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Short communication

Effect of *ABCB1* (*MDR1*) 3435C >T polymorphism on salivary secretion of carbamazepine

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Abstract:
The aim of the present study was to evaluate the effects of *ABCB1* (*MDR1*) gene polymorphism on salivary secretion of carbamazepine. The study was carried out on 51 patients diagnosed with epilepsia medicated with carbamazepine. *ABCB1* polymorphism was evaluated using PCR-RFLP methods. Carbamazepine concentrations were measured in blood serum as well as in saliva using FPIA method. Evaluation of the impact of *ABCB1* 3435C > T polymorphism on salivary carbamazepine secretion did not reveal any significant influence of the genotype. Mean value of Pearson's correlation coefficient was 0.787. There was a trend towards higher values of the coefficient in *ABCB1* gene 3435CC carriers (0.855) as compared to 3435CT (0.684) and 3435TT (0.672) subjects. It can be stated that *ABCB1* gene polymorphism does not affect salivary carbamazepine secretion.

Key words:
ABCB1 polymorphism, P-glycoprotein, carbamazpine, salivary secretion

Abbreviations: *ABCB1* – ATP-binding cassette, sub-family B, member 1, *MDR1* – multidrug resistance-1, PCR– polymerase chain reaction, P-gp – P-glycoprotein, TDM – therapeutic drug monitoring

Introduction

Appropriate monitoring of anticonvulsant drugs has become the standard practice and can improve treatment of epilepsy. This is, however, only valid if the total drug concentration measured in serum reflects the therapeutically active component. Blood collection for therapeutic drug monitoring (TDM) has got its antecedent problems. As an alternative, saliva has been used previously for measurement of drug levels. The major advantage of saliva is that it can be obtained in non-invasive manner. Carbamazepine is a drug of choice for the treatment of seizures and in adults and children [12]. Many studies have shown a good correlation between serum and salivary levels of carbamazepine [10, 11]. However, TDM of carbamazepine in saliva is not routinely practiced, at least partly due to a wide interindividual differences of blood/saliva drug concentration ratio, which limits clinical application of salivary drug measurements for therapeutic drug monitoring [3, 8]. Among many potential factors which might contribute to interindividual differences of salivary drug secretion is drug active transport. Some drug transporters were identified in salivary glands. As reported by Uematsu et al. [15] and in our previous study [1], expression of P-

glycoprotein (product of *ABCB1* – formerly *MDR1* gene), multidrug resistance-associated protein (MRP1), MRP2/canalicular multispecific organic anion transporter (cMOAT) and lung-resistance-related protein (LRP) were detected in salivary glands. However, there is no available direct data on active drug transport in salivary glands. Some reports indicate that carbamazepine is a substrate of P-glycoprotein, a *ABCB1* gene product [9, 13]. Single nucleotide polymorphisms (SNPs) of *ABCB1* gene have been identified including one which was localized in the position 3435C > T of exon 26 [4]. This SNP is supposed to be related to altered expression of *ABCB1* gene and P-gp activity. In homozygous TT-allele, the P-gp expression is lower in comparison with heterozygous CT and homozygous non-mutated CC subjects [4, 5]. The 3435C > T mutation is a silent mutation that does not cause amino acid substitution and is suggested to be linked, in a majority of subjects, with the mutation in exon 21, position 2677 (G2677T/A), producing Ala893Thr and Ala893Ser, respectively [14]. Individuals who were homozygous for 2677A,T had significantly decreased intestinal P-gp expression and function. Another explanation of the functional role of 3435C > T polymorphism was provided Wang et al., who demonstrated an effect of the polymorphism on mRNA stability. The authors revealed that the T allele was associated with lower mRNA levels [16].

So, polymorphism in *ABCB1* gene encoding P-glycoprotein may influence salivary concentrations of drugs being its substrates, i.e. carbamazepine, and thus may affect drug-related oral side effects or feasibility of TDM (using drug concentrations in saliva). The aim of the present study was to evaluate the effects of *ABCB1* gene polymorphism on salivary secretion of carbamazepine.

Materials and Methods

Patients

Fifty one unrelated Polish subjects of Caucasian origin diagnosed with epilepsy (including 12 cases of posttraumatic epilepsy), 28 males and 23 females, aged from 20 to 76 years (mean age 44.2 ± 15.7 years) were included in this study after giving informed consent. The patients were medicated with carbamazepine (Tegretol CR, Novartis Pharma AG, n = 19; Timonil, Desitin Arznemittel GmbH, n = 13; Neurotop retard, Gerot Pharmazeutika GmbH, n = 19) 300–1600 mg/24 h for at least 30 days. The Ethics Committee of the Pomeranian Medical University in Szczecin, Poland approved protocol of the study.

Carbamazepine measurement

Carbamazepine concentrations were measured in blood serum as well as in saliva in all study subjects. Blood and saliva were sampled simultaneously, with a 1-minute interval between onset of blood and saliva sampling (at least 30 days from the onset of carbamazepine medication), before morning drug administration. Blood was drawn from peripheral vein. In preparation for saliva sampling, the mouth was rinsed with 10–15 ml of tap water. Saliva was collected from a cotton swab containing citric acid (Salivette, Sarstedt, Germany) placed sublingually (for 30–45 s), which was then centrifuged. Carbamazepine concentrations in serum and saliva were measured by the fluorescence polarization immunoassay (FPIA) method using TDx apparatus (Abbott, USA). Reference interval for carbamazepine serum concentration determined by that method is 4.0–10.0 μg/ml.

Genotyping

Genomic DNA was extracted from 450 μl of whole blood samples using a non-organic and non-enzymatic extraction method [2]. Genotyping for the presence of 3435C > T SNPs was performed using previously described PCR-RFLP method applied by our laboratory [6, 7].

Statistical analysis

Genotype frequencies were calculated by direct counting and then dividing by the number of subjects or the number of chromosomes to produce genotype and allele distribution, respectively. The data were tested for Hardy-Weinberg equilibrium by calculating expected frequencies of genotypes and comparing them to the observed values using the Fisher exact test (Statistica 6.0, Statsoft). Saliva/serum carbamazepine concentration ratios were compared using the Kruskal-Wallis non-parametric ANOVA test and Mann-Whitney U test (non-normal distribution), and Pearson's coefficient was calculated.

Results and Discussion

One of limitations of carbamazepine therapeutic drug monitoring using saliva samples is interindividual variation in the drug serum/saliva concentration ratio [3, 8]. Experimental data suggest that carbamazepine is a substrate for P-glycoprotein, whose expression was revealed in salivary glands [1, 15]. The P-glycoprotein activity is genetically determined with the highest activity in MDR-1 3435CC as opposed to MDR-1 3435TT subjects [4, 14]. Therefore, MDR-1 gene polymorphism could be a factor contributing to interindividual variability of serum/saliva ratio in carbamazepine medicated subjects.

The aim of the study was to evaluate the effect of MDR-1 gene polymorphism on salivary carbamazepine secretion. Mean serum carbamazepine concentration of 8.2 ± 2.5 μg/ml (range 4.20–16.00 μg/ml) and salivary drug concentration from 1.47 to 9.68 μg/ml (mean 3.2 ± 1.6 μg/ml) were measured (Tab. 1). The ratio of the drug saliva/serum concentration ranged from 0.25 to 1.02 (mean 0.39 ± 0.16) (Fig. 1). The aforementioned observations are in keeping with previously reported studies, where mean saliva/plasma total concentration ratios ranged from 0.26 to 0.44; revised by Liu [8]. Mean values of Pearson's correlation coefficient was 0.787 in our study are slightly different from data reported by others, i.e. from 0.84 to 0.99; revised by Liu [8]. So, in the further step the effect of MDR-1 gene polymorphism was analyzed. The results of the present study demonstrated no influence of *ABCB1* 3435C > T polymorphism on salivary carbamazepine secretion. However, there was a trend towards higher values of the coefficient in *ABCB1* gene 3435CC carriers (0.855) as compared to 3435CT (0.684) and 3435TT (0.672) subjects (Fig. 2). Therefore, it seems that *ABCB1* 3435C > T polymorphism may have a slight impact on carbamazepine salivary secretion as comparison of 3435CC subjects against 3435CT and 3435TT cases was of borderline statistical significance ($p < 0.06$).

Tab. 1. Carbamazepine saliva/serum concentration ratio and correlation between saliva and serum drug levels in relation to ABCB1 3435CT genotypes in epileptic patients

	All patients n = 51	CC n = 10	CT n = 29	TT n = 12	p
Saliva/serum concentration ratio (mean ± SD)	0.39 ± 0.16	0.35 ± 0.04	0.40 ± 0.20	0.37 ± 0.07	NS
Pearson's correlation coefficient	0.787	0.855	0.684	0.672	NS

NS – no significant differences between the study groups.

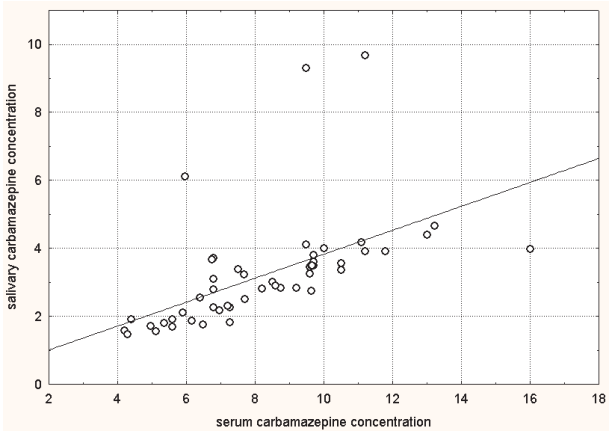

Fig. 1. Correlation between salivary and serum levels of carbamazepine in epileptic patients (n = 51)

Analysis of dose/serum concentration ratio of carbamazepine in patients administered different formulas of the drug did not reveal significant influence of *MDR1* genotype on the parameter studied. The mean values of dose/serum concentration ratio were comparable in all study subjects administered different drug formulas (Tab. 2). The Spearman's coefficient (r = 0.304 for all carbamazepine formulas) indicates low correlation between dose and serum concentration of the drug.

The distribution of *ABCB1* 3435C > T allele in the studied population was 3435C – 0.480, 3435T – 0.520, genotypes CC: n = 10 (19.6%), CT: n = 29 (56.9%) TT: n = 12 (23.5%). It was in concordance with Hardy-Weindberg equilibrium, and did not differ significantly from healthy controls from the same geographical region [6].

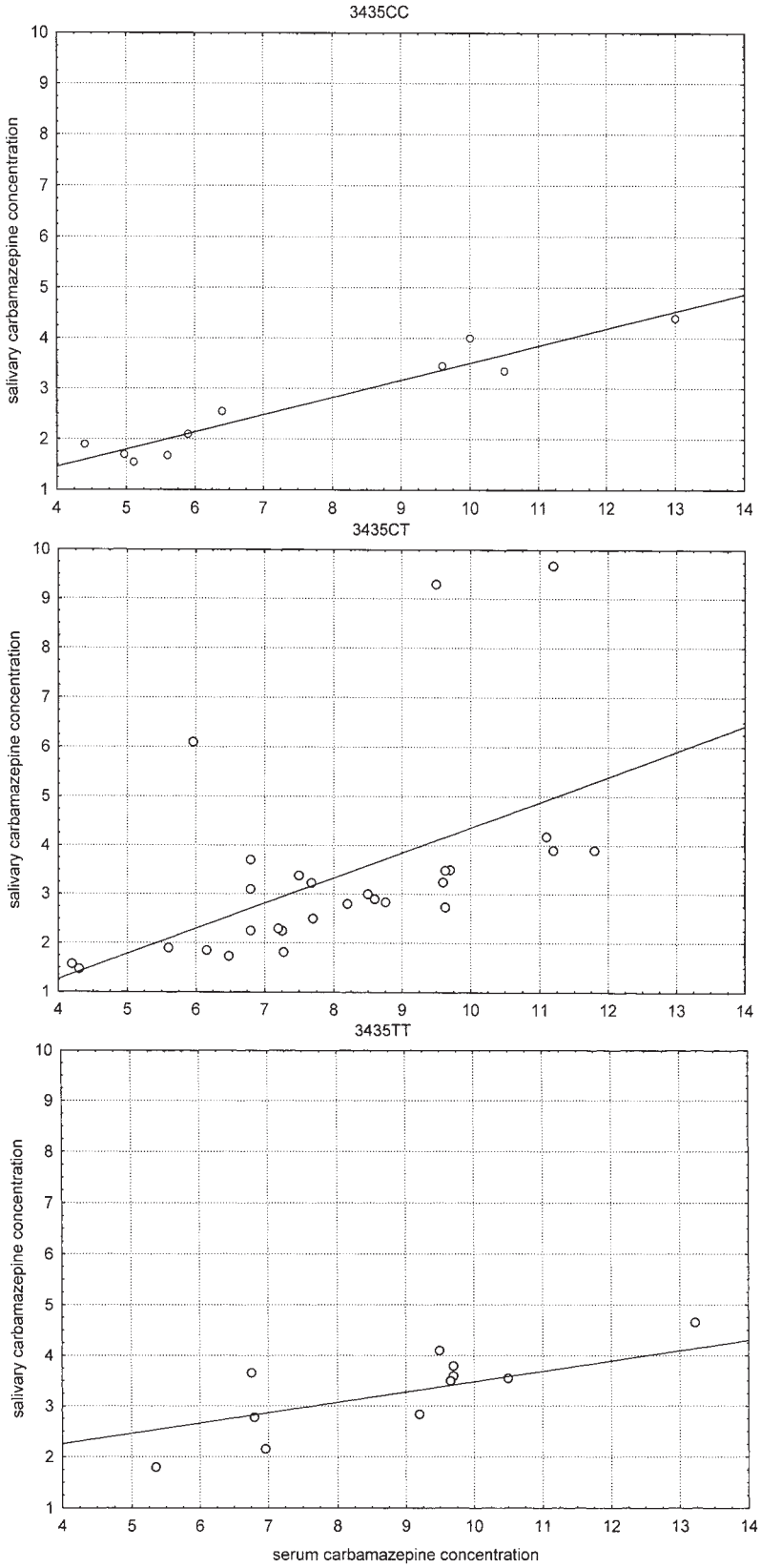

Fig. 2. Correlation between salivary and serum levels of carbamazepine in epileptic patients differing in *ABCB1* 3435CT genotype (3435CC n = 10; 3435CT n = 29; 3435TT n = 12)

Tab. 2. Carbamazepine dose/serum concentration ratio (mean ± SD) in relation to ABCB1 3435C>T genotypes in epileptic patients

	All patients	CC	CT	TT
Dose/serum concentration ratio				
all patients	n = 51 119.5 ± 48.6	n = 10 106.2 ± 43.2	n = 29 128.3 ± 55.9	n = 12 107.8 ± 26.9
Neurotop-treated patients	n = 19 117.1 ± 39.1	n = 5 119.9 ± 39.5	n = 10 117.3 ± 47.6	n = 4 113.4 ± 16.3
Tegretol-treated patients	n = 19 122.0 ± 64.1	n = 2 99.0 ± 26.1	n = 11 136.6 ± 78.0	n = 6 102.9 ± 37.1
Timonil-treated patients	n = 13 119.3 ± 37.2	n = 3 88.4 ± 62.9	n = 8 132.9 ± 23.4	n = 2 111.0 ± 4.6

No statistically significant differences were found as evaluated by means of Student's *t*-test, U-Mann-Whitney and Kruskall-Wallis tests

Conclusions

The *ABCB1* 3435C >T polymorphism does not significantly affect salivary carbamazepine secretion.

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