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Role of P-Glycoprotein Inhibition for Drug Interactions

Evidence from *In Vitro* and Pharmacoepidemiological Studies

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Abstract

Objectives: We determined *in vitro* the potency of macrolides as P-glycoprotein inhibitors and tested in hospitalised patients whether coadministration of P-glycoprotein inhibitors leads to increased serum concentrations of the P-glycoprotein substrates digoxin and digitoxin.

Methods: *In vitro*, the effect of macrolides on polarised P-glycoprotein-mediated digoxin transport was investigated in Caco-2 cells. In a pharmacoepidemiological study, we analysed the serum digoxin and digitoxin concentrations with and without coadministration of P-glycoprotein inhibitors in hospitalised patients.

Results: All macrolides inhibited P-glycoprotein-mediated digoxin transport, with concentrations producing 50% inhibition (IC_{50}) values of 1.8, 4.1, 15.4, 21.8 and 22.7 μmol/L for telithromycin, clarithromycin, roxithromycin, azithromycin and erythromycin, respectively. Coadministration of P-glycoprotein inhibitors was associated with increased serum concentrations of digoxin (1.3 ± 0.6 vs 0.9 ± 0.5 ng/mL, $p < 0.01$). Moreover, patients receiving macrolides had higher serum concentrations of cardiac glycosides ($p < 0.05$).

Conclusion: Macrolides are potent inhibitors of P-glycoprotein. Drug interactions between P-glycoprotein inhibitors and substrates are likely to occur during hospitalisation.

Background

The *ABCB1* (*MDR1*) gene encodes for the adenosine triphosphate-dependent efflux transporter P-glycoprotein. Its clinical relevance was first observed with the occurrence of multidrug resistance in cancer cells during chemotherapy.[1,2] It is now well known that the expression of P-glycoprotein in healthy tissues plays an important role in drug disposition and drug interactions.[3-5] In humans, P-glycoprotein is expressed in the apical membrane of epithelial cells in the intestine, the canalicular membrane of hepatocytes and the luminal membrane of proximal renal tubular epithelial cells, thereby modulating absorption and elimination of xenobiotics.[3-5] Moreover, it is an essential part of blood-tissue barriers, e.g. the blood-brain barrier, blood-testis barrier and placenta.[5-7] Both inhibition and induction of P-glycoprotein lead to drug interactions in humans. This is of particular clinical significance for drugs with a narrow therapeutic index, such as cardiac glycosides. For example, induction of P-glycoprotein by rifampicin (rifampin) leads to reduced plasma concentrations of orally administered digoxin, whereas P-glycoprotein inhibition by quinidine results in increased plasma concentrations of the cardiac glycoside.[8,9]

Among antibacterials, macrolides are very frequently prescribed drugs. In Germany, 52.6 million daily doses of macrolides were prescribed in 2004.[10] A broad variety of drug interactions with macrolides have been reported, which are mainly attributed to inhibition of CYP3A4.[11,12] However, there have been reports of increased serum concentrations and incidental toxicity of the P-glycoprotein substrates digoxin and digitoxin, which are not metabolised to a major extent in humans, during concomitant therapy with macrolides.[13-22] In addition, *in vitro* data indicate that erythromycin is a potent P-glycoprotein inhibitor.[23] Very few *in vitro* data are available on potential P-glycoprotein inhibition by other macrolides (e.g. roxithromycin) and ketolids such as telithromycin[24] and their relative potencies tested in the same *in vitro* system.

In spite of the increasingly recognised role of P-glycoprotein for drug interactions,[3-5,8,9,23,25] there is very little information as to whether P-glycoprotein-mediated drug interactions (e.g. with cardiac glycosides) are of relevance during routine care of hospitalised patients.[26] Moreover, factors influencing serum digoxin concentrations in routine patient care are still poorly understood. Recent data have indicated that in women with heart failure, digoxin serum concentrations of >1.2 ng/mL seem to be harmful.[27]

We therefore investigated the potential P-glycoprotein inhibitory effect of frequently used macrolides and the ketolid telithromycin *in vitro* using digoxin as a substrate on monolayers of the human colon carcinoma cell line Caco-2, which express P-glycoprotein in their apical membrane.[9,28,29] In the second part of the study, we evaluated whether digoxin and/or digitoxin serum concentrations are altered in hospitalised patients treated with macrolides or other P-glycoprotein inhibitors.

Methods

In Vitro Transport Studies in Caco-2 Cells

[^{3}H]digoxin (19 Ci/mmol) and [^{3}H]inulin (3.3 mg/mCi) were supplied by NEN Life Science Products (Boston, MA, USA). Unlabelled digoxin and erythromycin were purchased from Sigma Chemie (Deisenhofen, Germany). Unlabelled azithromycin, clarithromycin and roxithromycin were obtained from Chemos GmbH (Regenstauf, Germany). Unlabelled telithromycin was obtained after extraction of Ketek®[1] tablets (Sanofi-Aventis Deutschland GmbH; Bad Soden, Germany) using ethyl acetate

1 The use of trade names is for product identification purposes only and does not imply endorsement.

and crystallisation from ethyl acetate/hexane (8 : 2 v/v). Purity was assayed by high-performance liquid chromatography with UV detection and found to be >99%.

Inhibition of *In Vitro* Drug Transport

The potential inhibition of P-glycoprotein function by macrolides and telithromycin was investigated using monolayers of P-glycoprotein-expressing Caco-2 cells (a human colon carcinoma cell line) as previously described.[9,29,30] In brief, Caco-2 cells were obtained from the American Type Culture Collection. The cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine 2 mmol/L, penicillin 100 U/mL, streptomycin 100 µg/mL, 1% sodium pyruvate and 1% nonessential amino acids at 37°C and 5% CO_2. All cell culture media were obtained from Invitrogen GmbH (Karlsruhe, Germany). For transport studies, cells (passage number 43–65) were plated onto Transwell™ filters (Costar®; Corning Inc., Acton, MA, USA) and monolayers were used for experiments 7 days later. To ensure the integrity of the monolayer, the transepithelial resistance was measured after replacing the medium in each compartment by serum-free medium (Optimem™, Gibco-Invitrogen GmbH; Karlsruhe, Germany). Only wells with a resistance of ≥200Ω (≥182 Ω/cm^2) after correction for the resistance obtained in control blank wells were used. For transport experiments, the medium in each compartment was then replaced with 800µL of test solution. This solution consisted of a serum-free medium with addition of tracer [^{3}H]digoxin and 5 µmol/L of unlabelled digoxin as a P-glycoprotein substrate on the basal or apical side of the monolayer. In both compartments, the test solution also contained one of the macrolides as a putative inhibitor in concentrations of up to 500 µmol/L. For control experiments, the well known P-glycoprotein inhibitor valspodar (PSC-833; 1 µmol/L) [kindly provided by Novartis, Basel, Switzerland] was used instead of the macrolides. After 1, 2, 3 and 4 hours of incubation, the percentage of administered radioactivity appearing in the opposite compartment (basal or apical) was determined. Aliquots were analysed by liquid scintillation counting (Perkin Elmer Life Sciences GmbH; Rodgau-Jügesheim, Germany) after the addition of Ultima Gold™ (Perkin Elmer Life Sciences GmbH). All *in vitro* experiments were repeated at least six times. To control for the integrity of the monolayer after 4 hours and to rule out an influence of the macrolides on the integrity of the monolayer, the transport of [^{3}H]inulin was determined in additional experiments in the presence of maximum concentrations of the putative inhibitors after 4 hours of incubation.

Data Analysis for *In Vitro* Experiments

The apparent permeability coefficients (P_{app}) from the cumulative basal-to-apical and apical-to-basal transport rates were determined according to equation 1:

$$P_{app}\,(\text{cm/sec}) = \frac{dQ}{dt} \times \frac{1}{(A \times C_0)} \qquad \text{(Eq. 1)}$$

where dQ/dt (mmol/sec) is the transport rate at 4 hours, C_0 (mmol/cm^3) is the initial substrate concentration in the donor compartment, and A (cm^2) is the surface area of the monolayer (1.1 cm^2). Basal-to-apical net transport was calculated after 4 hours by subtracting the apical-to-basal transport rate from the basal-to-apical transport rate. The percentage transport in inhibition experiments refers to control experiments in the absence of macrolides. The corresponding concentrations producing 50% inhibition (IC_{50} values) for inhibition of P-glycoprotein-mediated digoxin transport were calculated by fitting the basal-to-apical net transport at different inhibitor concentrations to a sigmoidal dose responsive regression curve (Prism 4.01 2004, GraphPad Software, Inc., San Diego, CA, USA).

Data Acquisition for Pharmacoepidemiological Study

The patients included in this study were recruited from pharmacoepidemiological study cohorts in the Department of Internal Medicine at University Hospital Erlangen-Nuremberg, the Department of Geriatric Medicine at Waldkrankenhaus Erlangen and the Department of Gastroenterology at University Hospital Regensburg, Germany. Within these cohorts, the patients' demographic, medication and laboratory data were collected using software specifically developed for pharmacovigilance studies.[31-33]

Data Analysis for Pharmacoepidemiological Study

In these cohorts, a total of 458 patients received cardiac glycosides for treatment of heart failure or atrial fibrillation. To these patients, the following inclusion criteria were applied: (i) routine therapeutic drug monitoring (trough concentrations) for cardiac glycosides had been performed; and (ii) steady-state concentrations had been reached. All patients who met the inclusion criteria were screened for administration of P-glycoprotein inhibitors[4] or macrolides/telithromycin as included in our *in vitro* experiments. Patients receiving at least one of these inhibitors were included in the case groups. Patients who received no P-glycoprotein inhibitor were included in the control groups. In an initial analysis, only the first hospital stay was considered for evaluation. In an additional step, patient data from subsequent hospital stays were also included in the analysis if any of the following parameters had changed since their first hospital stay: the administered cardiac glycoside; the cardiac glycoside daily dose; or the comedication with P-glycoprotein inhibitors. The patients' daily digoxin dosages were transferred to molar values to account for molecular weight differences between α-methyldigoxin and β-acetyldigoxin.

Statistical Analysis

In vitro data are presented as the mean ± 1 standard error of the mean. Multiple comparisons were analysed by ANOVA with a subsequent Tuckey test by using Prism 4.01 2004 (GraphPad Software, Inc.).

For the *in vivo* pharmacoepidemiological study, the database was screened with correlation analysis for related variables and possible covariates. The mean digoxin- and digitoxin serum values for each group were calculated on the basis of regression estimates using the general linear model in SPSS version 13 software (SPSS Inc., Chicago, IL, USA).

Serum digoxin/digitoxin values from the case and control groups were compared in a univariate analysis, taking into account the possible covariates of age, bodyweight, height, renal function (creatinine clearance determined according to the Cockcroft and Gault formula), hepatic function (AST, ALT and serum bilirubin), the daily dose of cardiac glycosides and the number of P-glycoprotein inhibitors. The data are presented as mean values ±1 standard deviation (SD) or with 95% confidence intervals (CIs). A p-value of <0.05 was required for statistical significance. In case of variance inhomogeneity, the level of significance was increased from $p < 0.05$ to $p < 0.01$.

Results

Inhibition of P-Glycoprotein by Macrolides

In Vitro Experiments

In control experiments, the directional basal-to-apical net transport of the P-glycoprotein substrate digoxin across the Caco-2 cell monolayers was confirmed. The respective P_{app} values (basal-to-apical $9.3 \pm 2.2 \times 10^{-6}$ cm/sec, apical-to-basal $2.4 \pm 0.7 \times$

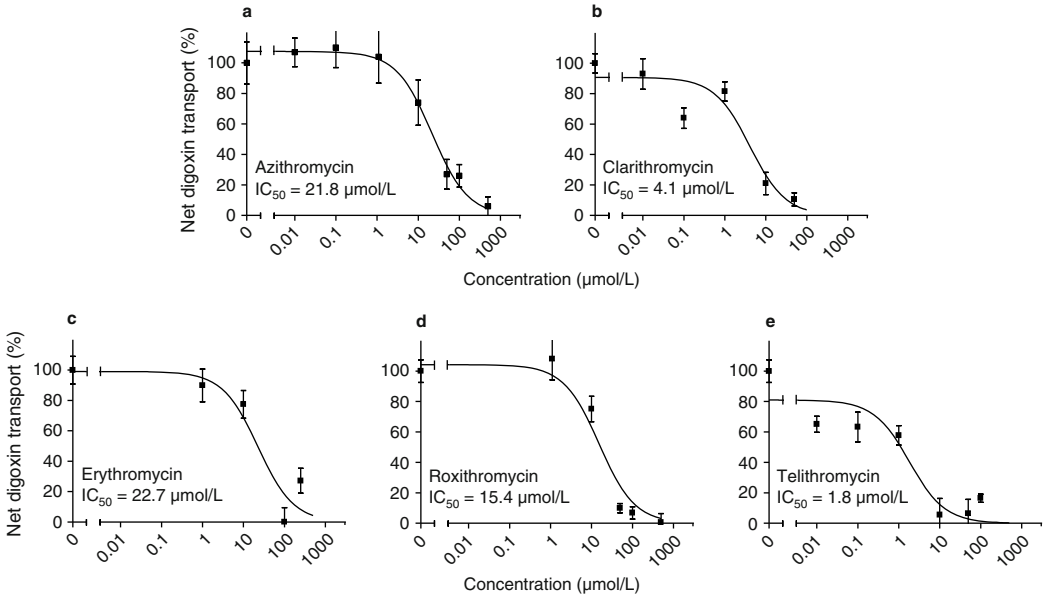

Fig. 1. Inhibition of P-glycoprotein-mediated digoxin net transport in Caco-2 monolayers by different concentrations of macrolides: (**a**) azithromycin; (**b**) clarithromycin; (**c**) erythromycin; (**d**) roxithromycin; and (**e**) telithromycin. Basal-to-apical net transport of [^{3}H]digoxin (5 μmol/L) is presented at increasing macrolide concentrations. The basal-to-apical net transport mediated by P-glycoprotein was obtained by subtracting the apical-to-basal transport rate from the basal-to-apical transport rate. The concentrations producing 50% inhibition (IC_{50} values) were calculated by fitting the data to a sigmoidal dose responsive regression curve. The data are shown as the percentage of control experiments (in the absence of macrolides) and expressed as the mean ± 1 standard error of the mean from ≥6 experiments.

10^{-6} cm/sec, $p < 0.001$) were in good agreement with previously reported values.[13,29,34] As previously published,[13] this directional transport could be inhibited completely by valspodar (data not shown). All macrolides showed significant inhibition of P-glycoprotein-mediated digoxin transport (figure 1). The following sequence of inhibitory potency was determined: telithromycin > clarithromycin > roxithromycin > azithromycin > erythromycin, with corresponding IC_{50} values of 1.8 μmol/L, 4.1 μmol/L, 15.4 μmol/L, 21.8 μmol/L and 22.7 μmol/L, respectively (figure 1). The transepithelial translocation of [^{3}H]inulin was not higher than 1%/hour in the absence and presence of azithromycin, clarithromycin, erythromycin, roxithromycin and telithromycin at the maximum tested concentrations, indicating a lack of effect of macrolides and telithromycin on the integrity of the cell monolayers.

Influence of P-Glycoprotein Inhibitors on Serum Concentrations of Digoxin and Digitoxin

Pharmacoepidemiological Study

Of 458 datasets, the inclusion criteria were met by 157 patients on cardiac glycosides (80 men and 77 women). Of these 157 patients, 77 received digitoxin and 80 received digoxin. The demographic data and blood chemistry values of both patient groups are summarised in table I. As expected, patients treated with digitoxin had significantly reduced creatinine clearance compared with the digoxin group (table I). Altogether, ten different P-glycoprotein inhibitors were applied to 38 patients in the digitoxin group and 31 patients in the digoxin group, both representing the case groups (table II). On average, 1.3 and 1.5 P-glycoprotein inhibitors per patient were administered in the case groups,

Table I. Demographic and laboratory parameters of patients[a]

Parameter	Digitoxin	Digoxin
No. of patients	77	80
Sex		
male (n)	49	31
female (n)	28	49
Age (y)	75 ± 9.7	77 ± 10.0
Bodyweight (kg)	68.2 ± 14.1	70.2 ± 17.1
Creatinine clearance (mL/min)	51.8 ± 30.1	62.2 ± 24.5[b]
AST (U/L)	34.7 ± 40.5	39.0 ± 46.2
ALT (U/L)	32.9 ± 46.2	43.3 ± 34.7
Serum bilirubin (mg/dL)	0.76 ± 0.50	0.82 ± 0.83
Daily cardiac glycoside dose (μmol)	0.099 ± 0.033	0.206 ± 0.085
Patients with concentrations above the therapeutic serum cardiac glycoside range [n (%)][c]	12 (16)	16 (20)
Patients treated with P-glycoprotein inhibitors [n (%)]	38 (49)	31 (39)

a Values are expressed as the mean ± SD unless specified otherwise.

b Significant group difference, unpaired t-test ($p < 0.05$).

c Serum digitoxin >25 ng/mL; serum digoxin >1.5 ng/mL.

together with digitoxin and digoxin, respectively. A subgroup analysis of patients with serum cardiac glycoside concentrations above the therapeutic range (see table I) revealed that 33% (digitoxin) and 56% (digoxin) were additionally treated with P-glycoprotein inhibitors. In the presence of P-glycoprotein inhibitors, the odds ratio for elevated serum cardiac glycoside concentrations using digoxin instead of digitoxin was 3.48 (95% CI 0.95, 12.69). Without inhibitors, the odds ratio was <1 (0.65; 95% CI 0.21, 1.97).

Pharmacoepidemiological Study: Digitoxin

Patients receiving concomitant P-glycoprotein inhibitors had serum digitoxin concentrations similar to those of the control patients (18.9 ± 6.9 vs 18.6 ± 6.5 ng/mL) [figure 2a]. This result remained unchanged by inclusion of values from subsequent hospital stays under changed medication and consideration of possible covariates such as hepatic function (AST, ALT and serum bilirubin) [figure 2b].

Table II. Number of P-glycoprotein inhibitors administered in the digitoxin and digoxin case groups

P-glycoprotein inhibitor	Patients receiving digitoxin plus a P-glycoprotein inhibitor (n)[a]	Patients receiving digoxin plus a P-glycoprotein inhibitor (n)[a]
Amiodarone	2	3
Atorvastatin	1	5
Carvedilol	5	8
Ciclosporin	1	0
Flupentixol	1	0
Macrolides (clarithromycin, erythromycin)	4	8
Propafenone	0	1
Spironolactone	15	11
Verapamil	19	10
Total	**48**	**46**

a Any patient who received two P-glycoprotein inhibitors is listed twice in this table.

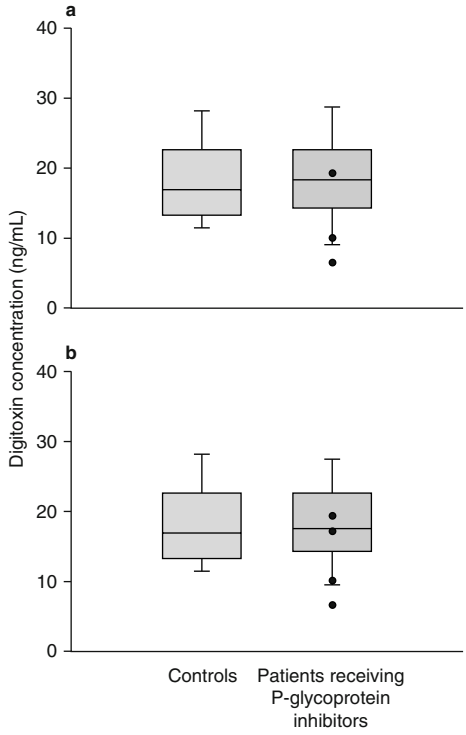

Fig. 2. Serum digitoxin concentrations in patients receiving P-glycoprotein inhibitors compared with controls (**a**) and also taking into account data from subsequent hospital stays under changed medication (**b**). The dots in the P-glycoprotein inhibitor group correspond to data from single patients receiving macrolides [n = 3 in (a) and n = 4 in (b)]. The central line in each box represents the median value, the lower and upper lines represent the 25th and 75th percentiles, respectively, and the whisker represents the 10th and 90th percentiles.

Pharmacoepidemiological Study: Digoxin

Because the daily digoxin dose and the patients' mean creatinine clearance values differed between the case group and the control group, these variables were included as covariates in the univariate analysis of variance. Patients receiving concomitant P-glycoprotein inhibitors had higher serum digoxin concentrations than those not receiving them. The result was confirmed by adding covariates such as creatinine clearance and the daily digoxin dose (1.3 ± 0.6 vs 0.9 ± 0.5 ng/mL, $p < 0.01$) [figure 3a]. Addition of patient data from subsequent hospital stays did not affect the observed difference ($p = 0.01$) [figure 3b].

Pharmacoepidemiological Study: Macrolides

Data analysis of normalised cardiac glycoside serum concentrations (percentage values based on group means) revealed that patients receiving concomitant macrolides (erythromycin in six patients, clarithromycin in six patients) had higher serum cardiac glycoside concentrations than control patients receiving no P-glycoprotein inhibitor (Moses rank test $p < 0.05$) [figure 4a]. In a further analysis, this effect was significant for the digoxin subgroup only (serum digoxin concentration control vs

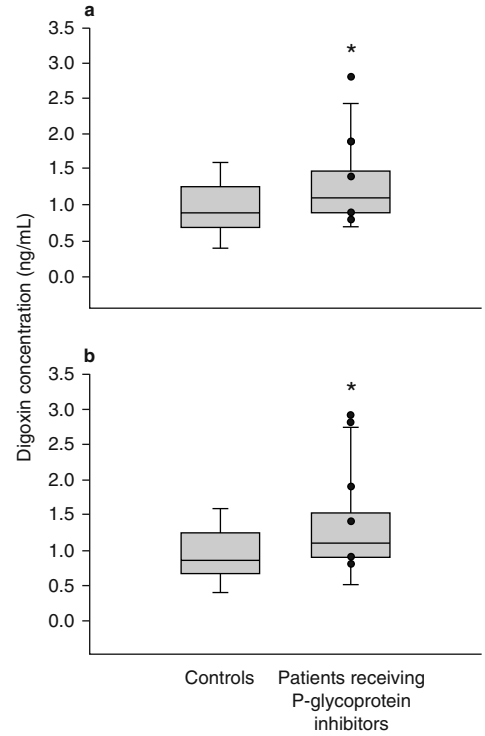

Fig. 3. Elevated serum digoxin concentrations in patients receiving P-glycoprotein inhibitors compared with controls (**a**) and also taking into account data from subsequent hospital stays under changed medication (**b**). The dots in the P-glycoprotein inhibitor group correspond to data from single patients receiving macrolides [n = 7 in (a) and n = 8 in (b)]. The central line in each box represents the median value, the lower and upper lines represent the 25th and 75th percentiles, respectively, and the whisker represents the 10th and 90th percentiles. * $p < 0.01$ vs controls.

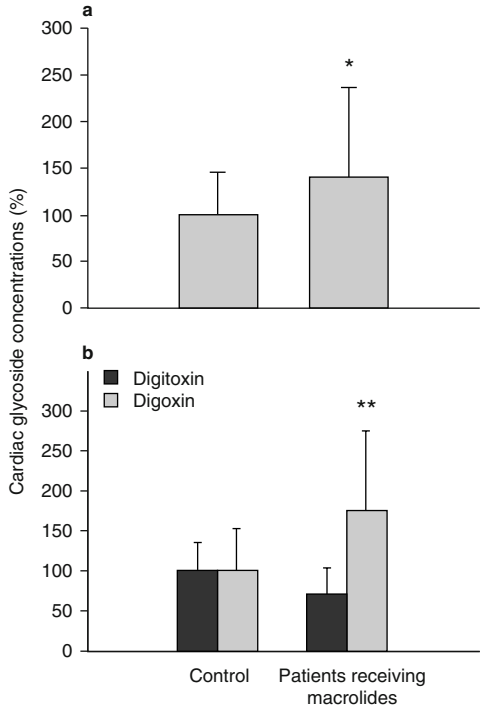

Fig. 4. (**a**) Serum cardiac glycoside concentrations in patients receiving macrolides compared with controls. Pooled data from all patients are shown (normalised to the mean values of each control group; the data are shown as the mean percentage ± SD). (**b**) Serum digoxin and digitoxin plasma concentrations in patients receiving macrolides compared with controls treated with digoxin or digitoxin only. The data are normalised to the mean values of each control group. The effect of macrolides on cardiac glycoside serum levels was significant in the digoxin group. * $p < 0.05$, ** $p < 0.01$ vs controls.

macrolide 100.0 ± 52.9% vs 175.0 ± 100.1%, Moses rank test $p < 0.01$) [figure 4b].

Discussion

Cardiac glycosides play an important role in the treatment of cardiovascular diseases such as heart failure or tachyarrhythmia. Because of their narrow therapeutic range, it is essential to understand factors determining interindividual differences in serum concentrations of cardiac glycosides. For example, recent data have indicated that women with heart failure and high serum digoxin concentrations were at increased risk of death.[27,35]

In this study, we observed the P-glycoprotein inhibitory effect of four commonly used macrolides and telithromycin *in vitro* using monolayers of P-glycoprotein-expressing Caco-2 cells. We found that all compounds inhibited P-glycoprotein-mediated digoxin transport, with IC_{50} values of 1.8–22.7 μmol/L. It is very likely that these concentrations are reached at the apical membrane of enterocytes, where P-glycoprotein is an important part of the barrier against entry of xenobiotics.

In our pharmacoepidemiological study of patients from routine clinical management, we showed that coadministration of P-glycoprotein inhibitors was associated with increased serum concentrations of digoxin, whereas no significant effect of P-glycoprotein inhibitors on serum concentrations of digitoxin was detected. Our findings on digoxin are consistent with those of a previous retrospective study[26] in which the possible influence of P-glycoprotein inhibitors on serum digoxin concentrations, but not on digitoxin concentrations, was assessed in comparison with controls in a parallel-group design. In line with our data, the investigators demonstrated that serum concentrations of digoxin were increased in the case group in comparison with controls, using a univariate and multivariate approach.[26] In accordance with our *in vitro* data, we were also able to show that concomitant administration of the macrolides clarithromycin and erythromycin was associated with significantly increased cardiac glycoside serum concentrations in comparison with controls who did not receive macrolides.

Our *in vitro* data are in line with previous studies of erythromycin, clarithromycin and roxithromycin.[17,23,24] Pachot et al.[24] showed qualitatively that 100 μmol/L (but not 1 μmol/L) of roxithromycin and telithromycin significantly inhibited basal-to-apical verapamil translocation across Caco-2 monolayers. Unfortunately, no IC_{50} values were determined in this study.[24] The IC_{50} values for P-glycoprotein inhibition obtained in our study were

close to the therapeutic serum concentrations of the investigated compounds in humans. Since the highest inhibitor concentrations are reached in the gut lumen, it is likely that inhibition of intestinal P-glycoprotein plays the major role in the observed drug interactions. This is in line, for example, with the increased bioavailability of digoxin when coadministered with clarithromycin compared with the moderate effect on digoxin renal clearance.[17,19,20] In addition, there have been a number of case reports of interactions of macrolides (erythromycin or clarithromycin) and digoxin, where P-glycoprotein inhibition is likely to be one important underlying mechanism.[14,17,36-39]

We observed increased cardiac glycoside serum concentrations in patients receiving macrolides. Interestingly, the patient with the highest serum digoxin concentration during administration of the P-glycoprotein inhibitor clarithromycin (2.9 ng/mL [figure 3b], which is in the toxic range), had had a previous hospital stay without any coadministration of a P-glycoprotein inhibitor. During that previous hospital stay, the patient's serum digoxin concentrations had been in the therapeutic range (0.8 ng/mL). These data indicate that cardiac glycosides serum concentrations need to be carefully monitored in the clinical setting if these macrolides are coadministered.

There are several lines of evidence from *in vivo* studies for a major role of P-glycoprotein in digoxin disposition in humans. First, following intravenous application to healthy subjects, about 11% of the digoxin dose was found in the gut lumen, demonstrating the secretory function of intestinal cells.[25] Second, this efflux transport could be inhibited by luminally administered P-glycoprotein inhibitors.[25] Increased bioavailability of digoxin was observed when it was coadministered orally with quinidine, a potent P-glycoprotein inhibitor.[40] Third, reduced biliary clearance was observed when digoxin and quinidine were administered simultaneously.[41] Fourth, animal studies and studies in humans also showed reduced renal clearance of digoxin when quinidine was coadministered.[40,42] In addition, *in vitro* and *in vivo* data have indicated a potential role of P-glycoprotein in digitoxin disposition in humans.[13,43,44]

In our pharmacoepidemiological study, we found increased serum digoxin concentrations but no change in digitoxin serum concentrations compared with the respective control groups. The latter finding was unexpected since no obvious differences for both P-glycoprotein substrates have been reported from *in vitro* experiments.[13] One possible explanation is the different pharmacokinetic properties of both cardiac glycosides. Whereas digitoxin has nearly maximal oral bioavailability (90%) in humans, the lower bioavailability of digoxin (60–80%) could lead to a more pronounced increase in serum digoxin concentrations if P-glycoprotein inhibitors are coadministered.[45,46] This indicates that the effect of P-glycoprotein inhibition on digitoxin serum concentrations will be smaller than with digoxin.

The present study may have underestimated the effect of digitalis drug interactions because of other confounders in the hospital setting (e.g. underlying diseases, partial noncompliance, comedications not being mentioned to the physician, e.g. St John's wort). On the other hand, clinicians need information about drug interactions that reflect 'real life' in the hospital, e.g. studying drug interactions in steady-state conditions. We therefore believe that the present study contributes to this knowledge.

Conclusion

By use of a combined *in vitro* and *in vivo* approach, we have provided some insight into determinants of cardiac glycoside serum concentrations. First, a direct *in vitro* comparison of four frequently used macrolides and telithromycin as P-glycoprotein inhibitors was conducted. Second, the relevance

of P-glycoprotein inhibition to routine hospital care was shown, since P-glycoprotein inhibitors increased serum digoxin concentrations. Finally, in accordance with the *in vitro* data, patients receiving macrolides had significantly increased serum concentrations of cardiac glycosides.

In view of recent data showing that serum digoxin concentrations of >1.2 ng/mL seem to be harmful in women with heart failure,[27] particular attention should be given to transporter-mediated drug-drug interactions in the clinical setting.

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