The Impact of the Induction of Multidrug Resistance Transporters in Therapies by Used Drugs: Recent Studies

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Abstract: Multidrug resistance (MDR) against groups of therapeutic drugs emerged to a central problem in the treatment of various diseases, i.e. cancer and infectious diseases like HIV or malaria. ABC transporters namely P-glycoprotein (P-gp) and various multidrug resistance associated proteins (MRPs) mainly contribute to the MDR phenomenon in cancer treatment and HIV therapy. Their cellular expression in respective cells like cancer cells lowers the intracellular drug concentrations and thus reasons the cellular resistance. The induction of such efflux pumps occurs during the therapy with drugs which will be affected by the MDR phenomenon as a consequence of the induction. In this review studies which report such drugs-caused inductions will be viewed. The review will cover the literature of recent years and attract attention to this important question in drug resistance. Finally, the discussion will suggest possible strategies to overcome the problem, i. e. by using non-inducing drugs.

Keywords: Drug therapy, efflux pumps, multidrug resistance, MDR modulators, transporter induction studies, transporter substrates.

INTRODUCTION

MDR emerged as a central problem in the treatment of various diseases [1-4]. Formerly effective drugs lose their efficiency in early states of drug therapy [5,6]. Consequently higher drug dosages cause toxic side effects so that a therapy regime has to be changed by the use of other mainly novel drugs with a different structure or a modified mode of action within the disease progression [4,7,8]. In the case of cancer the addressing of novel target structures with novel drugs like tyrosine kinase inhibitors or the use of non-peptidic drugs in the case of the antiretroviral treatment of HIV with HIV-1 protease inhibitors was disappointing because the novel drugs were also affected by the MDR phenomenon [2,3,9-11].

One main contribution to the MDR problem is made by the overexpression of transmembrane efflux pumps. They transport drugs out of the cells so that the intracellular therapeutically relevant drug levels are not reached [5,6,12]. The main MDR-causative efflux pumps have been P-glycoprotein (ABCB1), the multidrug resistance associated protein (MRP) 1 (ABCC1) and the breast cancer resistance protein (BCRP), all belonging to the group of ABC transporters [6,12,13]. These efflux pump proteins transport structurally different drugs out of the cells including novel therapeutics also [2,14]. ABC transporters are found in many human tissues. They are responsible for excretion processes and the detoxification of drugs or metabolites. ABC transporters are also involved in cellular distribution processes [13,15-17].

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The number of novel drugs is limited and novel drugs were furthermore shown to fail in the treatment of MDR-affected diseases so that one strategy to overcome the MDR phenomenon has been the development of modulators of the efflux pump activities [12,18,19]. Early MDR modulators which inhibited the activity of efflux pumps showed undesired pharmacological effects. They belonged to various drug families like verapamil and nifedipine as antihypertensive agents or cyclosporine A as an immunosuppressive drug. Such MDR modulators have been structurally modified to reduce their originally pharmacological effects [18-21]. Thus their usage in clinical studies was strongly dose-limited [7,8,18-20]. Another problem was the limited selectivity of the ABC transporter inhibition that led to an enhanced and critical involvement in the excretion of conjugated drugs by related transporters of the ABCC family like MRP2 (ABCC2) or MRP3 (ABCC3) [21-23]. Recently reviewed MDR modulators like zosuquidar or tariquidar presently undergo clinical trials but partly suffer from toxicity problems [19, 24].

One reason for the emerging resistance under drug therapy has been the induction of the transport protein expression by the drugs themselves [25-27]. In the following, recent studies which address the drug induction problems will be viewed. The mode of the transcriptional control of ABC transporters and various mechanisms in drug-resistant cancer chemotherapy have been reviewed in recent reports [2,25,28,29]. However, the ABC transporter inducing properties by the drugs used in therapy have still not been investigated. The following look at the recent years of reported induction studies reveals that some substrates of ABC transporters also act as inducers. Most ABC transporter substrates have not been investigated to induce relevant transport proteins. So the contribution of possible drug induction to occurring drug resistance is not clear. The following review will show that

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the induction-phenomenon plays a central role in the occurring drug resistance under drug therapies.

HIV THERAPY

HIV-1 Protease Inhibitors

HIV-1 protease inhibitors play an important role in the therapy of the HIV-1 infection [3]. One central problem in the HIV therapy is the occurence of viral sanctuaries in brain and in the testis tissue [3]. The expression of ABCB1 at the blood brain barrier and in the testis tissue was found as reason for a limited penetration of the HIV-1 protease inhibitors into both the brain and the testes [30,31]. The fact that all HIV-1 protease inhibitors have been substrates of ABCB1 was demonstrated by the use of effective ABCB1 inhibitors like cyclosporine A or PSC 833, a less immunosuppressive derivative of cyclosporine A [32]. Ritonavir was early identified as a strong ABCB1 inhibitor (Fig. **1**) [33]. Ritonavir gained an established role in the HIV-1 therapy as coadministered agent because of its ABCB1-inhibiting and CYP3A4-inhibiting properties [34-37]. Thus, an increase in the cellular and intestinal uptake of other HIV-1 protease inhibitors could be observed under ritonavir coadministration. These increases resulted from the ABCB1 inhibition and the inhibition of the intestinal metabolism by ritonavir [34,38]. However, ritonavir disappointed in *in vivo* studies. Earlier cellular studies demonstrated a ritonavir induction of ABCB1 parallel to the inhibition of ABCB1 [31,39]. Western blot analysis proved increased protein levels. An increase of cellular RNA levels was shown by northern blot analysis [39]. Recent studies of Perloff *et al.* in bovine brain microvessel endothelial cells demonstrated a concentration dependent decrease of the intracellular uptake of the ABCB1 substrate rhodamine 123 by fluorescence microscopy [40]. An increase in the expression of ABCB1 was demonstrated by Western blot analysis thus proving a dependency of a lowered ABCB1 substrate uptake and the increasing cellular ABCB1 levels caused by the increasing ritonavir concentrations in the pretreated cells. So the HIV-1 protease inhibitor induction of ABCB1 both at the blood brain barrier and in the testis tissues may play an important role in the occurring inhibitor resistance.

In following studies of Zastre *et al.* the effects of atazanavir, a newer HIV-1 protease inhibitor, was investigated in various efflux transporter-overexpressing cell lines. The studies included the human brain microvessel endothelial cell line hCMEC/D3 as an *in vitro* model for the human blood brain barrier [41]. Ritonavir has been used for comparison. In both ABCB1 and ABCC1 overexpressing cell lines, namely MDA435/LCC6-MDR1 and HeLa-MRP1, a lowered cellular accumulation of atazanavir and ritonavir was observed if compared to the uptake into the non-overexpressing cell lines.

These results proved that the cellular uptake of atazanavir is also mediated by the transporter efflux pumps ABCB1 and ABCC1.

The influence of the ABCB1 inhibitor PSC 833 and of the ABCC inhibitor MK571 was investigated in the hCMEC/D3 brain microvessel endothelial cell line which expresses both ABCB1 and ABCC1. The inhibitors showed different effects. While the cellular accumulation of both HIV-1 protease inhibitors was increased by the use of PSC 833 the used ABCC inhibitor MK571 showed no effects.

Long term induction studies of the transport proteins over 72 h have been carried out with the hCMEC/D3 brain microvessel endothelial cell line.

The HIV-1 protease inhibitors and each rifampin and SR12813 as ligands of the pregnane x receptor (SXR) were used for preincubation. SXR is kown to be involved in the induction of ABCB1 by earlier studies done in the human intestinal adenocarcinoma cell line LS180 [42]. Both, the used drugs and the ligands led to increased protein levels of ABCB1 determined by Western blot analysis. So a role of the ligands and the protease inhibitors in the overexpression of ABCB1 was documented for the brain microvessel endothelial cell line which is suggested to be mediated by SXR. No effects have been observed in the expression rates of ABCC1.

Finally, the potential of the HIV-1 protease inhibitors atazanavir and ritonavir was tested to increase the cellular accumulation of the fluorescent ABCB1 substrate rhodamin 6G in the ABCB1 induced overexpressing cell line hCMEC/D3. However, both effective inhibitors in the non-overexpressing cell line lose their potential in the induced cell line. An inhibition of ABCB1 was only reached by the use of PSC 833.

These results document that the effect of an ABCB1 inhibition by a known ABCB1 inhibitor is lowered after long-term treatment with the inhibitor due to the induction of ABCB1. So the question of a concentration dependent effect on both the inhibition and the induction of transport proteins has to be considered. Another consequent aspect may be a restoration of the inhibitory effects by the use of increasing inhibitor concentrations which mainly depends on critical toxic effects of the used inhibitor.

Non-Nucleoside Reverse Transcriptase Inhibitors

Weiss *et al.* also investigated induction studies for efavirenz to a wide range of ABC drug transporters [43]. Efavirenz is also used as an agent in the highly active antiretroviral therapy (HAART) against HIV-1 infection. It is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and is mostly combined with two nucleoside reverse transcriptase inhibitors (NRTIs). The combination of different drugs results in a strong antiretroviral activity. Changes in plasma level of the drugs caused by a non-compliance of the patients or by cellular resistance mechanisms imperils the success of the antiretroviral therapy. Weiss *et. al.* investigated the time-dependent effect on mRNA levels of different drug transporters using 10 μM of efavirenz which is also a well known inducer of cytochrome P450 enzymes (CYPs). After one week changes in the ABCB1, the breast cancer resistance protein (BCRP) ABCG2 expression and the CYP3A4 expression were observed with increasing mRNA levels by the two- to six-fold. After 4 weeks of efavirenz treatment increased mRNA levels were measured also for other drug transporters like ABCC1-5. However, the changes in transporter expressions did not alter the intracellular efavirenz concentration because it is known that efavirenz does not act

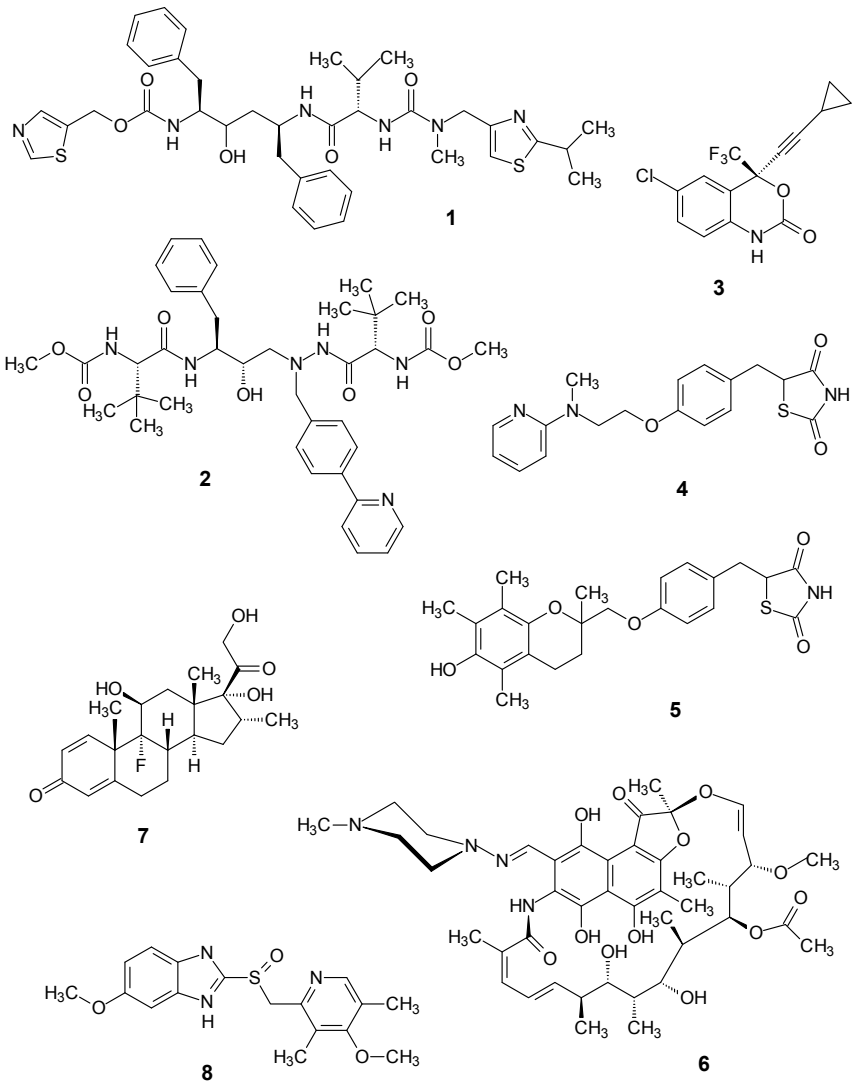

Fig. (1). Structures of efflux pump-inducing drugs namely ritonavir (**1**), atazanavir (**2**), efavirenz (**3**), rosiglitazone (**4**), troglitazone (**5**), rifampicine (**6**), dexamethasone (**7**), omeprazole (**8**) and delavirdine (**9**).

as a substrate for transporters like ABCB1 und ABCC1 [44]. It is more critical that the altered transporter expressions can influence the kinetics and efficiency of coadministered drugs like HIV-1 protease inhibitors which are well known substrates for ABCB1. Ritonavir as an important member of the HIV-1 protease inhibitor group is often coadministered with other HIV drugs to increase plasma levels by inhibiting the ABCB1 efflux pump activity as discussed. Delavirdine is another NNRTI which turned out to induce ABCB1 expression in LS180 cells as reported by Weiss *et al*. The observed increase in the cellular ABCB1 activity was about five-fold if compared to the untreated non-induced control after long-term incubation of 168 h. However, the necessary amounts of delavirdine for such ABCB1-inducing effects was 100 μM. This concentration is a non-realistic one with respect to *in vivo* studies [45].

In recent clinical studies earlier described effects of an ABC transporter induction has been discussed under the ongoing application of ritonavir and tipranavir, a nonpeptidic HIV-1 protease inhibitor. The co-medication of both HIV-1 protease inhibitors with the ABCB1 substrates loperamide and digoxin led to main decreases in the bioavailibilities of loperamide (51% under ritonavir and 63% under tipranavir co-medication, determined as AUC (area under the curve)) and of digoxin determined as AUC and C_{max} as highest plasma concentrations [46,47]. The discussed ABCB1 inducing effects have been attributed to tipranavir because ritonavir alone led to increased levels of loperamide (121%) under the same medication conditions.

OTHER EFFLUX PUMP INDUCING DRUGS

Cellular Studies

Beside antiretroviral drugs also other classes of medication contribute to ABC transporter inducing effects. Thiazolidinediones (glitazones) used as antidiabetic drugs are known inhibitors of the breast cancer resistance protein (BCRP) ABCG2 and of ABCB1 (Fig. **1**) [48,49].

Recently, it was demonstrated that a 4-days exposure of HuH-7 cells to the thiazolidinediones rosiglitazone and troglitazone induce a concentration-dependent mRNA expression of BCRP (ABCG2) [49]. No changes were observed in the extent of ABCB1 expression. Although the ABCG2 transcription is also regulated by other transcription factors Szatmari *et al.* demonstrated that the induction of ABCG2 by glitazones is most likely mediated by the nuclear peroxisome proliferator-activated receptor [50]. It is also possible that the pharmacokinetics of other drugs will be influenced through changes in the ABCG2 expression. Especially other antidiabetic drugs like biguanides and sulfonylureas as well as antihypertensive and lipid-lowering drugs are often used in combination-drug regimes. Thus, glitazones with increasing effects on the ABCG2 expression have a higher potential for drug-drug interactions.

Beside ABCB1 and ABCG2 members of the ABCC family are also involved in MDR. Most notably the MRP1 (ABCC1) protein which is encoded by the ABCC1 gene has been demonstrated to transport glutathione conjugates of many toxic xenobiotics [51]. It was also shown that ABCC1 is widely overexpressed in tumour cells and causes resistance to anticancer agents [52]. The inducibility of ABCC1 and also of MRP2 (ABCC2) and of ABCB1 was investigated by Nishimura *et. al.* using primary cultures of human and cynomolgus monkey hepatocytes [53]. Changes in mRNA levels following exposure to different concentrations of rifampicin, dexamethasone and omeprazole were measured. After a short incubation time of 24 h an increase in the ABCB1, ABCC1 and ABCC2 mRNA levels up to 3-fold was observed under rifampicin and omeprazole treatment. For cell exposure to dexamethasone only poor effects on the MRP2 gene expression were measured. Omeprazole has been investigated as an effective co-medication to prevent chemotherapy-induced gastroduodenal injuries [54]. An omeprazol co-mediaction with anticancer drugs may be a risk for the anticancer therapy. Many anticancer drugs, like doxorubicin, etoposid, mitomycin, mitoxantrone or paclitaxel function as substrates for the ABC transporter ABCB1. An up-regulation of this transporter could lead to MDR mediated by the increased efflux pump activity as a consequence of the induction.

Inhibitor Inducing Studies

Up to now much more drugs and inhibitors of the efflux pump activities have been identified than inducers [55]. However, until now the number of known inducers is strongly limited. This makes it rather difficult to predict whether a new drug or a used inhibitor may act as a potential inducer of an efflux pump protein formation. Additionally, some studies still concentrated on the mRNA levels without an investigation of the actual protein amount increases and without determining the changes in the activity of the important and decisive extracellular drug transport and thus the protein functionality.

The limited number of characterized inducing drugs mainly weakened the results from a recent in silico analysis of ABCB1 inducers [55]. The analysis of the very few drugs concluded the following structure-activity relationships (SARs): A certain number of aromatic rings and a sufficient lipophilicity characterized as logD value with about 4.0 both are favourable molecular properties of inducing compounds [55].

The summarized situation of limited precise knowledge about certain compound characteristics for induction makes it necessary to analyse new inhibitors concerning their properties to effectively inhibit an efflux pump target protein, to induce the protein and finally consider possible substrate properties. In cases where an inducing agent is a substrate of an efflux pump its pharmacokinetic is influenced by this fact because the clinical usage will be limited by an increased cellular excretion from the transport protein expressing cells and, moreover, from the induced cells by an increased extracellular transport.

ADVANCES WITH NOVEL NON-INDUCING DRUGS

Recently a novel series of MDR modulators has been developed by Hilgeroth *et al.*. Structurally they are cage dimeric 1,4-dihydropyridines given by double reactions of the 1,4-dihydropyridine double bonds [56]. Early derivatives have been reported by us to inhibit the activity of the efflux pump ABCB1 in an ABCB1-overexpressing mouse T lymphoma cell line in comparison to the non-expressing cell line. We have shown that the effect of ABCB1 inhibition was a transporter-specific effect because the resistance-mediating human MDR1 gene had been introduced into the cell line by a retrovirus-gene transfection. The effects of ABCB1 inhibition were more effective than those observed for cyclosporine A in a gastric carcinoma cell line (EPG85-257P) proved by an increased uptake of the fluorescent cytostatic agent daunorubicin as a known ABCB1 substrate [27]. Recent derivatives proved to selectively inhibit ABCB1. Hydrogen bond acceptor functions and their positioning within the molecular framework as well as lipophilicity patterns were characterized as important features for influencing SARs of the ABCB1 inhibition [13]. The inhibition of related efflux pumps of the ABCC family like ABCC1 and ABCC2 was poor and had been determined in overexpressing cell lines (A27080CIS) given by induction treatments with cisplatin and the different uptake rates of the ABCC substrate carboxyfluorescein in comparison to the untreated control cell line [12]. Furthermore, the affection of ABCG2 was investigated in an ABCG2-overexpressing cell line (EPG85-257NOV). The strongest ABCB1 inhibitors did not act as an ABCBG2 inhibitor, so that a transporter-specific inhibition was proved.

We selected the most effective ABCB1 inhibitors H17 and JW41 to investigate their ability to induce ABCB1 in various cancer cell lines (Fig. **2**) [27].

First the MDR1 gene mRNA was quantified using the real-time quantitative polymerase chain reaction (RTQ-PCR) technique. An ABCB1 non-expressing gastric carcinoma cell line (EPG85-257P), a colon carcinoma cell line with a known ABCB1 expression and an already ABCB1-induced cancer cell line (EPG85-257RDB) cell line were investigated [27]. The induced gastric carcinoma cell line proved to have a significantly higher count of MDR1 gene transcripts by a factor of about 2000 if compared to the untreated control cell line (EPG85-257P) [27]. The known ABCB1-expressing

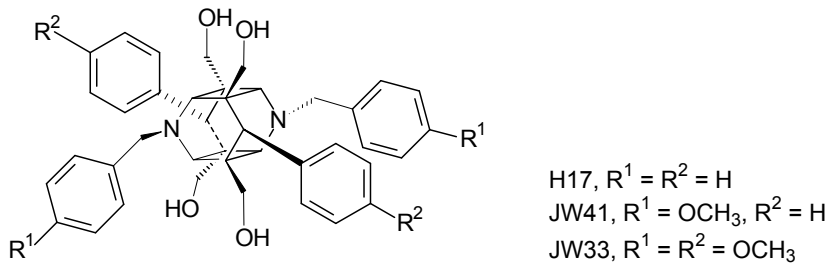

Fig. (2). Structures of MDR modulating agents without efflux pump-inducing properties, namely cage dimeric 1,4-dihydropyridines H17, JW 41 und JW33.

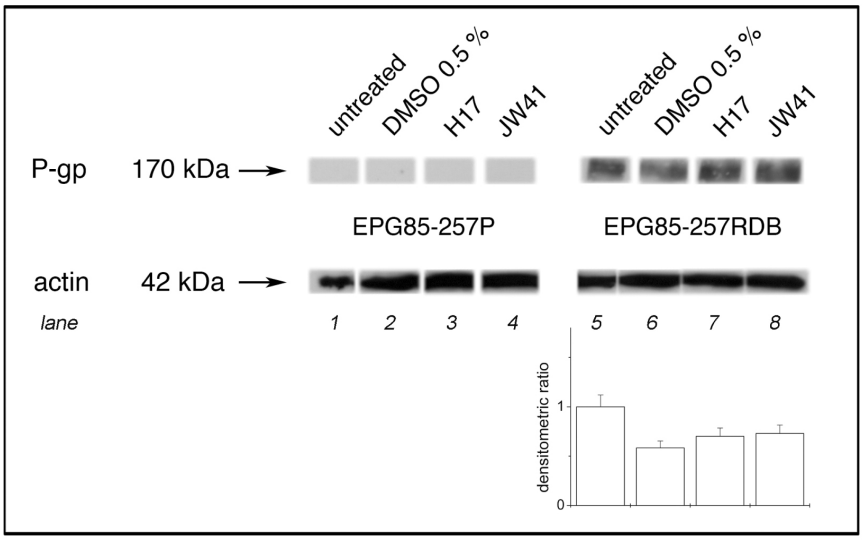

Fig. (3). Western blot analyses of the gastric carcinoma cell line (EPG85-257P) before induction (lane 1), with 0.5% DMSO (lane 2), after incubation with compounds H17 (lane 3) and JW41 (lane 4), respectively, after induction with daunorubicin (lane 5) and after treatment of the induced cell with 0.5% DMSO (line 6), incubation with compound H17 (lane 7) and with JW41 (lane 8), respectively [27]. A densitometric analysis of the bands is given below.

colon carcinoma cell line showed lowered MDR1 gene-transcript levels if compared to the already induced gastric carcinoma cell line. Then induction studies have been carried out with compounds H17 and JW41 for 72 h known from earlier studies as a long time of preexposure. The MDR1 gene expression rates were determined using RTQ-PCR technique and proved no induction for both derivatives in all cell lines. The mRNA ratios were unchanged and even lower than those obtained for the used DMSO control. These results were confirmed by Western blot analysis for all cell lines [30]. In the non-ABCB1-expressing gastric carcinoma cell line there were no detectable amounts of ABCB1 after pretreatment with the compounds JW17 und JW41 (Fig. **3**).

A strong ABCB1 band was detected in the induced cell line (EPG85-257RDB). The preincubation with both compounds led to almost unchanged amounts of detectable ABCB1 which were proved by densitometric analysis. Due to the lowered mRNA levels in the colon carcinoma cell line the detectable amounts of ABCB1 appeared as a smaller band in the Western blots (Fig. **4**).

Also the preincubation with both compounds H17 and JW46 in this cell line led to almost unchanged amounts of detectable protein which were also demonstrated by densitometric analysis. The later results have additionally been proved by a Northern blot analysis of the pretreated colon carcinoma cell line which resulted in similar RNA levels for the untreated cell line if compared with the compound pretreated cells [27].

The studies with the new cage dimers finally included a functionality assay with the colon carcinoma cell line for different concentrations of the compounds during the preincubation period. The effect of the cellular uptake of the fluorescent ABCB1 substrate rhodamine 123 was compared and it was found almost unchanged for the different concentrations which meant that no concentration-dependent induction occurred because such a concentration-dependent induction would have led to lowered uptake ratios of the fluorescent ABCB1 substrate due to increased efflux rates from the cells [27].

The question of being substrates of an efflux pump is important with reference to possible lowered activities of an inhibitor in the case of protein induction as has been discussed and shown in our recent studies by Coburger *et al.* with ritonavir [4]. Cage dimeric 1,4-dihydropyridines have been documented to be potential HIV-1 protease inhibitors [57]. They may dually act both as HIV-1 protease inhibitors and as ABCB1 inhibitors in HIV therapy regimes similar to ritonavir. The central question of their benefit in such an

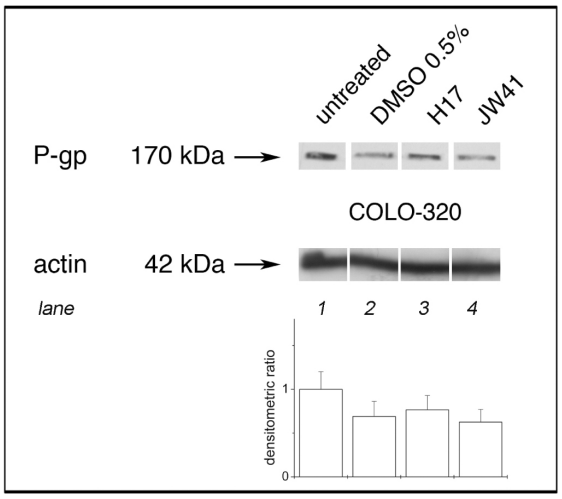

Fig. (4). Western blot analyses of the colon carcinoma cell line (COLO-320) before induction (lane 1), with 0.5% DMSO (lane 2) and after incubation with compounds H17 (lane 3) and JW41 (lane 4), respectively [27]. A densitometric analysis of the bands is given below.

application mainly depends on the question of being substrates of the efflux pump and their toxicity which may limit their application as has been reported for ritonavir [39].

Our cytotoxic studies determined in the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay proved that one of the relevant candidates JW33 with highest ABCB1 inhibiting properties if compared to ritonavir showed no toxic effects in various cell line models like hepG2 cells, drug-sensitive Jurkat cells and the gastric carcinoma cells EPG85-257P. The ABCB1 substrate properties were investigated in a model of two gastric carcinoma cell lines by the comparison of the cytotoxic effects of the tested compounds. The difference between the two cell lines was the only expression of ABCB1 which has been induced by the pretreatment of the cell line EPG85-257P with daunorubicin. Ritonavir which is a known ABCB1 substrate in literature was less toxic in the ABCB1-expressing cell line due to the fact that it is transported out of this cell thus affording a higher application concentration to achieve a toxic effect determined as IC_{50} value of a reduced cell viability.

Some of our investigated cage dimers including H17 and JW41 showed no ABCB1 substrate properties because the application concentrations for receiving the same cytotoxic effects were similar in both the ABCB1-expressing cell line and the non-expressing cell line.

So it may be expected that a failure of selected cage dimeric 1,4-dihydropyridines in further *in vivo* studies will not be observed due to their favourable properties of non-inducing the relevant ABCB1 efflux pump and not being transported by ABCB1.

DISCUSSION

The knowledge of the induction of ABC transporters increased during the last years. So beside known mechanisms of a discussed protein kinase C (PKC) activation and of cellular stress components the influence of SXR was further investigated. The demonstrated inductions of cytostatic drugs on both the mRNA and the protein level convincingly proved a role of SXR in the drug-inducing processes. It would be very helpful to investigate SARs of transport protein-inducing drugs which act as SXR ligands. If there is closer insight what structural properties in such drugs are favourable for binding to this receptor structural modifications may be investigated to reduce the receptor affinity and thus the drug potential to induce the corresponding transport protein. Interestingly, the additional role of SXR in the induction of the metabolizing enzyme CYP3A4 emphasizes that not only an induction of an ABC transporter is a problem for an effective MDR therapy with an inducing drug. Also the occurring induction of metabolizing enzymes is critical because a changed metabolism for co-administered drugs will result and cause changed pharmacokinetic properties of drugs and partly toxic effects in anticancer therapies by increasing concentrations of toxic drug metabolites. So there will be a great benefit from such SARs investigations because the negative influence of drugs on the undesired induction of metabolizing enzymes may also be reduced.

Many substrates of ABC transporters are known and have been considered. It will be helpful to analyse their transporter inducing properties by bioanalysis on both the RNA and protein level to get complex insight in the so far unknown role of transporter substrates as inducers. More experimental data will help to analyse SARs and molecular properties for inducing an ABC transporter.

It became obvious that the induction of ABC transporters may be affected by differently acting drugs. If theoretical calculations are made to predict drug activities they are based on a given set of compounds which have to be tested in the same test model or system. Drugs can only be compared if their mode of action towards an efflux pump target structure like the induction is the same. Attempts to predict induction properties for anticancer drugs are likely to fail because we know that different modes of influencing the drug induction process are possible.

The role of the induction of ABC transporters during the antiretroviral HIV therapy has attracted main attention because of an increasing prevalence of HIV dementia [61]. The induction of ABCB1 at both the mRNA level and the protein level *in vitro* and *in vivo* by the use of HIV-1 protease inhibitor shows the emerging problem of transporter induction during therapy resulting in the formation of the central nervous system as a viral sanctuary site. Moreover, a non-nucleoside transcriptase inhibitor proved to induce several transporters including ABCC4 which is made responsible for a resistance development against nucleoside transcriptase inhibitors.

The difficult treatment of HIV with several drugs in changing drug regimes is a great problem. The analysed induction problem demands novel drug therapeutics which do not induce the corresponding ABC transporters.

So far unknown ABC genes are under discussion to be involved in anticancer drug resistance developments so that

the resistance problem becomes more complicated. New strategies have not been established and are suspected to be difficult to realize. Antisense oligonucleotides are effective to inhibit ABCB1 expressions in higher concentrations which will be difficult to reach in clinical studies [23]. Also short hairpin RNA treatment was successful in sequence-specific gene silencing to suppress an ABCB1 expression [59]. The realization of such concepts in clinical trials is under discussion, but it will also be difficult to reach effective reductions of protein expression.

So finally, the solution of the induction problem may be non-inducing drugs which selectively inhibit transmembrane efflux pumps in overexpressing cells and can be used in non-toxic therapeutically sufficient concentration ranges. Cage dimeric 1,4-dihydropyridines have been documented to not induce ABCB1 in various human cell lines. They cause no toxicity problems in ABCB1-inhibiting concentration ranges and have also been profiled in the drug-sensitive lymphocyte cell line Jurkat. Lymphocytes are known to be mainly affected by HIV infections and thus they are target cells for the novel MDR modulators. One main weakness of many studies in the recent past has been the exclusive documentation of induction on the mRNA level using the RTQ-PCR technique. So future studies have to include the transporter inductions also on the protein level assisted by functional transporter studies which have been shown for the novel cage dimeric MDR modulators. Only such a complex analysis will provide sufficient information to estimate the potential of a drug to act as a transport protein inducer in further clinical studies. The novel cage dimeric MDR modulators which have been fully characterized as non-inducing agents are a perspective class of innovative drugs for the treatment of MDR either in cancer therapy or with their HIV-1 protease inhibiting properties in antiretroviral therapies.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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REFERENCES

[1] Gottesman, M.M.; Ling, V. The molecular basis of multidrug resistance in cancer: The early years of P-glycoprotein research. *FEBS Letters*, **2006**, *580*, 998-1009.

[2] Lage, H. An overview of cancer multidrug resistance: a still unsolved problem. *Cell Mol. Life Science*, **2008**, *65*, 3145-3167.

[3] Pomerantz, R.J. Reservoirs, Sanctuaries, and Residual Disease: The Hiding Spots of HIV-1. *HIV Clin. Trials*, **2003**, *4*, 137-143.

[4] Coburger, C.; Lage, H.; Molnár, J.; Langner, A.; Hilgeroth, A. MDR reversal properties and cytotoxic evaluation of representatives of a novel class of HIV-1 protease inhibitors. *J. Pharm. Pharmacol.*, **2010**, *62*, 1704-1710.

[5] Huang, Y.; Sadee, W. Membrane transporters and channels in chemoresistance and -sensitivity of tumor cells. *Cancer Lett*., **2006**, *239*, 168-182.

[6] Choi, C.H. ABC transporters as multidrug resistance mechanisms and the development of chemosensitizers for their reversal. *Cancer Cell Int.*, **2005**, *5*, 30-42.

[7] Eckford, P.D.; Sharom, F.J. ABC multidrug transporters: structure, function and the role in chemoresistance. *Pharmacogenomics* **2008**, *9*, 105-127.

[8] Dean, M.; Rzhetsky, A.; Allikments, R. The human ATP-binding cassette (ABC) transporter superfamily. *Genom Res*., **2009**, *11*, 1156-1166.

[9] Mahon, F.X. ; Belloc, F., Lagarde, V. ; Chollet, C. ; Moreau Gaudry, F. ; Reiffers, J.; Goldman, J.M.; Melo, J.V. MDR1 gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. *Blood*, **2003**, *101*, 2368-2373.

[10] Matsui, H.; Takeshita, A.; Naito, K.; Shinjo, K.; Shigeno, K.; Maekawa, M.; Yamakawa, Y.; Tanimoto, M.; Kobayashi, M.; Ohnishi, K.; Ohno, R. Reduced effect of gemtuzumab ozogamicin (CMA-676) on P-glycoprotein and/or CD34-positive leukemia cells and its restoration by multidrug resistance modifiers. *Leukemia*, **2002**, *16*, 813-819.

[11] Bonavida, B. Rituximab-induced inhibition of antiapoptotic cell survival pathways: implications in chemo/immunoresistance, rituximab unresponsiveness, prognostic and novel therapeutic interventions. *Oncogene*, **2007**, *26*, 3629-3636.

[12] Coburger, C.; Wollmann, J.; Krug, M.; Baumert C., Seifert, M.; Molnár, J.; Lage, H.; Hilgeroth, A. Novel structure-activity relationships and selectivity profiling of cage dimeric 1,4-dihydropyridines as multidrug resistance (MDR) modulators. *Bioorg. Med. Chem.*, **2010**, *18*, 4983-4990.

[13] Sharom, F.J. ABC multidrug transporters: structure, function and role in chemoresistance. *Pharmacogenomics*, **2008**, *9*, 105-127.

[14] Livingston, D.M.; Silver, D.P. Crossing over to drug resistance. *Nature*, **2008**, *451*, 1066-1067.

[15] Rees, D.C.; Johnson, E.; Lewinson, O. ABC transporter: the power to change. *Nat. Rev. Mol. Cell Biol.*, **2009**, *10*, 218-227.

[16] Dean, M.; Annilo, T. Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. *Annu. Rev. Genomics Human Genet.*, **2005**, *6*, 123-142.

[17] Bakos, E.; Homolya, L. Portrait of multifaceted transporter, the multidrug resistance-associated protein 1 (MRP1/ABCC1). *Pflugers Arch.*, **2007**, *453*, 621-641.

[18] Avendanó, C.; Menéndez, J.C. Recent Advances in Multidrug Resistance Modulators. *Med. Chem. Rev.-Online*, **2004**, *1*, 414-444.

[19] Baumert, C.; Hilgeroth, A. Recent Advances in the Development of P-gp Inhibitors. *Anticancer Agents Med. Chem.*, **2009**, *9*, 415-436.

[20] Tan, B.; Piwnica-Worms, D.; Ratner, L. Multidrug resistance transporters and modulation. *Curr. Opin. Oncol.*, **2000**, *12*, 450-458.

[21] Leschziner, G.D.; Andrew, T.; Pirmohamed, M.; Johnson, M.R. ABCB1 genotype and PGP expression, function and therapeutic drug response: a critical review and recommendations for future research. *Pharmacogenomics J.*, **2007**, *7*, 154-179.

[22] Tain, Q.; Zhang, J.; Chan, E.; Duan, W.; Zhou, S. Multidrug resistance proteins (MRPs) and implication in drug development. *Drug Development Res.*, **2005**, *64*, 1-18.

[23] Gillet, J.P.; Efferth, T.; Remacle, J. Chemotherapy-induced resistance by ATP binding cassette transporter genes. *Biochim. Biophys. Acta*, **2007**, *1775*, 237-262.

[24] Kelly, R.J.; Draper, D.; Chen, C.C. A Pharmacodynamic Study of Docetaxel in Combination with the P-glycoprotein Antagonist Tariquidar (XR9576) in Patients with Lung, Ovarian, and Cervical Cancer. *Clin. Cancer Res.*, **2011**, *17*, 569-580.

[25] Harmsen, S.; Meijerman, I.; Febus, C.L.; Maas-Bakker, R.F.; Beijnen, J.H.; Schellens, J.H. PXR-mediated induction of P-glycoprotein by anticancer drugs in a human colon adenocarcinoma-derived cell line. *Cancer Chemother. Pharmacol.*, **2009**, *66*, 765-771.

[26] Shitl, A.A. Signal transduction pathways and transcriptional mechanisms as targets for prevention of emergence of multidrug resistance in human cancer cells. *Curr. Drug Tragets*, **2001**, *2*, 57-77.

[27] Coburger, C.; Lage, H.; Molnár, J.; Hilgeroth, A. Impact of Novel MDR Modulators on Human Cancer Cells: Reversal Activities and Induction Studies. *Pharm. Res.*, **2009**, *26*, 182-188.

[28] Luqmani, Y.A. Mechanisms of Drug Resistance in Cancer Chemotherapy. *Med. Princ. Pract.*, **2005**, *14 (Suppl.1)*, 35-48.

[29] Scotto, K.W. Transcriptional regulation of ABC drug transporters. *Oncogene*, **2003**, *22*, 7496-7511.

[30] Kim, R.B.; Fromm, M.F.; Wandel, C.; Leake, B.; Wood, A.J.J.; Roden, D.M.; Wilkinson, B.R. The drug transporter P-glycoprotein limits oral absorption and the brain entry of HIV-1 protease inhibitors. *J. Clin. Invest.*, **1998**, *101*, 289-294.

[31] Huisman, M.T.; Smit, J.W.; Wiltshire, H.R.; Hoetelmans, R.M.; Beijnen, J.H.; Schinkel, A.H. P-Glycoprotein limits oral availability, brain and fetal penetration of saquinavir even with high doses of ritonavir. *Mol. Pharmacol.*, **2001**, *59*, 806-813.

[32] Ronaldson, P.T.; Lee, G.; Dallas, Sh.; Bendayan, R. Involvement of P-Glycoprotein Transport of Saquinavir and Indinavir in Rat Brain Microvessel Endothelial and Microglia Cell Lines. *Pharm. Res.*, **2004**, *21*, 811-818.

[33] Drewe, J.; Gutman, H.; Fricker, G.; Torok, M.; Beglinger, C.; Huwyler, J. HIV protease inhibitor ritonavir: a more potent inhibitor of P-glycoprotein than the cyclosporine analog SDZ PSC 833. *Biochem. Pharmacol.*, **1999**, *57*, 1147-1152.

[34] Usansky, H.H.; Hu, P.; Sinko, P.J. Differential Roles of P-Glycoprotein, Multidrug Resistance-Associated Protein 2, and CYP3A on Saquinavir Oral Absorption in Sprague-Dawley Rats. *Drug Metab. Disp.*, **2008**, *36*, 863-869.

[35] Cooper, C.L.; van Heeswijk, R.P.G.; Gallicano, K.; Cameron, D.W. A review of low-dose ritonavir in protease inhibitor combination therapy. *Clin. Infect. Dis.*, **2003**, *36*, 1585-1592.

[36] Youle, M. Overview of boosted protease inhibitors in treatment-experienced patients. *J. Antimicrob. Chemother.*, **2007**, *60*, 1195-1205.

[37] Busse, K.H.; Penzak, S.R. Pharmacological enhancement of protease inhibitor with ritonavir: An update. *Expert Rev. Clin. Pharmacol.*, **2008**, *1*, 533-545.

[38] Richter, M.; Gyémant, N.; Molnár, J.; Hilgeroth, A. Comparative Effects on Intestinal Absorption *in Situ* by P-glycoprotein-Modifying HIV Protease Inhibitors. *Pharm. Res.*, **2004**, *21*, 1862-1866.

[39] Perloff, M.D.; Von Moltke, L.L.; Marchand, J.E.; Greenblatt, D.J. Ritonavir induces P-glycoprotein expression, multidrug resistance-associated protein (MRP1) expression, and drug transporter-mediated activity in a human intestinal cell line. *Pharm. Sci.*, **2001**, *90*, 1829-1837.

[40] Perloff, M.D.; Von Moltke, L.L.; Fahey, J.M.; Greenblatt, D.J. Induction of P-glycoprotein expression and activity by ritonavir in bovine brain microvessel endothelial cells. *J. Pharm. Pharmacol.*, **2007**, *59*, 947-953.

[41] Zastre, J.A.; Chan, G.N.Y.; Ronaldson, P.T.; Ramaswamy, M.; Couraud, P.O.; Romero, I.A.; Weksler, B.; Bendayan, M.; Bendayan, R. Up-regulation of P-glycoprotein by HIV protease Inhibitors in a Human Brain Microvessel Endothelial Cell Line. *J. Neurosci. Res.*, **2009**, *87*, 1023-1036.

[42] Gupta, A.; Thummel, K.E.; Unadkat, J.D. Induction of CYP3A4 and MDR1 by anti-HIV protease inhibitors in human intestinal adenocarcinoma cell line. *Drug. Metab. Rev.*, **2006**, 38, 111-112.

[43] Weiss, J.; Herzog, M., König, S.; Storch, C.H.; Ketabi-Kiyanvash, N.; Haefeli, W.E . Induction of multiple drug transporters by efavirenz. *J. Pharmacol. Sci.*, **2009**, *109*, 242-250.

[44] Almond, L.M.; Hoggard, P.G.; Edirisinghe, D.; Khoo, S.H.; Back, D.J. Intracellular and plasma pharmacokinetics of efavirenz in HIV-infected individuals. *J. Antimicrob. Chemother.*, **2005**, *56*, 738-744.

[45] Weiss, J.; Weis, N.; Ketabi-Kiyanvash, N.; Storch, C.H.; Haefeli, W.E. Comparison of the induction of P-glycoprotein activity by nucleotide, nucleoside, and non-nucleoside reverse transcriptase inhibitors. *Eur. J. Pharmacol.*, **2007**, *579*, 104-109.

[46] Mukwaya, G.; MacGregor, T.; Hoelscher, D.; Heming, Th.; Legg, D.; Kavanaugh, K.; Johnsom, Ph.; Sabo, J.P.; McCallister, S. Interaction of ritonavir-boosted tipranavir with loperamide does not result in loperamide-associated neurologic side effects in healthy volunteers. *Antimicrob. Agents*, **2005**, *49*, 4903-4910.

[47] Vourvahis, M.; Kashuba, A.D.M. Mechanisms of pharmacokinetic and pharmacodynamic drug interactions associated with ritonavir-enhanced tipranavir. *Pharmacother.*, **2007**, *27*, 888-909.

[48] Solbach, T.F.; Paulus, B.; Weyand, M.; Eschenhagen, T.; Zolk, O.; Fromm, M.F. ATP-binding cassette transporters in human heart failure. *Naunyn Schmiedebergs Arch. Pharmacol.*, **2008**, *377*, 231-243.

[49] Weiss, J.; Sauer, A.; Herzog, M.; Böger, R.H.; Haefeli, W.E.; Benndorf, R.A. Interaction of Thiazolidinediones (Glitazones) with the ATP-Binding Cassette Transporters P-Glycoprotein and Breast Cancer Resistance Protein. *Pharmacology*, **2009**, *84*, 264-270.

[50] Szatmari, I.; Vamosi, G.; Brazda, P.; Balint, B.L.; Benko, S.; Szeles, L.; Jeney. V.; Ozvegy-Laczka, C.; Szanto, A.; Barta, E.; Balla, J.; Sarkadi, B.; Nagy, L. Peroxisome proliferator-activated receptor gamma-regulated ABCG2 expression confers cytoprotection to human dendritic cells. *J. Biol. Chem.*, **2006**, *281*, 23812-23823.

[51] Borst, P.; Elferink, R.O. Mammalian ABC transporters in health and disease. *Annu. Rev. Biochem.*, **2002**, *71*, 537-592.

[52] Dean, M. ABC transporters, drug resistance, and cancer stem cells. *J. Mammary Gland Biol. Neoplasia*, **2009**, *14*, 3-9.

[53] Nishimura, M.; Koeda, A.; Morikawa, H.; Satoh, T.; Narimatsu, S.; Naito, S. Comparison of inducibility of multidrug resistance (MDR)1, multidrug resistance-associated protein (MRP)1, and MRP2 mRNAs by prototypical microsomal enzyme inducers in primary cultures of human and cynomolgus monkey hepatocytes. *Biol. Pharm. Bull.*, **2008**, *31*, 2068-2072.

[54] Sartori, S.; Trevisani, L.; Nielsen, I.; Tassinari, D.; Panzini, I.; Abbasciano, V. Randomized Trial of Omeprazole or Ranitidine Versus Placebo in the Prevention of Chemotherapy-induced Gastroduodenal Injury. *J. Clin. Oncol.*, **2000**, *18*, 463-467.

[55] Aszalos, A. Drug-drug interactions affected by the transporter protein, P-glycoprotein (ABCB1, MDR1) I. Preclinical aspects. *Drug Disc. Today*, **2007**, *12*, 833-837.

[56] Hilgeroth, A.; Molnár, J.; Declercq, E. Using Molecular Symmetry to Form New Drugs: Hydroxymethyl-substituted 3,9-Diazatetraasteranes as the First Class of Symmetric MDR Modulators. *Angew. Chem. Int. Ed.*, **2002**, *114*, 3772-3775.

[57] Wollmann, J.; Baumert, C.; Erlenkamp, G.; Sippl, W.; Hilgeroth, A. Novel Insight into Inhibitor Binding of Highly Symmetric HIV-1 Protease. *ChemBioChem*, **2008**, *9*, 874-878.

[58] Ronald, E.; Langford, D.; Eliezer, M. HIV and antiretroviral therapy in the brain: neuronal injury and repair. *Nature Rev. Neurosci.*, **2007**, *8*, 33-44.

[59] Krühn, A.; Wang, A.; Fruehauf, J.H.; Lage, H. Delivery of short hairpin RNAs by transkingdom RNA interference modulates the classical ABCB1-mediated multidrug-resistant phenotype of cancer cells. *Cell Cycle*, **2009**, *20*, 3349-3354.

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