ABSTRACT
Pharmacokinetic drug-drug interactions often occur at the level of P-glycoprotein (Pgp). To study possible interactions caused by the newer antidepressants we investigated citalopram, fluoxetine, fluvoxamine, paroxetine, reboxetine, sertraline, and venlafaxine and their major metabolites desmethylcitalopram, norfluoxetine, paroxetine-metabolite (paroxetine-M), desmethylsertraline, N-desmethylvenlafaxine, and O-desmethylvenlafaxine for their ability to inhibit Pgp. Pgp inhibition was studied by a fluorometric assay using calcein-acetoxymethylester as Pgp substrate and two different cell systems: L-MDR1 cells (model for human Pgp) and primary porcine brain capillary endothelial cells (pBCECs, model for the blood-brain barrier). Both cell systems proved to be suitable for the evaluation of Pgp inhibitory potency of drugs. All antidepressants tested except O-desmethylvenlafaxine showed Pgp inhibitory activity with sertraline, desmethylsertraline, and paroxetine being the most potent, comparable with the well known Pgp inhibitor quinidine. In L-MDR1 cells fluoxetine, norfluoxetine, fluvoxamine, reboxetine, and paroxetine-M revealed intermediate Pgp inhibition and citalopram, desmethylcitalopram, venlafaxine, and N-desmethylvenlafaxine were only weak inhibitors. The ranking order was similar in pBCECs. The fact that some of the compounds tested exert Pgp inhibitor effects at similar concentrations as quinidine suggests that pharmacokinetic drug-drug interactions between the newer antidepressants and Pgp substrates should now be thoroughly studied in vivo.
Materials and Methods

Materials. Culture media, fetal calf serum, medium supplements, antibiotics, and Hanks’ balanced salt solution were purchased from Invitrogen (Karlsruhe, Germany); collagenase/disperse and dispase were from Roche Diagnostics (Mannheim, Germany); collagen-R was from Serva (Heidelberg, Germany); DMSO and Triton X-100 were from AppliChem (Darmstadt, Germany); dextran was from Sigma-Aldrich (Taufkirchen, Germany); Percoll was from Amersham Biosciences, Inc. (Freiburg, Germany); calcein-AM was from MoBiTec (Göttingen, Germany); vincristine was from Calbiochem (Darmstadt, Germany); and 96-well microtiter plates were from NUNC GmbH & Co. KG (Wiesbaden, Germany).

Drugs. Citalopram hydrobromide and desmethylcitalopram hydrochloride were kind gifts from Lundbeck (Valby, Denmark); fluvoxamine maleate was from Solvay (Hannover, Germany); LY335979 was obtained from Eli Lilly & Co. (Bad Homburg, Germany); paroxetine hydrochloride and venlafaxine hydrochloride were from Wyeth (Münstet, Germany). Fluoxetine hydrochloride, norfluoxetine hydrochloride, and verapamil hydrochloride were purchased from Sigma-Aldrich and quinidine was from Roth (Karlsruhe, Germany).

LLC-PK1 and L-MDR1 Cells. As model for human Pgp we used L-MDR1 cells, a cell line generated by transfection of the porcine kidney epithelial cell line LLC-PK1 with the human MDR1 gene (Schinkel et al., 1996) and the parental cell line LLC-PK1 (American Type Culture Collection, Manassas, VA) as a control. The L-MDR1 cell line was kindly provided by Dr. A. H. Schinkel (The Netherlands Cancer Institute, Division of Experimental Therapy, Amsterdam, The Netherlands). The cells were cultured under standard cell culture conditions with medium M199 supplemented with 10% heat inactivated fetal calf serum, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin sulfate. To maintain Pgp expression, the culture medium for L-MDR1 was supplemented with 0.64 μM vinblastine.

Isolation of Porcine Brain Capillary Endothelial Cells. The isolation of pBCECs was essentially based on the method described by Audus et al. (1996). Medium M199 supplemented with l-glutamine (0.7 mM), streptomycin sulfate (100 μg/ml), penicillin G (100 U/ml), gentamycin (100 μg/ml), and HEPES (10 mM) was used for all preparation steps. Briefly, cortical gray matter from seven to eight fresh porcine brains, which were obtained from a local slaughterhouse, was isolated, cut into very small pieces, and digested enzymatically using 0.5% dispase (in preparation medium). After centrifugation (1,000g, 4°C, 10 min), the pellets were resuspended in 13% dextran solution in preparation medium and centrifuged again (5,800g, 4°C, 12 min). The pellet containing the cerebral microvessels was subsequently incubated in preparation medium containing 0.1% (w/v) collagenase/dispase. The resulting cell suspension was filtered, and the brain capillary endothelial cells were separated on a discontinuous Percoll gradient (densities 1.03 and 1.07, centrifugation at 1,250g, 5 min, 20°C), washed, and filtered again before being seeded on collagen-coated microtiter plates in a density of 100,000 cells/cm². Cells were cultured under standard cell culture conditions with medium M199 containing l-glutamine (0.7 mM), streptomycin sulfate (100 μg/ml), penicillin G (100 U/ml), HEPES (10 mM), and 10% heat-inactivated horse serum. Eight days after seeding the confluent monolayers were used for the calcein assay.

The isolation method for the pBCECs was validated by immunohistochemistry. The isolated cells were positive for the endothelium-specific factor VIII-related protein (von Willebrand factor) and for Pgp. Potential contamination with astrocytes was assessed by staining with an antibody against glial fibrillary acidic protein and was negligible (<5%). Staining with an antibody against neurofilaments (as a marker for neurons) was absent.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) for the Detection of Pgp Expression at the mRNA Level. Expression of human Pgp and/or porcine ppgp1A (ppgp1A) at the mRNA level in the cell lines used was verified by reverse transcription of RNA followed by polymerase chain reaction. RNA was isolated using the RNeasy mini kit (QIAGEN, Hilden, Germany). First-strand cDNA synthesis was performed with the First-Strand cDNA synthesis kit for RT-PCR (Roche Diagnostics) with random hexamer primers according to the manufacturer’s instructions.

Primers used for the amplification of human Pgp were 5′-GGTGCTGGTGTGCTTTACAT-3′ (sense) and 5′-CCCGTGAAAATGTTCGCAAA-3′ (antisense). For ppgp1A, primers were used according to Childs and Ling (1996). All primers were synthesized by MWG Biotech AG (Ebersberg, Germany). PCR was performed on the LightCycler (Roche Diagnostics) in a total volume of 10 μl using the LightCycler-FastStart DNA Master SYBR Green I kit (Roche Diagnostics), 0.5 mM of each primer, and 3 mM MgCl₂. PCR fragment size was determined by 1.5% agarose gel electrophoresis.

Stock Solutions. Stock solutions of test compounds were prepared strictly following the manufacturers’ instructions. Most compounds were soluble in aqueous bident. Only sertraline, desmethylsertraline, O-desmethylvenlafaxine, quinidine, and verapamil hydro-
The calcein-AM uptake assay was performed in 96-well plates. All incubation steps and the cell lysis were conducted at 37°C on a rotary shaker at 450 rpm. Before the uptake assay, the cells were washed with prewarmed Hanks’ balanced salt solution supplemented with 10 mM HEPES (HHBSS) and preincubated with HHBSS for 30 min and subsequently with the test compound for 10 min in octetuplet. After preincubation, calcein-AM was added (final concentration 1 μM) and the cells were incubated for 60 min. The uptake was then stopped by transferring the plates on ice and washing the cells twice with HHBSS precooled to 4°C. Subsequently, cells were lysed in 1% Triton X-100 for 15 min. The fluorescence of the calcein generated with the supernatant.

Quenching Test. Each test compound was screened for possible quenching effects on the calcein fluorescence. Because calcein-AM is nonfluorescent and cannot be used for a quenching test, we generated the fluorescent dye calcein by incubating LLC-PK1 cells with 1 μM calcein-AM for 60 min at 37°C on a rotary shaker at 450 rpm. Increasing concentrations of the test compounds were added to aliquots of the cell lysate and the fluorescence was compared with control wells without test compounds.

Cytotoxicity Assay. Each test compound was screened for possible cytotoxic effects with the cytotoxicity detection kit (Roche Diagnostics). A colorimetric assay for the quantification of lactate dehydrogenase activity released from the cytosol of damaged cells into the supernatant. 

Statistical Analysis. For calculation of the inhibitor effects, a nonlinear four-parameter fit was used (Graphit, version 4; Erithacus Software, Middlesex, UK) according to the sigmoidal model with the following formula: 

\[
I = \frac{(I_{\text{max}} - \text{background}) (1 + (s/IC_{50})^n)}{1 + (s/IC_{50})^n} + \text{background},
\]

where \(I_{\text{max}}\) is the maximal inhibition, IC_{50} is the concentration leading to half-maximal inhibition of the calcein-AM transport, and \(s\) is the slope factor.

Due to solubility problems and/or cytotoxic influences, plateau effects and thus IC_{50} values were only obtained for some of the compounds tested. The curves were therefore also evaluated with two additional methods that are not based on plateau effects. In the first analysis the concentration of the test compound needed to double baseline fluorescence was derived from the concentration-response curve (f2) (Fig. 1). The second calculation quantifies the inhibitory effect of the compound on calcein-AM efflux at a fixed concentration (50 μM for all compounds except SDZ-PSC833 (2.5 μM) and LY335979 (1 μM)) and normalizes this value to the maximal effect of verapamil control in the same assay.

The resulting IP_{50relv} was calculated with the following formula (Bogman et al., 2001): 

\[
\text{IP}_{50\text{relv}} = \frac{\Delta F_{\text{test compound at 50 μM}}}{\Delta F_{\text{VPL at 200 μM}}} \times 100, \text{ where } \Delta F\text{ is the difference in calcein fluorescence in the absence and presence of the Pgp inhibitor.}
\]

\(p\) values were determined by analysis of variance with Dunnett’s multiple comparison test for post hoc pairwise comparison with the control results obtained with verapamil or with the Wilcoxon matched pairs test (GraphPad InStat, version 3.05; GraphPad Software, Inc., San Diego, CA). A \(p\) value of \(\leq 0.05\) was considered significant.

Results

Method Validation. RT-PCR demonstrated the expression of mRNA of human Pgp and/or porcine ppgp1A (Fig. 2). These results were verified by Western blot and immunohistochemistry (data not shown). The different Pgp levels of L-MDR1 and LLC-PK1 were also confirmed in functional experiments by differences in calcein accumulation and its inhibition by verapamil, a typical Pgp inhibitor (Fig. 3).

To exclude a possible involvement of the multidrug resistance-associated proteins (MRPs) 1 and 2, which also transport calcein-AM, we tested probenecid up to 500 μM in the calcein assay. Probenecid has been shown to inhibit the transport of calcein-AM and calcein in MRPs but not in Pgp-overexpressing cell lines (Feller et al., 1995; Gallapudi et al., 1997). In L-MDR1 cells, probenecid had no influence on the calcein accumulation up to 500 μM (\(n = 3\) experiments in
octuplets), and in pBCECs only a minor effect could be seen ($n = 3$ experiments in octuplets, $f_2$ was not reached). Similarly, the specific MRP inhibitor MK571 had no effect in L-MDR1 cell up to 2.5 μM and only a minor effect in pBCECs.

None of the test compounds showed any quenching effect in the concentration range tested on the fluorescence of calcein or an autofluorescence at the excitation wavelength used to measure calcein fluorescence.

In the upper concentration range, most of the antidepressants tested showed cytotoxic effects. The corresponding values leading to a decline in the concentration-response curve were not included in the analysis of the calcein assay. The four control compounds SDZ-PSC833, LY335979, verapamil, and quinidine proved not to be cytotoxic for any of the cell lines even in the highest concentrations used.

The ranking order of the three control compounds SDZ-PSC833, verapamil, and quinidine confirmed the results of the calcein assay conducted by Tiberghien and Loor (1996). The prototype Pgp substrate digoxin showed no inhibition in L-MDR1 cells up to 500 μM and only weak inhibition in pBCECs ($f_2 = 76.4 \pm 15.9$ μM, $n = 2$ experiments in octuplets, concentration of calcein-AM = 0.5 μM).

**Evaluation of the Pgp Inhibitory Potency of the Newer Antidepressants.** All concentration-response curves reaching a plateau and thus enabling the calculation of IC$_{50}$ values are shown in Fig. 4. In addition to three of the four control compounds (Fig. 4a), this applied to sertraline, paroxetine, and fluoxetine in L-MDR1 cells (Fig. 4b; Table 1). The potency of sertraline and paroxetine was comparable with the potency of quinidine (Tables 1 and 2). For most compounds plateau effects were not reached either because of cytotoxicity or limited dissolution. To permit a comparison between all compounds tested, IP$_{50\text{rel}}$ and $f_2$ values were calculated (Tables 1 and 2). Only venlafaxine and its metabolites (in both cell lines) and fluoxetine (in pBCECs) did not reach $f_2$. Nevertheless, except for O-desmethylvenlafaxine the highest concentrations analyzed (100 μM for fluoxetine and O-desmethylvenlafaxine, 500 μM for venlafaxine and N-desmethylvenlafaxine) produced significant increases in baseline fluorescence ($p < 0.0001$, Wilcoxon matched pairs test), thus confirming Pgp inhibition.

Independent from the calculation used and the cell line tested, LY335979, SDZ-PSC833, and verapamil proved to be the most effective Pgp inhibitors followed by quinidine, sertraline, desmethylsertraline, and paroxetine, which were all similar (Tables 1 and 2; Fig. 5). In L-MDR1 cells fluoxetine, norfluoxetine, fluvoxamine, reboxetine, and paroxetine-M showed intermediate and citalopram, desmethylcitalopram, venlafaxine, and N-desmethylvenlafaxine only weak inhibi-
No inhibition was found for O-desmethylvenlafaxine. The results obtained in pBCECs were similar (Table 2).

**Discussion**

The activity of the efflux transporter Pgp affects the pharmacokinetic parameters of many drugs and contributes to numerous pharmacokinetic drug-drug interactions (Yu, 1999). Hitherto, the role of Pgp for the bioavailability, distribution, and excretion of the newer antidepressants and for their interaction with coadministered drugs has not been elucidated thoroughly. For paroxetine, venlafaxine, and fluoxetine the data indicate that they might be Pgp substrates; for citalopram the data are conflicting (Rochat et al., 1999; Uhr et al., 2000; Uhr, 2002).

Even less is known about potential inhibitory characteristics of newer antidepressants on Pgp. In theory, Pgp inhibition by drugs may play an important role in drug safety, because it may increase plasma and brain concentrations of coadministered drugs and thus cause adverse drug reactions. Thus far, only fluoxetine has been tested in this regard. In line with the absence of Pgp-substrate characteristics (Uhr et al., 2000) and in agreement with our results no evidence for a potent interaction was found (Ekins et al., 2002).

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>n</th>
<th>IC50 μM</th>
<th>IP50 (for SDZ-PSC833, IP2, test) μM</th>
<th>Concentration Needed to Double Baseline Fluorescence (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDZ-PSC833</td>
<td>3</td>
<td>0.02 ± 0.01**</td>
<td>0.98 ± 0.14</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>LY335979</td>
<td>3</td>
<td>0.01 ± 0.002**</td>
<td>1.10 ± 0.33</td>
<td>0.003 ± 0.001</td>
</tr>
<tr>
<td>Verapamil</td>
<td>4</td>
<td>2.01 ± 0.04</td>
<td>1.09 ± 0.21</td>
<td>1.90 ± 1.53</td>
</tr>
<tr>
<td>Quinidine</td>
<td>3</td>
<td>3.16 ± 1.24</td>
<td>0.69 ± 0.12**</td>
<td>2.55 ± 1.59</td>
</tr>
<tr>
<td>Citalopram</td>
<td>4</td>
<td>N.D.</td>
<td>0.17 ± 0.03**</td>
<td>63.0 ± 12.9**</td>
</tr>
<tr>
<td>Desmethylcitalopram</td>
<td>4</td>
<td>N.D.</td>
<td>0.13 ± 0.03**</td>
<td>133.1 ± 29.1**</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>5</td>
<td>N.D.</td>
<td>0.14 ± 0.05**</td>
<td>N.D.</td>
</tr>
<tr>
<td>Norfluoxetine</td>
<td>4</td>
<td>N.D.</td>
<td>0.21 ± 0.05**</td>
<td>39.6 ± 2.9</td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>3</td>
<td>N.D.</td>
<td>0.23 ± 0.04**</td>
<td>51.8 ± 13.8</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>3</td>
<td>N.D.</td>
<td>0.56 ± 0.04**</td>
<td>14.0 ± 5.3</td>
</tr>
<tr>
<td>Paroxetine-M</td>
<td>4</td>
<td>N.D.</td>
<td>0.21 ± 0.05**</td>
<td>47.5 ± 15.1</td>
</tr>
<tr>
<td>Reboxetine</td>
<td>3</td>
<td>N.D.</td>
<td>0.23 ± 0.01**</td>
<td>34.9 ± 4.1</td>
</tr>
<tr>
<td>Sertraline</td>
<td>3</td>
<td>N.D.</td>
<td>0.62 ± 0.14**</td>
<td>14.4 ± 9.3</td>
</tr>
<tr>
<td>Desmethylsertraline</td>
<td>5</td>
<td>N.D.</td>
<td>0.25 ± 0.11**</td>
<td>8.3 ± 4.3</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>4</td>
<td>N.D.</td>
<td>0.05 ± 0.03**</td>
<td>N.D.</td>
</tr>
<tr>
<td>N-desmethyl-venlafaxine</td>
<td>3</td>
<td>N.D.</td>
<td>0.01 ± 0.01</td>
<td>N.D.</td>
</tr>
<tr>
<td>O-desmethyl-venlafaxine</td>
<td>2</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D., not definable; n, number of experiments, each performed in octuplet.

*p < 0.05; **p < 0.01.
interactions with the newer antidepressants at the level of Pgp.

The aim of the present study was to clarify whether the widely used newer antidepressants and their major metabolites inhibit Pgp in vitro as a marker of potential drug-drug interactions in vivo. Another objective was the comparison of the pBCECs and L-MDR1 cells concerning their suitability for a Pgp inhibition assay with calcein-AM as Pgp substrate.

We used an in vitro assay to characterize compounds concerning their ability to inhibit the transport of the Pgp substrate calcein-AM. The fact that all four well characterized Pgp inhibitors (LY335979, SDZ-PSC833, verapamil, and quinidine) were also potent Pgp inhibitors in these assays confirms the applicability of the calcein assays for the evaluation of Pgp-modulating drug effects. The two cell systems used offer different advantages. L-MDR1 cells overexpress human Pgp and can be compared with their parental cell line LLC-PK1. Effects only seen in the transfected cell line can thus be attributed to functional human Pgp. Effects observed in both cell lines can be ascribed to porcine pgp1A, which is expressed in both cells or to other shared characteristics. Indeed, the substantial difference in baseline fluorescence and the fact that verapamil had nearly no effect in LLC-PK1 cells confirms the expected difference in the Pgp activity between parental and transfected cell line (Fig. 2) and emphasizes the suitability of this cell system.

For all drugs tested, the influence on calcein fluorescence in LLC-PK1 cells was either absent or much less pronounced than in the Pgp-overexpressing cell line, indicating that the enhancement of the calcein fluorescence was based on inhibition of human Pgp. This finding indicates that MRP1 and 2 do not play a substantial role in this assay, particularly because the MRP inhibitors probenecid and MK571 had no effects in L-MDR1 cells and only minor effects in pBCECs on the calcein accumulation. This conclusion was also drawn by

Eneroth et al. (2001), evaluating a Pgp-overexpressing Caco-2 cell line in a calcein assay.

Interestingly, the maximal fluorescence obtained in L-MDR1 cells was greater than in LLC-PK1 cells (Fig. 2), most likely because L-MDR1 cells are roughly 60% thicker than LLC-PK1 cells and thus accumulation of calcein in the transfected cell line may be greater. Another possibility for such a finding might be differences within the intracellular milieu, e.g., involving different esterase activities.

For comparison, primary cell cultures of (porcine) pBCECs as a second, independent cell system were studied. These cells are sumptuous to isolate, differ slightly from preparation to preparation, and are more sensitive to cytotoxic effects. They exhibit a constant pgp1A expression (Hegmann et al., 1992; Huwyler et al., 1996) and due to the lower Pgp expression level compared with L-MDR1 cells they seem particularly suited to detect minor effects of weak inhibitors.

The similarity of the ranking order of inhibition in both cell systems suggests that both are suitable for the evaluation of Pgp-modulating effects. However, it is common experience that absolute values cannot be compared between different cell systems (Tables 1 and 2), especially if Pgp expression levels are different. Therefore, it is conceivable that different concentrations of a compound are needed in the two cell systems to reach similar effects.

Whenever possible, IC_{50} values were used to compare inhibitory characteristics. If no plateau effect was reached and solubility or cytotoxic effects precluded further increases of the concentration, we also calculated IP_{50rel} (Bogman et al., 2001) and the concentration needed to increase basal fluorescence 2-fold (f2). The IP_{50rel} features the advantage of normalizing all values to a control (e.g., verapamil) and thus compensates for interassay variability. However, the concentration at which the effect is compared is predefined arbitrarily, neglecting the fact that the slope factors of different concentration-effect curves may differ and that the potency of different compounds may vary by orders of magnitude. Moreover, the meaningful comparison of compounds with substantially differing maximum effects (efficacy) is not possible, and the IP_{50rel} does not give clear evidence at which concentration range a compound is active.

Hence, we introduced another assessment method (determination of f2) that also takes the different shapes of the respective concentration-response curves into consideration. Only for very weak inhibitors, which do not lead to a 2-fold increase in basal fluorescence, this method is not suitable. However, such minor effects are normally negligible and may not have importance for the in vivo situation. As an example, in the calcein assay the prototype Pgp substrate digoxin has only minor effects. This is perfectly in line with extensive clinical experience with this drug with only insignificant and rare alteration of the pharmacokinetics of coadministered substrates (Rameis, 1985). Despite the individual advantages and disadvantages of the different cell systems applied and independent of the calculation methods and the cell line used, this series of experiments for the first time shows that the newer antidepressant are inhibitors of Pgp. Indeed, sertraline, desmethylsertraline, and paroxetine had an effect similar to one of the most potent inhibitors (quinidine), whereas citalopram, desmethylcitalopram, venlafaxine, and N-desmethylvenlafaxine exerted only very weak inhibition.

Based on these in vitro data, sertraline and paroxetine...
bear the apparently largest potential to influence the pharmacokinetics of coadministered drugs at the level of Pgp. However, at usual therapeutic doses, the IC_{50} value for inhibition of Pgp is around 250-fold higher than the plasma concentration for paroxetine and around 500-fold higher for sertraline (Preskorn, 1997). Von Molkte et al. (1998) recently suggested a model to predict in vivo drug interactions based on in vitro data, which they applied to estimate the inhibitor potential of SSRI on cytochrome P450 CYP2D6. Provided that blood/liver concentration ratios were considered, which amount to 1:36 for sertraline, and not the unbound SSRI plasma concentration, the model yielded good predictions of the in vivo situation. For sertraline, with effective plasma levels of about 65 nM (Preskorn, 1996), this would implicate concurrent liver concentrations of about 2 μM. However, these concentrations are roughly 1 order of magnitude below the concentrations that were found to inhibit Pgp in our assays, suggesting that even if the accumulation of the drugs within the cell (e.g., in the biliary or renal system) is taken into account, the Pgp inhibition observed in vitro might not be clinically relevant.

This is substantiated by the fact that both sertraline (Rapeport et al., 1996) nor fluvoxamine (Ochs et al., 1989) nor citalopram (Larsen et al., 2001) had a clinically relevant influence on the pharmacokinetic parameters of digoxin, a Pgp prototype substrate. There is only one case report describing a possible increase of serum digoxin levels after treatment with fluoxetine (Leibovitz et al., 1998). On the other hand, in addition to being an inhibitor of CYP2D6, paroxetine is a substrate of this isozyme, whose activity is regulated by a genetic polymorphism. In the absence of active enzyme (poor metabolizer) plasma paroxetine concentrations are up to 25-fold higher than in extensive metabolizers (Sindrup et al., 1992). Accordingly, it cannot be excluded that in poor metabolizer patients administration of high paroxetine doses may translate into clinically relevant modulation of the pharmacokinetics of concomitantly administered Pgp substrates.

In conclusion, the present study demonstrates that not only the widely used L-MDR1 cells are well suited to evaluate drug-induced Pgp inhibition with calcine-AM but also the pBCEC primary cell cultures, which are a well established model to pharmacologically characterize the pharmacological properties of the blood-brain barrier. This is the first study that comprehensively quantified inhibitory effects of the newer antidepressants, some of which exerted substantial Pgp inhibition. It remains to be investigated whether this property of the newer antidepressants might lead to drug-drug interactions in patients. Such interactions might, for instance, be relevant when drugs with low oral bioavailability due to substantial transport back into the gut lumen are to be coadministered, as it has been shown for loperamide when given in combination with quinidine (Sadeque et al., 2000).

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References


Stockley IH (1999) Drug Interactions, A Source Book of Adverse Interactions, Their...


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