

MDR1 Gene Polymorphism and Phenytoin Pharmacokinetics in Epilepsy

Adel Ali Alhazzani, MD* Murali Munisamy, PhD**
Gauthaman Karunakaran, PhD***

Background: Pharmacokinetics is a widely used anti-epileptic drug phenytoin, which exhibits noticeable inter-individual variations in efficacy. Genetic factors, such as MDR1 gene polymorphism may play a crucial role in drug response.

Objective: To investigate the influence of MDR1 variant genotypes on Phenytoin Pharmacokinetics in epileptic patients.

Design: A Case-Control Genetic Study.

Setting: College of Medicine and Pharmacy, King Khalid University, Abha, Saudi Arabia.

Method: Twenty-five epileptic patients non-responders to phenytoin monotherapy and 25 epileptic patients' responders to phenytoin monotherapy were recruited. DNA was isolated by conventional phenol-chloroform method. MDR1 (3435C>T) gene polymorphism was assessed using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms (PCR-RFLP) method. Allelic and genotypic frequency were calculated. Reversed-phase High-Performance Liquid Chromatography (HPLC) method was used to determine the plasma levels of Phenytoin drug. PK Solutions was used for non-compartmental analysis to estimate the pharmacokinetic parameters.

Result: The MDR1 (3435C>T) polymorphism was found to be in Hardy-Weinberg equilibrium and displayed significant allelic and genotypic association between non-responders and responders to phenytoin ($P < 0.01$). The finding of pharmacokinetics analysis demonstrated that longer half-life ($t_{1/2} = 33.26$ hours) and less clearance rate ($CL = 0.42$ L/hour) in the homozygous variant group compared to wild-type genotype group ($t_{1/2} = 19.2$ hrs, $CL = 0.8$ L/hour).

Conclusion: The finding suggests that the genetic polymorphism in the C3435T location of MDR1 gene might determine pharmacokinetics variability of phenytoin drug. Therefore, pharmacokinetics parameters along with genotyping of MDR1 (C3435T) genotype might be valuable in the perspective of personalized medicine in epileptic patients.

Bahrain Med Bull 2017; 39(1): 29 - 32

Epilepsy is considered one of the major public health problems and could have serious psychological and physical consequences, including mental health disorders, traumatic injury and premature death¹. Epilepsy is a chronic and disabling neurologic disorder and affects approximately 1% of the population globally, especially in developing countries^{1,2}. Although good prognosis has been seen in most patients with epilepsy, approximately 20% to 30% of patients with epilepsy do not remain seizure free despite the intervention of multiple antiepileptic drugs (AEDs)^{3,4,5}.

The majority of epilepsy phenotypes result from the interaction between genes and environmental factors. In Saudi Arabia, prevalence for active epilepsy was found to be 6.54/1000 population⁶. Multidrug-resistant protein 1 (MDR1), also named P-glycoprotein (P-gp), is an ATP-binding cassette sub-family

B 1 (ABCB1) membrane transporter protein that catalyzes the ATP-dependent efflux of a broad range of substrates from cells⁷. MDR1 is highly expressed in the luminal membrane of the blood-brain barrier's (BBB) endothelial cells; thus, MDR1 contributes in the regulation of the BBB's permeability to drugs⁸. MDR1 located on chromosome 7q21.1 and is composed of 28 exons. Among the 50 SNPs of the MDR1 gene, more attention has been focused on C3435T mutation in exon 26 of the MDR1 gene, which is a silent mutation encoding the amino acid isoleucine, this polymorphism affects the expression and function of the P-gp in many ways⁹.

Several studies demonstrated the relationship between polymorphism at C3435T location of ABCB1 gene and AEDs drug response; however, the results suggest inconsistent evidence. Ethnic differences, selection bias, differences in

* Assistant Professor of Neurology
Department of Neurology, College of Medicine
** Assistant Professor
Department of Pharmaceutics, College of Pharmacy
*** Associate Professor
Department of Pharmacology, College of Pharmacy
King Khalid University, Abha
Kingdom of Saudi Arabia
E-mail: alhazzani@kku.edu.sa, aalhazzani@yahoo.ca

study design and inadequate statistical power may explain the variability in the results.

The aim of the study is to evaluate the possible association of MDR1 variant genotypes on phenytoin pharmacokinetics in epileptic patients.

METHOD

Fifty patients suffering from epilepsy using 300 mg therapeutic dose of phenytoin monotherapy were reviewed between 1 December 2014 to 31 November 2015. Informed consent was obtained from all the study participants. The diagnosis of epilepsy was based on the classification system for seizure types provided by the International League Against Epilepsy (ILAE). The patients were divided into two groups:

Group I: 25 non-responder epileptic patients under phenytoin monotherapy.

Group II: 25 responder epileptic patients under phenytoin monotherapy.

The responders were completely free of seizures for phenytoin monotherapy and therapeutic efficacy of plasma drug levels (10-20 mcg/ml). Non-responders had treatment failure for phenytoin, occurrence of seizures and lack of therapeutic efficacy. Patients who had other antiepileptic drugs, H2 histamine receptor antagonist, diazepam, haloperidol and antipsychotics were excluded. Patients were excluded if they were diagnosed with diabetes mellitus (DM) and severe kidney/liver disease. Patients having phenytoin drug for <1 month were also excluded.

Venous blood samples (8 ml) were collected from all patients. DNA was isolated from samples stored in EDTA tubes using phenol-chloroform isolation method.

MDR1 (C3435T) polymorphism, exon 26 was amplified with the designed primers. The PCR was performed in 25 µl reaction volume containing 20 pmol of forward and reverse primer, 1 ul of 100 to 250 ng of template DNA and 0.5 mM of dNTP; 1/10 of reaction buffer contained 2 mM of MgCl₂, 1U of Taq polymerase.

Fifty patients with epilepsy were recruited for pharmacokinetic study and blood samples were obtained from each patient showing poor metabolizers (PM), intermediate metabolizers (IM) and extensive metabolizers (EM) for polymorphism at (C3435T) location of MDR1 gene in the non-responders groups over 12 hours had reached the steady-state concentration.

DNA was isolated by conventional phenol-chloroform method. MDR1 (3435C>T) gene polymorphism was assessed using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms (PCR-RFLP) method. Allelic and genotypic frequency were calculated. Reversed-phase High-Performance Liquid Chromatography (HPLC) method was used to determine the plasma levels of Phenytoin drug. PK Solutions was used for non-compartmental analysis to estimate the pharmacokinetic parameters.

Chi-square test was used to calculate odds ratio (OR) and 95% confidence interval (CI). Pharmacokinetic parameters were analyzed using one-way NOVA. P-value of less than 0.05 was considered significant. SPSS, version 11.5 was used for all analyses.

RESULT

Personal and clinical characteristics of patients with epilepsy enrolled in our study are shown in table 1. PCR-RFLP method was used to examine MDR1 (3435C>T) genotype distributions in epileptic patients showing non-responders and responders to phenytoin, see figure 1. This polymorphism was polymorphic in our study population and were found to be under the Hardy-Weinberg equilibrium (cut off P<0.01). The allelic and genotypic frequency for MDR1 (3435C>T) showed a significant association between the phenytoin non-responders and responders groups, see tables 2 and 3. Allelic Chi-square=9.76, P-value=0.0017; OR: 3.14 (1.42-7.05), genotypic Chi-square= 4.37, P-value= 0.036: OR: 3.69 (0.90-5.81).

Table 1: Baseline Characteristics of Study Population

| Demographic Characteristics | Phenytoin Responders (N=25) | Phenytoin Non-Responders (N=25) |
|-----------------------------|-----------------------------|---------------------------------|
| Mean Age + SD (Years) | 24.2 ±8.4 | 26.8±11.9 |
| Sex (male:female) | 19:6 | 21:4 |
| Type of Seizures | GTCS ^a | 17 (68%) |
| | SPS ^b | 6 (24%) |
| | SPS sec gen ^c | 2 (8%) |
| | Mean Weight + SD Kgs | 48.2 ±6.83 |
| Phenytoin Therapeutic Dose | 300 mg | 300mg |

^a Generalized tonic-clonic seizures.

^b Simple partial seizures.

^c Simple partial seizures with secondary generalization.

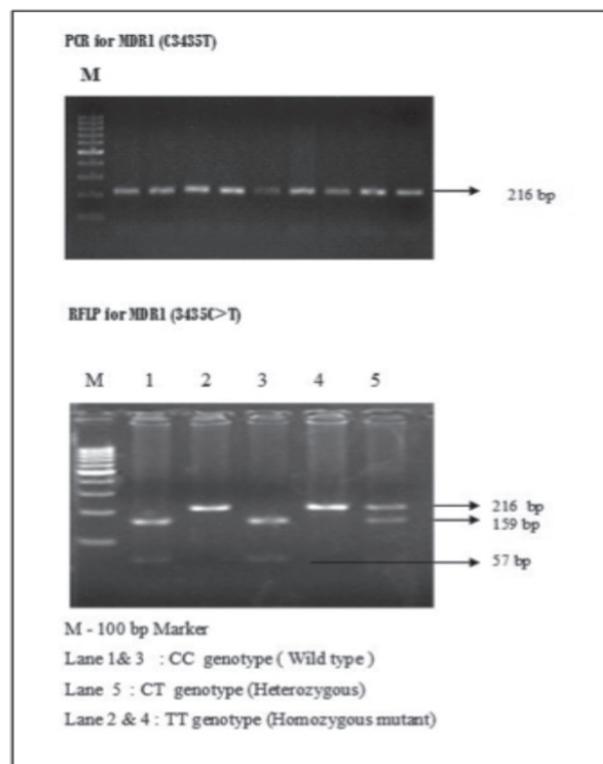


Figure 1: PCR-RFLP for MDR1 (C3435T) Gene Polymorphism

Table 2: Allelic Frequency of MDR1 (C3435T) Polymorphism

| Gene | Polymorphism | Allelic Frequency | | Allelic Chi-square Value, P value, Odds Ratio & 95 % CI |
|------|--------------|---------------------------------|----------------------------|--|
| | | Phenytoin Non-Responders, N =25 | Phenytoin Responders, N=25 | |
| MDR1 | 3435C>T | C = 0.70 T = 0.30 | C = 0.88 T = 0.12 | Non-Responders versus Responders: 9.76, 0.0017, 3.14 (1.42-7.05)* |

Table 3: Genotypic Frequency of MDR1 Polymorphism

| Gene | Polymorphism | Genotypic Frequency | | Allelic Chi-square Value, P value, Odds Ratio & 95% CI |
|------|--------------|---------------------------------|----------------------------|--|
| | | Phenytoin Non-Responders, N =25 | Phenytoin Responders, N=25 | |
| MDR1 | 3435C>T | CC = 13 CT+TT= 12 | CC = 20 CT+TT= 5 | Non Responders versus Responders : 4.37, 0.036, 3.69 (0.90-5.81) * |

Metabolizers were classified into extensive metabolizers (EMs) carrying both wild-type alleles (wt/wt), intermediate metabolizers (IMs) carrying one normal allele and one variant allele (wt/mt) and poor metabolizers (PMs) carrying both variant allele (mt/mt) according to their genotypes of MDR1 transporter enzymes. Pharmacokinetic profile of phenytoin is shown in table 4. PK Solutions was used to determine pharmacokinetic parameters from plasma concentration-time curve. The C-max was 20.4 ± 0.29 $\mu\text{g/mL}$ in EM, 28 ± 0.10 $\mu\text{g/mL}$ in the IM and 32 ± 0.16 $\mu\text{g/mL}$ in the PM after oral administration of phenytoin. Similarly, T-max was 6 \pm 0.12 h in the EM, 6 \pm 0.08 h in the IM and 6 \pm 0.08 h in the PM group. The area under curve (AUC) at 0–12 h elimination half-life (t_{1/2}) of the drug phenytoin data analysis demonstrated significant differences among metabolizer groups in the C-max, AUC, t_{1/2} and CL are shown in table 4.

Table 4: Pharmacokinetic Profile – Phenytoin

| Pharmacokinetic Parameters | Extensive Metabolizers (EM) Mean \pm S.E.M | Intermediate Metabolizers (IM) Mean \pm S.E.M | Poor Metabolizers (PM) Mean \pm S.E.M | P-value One-way ANOVA |
|-------------------------------|--|---|---|-----------------------|
| C max (mg/ml) | 20.4 \pm 0.29 | 28 \pm 0.10 | 32 \pm 0.16 | P < 0.01 |
| T max | 6 \pm 0.12 | 6 \pm 0.08 | 6 \pm 0.08 | P > 0.05 |
| AUC (0-12 hr) mg-hr/ml | 302 \pm 0.45 | 545 \pm 0.71 | 631 \pm 0.48 | P < 0.01 |
| Half-life (t _{1/2}) | 19.27 \pm 0.36 | 30.2 \pm 0.12 | 33.26 \pm 0.14 | P < 0.01 |
| Clearance (L/hr) | 0.80 \pm 0.11 | 0.53 \pm 0.04 | 0.42 \pm 0.21 | P < 0.01 |

DISCUSSION

Pharmacoresistance is a well-known complication in clinical practice. Despite the intake of some AEDs, several patients develop drug resistance and toxic reactions. The frequency of non-responders has been reported to be 30% to 40%^{11,12}. No single definition or validated method has been defined for AED resistance except seizure recurrence and measurement of drug concentration in serum; the reported prevalence varies according to ethnicity. It has been observed that many patients resistant to AED treatment are resistant to a broad range of AEDs with a different mechanism of actions, and resistance occurs across a wide range of epilepsy types¹³.

The variability in AED response could be attributed to genetic polymorphisms in the transporters. Among all the drug

transporters, PgP encoded by ABCB1 is the most extensively studied transporter as several AEDs are common substrates of ABCB1¹⁴.

Siddiqui et al found the association of C3435T in ABCB1 gene with refractory epilepsy and suggested that drug resistance in epilepsy might be genetically determined¹⁵. Studies have investigated the association of ABCB1 gene polymorphism C3435T and drug response in diverse ethnic populations^{11-13,16}. However, there is a discrepancy in the results of these studies.

In our study, we found a significant association of TT genotype with seizure recurrence and drug non-responders in epilepsy. Non-responders recurrence of seizure was found to be higher compared to responders. TT genotype of MDR1 polymorphism might be influencing the oral bioavailability of AEDs. In our study, the frequency of a variant allele (0.30) was observed higher in the non-responders compared to the responder's group (0.12), both less than that of the Caucasian population (0.34). Furthermore, the allelic frequency of 3435T in the healthy Saudi population was found to be 0.45, which was similar to that in Caucasians and Asians, but higher than Africans¹⁷.

Phenytoin (5,5-diphenylhydantoin), a widely used anticonvulsant, exhibits non-linear pharmacokinetics in humans. Phenytoin exhibits a non-linear pattern of pharmacokinetics action. It follows the zero-order kinetics at the concentration of <10 $\mu\text{g/mL}$ ¹⁸. However, in elimination, it follows first-order kinetics at therapeutic levels. Half-life also differs with phenytoin concentration and dosage. Phenytoin exhibit a dose or concentration dependent CL due to non-linear pharmacokinetic behavior. It indicates that the restricted metabolic capacity causes a disproportional increase in the drug level in the serum after a small increase in drug concentration. Defective isoenzymes functioning may result in adverse clinical outcomes, which lead to altered efficacy of drugs or toxicity. It has been observed that optimal plasma half-life of phenytoin drug may differ from 7 to 60 hours, but in the majority of cases remains between 15 and 20 hours. The variation in the half-life of phenytoin is also supported by the information that the dose-dependent kinetics determines the elimination of the drug. Studies suggest that plasma CL value ranges from 0.0081 to 0.0551 per h kg^{19,20}. Ethnic difference in phenytoin clearance rate values has been reported²¹.

The study findings on the pharmacokinetic parameters showed that PM group exhibit a longer removal half-life of a therapeutic agent (t_{1/2}=33.26 h) and relatively smaller CL (CL=0.42 L/h) compared to IM group (t_{1/2} = 30.2 h, CL = 0.53 L/h) and EM (t_{1/2}=19.2 h, CL=0.80L/h). The findings of our study demonstrated statistically significant differences among the metabolizers of phenytoin pharmacokinetics profile.

CONCLUSION

Polymorphism at C3435T location of MDR1 gene might be related to Pharmacokinetics of the Phenytoin drug. Therefore, MDR1 (C3435T) genotyping along with pharmacokinetic measurement might be valuable in personalized medicine for improving efficacy of phenytoin drug in epileptic patients. Further studies are advised with larger sample to validate our findings.

Author Contribution: All authors share equal effort contribution towards (1) substantial contribution to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of manuscript version to be published. Yes.

Potential Conflicts of Interest: None.

Conflict of interest: None.

Sponsorship: None.

Acceptance Date: 12 February 2017.

Ethical Approval: Approved by the College of Pharmacy, King Khalid University, Saudi Arabia.

REFERENCES

1. Duncan JS, Sander JW, Sisodiya SM, et al. Adult Epilepsy. *Lancet* 2006; 367(9516):1087-100.
2. Brodie MJ, Shorvon SD, Canger R, et al. Commission on European Affairs: Appropriate Standards of Epilepsy Care Across Europe. *ILEA. Epilepsia* 1997; 38(11):1245-50.
3. Bhalla D, Lotfalinezhad E, Timalisina U, et al. A Comprehensive Review of Epilepsy in the Arab World. *Seizure* 2016; 34:54-9.
4. Brodie MJ, Dichter MA. Antiepileptic Drugs. *N Engl J Med* 1996; 334(3):168-75.
5. Ben-Menachem E. Epilepsy in 2015: The Year of Collaborations for Big Data. *Lancet Neurol* 2016; 15(1):6-7.
6. Al Rajeh S, Awada A, Bademosi O, et al. The Prevalence of Epilepsy and Other Seizure Disorders in an Arab Population: A Community-Based Study. *Seizure* 2001; 10(6):410-4.
7. Ebinger M, Uhr M. ABC Drug Transporter at the Blood-Brain Barrier: Effects on Drug Metabolism and Drug Response. *Eur Arch Psychiatry Clin Neurosci* 2006; 256(5):294-8.
8. Wijnholds J, deLange EC, Scheffer GL, et al. Multidrug Resistance Protein 1 Protects the Choroid Plexus Epithelium and Contributes to the Blood-Cerebrospinal Fluid Barrier. *J Clin Invest* 2000; 105(3):279-85.
9. Hoffmeyer S, Burk O, von Richter O, et al. Functional Polymorphisms of the Human Multidrug-Resistance Gene: Multiple Sequence Variations and Correlation of one Allele with P-Glycoprotein Expression and Activity in Vivo. *Proc Natl Acad Sci USA* 2000; 97(7):3473-8.
10. Bhatti MM, Hanson GD, Schultz L. Simultaneous Determination of Phenytoin, Carbamazepine, and 10,11-Carbamazepine Epoxide in Human Plasma by High-Performance Liquid Chromatography with Ultraviolet Detection. *J Pharm Biomed Anal* 1998; 16(7):1233-40.
11. Vahab SA, Sen S, Ravindran N, et al. Analysis of Genotype and Haplotype Effects of ABCB1 (MDR1) Polymorphisms in the Risk of Medically Refractory Epilepsy in an Indian Population. *Drug Metab Pharmacokinet* 2009; 24(3):255-60.
12. Ponnala S, Chaudhari JR, Jaleel MA, et al. Role of MDR1 C3435T and GABRG2 C588T Gene Polymorphisms in Seizure Occurrence and MDR1 Effect on Anti-Epileptic Drug (Phenytoin) Absorption. *Genet Test Mol Biomarkers* 2012; 16(6):550-7.
13. Kim YO, Kim MK, Woo YJ, et al. Single Nucleotide Polymorphisms in the Multidrug Resistance 1 Gene in Korean Epileptics. *Seizure* 2006; 15(1):67-72.
14. Potschka H, Löscher W. Multidrug Resistance-Associated Protein is Involved in the Regulation of Extracellular Levels of Phenytoin in the Brain. *Neuroreport* 2001; 12(11):2387-9.
15. Siddiqui A, Kerb R, Weale ME, et al. Association of Multidrug Resistance in Epilepsy with a Polymorphism in the Drug-Transporter Gene ABCB1. *N Engl J Med* 2003; 348(15):1442-8.
16. Sisodiya SM, Lin WR, Harding BN, et al. Drug Resistance in Epilepsy: Expression of Drug Resistance Proteins in Common Causes of Refractory Epilepsy. *Brain* 2002; 125(Pt 1):22-31.
17. Ameyaw MM, Regateiro F, Li T, et al. MDR1 Pharmacogenetics: Frequency of the C3435T Mutation in Exon 26 is Significantly Influenced by Ethnicity. *Pharmacogenetics* 2001; 11(3):217-21.
18. McNamara JO. Drugs Effective in the Therapy of the Epilepsies. In: Hardman JG, Limbard LE, Gilman AG, eds. *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*. New York: McGraw-Hill, 2001; 528-530.
19. Bochner F, Hooper WD, Tyrer JH, et al. Effect of Dosage Increments on Blood Phenytoin Concentrations. *J Neurol Neurosurg Psychiatry* 1972; 35(6):873-6.
20. Deleu D, Aarons L, Ahmed IA. Estimation of Population Pharmacokinetic Parameters of Free-Phenytoin in Adult Epileptic Patients. *Arch Med Res* 2005; 36(1):49-53.
21. Klotz U. The Role of Pharmacogenetics in the Metabolism of Antiepileptic Drugs: Pharmacokinetic and Therapeutic Implications. *Clin Pharmacokinet* 2007; 46(4):271-9.