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Inhibitory Effect of Valproic Acid on Cytochrome P450 2C9 Activity in Epilepsy Patients

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Abstract: Drug interactions constitute a major problem in the treatment of epilepsy because drug combinations are so common. Valproic acid is a widely used anticonvulsant drug with a broad therapeutic spectrum. Case reports suggest interaction between valproic acid and other drugs metabolized mainly by cytochrome P450 isoforms. The aim of this study was to evaluate the inhibitory effect of valproic acid on cytochrome P450 2C9 (CYP2C9) activity by using losartan oxidation as a probe in epilepsy patients. Patients were prescribed sodium valproate (mean 200 mg/day for the first week and 400 mg/day in the following period) according to their clinical need. A single oral dose of 25 mg losartan was given to patients before and after the first dose, first week and 4 weeks of valproic acid treatment. Losartan and E3174, the CYP2C9-derived carboxylic acid metabolite of losartan in 8 hr urine were assayed by using high pressure liquid chromatography. Urinary losartan/E3174 ratio did not change significantly on the first day (0.9, 0.3–3.5; median, range), and first week (0.6, 0.2–3.8; median, range), while a significant increase was observed after 4 weeks of valproic acid treatment (1.1, 0.3–5.7; median, range) as compared to that of measured before valproic acid administration (0.6, 0.1–2.1; median, range) (P = 0.039). The degree of inhibition was correlated with the steady-state plasma concentrations of valproic acid (r^2 = 0.70, P = 0.04). The results suggest an inhibitory effect of valproic acid on CYP2C9 enzyme activity in epilepsy patients at steady state. The risk of pharmacokinetic drug–drug interactions should be taken into account during concomitant use of valproic acid and CYP2C9 substrates.

The high risk of drug–drug interactions in the treatment of epilepsy is generally attributed to the long-term treatment of the disease and common use of drug combinations. Valproic acid is a first-line drug for the treatment of primary generalized tonic–clonic, absence and partial seizures. It has also been used in the treatment of psychiatric disorders due to its modulator effect on serotoninergic and dopaminergic transmission [1].

Cytochrome P450 2C9 (CYP2C9) is a polymorphic enzyme that catalyses the major pathways in the metabolism of a large number of clinically used drugs such as phenytoin, oral anticoagulants, oral antidiabetics and nonsteroidal anti-inflammatory drugs [2]. The *CYP2C9*2* and *CYP2C9*3* variants code for enzymes with decreased activity, compared to the enzyme coded by the common variant *CYP2C9*1* allele [3]. Losartan, a selective angiotensin II receptor antagonist, is converted to its active metabolite, E3174, by CYP2C9 [4]. Urinary losartan/E3174 ratio after a single oral dose of losartan (25 mg) has been suggested as a useful marker for measuring CYP2C9 activity in human beings [5,6]. Although the cytochrome P450 enzymes do not catalyse the major metabolic pathway for valproic acid, it has been shown that CYP2C9 and CYP2A6 catalyse the formation of its hepatotoxic metabolite 4-ene-valproic acid [7]. Additionally, the formation of 4-ene-valproic acid, 4-OH-valproic acid and 5-OH-valproic acid were decreased in human liver microsomes expressing the *CYP2C9*2* and **3* variants compared to *CYP2C9*1* [8].

Much of the evidence indicating that valproic acid is an inhibitor of cytochrome P450 enzymes derives from clinical observations. Valproic acid has been reported to inhibit the drug oxidation and elevate the plasma levels of several drugs including phenytoin, phenobarbital, diazepam and lorazepam [9–12]. These drugs are mainly metabolized by CYP2C9, CYP2C19, CYP2D6, CYP3A4 isoforms of cytochrome P450 system and glucuronidation. An *in vitro* study has shown that valproic acid inhibits CYP2C9 at clinically relevant concentrations in human liver microsomes [13]. However, there is no evidence in the literature that valproic acid is an inhibitor of CYP2C9 activity *in vivo*. Therefore, we aimed at evaluating the effect of valproic acid on CYP2C9 activity by using losartan oxidation as a probe in epilepsy patients.

Materials and Methods

Patients. Eleven patients suffering from epilepsy (six women, five men, aged between 18 and 62 years, with 3- to 15-year history of generalized or partial-seizure), were included in the study and followed at the Department of Neurology, Gazi University Hospital, Ankara, Turkey, during the study. The patients were not treated with valproic acid previously and did not receive other anti-epileptics or drugs known to be metabolized mainly by CYP2C9 or any other drug that may affect CYP2C9 activity, during the study period. None of them had hepatic or renal dysfunction.

The study was performed in accordance with the Declaration of Helsinki and approved by the local Ethics Committee at Gazi University Hospital, Ankara, Turkey. Written informed consent was obtained from the patients before participation in the study.

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Table 1.

Major patient characteristics (age and seizure history represented as years) and genotype, change in metabolic ratios and plasma valproic acid concentrations (μg/ml) of the patients.

Patient no.	Age	Gender	Seizure history	CYP2C9 genotype	Fold change in metabolic ratio*	Plasma valproic acid concentration
1	25	F	2	*1*1	3.5	89.5
2	18	M	2	*1*2	1.4	51.1
3	62	F	1	*1*2	0.5	56.6
4	29	M	1	*1*3	4.8	83.1
5	25	F	2	*1*2	1.1	69.1
6	23	M	14	*1*2	5.0	117.4
7	23	F	5	*1*1	NA	NA
8	18	F	5	*1*2	5.3	82.9
9	42	F	3	*1*3	NA	NA
10	44	M	15	*1*2	1.8	75
11	19	M	3	*1*1	1.9	38.7

*Alteration of the metabolic ratios calculated as the fold change in losartan/E3174 ratios after 4 weeks of valproic acid treatment as compared to the values prior to the treatment. Plasma levels of valproic acid at the end of 4 weeks of treatment correlated well with the fold change in metabolic ratios ($r^2 = 0.70$, P = 0.04). NA denotes for 'not available'.

The patients were prescribed sodium valproate according to their clinical need (mean 200 mg/day for the first week and doubled in the following period). A single oral dose of 25 mg losartan (Cozaar®, Merck Sharp & Dohme, Haarlem, The Netherlands) was given to the patients at least 2 days prior to the valproic acid treatment and after the first dose, first week and 4 weeks of valproic acid treatment 3 hr after the valproic acid dose. Urine was collected during the following 8 hr. An aliquot of 10 ml was stored at −20°C until analysis. The ratio of losartan/E3174 before the valproic acid treatment was compared to those for each patient during the treatment.

High pressure liquid chromatography analysis of losartan and its carboxy metabolite. Analysis of losartan and its carboxy metabolite, E3174, in urine was performed by high pressure liquid chromatography (HPLC) system with a fluorescence detection as described previously [14]. In brief, urine samples were mixed with 0.5 M citrate buffer and isopropanol (4/1/1:v/v/v, respectively) to yield a pH of 4.3. Twenty microlitres of this mixture was injected to the HPLC instrument through a Zorbax SB-Phenyl column (4.6 mm ID × 25 cm). The mobile phase (pH: 2.3) consisted of 10 mM sodium di-hydrogen phosphate, acetonitrile (64/36:v/v, respectively) with a flow rate of 1.5 ml/min. The limits of detection for losartan and E3174 were 20 nM and 10 nM, respectively. The coefficients of variation were less than 10% for losartan and the metabolite. Urinary losartan/E3174 ratio was calculated from the recoveries of losartan and E3174 in the 8 hr urine sample.

Plasma valproic acid levels. Blood samples for the determination of valproic acid plasma concentrations were obtained between 8:00 a.m. and 9:00 a.m., approximately 12 hr after the valproic acid intake after 4 weeks of treatment. The plasma concentration of valproic acid was analysed by florescence polarization immunoassay method using TDx (Abbott, Abbott Park, IL, USA) commercial kit according to the recommendations of manufacturer. The inter-day co-efficient of variation was less than 5% and the lowest limit of quantitation was 0.70 μg/ml.

CYP2C9 genotyping. Genomic DNA was isolated from peripheral blood cells using QIAamp DNA blood kit (QIAGEN, Hilden, Germany). The patients were genotyped with respect to the *CYP2C9*1*, *CYP2C9*2* and *CYP2C9*3* alleles with a previously described polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method [5]. PCR amplification was performed using the following forward and reverse primers for *CYP2C9*2: 5′-TAC* AAA TAC AAT GAA AAT ATC ATG-3′ and 5′-CTA ACA ACC AGA CTC ATA ATG-3′, respectively. For *CYP2C9*3*, the forward and reverse primers were 5′-TGC ACG AGG TCC AGA GATGC-3′ and 5′-GAT ACT ATG AAT TTG GGA CTTC-3′, respectively [15]. The PCR products were digested by the endonucleases *AvaII* (5U) and *NsiI* (5U) for the determination of *CYP2C9*2* and *CYP2C9*3* alleles, respectively.

Statistics. The changes in the urinary losartan/E3174 ratio before and after valproic acid treatment were compared with Wilcoxon matched-pairs test. Sperman *r* correlation coefficient was used to test the correlation between the degree of inhibition in losartan oxidation and plasma steady-state valproic acid levels. A P-value of <0.05 was accepted as statistically significant.

Results

Of the 11 patients included to the study, nine patients completed the 4-week study protocol. Two patients dropped out after completing the first week of the study. Valproic acid was well tolerated by the patients, except for one patient who reported nausea and another who reported weight gain. None of the patients reported any adverse effects related to administration of a single oral dose of 25 mg losartan. The characteristics of patients (age, gender, seizure history and change in the metabolic ratio at the end of the 4-week valproic acid therapy together with valproic acid plasma levels) are summarized in table 1.

Effects of valproic acid treatment on CYP2C9 activity.

The losartan/E3174 metabolic ratio did not differ significantly after the first dose and the first week of valproic acid treatment compared to the pretreatment values. The median pretreatment metabolic ratio was 0.6 (0.1–2.1), while it was 0.9 (0.3–3.5) after the first dose and 0.6 (0.2–3.8) after the first week valproic acid treatment [values (median and range), P > 0.05]. The average (median and range) losartan/E3174 metabolic ratio was increased significantly from 0.6 (0.1–2.1) to 1.09 (0.3–5.7) after 4 weeks of valproic acid treatment (P = 0.039, fig. 1).

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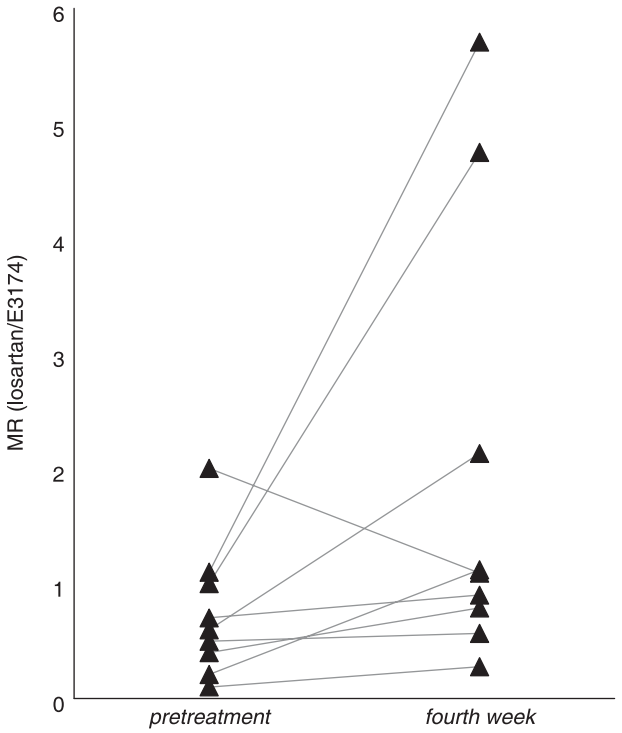


Fig. 1. Comparison of losartan/E3174 metabolic ratios (MR) before and after the 4-week valproic acid treatment (P = 0.039).

Plasma valproic acid level correlation with inhibiton.

The plasma levels of valproic acid at the end of the 4 weeks of valproic acid treatment correlated well with the change in metabolic ratios, calculated as the fold change in losartan/E3174 metabolic ratios after 4 weeks of treatment compared to metabolic ratios prior to the treatment, in patients completed the study ($r^2 = 0.70$, P = 0.04, table 1).

Three patients had the *CYP2C9*1*1* genotype, while six patients had *CYP2C9*1*2* and two had the *CYP2C9*1*3* genotype. The patients with the *CYP2C9*1*1* genotype had the lowest pretreatment losartan/E-3174 metabolic ratios while *CYP2C9*1*2* had intermediate and *CYP2C9*1*3* had the highest [0.4 (0.1–0.6), 0.6 (0.2–2.0) and 1.1 (median and range), respectively]. Among the nine patients that completed the 4 weeks of study protocol, the average degree of inhibition observed in the *CYP2C9*1*1*, **1*2* groups and a patient carrying *CYP2C9*1*3* genotype were 2.70, 2.52 and 4.80, respectively.

Discussion

Inhibition of CYP2C9 activity, measured by oxidation of losartan to its CYP2C9-derived metabolite E3174, was observed in patients treated with valproic acid for 4 weeks. Furthermore, the inhibition correlated with steady-state plasma levels of valproic acid.

The main limitation of this study was the small number of patients participating in the study, mainly due to the inclusion criteria allowing patients treated with only valproic acid in order to eliminate a possible influence of concomitant drugs on the enzyme activity. However, in the present study, enzyme activities of each patient were compared to the corresponding baseline values before valproic acid administration. A paired analysis allowed us to examine the alteration of metabolic ratios after valproic acid treatment in each individual separately.

Despite the difficulty of drawing strong conclusions due to the small number of patients, the results of the present study are in accordance with previous reports, suggesting an inhibitory effect of valproic acid on CYP2C9 activity. This inhibition was observed at the end of 4 weeks of valproic acid treatment, while there was no significant inhibition within the first week of the treatment in our study group. This could be explained by increased median daily dose of valproic acid from 200 mg to 400 mg at the end of first week of valproic acid treatment. Another reason could be the small number of patients participating in our study.

Interactions between valproic acid and CYP2C9 substrates, phenytoin, tolbutamide and warfarin, have been reported previously [16–18]. More than 90% of valproic acid binds to serum albumin and may displace other drugs such as phenytoin and warfarin. Due to the increase in unbound drug concentrations, the interactions were attributed to inhibition of metabolism, as well as displacement from plasma albumin. Phenytoin and warfarin are extensively metabolized in the liver by CYP2C9. Both drugs have narrow therapeutic indices and bind more than 90% to plasma albumin. Only 10% of losartan binds to plasma proteins, thus the interaction between losartan and valproic acid observed in the present study could not be explained by displacement from plasma albumin.

CYP2C9 metabolic activity can be assessed by using a number of probe drugs. Tolbutamide, flurbiprofen, phenytoin and warfarin have been suggested as alternative probe agents for phenotyping purpose [19]. Recently, urinary losartan/E3174 ratio after an oral dose of losartan has also been suggested as a safe and selective measure of CYP2C9 activity in Turkish patients, as well as in various studies performed in Beninese, Japanese, Spanish and Swedish populations [5,6,14,20,21]. In the present study, we used a single subtherapeutic dose of losartan as a probe for determination of CYP2C9 activity; no side effect was reported by the patients.

CYP2C9 is responsible for the metabolism of certain drugs with low therapeutic indices such as warfarin, acenocoumarol, phenytoin, glibenclamide and glipizide [2]. The concomitant use of valproic acid with CYP2C9 substrates can cause elevated plasma levels and adverse effects of these drugs. The nature of the inhibitory effect after long-term treatment of valproic acid also needs to be evaluated.

In conclusion, the present study in a small group of patients treated with valproic acid provides a preliminary *in vivo* evidence that valproic acid is an inhibitor of CYP2C9 activity at steady state. The degree of inhibition showed a good correlation with plasma concentration of vaproic acid. Further studies are needed to evaluate this finding in larger populations and more patients.

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