly acidified with citrate buffer (pH 1.5) and frozen until further processing. This approach prevents interconversion and hydrolysis of thalidomide enantiomers. The paracellular marker fluorescein isothiocyanate dextran (FITC dextran, MW 4000, Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) was used for monitoring the monolayer integrity after each experiment. FITC dextran transport was measured in a fluorescence reader (Perkin Elmer HTS Soft 7000 Plus).

Determination of thalidomide concentrations in the transport buffer samples was performed in triplicates by an enantioselective HPLC analysis. The HPLC system (LaChrom) and the software (D-7000 HPLC system manager) were from Merck Hitachi. The following components were integrated into the HPLC system: interface D-7000, diode array detector L-7455, column oven L-7300, autosampler L-7200, pump L-7100. For the separation of the thalidomide enantiomers, a chiral column (Chiralcel OJ, 250×46 mm, Daicel Chemical Industries) was used.

The temperature of the column was 40 °C. The mobile phase consisted of 65% ethanol and 35% hexane with a flow rate of 0.85 mL/min. Injection volume of the samples was 80 µL. UV detection of the substances was performed at a wavelength of 220 nm. (+)-Thalidomide and (–)-thalidomide were well separated with retention times of 11.15 and 14.75 min, respectively. The standard curves were linear (correlation coefficients > 0.999) in the concentration range of 20–2.0 µg/mL (corresponding to 77.45 nM–7.745 µM). The lower limit of quantification was 20 ng/mL (signal-to-noise ratio > 5) for both enantiomers. Precision and accuracy of the method were determined at 0.02, 0.5, and 2 µg/mL (corresponding to 77.45 nM, 1.94 µM, and 7.745 µM, respectively). The precision (coefficient of variation) and accuracy (deviation of the true value from the mean value) were below 15% for all concentrations (*n* = 5). All response values were based on the ratio of peak height of the thalidomide enantiomers to that of anthracene. Anthracene was used as internal standard with a final concentration of 2 µg/mL.

After addition of 5.0 mL of extraction medium [dichloromethane:hexane 1:1(v/v)], the samples were shaken for 20 min. The organic layers were separated by centrifugation (3000 rpm, 0°C, 10 min) and then transferred to another glass tube. The solvent was evaporated under a N₂ stream at 40°C for 12 min. The residue was re-dissolved in 200 µL of methanol by means of vortexing and ultra-sonic. The solution was then filtered through a 0.2 µm filter and 80 µL was injected into the HPLC system.

For the investigation of bi-directional (apical-to-basolateral and basolateral-to-apical) transport, the apparent permeability values (*P*_{app}) were calculated as:

$$P_{\text{app}} = \Delta Q / \Delta t \times 1/A \times 1/C_0.$$

Initial slope, $\Delta Q / \Delta t$, was determined by plotting the amount of transported thalidomide enantiomer (micro-moles) as a function of time (s). The slope of the line was calculated using linear regression. Time points were 5, 10, 15, and 30 min. A is the surface of the filter (cm^2) and C_0 is the thalidomide concentration (μM) in the donor compartment.

For the determination of dose-dependent transport, the amount of each thalidomide enantiomer (nanomoles) in the basolateral compartment after 10 min was plotted against the applied concentration in the apical compartment. The correlation coefficients were determined by linear regression.

Results

In LS180 cells, thalidomide treatment had no effect on MDR1 expression. Incubation with 10 and 100 μM thalidomide for 72 h did not change the MDR1 mRNA transcript number compared to control (Fig. 1). Rifampicin, as a positive control for MDR1 induction, increased the expression 2.5-fold. The expression of the housekeeping gene GAPDH was not altered under any of the treatments (data not shown).

The uptake of the P-gp substrate rhodamine 123 in CCRF-CEM/MDR1 cells was not significantly changed by thalidomide treatment compared to control (Fig. 2). Thalidomide concentrations of 10, 100 and 300 μM did not influence P-gp function, whereas verapamil showed strong P-gp inhibition as the intracellular fluorescence of rhodamine increased about fivefold. At all applied

concentrations, thalidomide and verapamil exhibited no quenching regarding the fluorescence of rhodamine 123 (data not shown).

The bi-directional transport of 100 μM thalidomide across Caco-2 cell monolayers indicates that the permeability (P_{app}) from apical-to-basolateral side (A–B) was similar to that from basolateral-to-apical side (B–A) (Fig. 3). For (+)-thalidomide the P_{app} values (cm/s) were 6.89×10^{-5} and 5.70×10^{-5} , for (–)-thalidomide 6.90×10^{-5} and 5.67×10^{-5} , respectively. There was a trend towards increased A–B transport, however it was not statistically significant ($p > 0.1$). The enantiomers showed no differences in their permeability. The ratios P_{app} (B–A) over P_{app} (A–B), as a measure for active transport, were 0.826 and 0.822 for (+)- and (–)-thalidomide, respectively. Dose-dependent transport of 10, 50, 100, and 250 μM thalidomide after 10 min appeared to be linear. The correlation coefficients were > 0.999 for both enantiomers (Fig. 4).

Discussion

Drug transporters, such as P-glycoprotein, are increasingly recognised as an important determinant of drug disposition [24]. Like cytochrome P450 enzymes, inhibition and induction of P-gp have been reported as the cause of drug–drug interactions [20]. Here we showed that the drug thalidomide is not capable of inhibiting P-gp function or inducing its expression. This knowledge may be of importance, because thalidomide is a drug that is increasingly used in clinical studies, mainly as an adjuvant therapy.

Fig. 1 Expression of MDR1 in LS180 cells. Cells were incubated for 72 h. Rifampicin served as a positive control for MDR1 induction. Values represent mean expression ratios (MDR1 expression/GAPDH expression) \pm SEM; $N = 3$

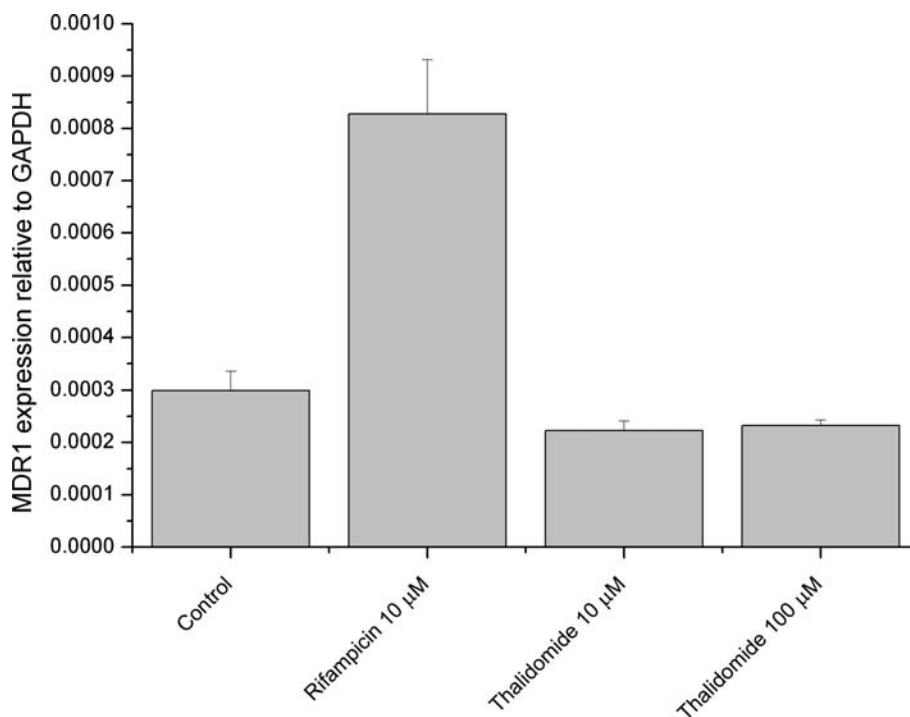


Fig. 2 Uptake of R123 in CCRF/MDR1 cells. Verapamil (100 μ M) was applied as a P-gp inhibitor. Values represent mean intracellular R123 fluorescence \pm SEM; $N = 4$

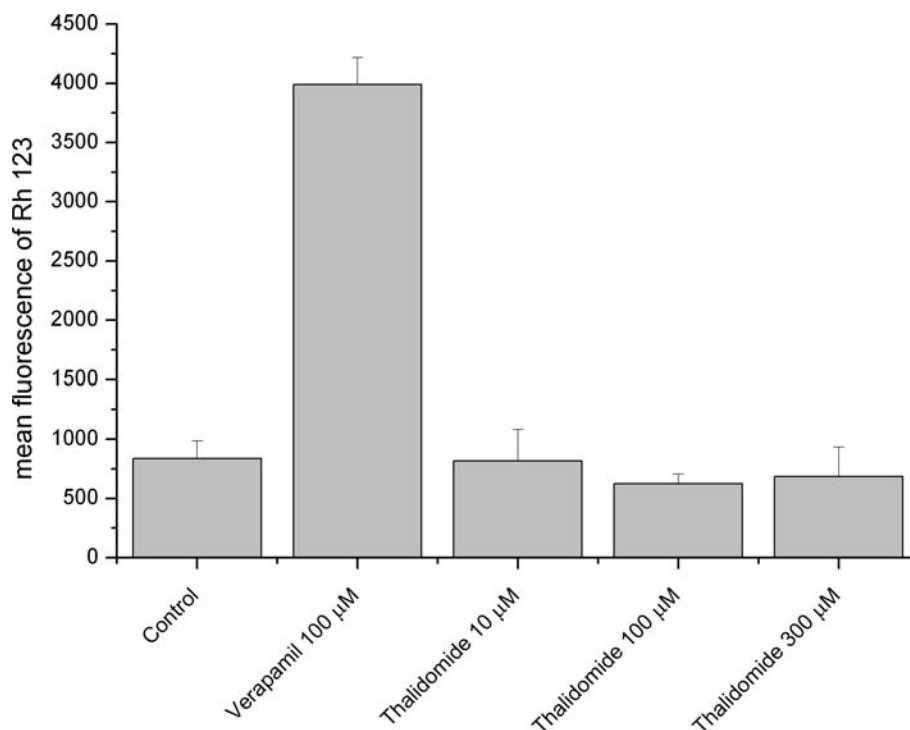
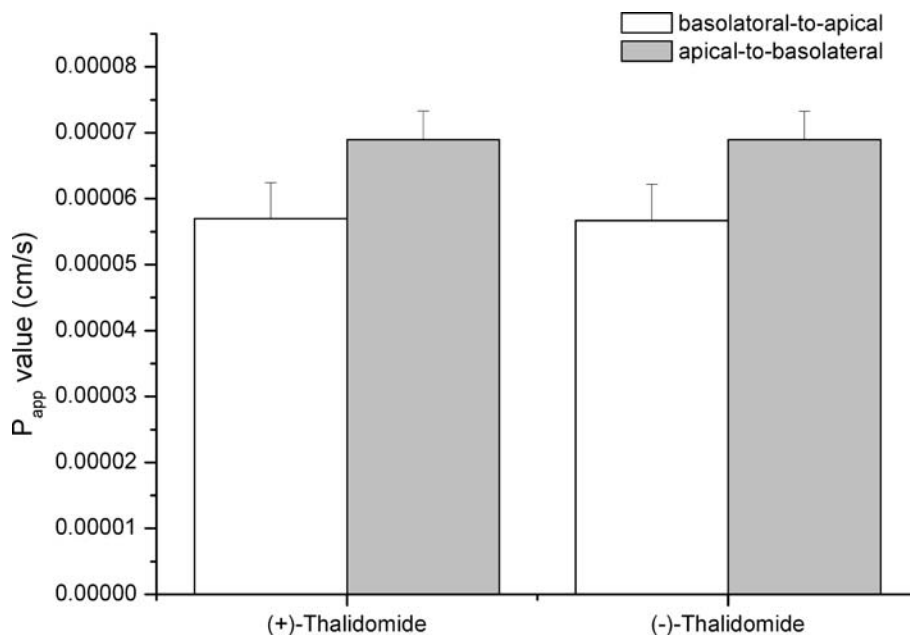


Fig. 3 Apparent permeability (P_{app}) of (\pm)-thalidomide (100 μ M) through Caco-2 cells. The drug was added to the basolateral side (B-A) or to the apical side (A-B) of the monolayers. Values represent mean \pm SEM; $N = 3$. Differences are not significant ($p > 0.05$)

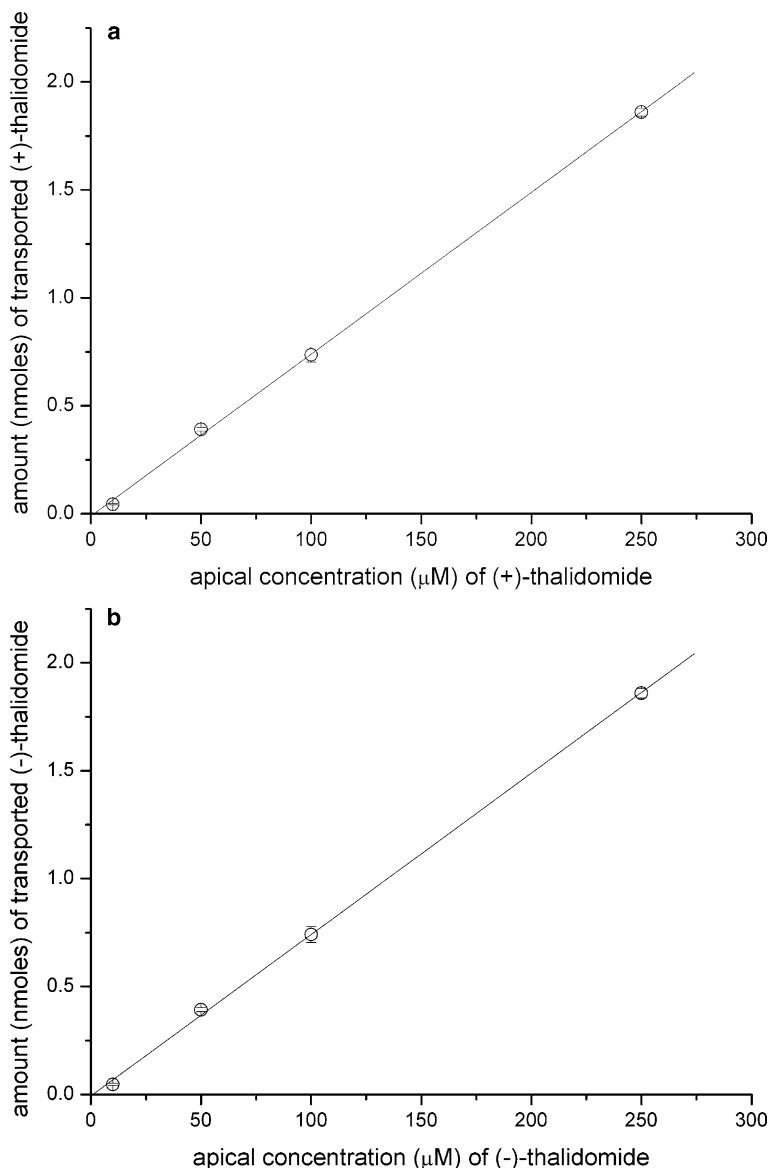


P-gp expression has been shown to be inducible by many xenobiotics such as rifampicin [25], phenobarbital [26], dexamethasone [27], and herbal extracts from St. John's wort [28, 29]. In this study, we used the LS180 cell line as an appropriate model to investigate P-gp induction. LS180 cells are derived from a human colon carcinoma cell line and it has been shown that several drugs such as rifampicin or phenobarbital could increase P-gp mRNA and protein content in these cells [30, 31]. Rifampicin, used as positive control in our experiments,

strongly induced MDR1 expression, whereas thalidomide did not change MDR1 mRNA levels.

Several cases of drug-drug interactions caused by P-gp inhibition have been reported. Elevated digoxin plasma concentrations were observed with cardiac drugs that are P-gp inhibitors such as verapamil or quinidine [32, 33]. Ketoconazole, a potent P-gp inhibitor, caused a marked increase in the CSF concentration of ritonavir and saquinavir [34]. By measuring the rhodamine 123 uptake in a cell line over-expressing MDR1, we used a

Fig. 4 Dose-dependent transport of thalidomide through Caco-2 cell monolayers. 10, 50, 100, and 250 μM (\pm)-thalidomide were added to the apical compartment. After 10 min the transported amounts (n moles) of (+)- and (–)-thalidomide were determined. Values represent means \pm SEM; $N = 3$



specific assay for the investigation of functional P-gp inhibition. Though this assay is not able to discriminate between substrates and inhibitors, it can be concluded that thalidomide does not interact with P-gp, neither as a substrate nor as an inhibitor. Verapamil, a known P-gp inhibitor *in vitro* and *in vivo*, was used as a positive control showing significant accumulation of rhodamine 123 in the cells.

At present, it is unclear whether transporters are involved in the absorption of thalidomide. We determined the bi-directional permeability and the dose-dependent transport of thalidomide enantiomers through Caco-2 cell monolayers. This cell line is generally accepted as *in vitro* model to study intestinal permeation [35]. Furthermore, Caco-2 cells are reported to express membrane transporters, such as P-gp and multi-drug associated proteins (MRPs) [30, 36]. Therefore, we used this model to assess if thalidomide underwent carrier-mediated transport and whether there is a difference in

the permeability between (+)- and (–)-thalidomide. The ratio P_{app} (B to A) over P_{app} (A to B) is a measure for active, apical directed transport. For paclitaxel, a known P-gp substrate, this ratio was reported to be 41.9 in Caco-2 cells [37]. For thalidomide, we observed equal transport for both directions of the monolayer leading to a P_{app} ratio of 0.82. In a previous study, where thalidomide transport through Caco-2 cells was also investigated, similar results were obtained [38]. In addition, they showed that verapamil had no effect on thalidomide permeability. A drawback of their study, however, was the lack of chiral separation. The present study is the first one investigating the transepithelial transport of thalidomide including chiral separation of the enantiomers.

Due to the fast chiral inversion at physiological pH, the stereoselective absorption of thalidomide can hardly be determined *in vivo*. In order to avoid racemisation, the samples in our *in vitro* experiments were instantly

acidified when drawn from the acceptor chamber [23]. Since both enantiomers share the same physical and chemical properties, different absorption rates could indicate an interaction of thalidomide with chiral structures such as proteins. Our results show, however, that there is no difference in the permeability of the enantiomers.

In conclusion, thalidomide exhibited no functional interaction with P-gp in our *in vitro* experiments. Furthermore, thalidomide did not induce P-gp expression. Drug–drug interactions due to these mechanisms are therefore unlikely. Transport studies showed linear permeability that appeared to be neither site-directed nor stereoselective. These results suggest that passive diffusion is predominantly involved in thalidomide absorption. Luminal efflux pumps like P-gp seem not to restrict oral thalidomide uptake, which could also explain the high bioavailability found *in vivo*. Moreover, the efficacy of thalidomide in cancer therapy is most likely not limited by P-gp over-expression in tumour tissues. Whether thalidomide has an affinity to other transporters cannot be ruled out by this study and further investigations are required.

References

- Sheskin J (1965) Further observation with thalidomide in lepra reactions. *Lepr Rev* 36:183–187
- Meierhofer C, Wiedermann CJ (2003) New insights into the pharmacological and toxicological effects of thalidomide. *Curr Opin Drug Discov Devel* 6:92–99
- Moreira AL, Sampaio EP, Zmuidzinas A, Frindt P, Smith KA, Kaplan G (1993) Thalidomide exerts its inhibitory action on tumor necrosis factor alpha by enhancing mRNA degradation. *J Exp Med* 177:1675–1680
- Rowland TL, McHugh SM, Deighton J, Dearman RJ, Ewan PW, Kimber I (1998) Differential regulation by thalidomide and dexamethasone of cytokine expression in human peripheral blood mononuclear cells. *Immunopharmacology* 40:11–20
- Kedar I, Mermershtain W, Ivgi H (2004) Thalidomide reduces serum C-reactive protein and interleukin-6 and induces response to IL-2 in a fraction of metastatic renal cell cancer patients who failed IL-2-based therapy. *Int J Cancer* 110:260–265
- Meierhofer C, Dunzendorfer S, Wiedermann CJ (2001) Theoretical basis for the activity of thalidomide. *BioDrugs* 15:681–703
- Singhal S, Mehta J (2002) Thalidomide in cancer. *Biomed Pharmacother* 56:4–12
- Franks ME, Macpherson GR, Figg WD (2004) Thalidomide. *Lancet* 363:1802–1811
- Eriksson T, Bjorkman S, Roth B, Fyge A, Hoglund P (1998) Enantiomers of thalidomide: blood distribution and the influence of serum albumin on chiral inversion and hydrolysis. *Chirality* 10:223–228
- Eriksson T, Bjorkman S, Hoglund P (2001) Clinical pharmacology of thalidomide. *Eur J Clin Pharmacol* 57:365–376
- Schumacher H, Smith RL, Williams RT (1965) The metabolism of thalidomide: the fate of thalidomide and some of its hydrolysis products in various species. *Br J Pharmacol* 25:338–351
- Eriksson T, Bjorkman S, Roth B, Bjork H, Hoglund P (1998) Hydroxylated metabolites of thalidomide: formation *in-vitro* and *in-vivo* in man. *J Pharm Pharmacol* 50:1409–1416
- Teo SK, Sabourin PJ, O'Brien K, Kook KA, Thomas SD (2000) Metabolism of thalidomide in human microsomes, cloned human cytochrome P-450 isozymes, and Hansen's disease patients. *J Biochem Mol Toxicol* 14:140–147
- Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM (1999) Biochemical, cellular, and pharmacological aspects of the multidrug transporter. *Annu Rev Pharmacol Toxicol* 39:361–398
- Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC (1987) Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci U S A* 84:7735–7738
- Cordon-Cardo C, O'Brien JP, Boccia J, Casals D, Bertino JR, Melamed MR (1990) Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. *J Histochem Cytochem* 38:1277–1287
- Takara K, Sakaeda T, Yagami T, Kobayashi H, Ohmoto N, Horinouchi M, Nishiguchi K, Okumura K (2002) Cytotoxic effects of 27 anticancer drugs in HeLa and MDR1-overexpressing derivative cell lines. *Biol Pharm Bull* 25:771–778
- Lee CG, Gottesman MM, Cardarelli CO, Ramachandra M, Jeang KT, Ambudkar SV, Pastan I, Dey S (1998) HIV-1 protease inhibitors are substrates for the MDR1 multidrug transporter. *Biochemistry* 37:3594–3601
- Borst P, Zelcer N, van Helvoort A (2000) ABC transporters in lipid transport. *Biochim Biophys Acta* 1486:128–144
- Lin JH (2003) drug–drug interaction mediated by inhibition and induction of P-glycoprotein. *Adv Drug Deliv Rev* 55:53–81
- Fanelli M, Sarmiento R, Gattuso D, Carillio G, Capacetti B, Vacca A, Roccaro AM, Gasparini G (2003) Thalidomide: a new anticancer drug?. *Expert Opin Investig Drugs* 12:1211–1225
- Juliano RL, Ling V (1976) A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta* 455:152–162
- Eriksson T, Bjorkman S (1997) Handling of blood samples for determination of thalidomide. *Clin Chem* 43:1094–1096
- Fromm MF (2000) P-glycoprotein: a defense mechanism limiting oral bioavailability and CNS accumulation of drugs. *Int J Clin Pharmacol Ther* 38:69–74
- Westphal K, Weinbrenner A, Zschiesche M, Franke G, Knoke M, Oertel R, Fritz P, von Richter O, Warzok R, Hachenberg T, Kauffmann HM, Schrenk D, Terhaag B, Kroemer HK, Siegmund W (2000) Induction of P-glycoprotein by rifampin increases intestinal secretion of talinolol in human beings: a new type of drug/drug interaction. *Clin Pharmacol Ther* 68:345–355
- Lu Y, Yan Y, Wang XF (2004) Antiepileptic drug-induced multidrug resistance P-glycoprotein overexpression in astrocytes cultured from rat brains. *Chin Med J (Engl)* 117:1682–1686
- Fardel O, Lecureur V, Guillouzo A (1993) Regulation by dexamethasone of P-glycoprotein expression in cultured rat hepatocytes. *FEBS Lett* 327:189–193
- Zhou S, Chan E, Pan SQ, Huang M, Lee EJ (2004) Pharmacokinetic interactions of drugs with St John's wort. *J Psychopharmacol* 18:262–276
- Pfrunder A, Schiesser M, Gerber S, Haschke M, Bitzer J, Drewe J (2003) Interaction of St John's wort with low-dose oral contraceptive therapy: a randomized controlled trial. *Br J Clin Pharmacol* 56:683–690
- Pfrunder A, Gutmann H, Beglinger C, Drewe J (2003) Gene expression of CYP3A4, ABC-transporters (MDR1 and MRP1-MRP5) and hPXR in three different human colon carcinoma cell lines. *J Pharm Pharmacol* 55:59–66
- Schuetz EG, Beck WT, Schuetz JD (1996) Modulators and substrates of P-glycoprotein and cytochrome P4503A coordinately up-regulate these proteins in human colon carcinoma cells. *Mol Pharmacol* 49:311–318
- Mordel A, Halkin H, Zulty L, Almog S, Ezra D (1993) Quinidine enhances digitalis toxicity at therapeutic serum digoxin levels. *Clin Pharmacol Ther* 53:457–462

33. Verschraagen M, Koks CH, Schellens JH, Beijnen JH (1999) P-glycoprotein system as a determinant of drug interactions: the case of digoxin-verapamil. *Pharmacol Res* 40:301–306
34. Khaliq Y, Gallicano K, Venance S, Kravcik S, Cameron DW (2000) Effect of ketoconazole on ritonavir and saquinavir concentrations in plasma and cerebrospinal fluid from patients infected with human immunodeficiency virus. *Clin Pharmacol Ther* 68:637–646
35. Artursson P, Palm K, Luthman K (2001) Caco-2 monolayers in experimental and theoretical predictions of drug transport. *Adv Drug Deliv Rev* 46:27–43
36. Gutmann H, Fricker G, Torok M, Michael S, Beglinger C, Drewe J (1999) Evidence for different ABC-transporters in Caco-2 cells modulating drug uptake. *Pharm Res* 16:402–407
37. Hugger ED, Audus KL, Borchardt RT (2002) Effects of poly(ethylene glycol) on efflux transporter activity in Caco-2 cell monolayers. *J Pharm Sci* 91:1980–1990
38. Zhou S, Li Y, Kestell P, Paxton JW (2003) Determination of thalidomide in transport buffer for Caco-2 cell monolayers by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 785:165–173