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Short communication

Impact of *ABCB1* (*MDR1*) gene polymorphism and P-glycoprotein inhibitors on digoxin serum concentration in congestive heart failure patients

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Abstract:
Digoxin, a drug of narrow therapeutic index, is a substrate for common transmembrane transporter, P-glycoprotein, encoded by *ABCB1* (*MDR1*) gene. It has been suggested that *ABCB1* polymorphism, as well as co-administration of P-glycoprotein inhibitors, may influence digoxin bioavailability. The aim of the present study was to evaluate the effects of *ABCB1* gene polymorphism and P-gp inhibitor co-administration on steady-state digoxin serum concentration in congestive heart failure patients. Digoxin concentrations as well as 3435C > T and 2677G > A,T *ABCB1* single nucleotide polymorphisms, were determined in 77 patients administered digoxin (0.25 mg daily) and methyldigoxin (0.50 mg daily), some of them co-medicated with known P-glycoprotein (Pgp) inhibitors. Significant differences were noted in digoxin serum concentrations ($C_{min,ss}$) between patients co-administered and not co-administered P-gp inhibitors: 0.868 ± 0.348 and 0.524 ± 0.281 for digoxin ($p < 0.002$), as well as 1.280 ± 0.524 and 0.908 ± 0.358 for methyldigoxin ($p < 0.02$), respectively. No influence of *ABCB1* 2677G > A,T and C3435C > T polymorphisms on digoxin concentration was noted. Although some of the previous studies have shown that digoxin pharmacokinetics might be affected by *ABCB1* genetic polymorphism, those modest changes are probably clinically irrelevant, and digoxin dose adjustment should include P-gp inhibitor co-administration rather than *ABCB1* genotyping.

Key words:
ABCB1 polymorphism, P-glycoprotein, digoxin, genetic polymorphism, inhibitors

Abbreviations: *ABCB1* – ATP-binding cassette, sub-family B, member 1; $C_{min,ss}$ – minimum concentration at steady state, Dx3 – digoxin; MDx3 – β-methyldigoxin, *MDR1* – multidrug resistance-1, PCR– polymerase chain reaction, P-gp – P-glycoprotein

Introduction

Digoxin (Dx3) is clinically used as an important drug for treatment of congestive heart failure, and it is subject to therapeutic monitoring, because the range of effective serum drug concentrations is very narrow. β-Methyldigoxin (MDx3) is a more absorbable digoxin derivative, and as it is mainly metabolized to Dx3, both glycosides can be monitored using the same immunoassay [5, 14].

As a substrate for common transmembrane transporter, P-glycoprotein, digoxin is one of the model drugs to study P-glycoprotein activity and function [13, 19]. Since P-gp is a drug efflux pump, widely expressed in all blood-tissue barriers, including intes-

tine, P-gp activity is an important factor, limiting bioavailability of digoxin. Some compounds, including drugs often co-administered with digoxin in congestive heart failure patients, act as P-gp inhibitors. Decreased transporter activity may lead to the increase in intestinal absorption of P-gp substrates [11, 17, 21]. Hence, one can expect that co-administration of P-gp inhibitors may influence digoxin serum concentration in steady-state patients.

Recently, several single nucleotide polymorphisms (SNPs) have been identified in *ABCB1* (*MDR1*) gene, including two potentially influencing P-gp activity: 3435C > T in exon 26 and 2677G > T,A in exon 21 [6]. In homozygous 3435TT individuals, the P-gp expression in digestive system is lower in comparison with heterozygous 3435CT and homozygous 3435CC subjects. The 3435TT homozygotes have been reported to have higher plasma concentrations of digoxin, due to decreased P-gp activity in the digestive tract [6]. The 3435C > T SNP is a silent polymorphism that does not cause amino acid substitution, but it appears to be the main factor in allelic variation of *ABCB1* mRNA expression by changing mRNA stability [22]. The 2677G > T,A SNP, leading to amino acid substitution (Ala893Thr and Ala893Ser) is suggested to be linked, in a majority of subjects, with the SNP in exon 26 [18]. Individuals who were homozygous for 2677A,T had significantly decreased intestinal P-gp expression and increased digoxin serum levels after oral administration [18]. However, the influence of *ABCB1* gene polymorphism on digoxin pharmacokinetics is still not clear, as the results of different studies (mainly in healthy individuals) are contradictory [1, 4, 6, 8, 11, 15, 20, 21].

The aim of the present study was to evaluate the effects of *ABCB1* gene polymorphism on steady-state digoxin serum concentration in congestive heart failure patients. Additionally, the influence of P-gp inhibitor co-administration was evaluated.

Materials and Methods

Patients

Seventy-seven unrelated Polish subjects of Caucasian origin diagnosed with congestive heart failure, 40 males and 37 females, aged from 30 to 85 years were included in this study after giving informed consent. The patients were medicated with digoxin 0.25 mg daily (Digoxin, Polfa-Warszawa; group 1, n = 39), and β-methyldigoxin 0.50 mg daily (Bemecor, Lek; group 2, n = 38) for at least 7 days to reach steady state. The subjects treated with each glycoside were subsequently divided into two subgroups: group 1A (n = 15) and 2A (n = 16) – subjects not taking any known P-glycoprotein inhibitors, and group 1B (n = 24) and 2B (n = 22) – patients co-administered P-glycoprotein inhibitors for at least 7 days (spironolactone, carvedilol, verapamil, famotidine, amiodarone, propaphenone, prednisone, atorvastatin, simvastatin). The Ethics Committee of the Pomeranian Medical University in Szczecin, Poland, approved protocol of the study.

Digoxin measurement

Blood samples were collected from peripheral vein at steady state (at least 7 days from the medication onset), before morning drug administration ($C_{min,ss}$). In all study subjects, digoxin concentrations were measured in blood serum by the fluorescence polarization immunoassay (FPIA) using TDx apparatus (Abbott, USA). Reference interval for digoxin serum concentration determined by the method is 0.8–2.0 ng/ml.

Genotyping

Genomic DNA was extracted from 450 μl of whole blood samples using an inorganic and non-enzymatic extraction method. Genotyping for the presence of *ABCB1* 3435C > T and 2677G > A,T SNPs was performed using previously described PCR-based assays applied by our laboratory [12].

Statistical analysis

Genotype and allele frequencies were calculated by direct counting of chromosomes. The data were tested for Hardy-Weinberg equilibrium by calculating expected frequencies of genotypes and comparing them to the observed values using the χ^2 test (Statistica 6.0, Statsoft). Serum digoxin concentrations were compared using the Kruskal-Wallis non-parametric ANOVA test and Mann-Whitney U test (non-normal results distribution). Subsequently, concentration values were log-transformed. The transformed data followed normal distribution, and were analyzed by

two-way ANOVA test in relation to *ABCB1* genotype and P-gp inhibitors co-administration.

Results

Mean serum digoxin levels (± SD) in group 1 (Dx3) were significantly lower compared to group 2 (MDx3): 0.725 ± 0.368 ng/ml and 1.123 ± 0.492 ng/ml, respectively ($p < 0.0001$). Significant differences were noted in digoxin serum concentration between patients co-administered and not co-administered P-gp inhibitors. Respective values were: 0.524 ± 0.281 ng/ml (group 1A – digoxin, no inhibitors), 0.868 ± 0.348 ng/ml (group 1B – digoxin, with inhibitors, $p < 0.002$), and 0.908 ± 0.358 ng/ml (group 2A – methyldigoxin, no inhibitors), 1.280 ± 0.524 ng/ml (group 2B – methyldigoxin, with inhibitors, $p < 0.02$).

The following *ABCB1* allele frequencies have been found in study subjects: 2677G – 0.591, 2677T – 0.370, 2677A – 0.039, 3435C – 0.474, 3435T – 0.526. They did not differ significantly from frequencies in general population [12], as evaluated by means of Fisher exact test. Evaluation of the impact of *ABCB1* 2677G > A,T and C3435C > T polymorphisms on digoxin concentration did not reveal any significant influence of the genotype (Table 1). ANOVA analysis of log-transformed data confirmed that only P-gp inhibitor co-administration ($p < 0.02$), but not *ABCB1* genotype ($p > 0.7$) could influence digoxin concentrations in congestive heart failure patients in both study groups (Dx3 and MDx3-administered).

Discussion

In the present study, no association was found between *ABCB1* polymorphism and steady-state digoxin serum concentration in congestive heart failure patients. The first study on the effect of *ABCB1* gene polymorphism on Dx3 pharmacokinetics was conducted by Hoffmeyer et al. in 2000, reporting an association of 3435C > T SNP with plasma concentration of digoxin after oral administration in healthy individuals [6]. In that study, 3435T allele was associated with lower P-gp expression, and maximum concentration of Dx3 at steady state in 3435TT homozygous subjects was found to be significantly higher compared to 3435CC homozygotes. In the subsequent years, clinical studies have been conducted all around the world, investigating an association between *ABCB1* polymorphism with P-gp expression, function

Tab. 1. Serum digoxin concentrations [ng/ml] ($C_{min,ss}$, mean ± SD) in congestive heart failure patients differing in *ABCB1* genotypes

	3435C > T				2677G > T,A			
	CC	CT	TT	p	GG	GT, GA	TT, TA	p
Digoxin								
All patients	n = 7 0.793 ± 0.210	n = 23 0.695 ± 0.399	n = 9 0.750 ± 0.405	0.873	n = 15 0.681 ± 0.245	n = 15 0.865 ± 0.380	n = 9 0.567 ± 0.464	0.082
Group 1A*	n = 3 0.655 ± 0.107	n = 9 0.405 ± 0.185	n = 3 0.703 ± 0.518	0.263	n = 4 0.555 ± 0.244	n = 6 0.550 ± 0.193	n = 5 0.436 ± 0.358	0.377
Group 1B**	n = 4 0.848 ± 0.230	n = 14 0.918 ± 0.378	n = 6 0.773 ± 0.392	0.566	n = 11 0.727 ± 0.244	n = 9 0.979 ± 0.371	n = 3 1.025 ± 0.644	0.209
Methyldigoxin								
all patients	n = 8 1.098 ± 0.366	n = 20 1.139 ± 0.570	n = 10 1.113 ± 0.453	0.638	n = 16 1.145 ± 0.582	n = 14 1.049 ± 0.403	n = 8 1.209 ± 0.482	0.781
Group 2A*	n = 5 1.026 ± 0.377	n = 6 0.882 ± 0.477	n = 5 0.820 ± 0.182	0.552	n = 7 0.930 ± 0.341	n = 6 0.943 ± 0.478	n = 3 0.783 ± 0.110	0.976
Group 2B**	n = 3 1.217 ± 0.408	n = 14 1.249 ± 0.586	n = 5 1.406 ± 0.462	0.604	n = 9 1.312 ± 0.690	n = 8 1.129 ± 0.348	n = 5 1.464 ± 0.429	0.211

All p values were calculated by means of Kruskal-Wallis non-parametric ANOVA test; * Group 1A and 2A – patients not taking P-gp inhibitors; ** Group 1B and 2B – patients co-administered P-gp inhibitors

and pharmacokinetics of P-gp substrates. However, there are still discrepancies in the results. Although some investigators confirmed the results of Hoffmeyer [8, 11, 20, 21], others found no association between *ABCB1* genotype and digoxin concentration [1, 4]. Moreover, several studies provided contrary results, as drug concentrations and P-gp expression were found significantly higher in 3435CC homozygotes [9, 15]. Similarly conflicting data have also been noted for the exon 21 2677G > T,A polymorphism, where some authors found 2677TT genotype associated with higher Dx3 concentration [10, 20], some found no significant differences [4], and others suggested an association of 2677G allele with increased digoxin bioavailability [7, 15].

Possibly, an impact of *ABCB1* polymorphism on P-gp expression and activity in steady-state digoxin level in congestive heart failure patients is very slight compared to other factors influencing P-gp-mediated transport, like dietary compounds, herbs, age, hormonal status, diseases, and especially co-administered drugs [1–3]. In the current study, we have observed a significantly higher digoxin concentration in patients co-administered P-gp inhibitors (about 1.5-fold elevation *vs*. subjects without P-gp inhibitors), which is in concordance with the results of previous studies [11, 17, 21]. As digoxin is characterized by a narrow therapeutic index, and Dx3 serum concentration is a subject to drug monitoring, the differences may be of clinical relevance. Although some of the previous studies have shown that digoxin pharmacokinetics might be affected by *ABCB1* genetic polymorphism, those modest changes are probably clinically irrelevant, and digoxin dose adjustment should be based on P-gp inhibitors co-administration rather than on *ABCB1* genotyping. However, influence of *ABCB1* genotype on steady-state digoxin serum concentration cannot be definitely excluded based on the current study, as the number of subjects in the study subgroups does not provide enough power to detect some minor effects.

In the recent, very extensive review by Sakaeda [16], it was concluded that *ABCB1* genotypes were generally little associated with the pharmacokinetics of drugs, since effects of intestinal P-gp on the intestinal absorption of substrates are minimal for commercially available oral drugs, including digoxin. The results of the present study confirm that conclusion, as no genotype-related differences were noted in serum digoxin levels among congestive heart failure patients.

The present study also indicated that medication with standard doses of digoxin (0.25 mg daily) resulted in lower serum digoxin concentrations than administration of methyldigoxin (0.5 mg daily), which were often below recommended range. This observations are keeping with the notion of more efficient oral absorption of methyldigoxin than digoxin [9].

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