ORIGINAL ARTICLE

THE INFLUENCE OF *CYP2C8*3* ON CARBAMAZEPINE SERUM CONCENTRATION IN EPILEPTIC PEDIATRIC PATIENTS

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ABSTRACT

The aim of the present study was to investigate the distribution of *CYP2C8* variants *3* and *5*, as well as their effect on carbamazepine pharmacokinetic properties, in 40 epileptic pediatric patients on carbamazepine treatment. Genotyping was conducted using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and allele-specific (AS)-PCR methods, and steady-state carbamazepine plasma concentrations were determined by high performance liquid chromatography (HPLC). The *CYP2C8 *3* and *5* polymorphisms were found at frequencies of 17.5 and 0.0%, respectively. After dose adjustment, there was a difference in daily dose in *CYP2C8*3* carriers compared to non carriers [mean ± standard deviation (SD): 14.19 ± 5.39 *vs.* 15.46 ± 4.35 mg/kg; $p = 0.5$]. Dose-normalized serum concentration of carbamazepine was higher in *CYP2C8*3* (mean ± SD: 0.54 ± 0.18 *vs.* 0.43 ± 0.11 mg/mL, $p = 0.04$), and the observed correlation between weight-adjusted carbamazepine dose and carbamazepine concentration after dose adjustment was significant only in *CYP2C8*3* non carriers ($r = 0.52$, $p = 0.002$). However, the population pharmacokinetic analysis failed to demonstrate any significant effect of *CYP2C8 *3* polymorphism on carbamazepine clearance [CL L/h = 0.215 + 0.0696*SEX + 0.000183*DD]. The results indicated that the *CYP2C8*3* polymorphism might not be of clinical importance for epilepsy treatment in pediatric populations.

Keywords: Carbamazepine pharmacokinetics; Children; *CYP2C8*3*; Population pharmacokinetics.

INTRODUCTION

Carbamazepine belongs to the older generation of anticonvulsants, which is almost completely metabolized in the liver through processes that involve several liver enzymes, including CYP2C8 [1-4]. To date, there are 16 different CYP2C8 alleles described http://www.cypalleles. ki.se/cyp2c8.htm), most of them associated with altered enzyme activity [5]. Although genes could affect the drug metabolism there is a general lack of evidence of influence of *CYP2C8* genetic variations on carbamazepine pharmacokinetics, especially in children [6]. Since the drug metabolism in the pediatric population is specific [7], the extrapolation of knowledge from adults, without prior evidence of how various factors influence drug metabolism, may lead to improper management of pediatric therapy [8]. Therefore, the main aim of this study was to investigate the effect of the *CYP2C8* genetic polymorphisms on carbamazepine dosing, serum concentration and clearance, in epileptic pediatric patients.

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MATERIALS AND METHODS

The study was conducted at the Clinical Centre, Kragujevac, Serbia, and involved 40 epileptic pediatric patients on ongoing therapy with carbamazepine [9]. Except for four patients, who were on comedication with valproate, all others were on monotherapy. The study was approved by the relevant Ethics Committee (approval No. 01-7848) and conducted in accordance with the Declaration of Helsinki and its subsequent revisions.

Blood samples for drug concentration analysis were collected twice, both times at the minimal concentration point (8-12 hours after the administration of the last dose): at the beginning of the study, and 4 weeks after dose adjustment. The steady-state carbamazepine serum concentrations were determined by validated high pressure liquid chromatography (HPLC) assay, as described by Jankovic et al. [10]. An additional blood sample was taken for CYP2C8 genotyping, and DNA was isolated using the PurelinkTM genomic DNA kit (Invitrogen, Carlsbad, CA, USA). CYP2C8*3 (416G>A, rs11572080) and CYP2C8*5 (475delA, rs72558196) were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and allele-specific (AS)-PCR methods, respectively, according to Nakajima et al. [11]. Primers and restriction enzyme were from Invitrogen and New England Biolabs (Ipswich, MA, USA), respectively, while all other reagents used were made by Thermo Scientific (Waltham, MA, USA) or Qiagen GmbH (Hilden, Germany). Electrophoresis on a 1.2% agarose gel, stained with Sybr® safe DNA gel stain (Invitrogen), was used to detect the obtained PCR products and restriction fragments.

To determine the factors affecting carbamazepine clearance, population pharmacokinetic modeling was employed, using the nonlinear mixed effect model (NONMEM) software, version 7.3.0 (Icon Development Solution, Hanover, MD, USA), and ADVAN 1 subroutine (within NONMEM) (one compartment model with no absorption). Carbamazepine serum concentrations, age, body weight, sex, total carbamazepine daily dose, CYP2C8 genotype and concomitant therapy with valproate, were included as covariates by the stepwise addition process in the model construction, and their significance was estimated by the likelihood ratio test. The final model was built through a backward deletion from the full model, using covariates that met criteria of the minimum objective function value difference of more than 6.6 for nominal $p <0.01$ and degree of freedom (df) = 1. Inter-patient varia-bility of the clearance and intra-patient (residual) variability in the concentration was estimated by exponential, additive, or proportional error model. Data distribution was assessed through the ratio of predicted (PRED) and measured the dependent variable (DV) concentrations of the drug, as well as the ratio in the weighted residuals (WRES) and PRED values of carbamazepine from the base to the final model. Bootstrapping analysis, as a preferable validation procedure for the small study sample size, was used to evaluate the predictive performance of the final model.

Statistical Analyses. The haplotype analysis was done by the population genetic software program Arlequin, version 3.11 (http://cmpg.unibe.ch/software/arlequin3), and Statistica, version 7.1 (StatSoft, Tulsa, OK, USA) was used for all other statistical analyses. The observed and expected allele frequencies were compared by the χ^2 test, and consistency of the data with the normal distribution was assessed by the Shapiro-Wilk test. The Spearman analysis was used to correlate doses and concentrations of carbamazepine, and the Student t-test for independent groups was used for assessment of dose requirements and carbamazepine serum concentrations in the CYP2C8*3 carrier and non carrier groups. A p value of <0.05 was considered significant.

RESULTS

The assessment included 24 male and 16 female pediatric patients, aged 4-16 years (median: 11 years), weighing 17 to 65 kg (median: 39 kg). All patients received daily doses of 260 to 1000 mg orally as tablets or syrup, and four of them were on concomitant therapy with valproate. Observed CYP2C8 genotype frequencies (Table 1) were in accordance with the Hardy-Weinberg equilibrium ($\chi^2 <1.111$, $p = 0.05$). As there was only one carrier of the CYP2C8*3/*3 genotype (no CYP2C8*5 was observed), all subjects were designated as either CYP2C8*3 carriers (CYP2C8*1A/*3 or CYP2C8*3/*3) or CYP2C8*3 non carriers (CYP2C8*1A/*1A).

After dose adjustment based on the serum concentration, the daily carbamazepine dose was

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Table 1. Nucleotide change, haplotype and genotype frequencies of *CYP2C8* in Serbian epileptic pediatric patients on carbamazepine treatment.

	Observed Frequency	**95% CI**
Nucleotide change:		
416G>A	0.100 (8/80)	0.050, 0.188
475delA	0.000 (0/80)	0.000, 0.056
Haplotype:		
*CYP2C8*1A*	0.900 (72/80)	0.812, 0.950
*CYP2C8*3*	0.100 (8/80)	0.050, 0.188
*CYP2C8*5*	0.000 (0/80)	0.000, 0.056
Genotype:		
*CYP2C8*1A/1A*	0.825 (33/40)	0.676, 0.861
*CYP2C8*1A/*3*	0.150 (6/40)	0.068, 0.245
*CYP2C8*3/*3*	0.025 (1/40)	0.000, 0.109

95% CI: 95% confidence interval.

found to be lower in *CYP2C8*3* carriers compared to *CYP2C8*3* non carriers, although the difference was not statistically significant [mean ± SD (standard deviation): 14.19 ± 5.39 mg/kg *vs.* 15.46 ± 4.35 mg/kg, *p* = 0.5]. The observed dose and *p* values remained exactly the same when valproate users were excluded from comparison. At the same time, higher dose-normalized serum concentration of carbamazepine was observed in *CYP2C8*3* carriers compared to *CYP2C8 *3* non carriers (mean ± SD: 0.54 ± 0.18 *vs.* 0.43 ± 0.11 mg/mL, *p* = 0.04) (Figure 1). The observed correlation between weight-adjusted carbamazepine dose and carba-mazepine concentration after dose adjustment was significant only in *CYP2C8*3* non carriers (*r* = 0.52, *p* = 0.002) (Figure 2).

The mean population value for carbamazepine clearance, estimated by the base population pharmacokinetics model, was 4.04 L/h. Inter- and intra-patient variability was best described by exponential model error, with the values of 41.37 and 22.64%, respectively. Out of six examined factors, only three met the minimum objective function value (MOF) difference requirement, and thus, were included in the full model: the total carbamazepine daily dose, sex and concomitant therapy with valproate. The process of backward deletion of covariates from the full model resulted in the following equation:

$$CL (L/h)=0.215+0.0696*SEX+0.000183*DD,$$

where SEX has a value of 1 if male and 0 if female, and DD is the total carbamazepine daily dose (mg/day). Value of MOF in the final model was
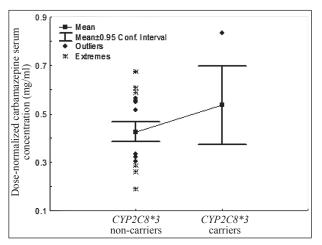

Figure 1. Comparison between C*YP2C8* genotype groups in terms of obtained dose-normalized serum concentration of carbamazepine after dose adjustment.
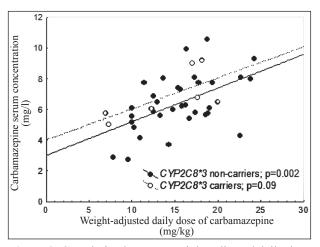

Figure 2. Correlation between weight-adjusted daily dose of carbamazepine and carbamazepine serum concentrations according to the *CYP2C8*3* genotype group.

Table 2. The final model parameter estimates.

Parameters	NONMEM		Bootstrap Analysis	
	Estimate	95% CI[a]	Estimate	95% CI[b]
Clearance (L/h)	0.215	0.176-0.254	0.207	0.189-0.225
Sex	0.0696	0.0545-0.0847	0.0698	0.0527-0.869
Daily dose of carbamazepine (mg/L)	0.000183	0.000079-0.000287	0.000194	0.000141-0.000247
Inter-individual variance of CL	0.0626	0.0371-0.0881	0.0669	0.058-0.0758
Residual variance (exponential)	0.0249	0.018-0.318	0.0262	0.023-0.0294

NONMEM: nonlinear mixed effect model.

[a] Estimate ± 1.96 × (standard error of the estimate).

[b] Percentile (2.5 and 97.5) of the ranked bootstrap parameter estimates.

148.759 units lower compared to the base model. The final model parameter estimates are presented in Table 2. Both inter- and intra-patient variability were decreased by 25.42 and 15.88%, respectively. The bootstrapping analysis that was conducted on 200 replicated data with replacement, resulted in similar values of carbamazepine clearance, effects of total carbamazepine daily dose, sex, and inter- and intra-patients variability, indicating a good precision and stability of the final model.

DISCUSSION

The present study investigated the distribution frequency of *CYP2C8* variations *3* (g.416G>A) and *5* (g.475delA), and their influence on carbamazepine dosing, serum concentration and clearance, in Serbian epileptic pediatric patients. Additionally, we estimated the effect of standard covariates such as body weight, age, sex, total daily dose of carbamazepine and other anticonvulsants on carbamazepine clearance. The results rendered sex and total carbamazepine daily dose relevant for carbamazepine treatment. The *CYP2C8* genetic polymorphism significantly affects carbamazepine metabolism, but its role seems not to be clinically important.

CYP2C8 is a phase I metabolizing enzyme involved in biotransformation of numerous drugs [12,13]. Although not the major role player in carbamazepine pharmacokinetics, it is considered to be of importance as it promotes conversion of the drug to its active metabolite carbamazepine-10,11-epoxide [14]. In addition, the CYP2C8 enzyme is inducible, with the induction mediated, among others, by pregnane X and the constitutive androstane receptor [15]. As both of these receptors might be activated by carba-

mazepine [16,17], it could be speculated that the contribution of CYP2C8 in carbamazepine metabolism is more complicated compared to its other substrates. Therefore, the polymorphism of the *CYP2C8* coding gene could partly explain observed inter-individual variation in response to carbamazepine treatment [18]. In Caucasians, *CYP2C8*3* (g.416G>A) is the most common nonsynonymous variant, frequently associated with decreased enzyme activity [12]. On the other hand, *CYP2C8 *5* (g.475delA) belongs to rare variations, but raises attention as one of the few that yield highly truncated and most probably completely inactive enzyme [19]. Based on the present study, the frequencies of *CYP2C8*3* and *CYP2C8 *5* polymorphisms in the Serbian population are in accordance with the data obtained from other Caucasian populations [5,20-22].

Effects of the *CYP2C8* polymorphism on drug metabolism have already been investigated [6,21-26]. Although the activity of the most frequent *CYP2C8* variant *3* appears to be substrate-dependent [6], its influence on carbamazepine pharmacokinetics was not explored. In the present study, there was a tendency toward lower daily dose and higher serum concentrations of carbamazepine in *CYP2C8*3* carriers, indicating decreased enzyme activity and slower metabolism of the drug. In addition, the observed correlation between carbamazepine dose and concentration was found to be significant only in carriers of the *CYP2C8* wild type allele. The lack of similar correlation in the presence of the *3* variant suggests the existence of other factors that might affect carbamazepine pharmacokinetics, *e.g.,* by altering the binding activity of the variant CYP2C8 enzyme [27]. Another plausible explanations might include the possible dose-dependent autoinduction of

carbamazepine CYP2C8-mediated metabolism [28], and/or the linkage disequilibrium between *3* and other *CYP2C8* alleles [18]. Regardless of the cause, the presented findings render *CYP2C8*3* carriers especially susceptible to an unpredictable reaction to carbamazepine, and therefore, good candidates for a closer follow-up during treatment, especially as the drug concentration in these patients proved not to be sufficient to guide the dose adjustment.

To further test whether *CYP2C8*3* genotyping should be considered as a routine analysis in patients on carbamazepine, population pharmacokinetic analysis was performed. Numerous pharmacokinetic models are already available from the literature dealing with carbamazepine clearance [29-32]. Bearing in mind that carbamazepine is the most frequently used anticonvulsant in Serbia [10], we considered it relevant to evaluate the *CYP2C8* genetic polymorphism in a pharmacokinetic model in Serbian epileptic pediatric patients. Population pharmacokinetic analysis, which included *CYP2C8*3* genotype as a covariate, failed to demonstrate a significant effect of genetic polymorphism on carbamazepine clearance. Unlike some other drug therapies investigated so far [21,22,24], our study showed that CYP2C8 might be of lesser clinical importance to carbamazepine treatment. Yet, the rather small sample size limited the generality of our findings, and additional studies, involving more subjects and also other populations, would be required for a better understanding of CYP2C8 polymorphism effects on epileptic patients' reaction to carbamazepine.

Other findings of our population pharmacokinetic analysis included sex and total carbamazepine daily dose as significant indicators of carbamazepine clearance. The observed effect of sex has been previously reported, with girls having lower values for clearance, most probably due to estrogen influence on microsomal enzymes activity [28,33,34]. However, most of the authors did not show such differences, thus the predictive value of sex for carbamazepine clearance remains controversial [10,30,35-38]. On the contrary, most of the studies denoted positive correlation of carbamazepine daily dose with the drug clearance [32,39,40], and our results confirm those reports.

In conclusion, our results do not support routine genotyping of *CYP2C8* in Serbian epileptic pediatric patients on carbamazepine treatment. Yet, significantly higher serum concentrations in *CYP2C8*3*

carriers confirm the importance of *CYP2C8* genetic polymorphism for carbamazepine pharmacokinetics, warranting further investigations.

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