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Article

ABCB1 Polymorphism Is Associated with Higher Carbamazepine Clearance in Children

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Abstract: Background/Objectives: The aim of our study was to investigate the role of *ABCB1* polymorphism in pharmacokinetics of carbamazepine (CBZ) in children. **Methods**: The study enrolled 47 Serbian pediatric epileptic patients on CBZ treatment. Genotyping for *ABCB1* 1236C<T (rs1128503), 2677G<A/T (rs2032582) and 3435C<T (rs1045642) was carried out using TaqMan method. Steady-state CBZ serum concentrations were available from the previous study, determined by high pressure liquid chromatography (HPLC) method. NONMEM software and one-compartment model were used for pharmacokinetic analysis. **Results**: *ABCB1* 1236C<T, 2677G<A/T and 3435C<T variations were found at the frequencies of 47.9%, 48.9% and 52.1%, respectively. The equation that described population clearance (CL) was CL (l/h) = 0.175 + 0.0403*SEX + 0.0332**ABCB1* + 0.0176*CYP1A2 + 0.000151*DD where SEX has a value of 1 if male and 0 if female, *ABCB1* has a value of 1 if C-G-C/T-T-T and 0 if any other *ABCB1* diplotype, CYP1A2 has a value of 1 if −163A/A and 0 if −163C/C or C/A, and DD is the total CBZ daily dose (mg/day). **Conclusions**: The presence of *ABCB1* 1236T-2677T-3435T haplotype is associated with increased clearance of CBZ in pediatric epileptic patients.

Keywords: *ABCB1*; genetic polymorphism; carbamazepine; population pharmacokinetics; children

1. Introduction

Carbamazepine (CBZ) is an old, well known and widely used anticonvulsant. Yet, it keeps to attract attention of medical and scientific community, due to pronounced inter-individual differences in patients' response, and its high propensity to drug interactions. Both seem to be the consequences of CBZ's complicated pharmacokinetics, whose features are only partly explained: drug absorption, metabolism and elimination in both adults and children have been well described almost half a century ago [1,2], but its transport over blood–brain barrier (BBB) is not fully clarified yet [3,4].

Multidrug resistance protein 1 (MDR1) is an ATP-binding cassette transporter that functions as a cellular efflux pump [5]. It is expressed at membranes, including BBB, and affects bioavailability and pharmacologic effects of many drugs [6], especially those whose action is dependent on BBB crossing [7]. However, the question whether MDR1 transports CBZ remains unanswered: arguments, gained mostly from animal and laboratory studies, exists for both sides of the coin [4]. Namely, it has been observed that inhibition of MDR1 in vivo in rats [8,9] and in vitro in canine kidney cells transfected with human MDR1 [10,11] increases the concentration of CBZ in the cortical extracellular

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fluid and in cells over-expressing MDR1, respectively, indicating that CBZ is a substrate of MDR1. On the other hand, no difference in brain penetration or anticonvulsant CBZ efficacy was observed between wild type and MDR1-deficient mice [12,13], or in transporting through Caco-2 cells with extensive MDR1 activity in the presence of MDR1 inhibitors [14]. At the same time, one human study analyzing resected brain tissue of drug resistant patients undergoing epilepsy surgery, while concluding that MDR1 is not responsible for resistance to anticonvulsants, revealed that co-administration of MDR1 inhibitor increased effectiveness of CBZ [15]. In addition, one case report described great improvement of overall seizure control in a CBZ-treated patient after addition of MDR1 inhibitor verapamil [16]. Currently, CBZ is classified as a probable substrate of MDR1 [17].

MDR1 is encoded by the *ABCB1* (*MDR1*) gene, comprised of 29 exons set within highly variable 209.6kb genomic region [6]. Previous studies have indicated that a number of *ABCB1* single nucleotide polymorphisms (SNPs), surprisingly including synonymous variants too, may result in a transporter with altered activity, expression level or substrate specificity [18]. The three most common and most frequently studied *ABCB1* coding SNPs rs1128503 (1236C>T), rs2032582 (2677G>A/T) and rs1045642 (3435C>T) happen to be the most controversial ones, as, in spite of numerous attempts to identify their impact on MDR1-dependent drug transport, the overall result is inconclusive [19]. The aim of our study was to investigate the possible role of *ABCB1* polymorphism in pharmacokinetics of CBZ in children.

2. Materials and Methods

Forty seven patients with epilepsy, treated with CBZ for at least 4 weeks, were enrolled in the study [20]. They were recruited from the pediatric department of the Clinical Centre, Kragujevac, Serbia, with the written informed assents and consents obtained from all patients and their parents. The study was approved by the ethics committee at the Clinical Centre, Kragujevac, Serbia (approvals No 01-7848 and No 01/20-401), and conducted in accordance with the Declaration of Helsinki and its subsequent revisions.

Genotyping for *ABCB1* 1236C<T (rs1128503), 2677G<A/T (rs2032582) and 3435C<T (rs1045642) was carried out on SaCycler-96 (Sacace Biotechnologies, Como, Italy), with the use of TaqMan Genotyping Master Mix 2X and the corresponding TaqMan DME genotyping Assays (Applied Biosystems, Foster City, CA). For forty patients, steady-state CBZ serum concentrations and CYP1A2 -163C>A genotype data and were available from the previous study [20].

Population pharmacokinetic analysis was performed using the non-linear, mixed-effects modeling program (NONMEM), version 7.3.0 (Icon Development Solution), with ADVAN1 subroutine that describes one-compartment model without absorption. The effects of 21 different covariates (including age, sex, body weight, total CBZ daily dose, co-therapy with valproate, CYP1A2 -163C>A genotype, as well as *ABCB1* data: genotypes, genotype groups and the most frequent diplotypes) were assessed based on the difference between the minimum of objective function (MOF) of the base and the incorporated covariate model. When a covariate was included in an univariate analysis, a decrease in MOF>3.84 ($p<0.05$, df=1) was considered statistically significant. To be considered as candidate for the full model, the influence of covariates was re-tested by the process of backward deletion with MOF difference greater than 6.6 ($p<0.005$, df=1). The stability and predictive performances of the final model were assessed through an internal validation process, using bootstrap analysis.

For haplotype analysis, Hardy-Weinberg equilibrium and linkage disequilibrium calculations, the population genetic software programs Arlequin, version 3.11 (http://cmpg.unibe.ch/software/arlequin3), and Haploview, version 4.2 (https://www.broadinstitute.org/haploview/haploview) were used. Subjects were assigned to genotype groups assuming dominant and recessive genetic models, which compare carriers of at least one, and carriers of both variant alleles, respectively, with other genotypes [21]. The normality of data distribution was confirmed by Shapiro-Wilk test. Doses of CBZ were presented as adjusted per weight, and the observed drug concentrations were normalized by dose per body weight. Comparisons among *ABCB1* genotypes, genotype groups and diplotypes in terms of CBZ doses and CBZ serum concentrations were made with one way ANOVA. Statistical analyses were carried out using Statistica, version 7.1 (StatSoft, Tulsa, OK, USA), and differences at $P<0.05$ were considered significant.

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3. Results

There were 29 male and 18 female patients (mean age ± SD: 10.32±3.06) included in the study, 40 of them diagnosed with idiopathic and 7 with symptomatic epilepsy. In addition to CBZ therapy, five patients were co-treated with valproate. The frequencies of observed *ABCB1* alleles, genotypes, and the most frequent diplotypes are presented in Table 1.

Table 1. Alelle, genotype, and diplotype frequencies of *ABCB1* in epileptic patients on carbamazepine treatment.

		Observed frequency	95% Confidence interval
Allele			
rs1128503 (1236T)		0.479 (45/94)	0.381, 0.579
rs2032582 (2677T/A)		0.489 (44/94)	0.391, 0.589
rs1045642 (3435T)		0.521 (49/94)	0.421, 0.619
Genotype			
rs1128503 (1236C>T)	CC	0.234 (11/47)	0.135, 0.375
	CT	0.574 (27/47)	0.433, 0.705
	TT	0.191 (9/47)	0.103, 0.329
rs2032582 (2677G>T/A)	GG	0.255 (12/47)	0.152, 0.397
	GT	0.511 (24/47)	0.373, 0.647
	GA	0.000 (0/47)	0.000, 0.092
	TT	0.191 (9/47)	0.103, 0.329
	AT	0.043 (2/47)	0.005, 0.152
	AA	0.000 (0/47)	0.000, 0.092
rs1045642 (3435C>T)	CC	0.149 (7/47)	0.072, 0.281
	CT	0.660 (31/47)	0.516, 0.778
	TT	0.191 (9/47)	0.103, 0.329
Diplotype 1236-2677-3435*			
C-G-C/C-G-C		0.064 (3/47)	0.016, 0.180
C-G-C/C-G-T		0.128 (6/47)	0.057, 0.257
C-G-C/T-T-T		0.362 (17/47)	0.240, 0.505
T-T-T/T-T-T		0.128 (6/47)	0.057, 0.257

* other diplotypes were deemed too rare to yield any significant association, thus were not included in statistical and population pharmacokinetics analyses.

All genotype frequencies were in accordance with the Hardy-Weinberg equilibrium ($p \geq 0.373$). Significant linkage disequilibrium was detected among all SNPs, with D' and r2 coefficients of 0.84 and 0.67, respectively, between 1236C<T and 2677G>A/T, 0.62 and 0.33 between 2677G>A/T and 3435C<T, and 0.56 and 0.28 between 1236C>T and 3435C<T (p values ≤ 0.0004).

There was no significant association between any of the examined *ABCB1* genotypes, genotype groups or diplotypes, and either weight-adjusted daily dose ($p \geq 0.10$), or dose-normalized serum concentration of CBZ ($p \geq 0.09$). However, the tendency towards lower doses ($p=0.14$) and higher serum concentrations ($p=0.09$) was observed in carriers of C-G-C/C-G-T, in contrast to C-G-C/T-T-T diplotype, where higher doses ($p=0.45$) and lower serum concentrations ($p=0.25$) were detected.

Of all covariates examined by population pharmacokinetics, only 6 showed to be of significance for the CBZ clearance: sex, total CBZ daily dose, CYP1A2 genotype, *ABCB1* C-G-C/T-T-T and C-G-C/C-G-T diplotypes, and co-therapy with valproate. The necessary additional decrease in the MOF value was not confirmed for the last two, and the final pharmacokinetic model was as follows:

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$$CL\ (l/h) = 0.175 + 0.0403*SEX + 0.0332*ABCB1 + 0.0176*CYP1A2 + 0.000151*DD$$

where SEX has a value of 1 if male and 0 if female, *ABCB1* has a value of 1 if C-G-C/T-T-T and 0 if any other *ABCB1* diplotype, CYP1A2 has a value of 1 if −163A/A and 0 if −163C/C or C/A, and DD is the total CBZ daily dose (mg/day).

Reduction of MOF value and both inter-individual and residual variability was notably recorded in the final model, with the values of 176.807, 20.97% and 16.22%, respectively. Parameter estimates of the final model and bootstrapping analysis were similar, suggesting good accuracy and stability of the final model (Table 2).

Table 2. The final model parameter estimates.

	NONMEM		Bootstrap Analysis	
Parameter	**Estimate**	**95% CI***	**Estimate**	**95% CI‡**
CL/F (l/h)	0.175	0.14 – 0.21	0.170	0.16 – 0.19
SEX	0.0403	0.0324 – 0.0482	0.0401	0.0269 – 0.0537
DD (mg/l)	0.000151	0.000097 – 0.000205	0.000154	0.00011 – 0.00019
CYP1A2	0.0176	0.0133 – 0.0219	0.0181	0.0163 – 0.0199
ABCB1	0.0332	0.0235 – 0.0429	0.0329	0.023 – 0.0367
ω^2_{CL}	0.0361	0.0216 – 0.0506	0.0385	0.0308 – 0.0415
σ^2	0.025	0.0191 – 0.0309	0.0257	0.022 – 0.0294

* (Estimate) ± 1.96 x (standard error of the estimate). ‡ 2.5th and 97.5th percentile of the ranked bootstrap parameter estimates. ω^2_{CL} - Interindividual variance of CL. σ^2 - Residual variance.

4. Discussion

In the present study, we investigated the effect of the three most important *ABCB1* coding SNPs, namely 1236C>T, 2677G>A/T and 3435C>T, on CBZ pharmacokinetics in pediatric epileptic patients. Our main finding is that the presence of *ABCB1* 1236T-2677T-3435T haplotype is associated with increased clearance of CBZ in children. To the best of our knowledge, this is the first population pharmacokinetic study to evaluate and report the effect of *ABCB1* polymorphism on CBZ clearance.

The overall MDR1 activity depends on the level of *ABCB1* expression and the functionality of *ABCB1*-coded protein, both being affected by *ABCB1* polymorphism [22]. The three most common *ABCB1* SNPs include two synonymous and one non-synonymous variation, namely 1236C>T (Gly412Gly), 3435C>T (Ile1145Ile) and 2677G>A/T (Ala893The/Ser), respectively [6]. The frequencies of these SNPs vary by ethnicity [6,23,24], yet seem to be rather uniform among Caucasians [24–31]. In the present study, we have genotyped Serbian epileptic patients, but our results do not differ significantly from previous observations in healthy Serbs [30,31], or other healthy Caucasian populations [24–29]. Similar distribution of *ABCB1* SNPs in epileptic patients and healthy subjects support the lack of association between *ABCB1* polymorphism and the risk of having epilepsy [32].

On the other hand, the available evidence, provided by systematic reviews and meta-analysis, suggests that *ABCB1* genetic variability, especially silent 3435C>T variation, affects the response to antiepileptic drugs [33,34]. It goes without saying that silent gene variations do not alter the primary structure of the protein. Yet, they have been linked with changes in protein function, which they could initiate either by altering messenger RNA splicing or protein stability, or due to linkage disequilibrium with another functional SNP [35]. Triallelic 2677G>A/T, which does cause an amino-acid change [36], is frequently linked to synonimous SNPs 1236C>T and 3435C>T [37,38]. All three *ABCB1* variations (both separately and co-existing on the same haplotype) have been associated with

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altered MDR1 function [39]. Yet, the results were conflicting and the effect was mostly drug-dependent [37,40–46]. In regard to antiepileptic treatment, the observations differed both by drug and by population [34,47].

Previous CBZ-related MDR1/*ABCB1* investigations yielded contradictory conclusions on both the role of MDR in CBZ transport, and the effect of *ABCB1* genetic polymorphism on CBZ pharmacokinetics or efficacy. The possibility of CBZ being a substrate for MDR1, introduced 25 years ago [48], have been supported [8,9,49], but more often opposed [11,13–15,50–52] over the years. While the proposed classification renders CBZ a probable substrate of MDR1 [17], currently there is no consensus on the subject [53]. Similarly, reports on association between *ABCB1* polymorphism and CBZ-based therapy outcome have been inconsistent too, results often depending on ethnicity [54] and including more efficient CBZ transport [55] and lower CBZ plasma concentrations [56] associated with 3435TT genotype, more frequent CBZ-resistant epilepsy observed in carriers of 3435TT, 2677TT or 1236T-2677T-3435T haplotype [57–60], as well as the lack of any significant effect of *ABCB1* genetic variability on CBZ pharmacokinetics [57,61,62] or CBZ-based treatment outcome [61,63–66].

In our study, the observed tendency towards lower serum CBZ concentration in the presence of all three examined *ABCB1* variant alleles has been supported by population pharmacokinetic analysis, which has predicted higher CBZ clearance in carriers of *ABCB1* 1236T-2677T-3435T haplotype. According to the model, the effect of *ABCB1* genetic polymorphism on CBZ pharmacokinetics seems to be even more pronounced than the already reported effects of CBZ dose and the presence of CYP1A2 −163C>A variation, latter explained by the dose-dependent auto-induction of CBZ metabolism [67] and the increased inducibility of CBZ-metabolizing enzyme CYP1A2 [20], respectively. However, the effect of *ABCB1* genetic variability is rather difficult to interpret, as neither pharmacokinetic nor pharmacogenetic-based connection between MDR1/*ABCB1* and CBZ has been firmly established yet.

Nevertheless, the most important CBZ metabolite CBZ-10,11-epoxide (CBZ-E) [68,69] is a confirmed substrate for MDR1 [11,70]. Since CBZ-E is equally active as its parent compound [1,2,71], previously reported association between *ABCB1* 1236T-2677T-3435T haplotype and higher CBZ resistance [57,72] could be due to increased MDR1-dependent efflux of CBZ-E at the BBB level and the consequent increase in CBZ-E plasma concentration. Similar to CBZ [1], CBZ-E induces several drug metabolizing enzymes [73], including the most important CBZ-metabolizing enzyme CYP3A4 [74,75]. Therefore, we hypothesize that the increased plasma CBZ-E concentration in the presence of all three variant *ABCB1* alleles is the reason for the observed increase in CBZ (metabolic) clearance. There are, of course, other possible explanations for the results of our study, including the possibility that CBZ is a substrate of MDR1 [8,9,48,49] and that the presence of *ABCB1* 1236T-2677T-3435T haplotype leads to decreased MDR1-dependent CBZ transport, and/or that the same haplotype somehow affects MDR1 induction by either CBZ [78] or potentially CBZ-E.

In the present study, the steady-state CBZ serum concentrations were available for only 40 patients, so the sample size was probably too small to reveal any statistically significant effect of the examined covariates. Our results are based on the population pharmacokinetic analysis, for which it has been demonstrated that can accurately estimate pharmacokinetic parameters from as few as five subjects having only one blood sample analyzed [76]. Therefore, while we believe our conclusions are sound, performing a replication study involving more participants and more than one blood sample would be advisable to confirm our findings. In addition, it should be noted that the production of CBZ-E and its contribution to CBZ anticonvulsant effect seem to be more pronounced in children [77,78], so the association between *ABCB1* polymorphism and higher CBZ clearance observed in our study might not be present in adult population.

In conclusion, our study reveals higher CBZ clearance in the presence of *ABCB1* 1236T-2677T-3435T haplotype in pediatric epileptic patients. What is the reason for the observed association, as well as whether the same can be expected in adults and in other ethnic populations, remains to be explored.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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