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Effect Of UGT2B7 Gene Polymorphisms rs77439366 (C802T) On Serum Concentration Of Valproic Acidin The Iraqi Epileptic Patients

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Abstract

Background Valproic acid (VPA) is commonly used to treat epilepsy in children. In the predominant pathway, it is metabolized by the uridine diphosphate glucuronosyl transferase 2B7 (UGT2B7) enzyme. UGT2B7 is a UGT isozyme that promotes carboxyl, hydroxyl, amino, and methoxyl glucuronidation and is a crucial metabolic enzyme that prevents the accumulation of potentially hazardous lipophilic chemicals and initiates their removal through the more hydrophilic biliary and renal systems. the effects of inheritable polymorphisms on human UGT-encoding genes has been well documented, and it has been shown to contribute for a fraction of the phenotypic heterogeneity in metabolism and excretion. in this study we seek to determine the effects of genetic variants in the UGT2B7 gene on serum VPA levels in steady-state condition among epileptic patients . UGT2B7 is agene on chromosome 4q13 16-kilobase gene with six exons. The proximal promoter region of this gene contains many polymorphisms. Exon 2 of UGT2B7 contains the C802T polymorphism , which is a common missense (MAF = 0.3349)

Aim. Investigated the relationship between serum VPA levels and UGT2B7 genotypes in order to determine how rs77439366C802Tvariation has impact on VPA pharmacokinetics.

Methodology Patients (n=113) were initially prescribed the ER formulations of valproate (Depakine Chrono) tablet and (depakine) syrup 10-15 mg/kg . The physician determined the dose,frequency of dosage, initial and

maintenance doses, and any additional increases or decreased basis of their seizures control, serum VPA concentration was measured after three and six months of treatment in 103 ,90 patients,respectively.The dosing regimen was continued for at least 2 weeks (>5 half-lives) to maintain a steady-state condition with respect to drugs pharmacokinetics. serum VPA was measured by chemiluminescent microparticle immunoassay (CMIA) technology for the quantitative measurement of valproic acid in human serum in vitrowith a linear range of (2 - 150 µg /mL). The UGT2B7rs77439366 (802C > T) in the coding regions were genotyped using polymerase chain reaction amplification .

Results After three and six months of regular valproic acid administration, patients with the T allele at UGT2B7 C802T had significantly lower adjusted VPA concentrations than those with the CC genotype**.**

Conclusion The findings suggested that UGT2B7 C802T may be an essential predictor of individual variability in VPA pharmacokinetics, and that individuals with the T allele may need to increase their VPA dose to achieve the therapeutic range of 50–100 µg /mL.

Keywordsvalproic acid (VPA), (UGT2B7),epilepsy, Adjusted concentration (AC),Genetic polymorphism, pharmacokinetics

Introduction

Valproic acid (VPA; 2-propylpentanoic acid) is an antiepileptic drug with a narrow therapeutic range (50–100 g/mL) for the treatment of epilepsy. Its pharmacokinetics and pharmacodynamics show significant individual variability due to high interindividual variability in steady-state serum concentration, which affects its therapeutic effects (1). Moreover, during clinical studies,in additional to drug resistance, VPA was observed to cause a variety of adverse effects including liver injury(2). As a result, dosage optimization played a crucial part in epilepsy treatment, and genetic polymorphisms that may have an impact on the pharmacokinetics and pharmacodynamics of VPA could contribute individualized treatment (3).

The biotransformation of VPA consists of three major metabolic pathways, including the uridine diphosphate glucuronosyltransferase (UGT) enzyme pathway, the mitochondria -oxidation pathway, and the cytochrome P450 (CYP) pathway, accounting for 50%, 40%, and 10%, respectively (4)The most common one is glucuronidation conjugation. Because glucuronide metabolites account for 50% of the VPA dose it plays a significant role in VPA metabolism

(5,6).

UGT enzymes are important metabolic proteins that prevent potentially hazardous lipophilic chemicals from accumulating and initiating their removal through the more hydrophilic biliary and renal systems. In humans, the UGT enzymes are divided into two groups: UGT1A and UGT2 (subdivided into UGT2A and UGT2B)(7) . The effect of inheritable polymorphisms on human UGT-encoding genes has been well

studied, and it has been shown to contribute for a portion of the phenotypic heterogeneity in metabolism and excretion (8)

UGT2B7 is a UGT isozyme that plays a key role in the formation of VPA glucuronides.UGT2B7 is a gene on chromosome 4q13 with a total length of just 16 kb,it is composed of six exons,the substrate binding domain is encoded by the first two exons, while the uridine 5'-diphosphate-glucuronic acid binding domain is encoded by the last four exons(9). UGT2B7 glucuronidates a wide range of endogenous and exogenous chemicals, including steroid hormones, bile acid, and nonsteroidal anti-inflammatory drugs (NSAIDs) (9,10)VPA has been shown to be a substrate for the enzymes UGT1A3, UGT1A4, UGT1A6, UGT1A9 and UGT2B7 enzymes(6). More specifically, regarding substrate specificity against carboxylic acid compounds, UGT2B7 shows the highest activity UGT1A6 and UGT1A9a have intermediate activity, while UGT1A3 and UGT1A4 have the lowest activity toward VPA (11)

According to the research, UGT2B7-catalyzed VPA glucuronidation has a low Km, a high Vmax, and a high intrinsic clearance. As a result, UGT2B7 is the enzyme with the greatest activity for VPA glucuronidation (13)**.**

Unlike other isoforms of the 2B family, UGT2B7 enhances carboxyl, hydroxyl, amino, and methoxyl glucuronidation. Evidence suggests that UGT2B7 isoforms are active in the glucuronidation of VPA (13,14).the UGT2B7 gene is approximately 16kb and has six exons. The proximal promoter region of this gene has numerous (15,16). rs77439366(C802T) polymorphism is a frequent missense (MAF = 0.3349) in exon 2 of UGT2B7 that causes an H268Y amino acid substitution (11,12)

In the present study, We investigated the association between serumVPA levels and the UGT2B7 genotype in order to determine whether the C802T variation has a significant impact on VPA pharmacokinetics.

Method and Material

Patients

This study is carried out at Imam AL-Hussein Medical City / Karbala –Iraq in Neurology Center, out clinic patients ,out laboratory and laboratories of College of Pharmacy / University of Kerbela. during the period from September 2020 to October 2021

one hundred thirty three (133) patients with newly diagnosed patient and other with previously failure antiepileptic treatment are chosen while seeking medical care, all of them are diagnosed as epileptics by senior neurologist based on the International League Against Epilepsy's criteria(ILAE)(18), and depending on clinical manifestations such semiology, available home recordings, and eyewitnesses, in combination with electroencephalograms , MRI , CT scan and family history of epilepsy, types of seizures were determined. the complete written informed permission taken from each patient after explain the nature of study.

Only one hundred and three patients (103) returned after three months of treatment to follow up and only nineteen patients (90)continuing treatment after six months and had doing follow up due to a

coronavirus pandemic and loss of follow up during treatment period so they were exclude from the study.

Patients were initially prescribed the ER formulations of valproate (Depakine Chrono) tablet and (depakine) syrup the median ER dose was10 mg/kg which may increase to 15 mg/kg at subsequent visits.the physician determined the dose ,frequency of dosage, initial and maintenance doses, and any additional increases or decreased basis of their seizures control. if a patient entered the study on antiepileptic drug an other than VPA, it should be tapered off within two weeks after the initiation the study .Patients were asked to visit department of Neurology, on monthly basis for monitoring of their clinical prognosis and to follow for adherence to valproic acid therapy. **Inclusion Criteria** patient diagnosed with epilepsy focal or generalized . Prescriped VPA monotherapy at a therapeutic dose and continuing treatment for 6 months durationthe dosing regimen was administered regular for at least 2 weeks (>5 half-lives) before samples collections to maintain a steady-state condition. **Exclusion Criteria** patients had severe adverse drug reactions and who had severe renal, hepatic, or cardiac dysfunction ,Patients who receiving other drugs that affect Valproic acid metabolism (drugs affected on UGT2B7 activity inducers or inhibitors) were excluded from the study.

Sample collection

Three blood samples were taken from each patient doing in laboratoryor in most cases blood draw doing by medical representative from laboratory went to patients s home in scheduled time. First sample before the treatment (5ml) was taken from each patients, (2 ml) was kept in EDTA tube stored on -20°C until used for DNA extraction and subsequent genotyping, and (3ml) was kept in a gel tube to isolate serum by centerfugation of the blood at 3000 rpm for 10 min. used for estimated liver enzymes before treatment.Second and third samples were draw after three and six months treatment, respectively. after regularly VPA administered for at least two weeks before blood sample collection to assurance the steady-state concentrations were achieved ,(3ml) venous blood sample draw from each patients (n=103 in the third month and n= 90 in the sixth month) at the morning and before the next dose of valproic acid (within 1 h prior to a scheduled dose) kept in a gel tubes the serum was separated for estimation serum concentration of valproic acid .

assay chemiluminescent microparticle immunoassay (CMIA) The ARCHITECT iValproic Acid (Abbott)technology is an in vitro using for the quantitative measurement of valproic acid in human serum with a linear range of (2-150 µg /mL)was used in this study .Due to large inter-individual variances, the VPA serum concentration at steady-state was adjusted by body weight and dosage of each patient and expressed as concentration to dose ratio of VPA (µg/mL per mg/kg).AC (adjusted concentration) was calculated by this equation (19):

AC (µg /mL per mg/kg) = serum VPA concentration (µg /mL)/[VPA daily dose (mg per day)/weight (kg)]

Ethics and data collection

The study's methodology was authorized by the College of Pharmacy / University of Kerbela's ethical committee, and each subject was given an informed signed permission form after being explained the

nature and objective of the study. After signing a written informed consent form, all patients were enrolled and asked to complete a specially crafted questionnaire.

All patients diagnosed with epileptics with normal liver function, were treated with VPA as monotherapy. The demographic and clinical characteristics of each patient was recorded at each therapeutic drug monitoring (TDM) visit, such as age, gender, body weight, types of epilepsy, treatment regimen, and concomitant medications.

Molecular Analysis

The DNA genome was obtained from a blood sample using a blood genomic DNA extraction kit (G- spin Total DNA extraction). This kit is acceptable for extracting DNA from whole blood after centrifuged at 2000 rpm for 1 minute to produce buffy coat.

Amplification refractory mutation system (ARMS-PCR): refers to a polymorphism detection method related to specific PCR primers, , in which a specific set of primers is used, which includes two forward primers (forward wild and forward mutant type) and two reveres primers that are complementary to the DNA template and include the region to be amplified.

Other components required for both approaches include DNA polymerase (Taq polymerase), deoxynucleotide triphosphates, and buffer solution, in addition to the DNA templet and primers.

PCR reaction was performed using a specific primer pairs designed for UGT2B7 (C802T) (rs 7349366) using primer blast software and depending on https://www.ncbi.nlm.nih.gov/ websites as in the table (1).

Table (1): Primers sequences of UGT2B7 (rs7439366) (C>T)

SNPs	Sequence	Product size (bp)
O-F	**TTTGCCTACATTATTCTAACCCCTTT**	**284**
O-R	**CTGAAAATTCAAAGCCAACAAAATAA**	**284**
I-F Allele C	**AACTCCTGGAATTTTCAGTTTCAAC**	**141**
I-R Allele T	**AAATCAACATTTGGTAAGAGTGGCTA**	**194**

lyophilized primers ordered from Bioneer, Korea, were dissolved in significant measure of nuclease free water15(0 µl) according to the manufacture to obtain a concentration of 100 pmol/µl stock solution. After that, a diluted solution was formed by adding 10 µl of each stock solution primer with 90 µl of nuclease-free water to obtain 10pmol/ µl as working solution .

The PCR reaction was done by mixing PCR components with DNA solution and primers using the optimized PCR programs as the following

1µL of each outer forward and outer reverse primer (10 poml/µL) , 0.5 µL of each inner forward and inner reverse primer (10 pmol/ µL), and 4 µL of extracted DNA in PCR premix tube

The volume was increased to 20 µL by adding 13 µL of nuclease-free water. and then centrifuged at 2000 rpm for 10 seconds in a micro centrifuge to mix the sample tubes before placing them in the thermocycler,the optimum thermal condition was obtain after several trial andshown in table (2).

Table (2) The thermal program UGT2B7 (C>T) (rs7439366)

Steps	Temperatures C	Time /second	Cycle
Denature template	95	3 minutes	1
Initial denaturation	95	30	35
Annealing	58	30	
Extension	72	55	
Final extension	72	5 minutes	1

Statistical analysis

The data of participants in this study were converted into a computerized database, revised for errors or inconsistencies, and then managed, processed, and analyzed by using the statistical package for social sciences (SPSS) version 26, IBM, US. Scale variables presented in mean, standard deviation (SD), Analysis of variances (one way ANOVA) was used to compare more than two means between groups

Result

demographic characteristics like age, sex, body weightmtypes of epilepsy, onset of illness, history of other antiepileptic drugs andbiochemical information of the epileptic patients such as liver enzyme (alanine transaminase (ALT) , aspartate aminotransferase (AST), Total Bilirubin, to assurance normal liver function before treatment and VPA doses, plasma VPA concentration, adjusted plasma VPA concentration (VPA ACs) are shown in table (3) and (4)

Table (3) and (4) demographic characteristics and clinical information of the epileptic patients

Table (3)

Variable	Group	Frequency	Percentage

Family history	Positive	47	45.6%
	Negative	56	54.4%
Consanguinity	No	47	42.7%
	Yes	56	57.3%
Etiology	Idiopathic	69	67%
	Symptomatic	26	25.2%
	Cryptogenic	8	7.8%
Seizure type	Simple partial	43	41.7%
	Complex partial	26	25.2%
	Generalized tonic clonic	20	19.4%
	Generalized atonic	5	4.9%
	Generalized myoclonic	9	8.8%
Onset of illness	10-18 years	22	21.4%
	5-10 years	31	30.7%
	From childhood	32	31.7%
	Adults	16	15.5%
history of other AED	Yes	44	42.7%
	No	59	57.3%

Table (4)

Characteristics	Values
Age, mean ± SD (year)	19.141 ± 3.26
Sex (male% /female%)	57.3% / 42.7%
Body weight, mean ± SD (kg)	51.91 ± 27.53
ALT (U/L)	25.08 ± 11.53
AST (U/L)	32.34 ± 11.18

Total bilirubin	0.55 ± 0.24	
VPA doses, mean ± SD	After 3 months	After 6 months
	599.03±330.33	669.9 ± 366.44
Plasma VPA concentration, mean ± SD (µg/mL)	49.68 ±22.25	56.53 ± 14.41
Adjusted plasma VPA concentration (VPA Cs), [(µg/mL)/(mg/kg)	4.6 ±1.81	5.1 ± 1.73

The distribution of detected genotypes of UGT2B7 (C> T) (rs7439366) in the Iraqi Epileptic patients

The frequency and percentage of detected genotypes of UGT2B7 (C> T) (rs7439366) in the epileptic patients , was detected mutant genotype (TT) in higher number and percentage (58.2%) ,wild (CC) in low frequency and percentage (17.5 %), another genotype also detected (CT) (24.3 %) which were heterozygous, as in table (5)and figure (2).

Table (5): The distribution of detected genotypes of UGT2B7 (C> T) (rs7439366) in the Epileptic patients

SNP	Genotypes	Frequency	Percentage
UGT2b7 genotypeUGT2B7 (C> T) (rs7439366),	CC	20	17.7 %
	CT	27	23.9 %
	TT	66	58.4 %
	Total	113	100 %

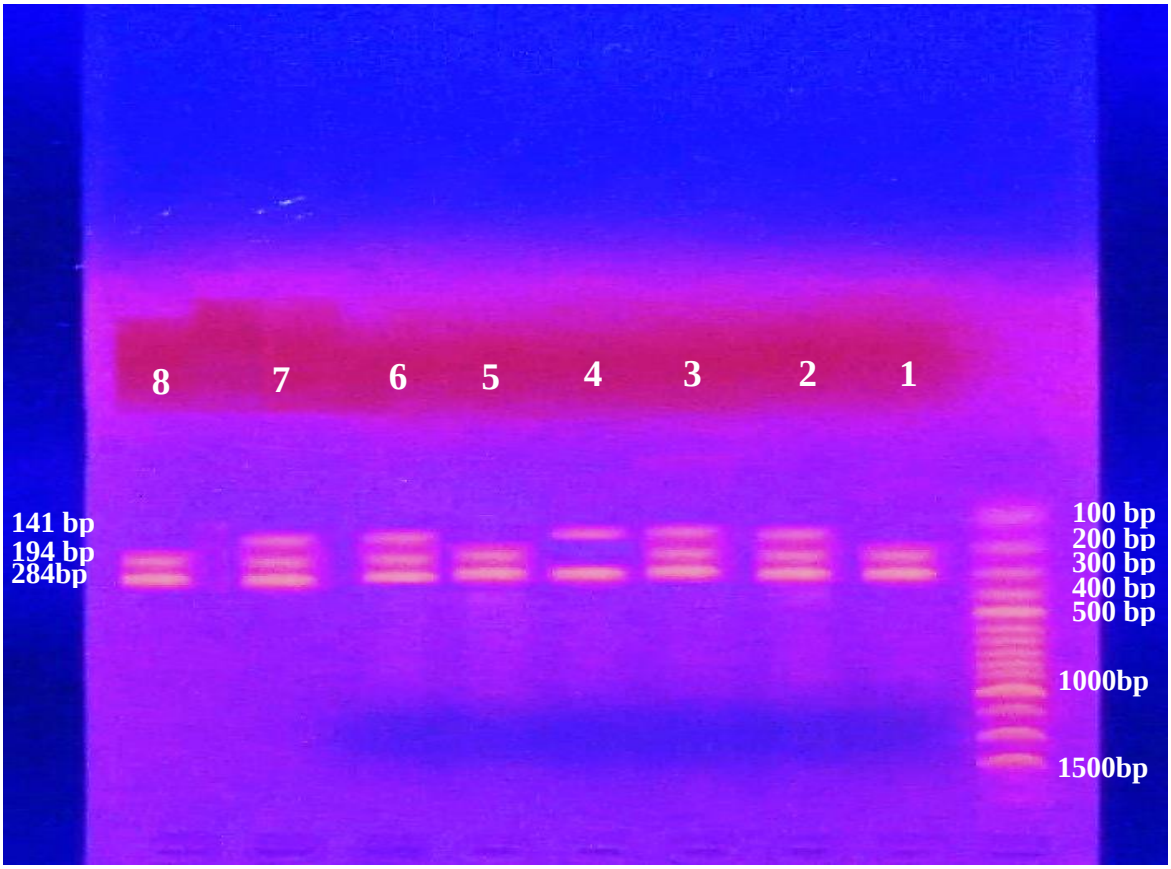

Figure (2): The amplification refractory mutation system (ARMS) of UGT2B7 (C>T) (rs7439366) genetic polymorphism showed: lane M: represented DNA ladder (100-1500 bp), lane 1,5and 8: represented (TT) genotype (mutant) were shown in (194 bp), lane 4: represented (CC) genotype (wild) were showed in (141bp) and lane 2,3,6,and 7: represented CT genotype (heterozygous).

To explore effects of the genetic factors on intraindividual variabilities in VPA concentrations in this study of epilepsy patients,.table (5) below showed that there were significant statistical association between valproic acid concentration and UGT2B7 polymorphism in three alleles The standardized adjustedconcentration of VPA was much lower in those patients with a T allele at UGT2B7rs7439366(C802T) than in those with the CC genotype after three and six months, (p 0.001) (p <0.001) respectively.

Table (5) Association between valproic acid adjusted concentration (AC) and UGT2B7 polymorphismafter three and six months of treatment.

	UGT2B7			p value
	CC	**CT**	**TT**	

VPA AC, [(µg/mL)/(mg/kg] After 3 months	5.83±1.9	3.94±2.13	4.13±1.41	**0.001 [S]**
VPA AC, [(µg/mL)/(mg/kg] After 6 months	6.78±1.91	4.35±1.94	4.17±1.35	**< 0.001 [S]**
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant				

Discussion

Pharmacogenetics is the study of how genes affect pharmacological response, which can range from a lack of the desired therapeutic effects to the adverse drug reaction.(9).despite advancements in the pharmacological treatment of epilepsy, pharmacoresistance continues to be a problem. The development of pharmacoresistant epilepsy could be explained by genetic changes in the activity of drug targeting and drug metabolizing enzymes.

this study is the first study to assess about how genetic variants of UGT2B7rs7439366(C802T) polymorphisms affect VPA metabolism in Iraqi epileptic patients.UGT2B7 is an important enzyme in the metabolism of VPA. C802T is one functional mutations in UGT2B7. The present study focused on UGT2B7 C802T because it may be important determinant of individual variability in VPA pharmacokinetics. after three and six months of valproic acid treatment, patients with the T allele at UGT2B7 C802T had significantly lower standardized trough plasma concentrations of VPA than those with the CC genotype. So these findings suggested that UGT2B7rs7439366(C802T) may be an important determinant of individual variability in the therapeutic range of VPA, It may be necessary to adjust VPA dose in individuals to ensure achievement of the therapeutic range of 50–100 µg/ml and reach to desired response.

And this result is consistent with several studies around the world that found that the T allele of C802T was associated with lower VPA CS in patients carrying one or more T alleles, suggesting that this SNP is a common locus for UGT2B7's pivotal role in VPA metabolism; gene mutations can cause both transcriptional and functional changes (14)

Our findings are also consistent with a study conducted in the china population, which found that plasma concentrations of VPA are higher in patients with the genotype UGT2B7(C802T) CC than in those with the genotypes CT or TT, implying that patients with the T genotype have a high metabolism (20).

Other studies on other UGT enzymes also found that gene polymorphisms affect enzyme activity and cause a decrease in VPA concentration in patients carrying mutant alleles. rs6759892 (T > G, UGT1A6, MAF = 0.260) and rs1105879 (A > C, UGT1A6*9, MAF = 0.262) are two missense mutations in exon that

result in increased enzyme activity. So these two variations may impair VPA metabolism, carriers may require a higher VPA dose(3,21,22)

rs7439366 (C > T, UGT2B7*2) is a missense variant of UGT2B7 is located in exon that changes the amino acid from Tyr to His. The C allele is the most frequent in Han Chinese (MAF = 0.722) and Japanese populations (MAF = 0.682), although the T and C alleles are equally common in northern and western European groups (MAF = 0.50, data from the 1000 Genomes database)(23). T allele is the major allele in this study, which is being conducted on the Iraqi population, and C allele is a minor allele.

Many previous studies on the effect of rs7439366 on VPA pharmacokinetics in epileptic patients showed that CC carriers had lower normalized plasma VPA concentrations than CT or TT genotypes which inconsistent with our findings(23,24,18). furthermore several other studies indicated that UGT2B7 C802T had no significant influence on plasma concentration of VPA(3,14,15) as a result , rs77439366 changes in UGT2B7enzyme activity were contradictory.

In conclusion, it may be essential to increase the VPA dose for Iraqi individuals carrying the T allele in order to obtain the therapeutic range of 50–100µg/ml. TDM during VPA therapy, as well as genotype screening for specific patients before to VPA administration, could improve the antiepileptic drug's safety and efficacy profile.

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