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RESEARCH ARTICLE

An analysis of the Michaelis-Menten pharmacokinetics of phenytoin in epileptic Indonesian adults

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Abstract

Background: Phenytoin metabolism has been shown to vary in different ethnic groups. Therefore, the authors studied phenytoin pharmacokinetics in Indonesian adult patients with epilepsy. **Objective:** The purpose of this study was to determine and analyse the pharmacokinetic parameters of Michaelis Menten Vmax (maximum velocity) and Km (the Michaelis constant) of phenytoin in adult epileptic patients. **Method:** Twelve adult epileptic patients were chosen on the basis that they had two reliable steady-state phenytoin serum levels at two different daily doses and they required an additional adjustment of phenytoin dosage monotherapy. **Result:** The Vmax and Km values for adult epileptic patients respectively, ranged from 3.78 to 9.65 mg/kg/day and from 0.71 to 5.58 mg/L and there was no correlation with age and weight ($p > 0.05$). The Vmax and Km values have shown individual variability. **Conclusion:** The optimal adjustment of phenytoin dose individually in adult epileptic patients based on Vmax and Km parameters needs to be done to get the desired steady-state level according to the clinical response.

Introduction

Epilepsy is a chronic disease with heterogeneous impairments, multifaceted disorders, characterised by recurrent seizures (two or more times), where involuntary movements involving either partial or whole body (*general*) occur, and often accompanied by loss of consciousness (Glauser *et al*., 2016). Clinically, individual differences in drug pharmacokinetics are common. These individual differences may include the process of absorption, distribution, metabolism, and excretion of drugs which ultimately lead to the variability of drug levels in plasma. Factors that can cause this variability include genetics, age, weight, drug interactions, nutrition, liver and kidney function disorders, or abnormalities (Rowland & Tozer, 2020; Shargel & Yu, 2022). AEDs (Anti Epileptic Drugs) are the primary therapy for most patient with epilepsy, such as phenytoin, valproic acid, carbamazepine, and phenobarbital, which are commonly used as a first-line for most seizure cases because they are as effective as the newest and significantly cheaper than others. Phenytoin (5,5-diphenylhydantoin, DPH) is a hydantoin group that is widely used in the treatment of epilepsy because it is effective for the treatment of generalised seizures, simple and complex partial seizures and does not have a central nervous system depressant effect (Lowenstein, 2017; Silvado *et al*., 2018).

Phenytoin also has a narrow therapeutic index (10-20 µg/ml). Phenytoin adverse effects such as nystagmus occur at phenytoin levels greater than 20 µg/ml and sub-therapeutic effects below 10 µg/ml (Wu & Lim, 2013). Therefore, monitoring phenytoin levels is very important to know the level of phenytoin in the blood. Therefore it is important to determine the dosage and to know the effectiveness of the therapy and the

frequency of seizures of the patients to ensure adherence (Lertsinudom *et al*., 2014).

Although it is the main choice from all antiepileptics, phenytoin has drawbacks such as a narrow therapeutic index and its kinetics in humans follow non-linear kinetics where there is a non-linear relationship between steady-state drug levels in blood/serum to dose. These conditions, as a result of saturation of the metabolic enzyme system of phenytoin, can vary the blood/serum drug levels even in the patients with the same dose, with very individual responses ranging from sub-therapeutic responses to highly toxic responses (Ozkaynacki *et al*., 2015; Shargel & Yu, 2022).

Several literature reviews showed that the variability between individual Michaelis-Menten pharmacokinetic parameters Vmax and Km is quite large. They concluded that genetic differences and the effect of pharmacokinetic saturation of phenytoin are more essential factors in determining steady serum phenytoin levels than other factors such as age, weight, height, and gender (Ismail & Rahman, 1990; Suzuki *et al*., 1994; Chanruang *et al*., 2021).

By looking at the variability of the Vmax and Km values that can cause variability in plasma drug levels and clinical response, it is necessary to individualise the phenytoin dose. Values of Vmax and Km can be used to adjust individual doses so that the appropriate therapeutic level (steady level) is obtained (Alqahtani *et al*., 2019; Shargel & Yu, 2022).

Based on the above background, the authors intended to determine the Michaelis-Menten pharmacokinetic parameters of phenytoin (Vmax and Km) for a number of adults with epilepsy.

Methods

Design

The subjects in this study were 12 adults with epilepsy who were undergoing outpatient treatment with the following criteria :

1. Receive phenytoin monotherapy with a dose according to the need of therapy
2. Two weeks before and during the study, patients were not allowed to take other drugs that could interfere with the metabolism of phenytoin (including phenylbutazone, sulfonamides, benzodiazepines, anticoagulants, phenobarbital, carbamazepine, valproic acid, isoniazid) (Horn, 2021).
3. Patients were male and female aged 21-30 years with average weight (not obese) and did not have liver and kidney function abnormalities.

The subjects in this study were Javanese, who were determined based on data from medical records and interviews. Patients were given the first dose based on body weight and clinical experience according to the patient's therapeutic needs for at least two weeks. Blood samples were taken in the morning on the 14th day, just before the next drug was taken. Then the patient was given a second dose according to the need for therapy for two weeks. Blood samples were retaken on day 14, and the clinical response was recorded. The sampling time was carried out in the morning, just before the next drug was taken. A one-millilitre blood sample was taken from the cubital vein sample collection time. The serum was separated by centrifuging at 2000 rpm. The serum obtained was stored at a temperature of -20^{o}C until the assay was carried out with Fluorescence Polarisation Immunoassay (FPIA/TDx analyser).

Assessment

The determination of the Michaelis-Menten kinetic parameters (Vmax and Km) was calculated using the formula (equation) (Shargel & Yu, 2022):

$$R = V\max - \frac{Km.R}{Css}$$

where :

Css: Steady level of drug in plasma (mg/L)

R: Dosage/day or rate of dosing (mg/day)

Vmax: The maximum rate of drug metabolism in the body (mg/day)

Km: Michaelis-Menten constant for drugs in the body (slope or tg; mg/L)

The dosing rate (R, mg/day), which at steady-state equals the elimination rate, is plotted as the Y-axis, while the dosing rate is divided by the steady-state plasma drug concentration (R/Css) (Clearance), litres/day) is plotted as the X-axis. The intercept on the Y-axis is the slope which is the negative value of Km (mg/L) (Shargel & Yu, 2022).

Results

This study was an observational study conducted cross-sectionally, which aims to determine the Michaelis-Menten pharmacokinetic parameters of phenytoin (Vmax and Km) for several adults with epilepsy. This study has received an ethical approval/statement from the Health Research Ethics Committee of Dr. Soetomo Hospital Surabaya. Twelve epileptic outpatients were using monotherapy of antiepileptic drug phenytoin were used as subjects. The subjects of this study

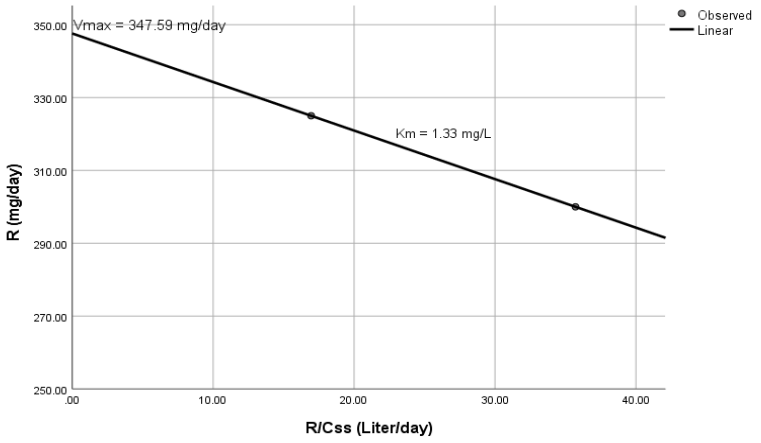
included ten men and two women, the majority of the subjects were between the ages of 21-30 years.

The Michaelis-Menten pharmacokinetic study of phenytoin has been carried out in adults with epilepsy in 21–30 years and a body weight range of 38–72 kg. The value of Vmax obtained ranged from 3.78 mg/kg/day to 9.65 mg/kg/day, and for Km, it ranged from 0.71 mg/L to 5.58 mg/L. Vmax and Km values can be seen in Table I. In this study, from statistical analysis with Pearson Correlation between sex, age, weight and Michaelis Menten Parameters (Vmax and Km), no correlation was found (*p*-value > 0.05). This could be due to the fact that this study was conducted in only one age group with small variations in age and weight data. However, this study found inter-subject variability in the values Vmax and Km.

Table I: Patient data and Vmax and Km value

Patients	Age (years)	Weight (kg)	Sex	Race	Vmax (mg/day)	Vmax (mg/kg/day)	Km (mg/L)
1	22	50	M	Java	328.18	6.56	0.71
2	29	39	F	Java	363.59	9.32	1.12
3	22	43	M	Java	162.55	3.78	1.97
4	26	38	F	Java	366.60	9.65	3.85
5	22	39	M	Java	347.59	8.91	1.33
6	23	50	M	Java	337.24	6.75	5.58
7	27	72	M	Java	470.13	6.53	4.39
8	23	45	M	Java	396.95	8.82	2.75
9	30	59	M	Java	388.74	6.59	1.08
10	21	46	M	Java	393.79	8.56	2.50
11	24	43	F	Java	302.61	7.04	1.60
12	21	67	M	Java	466.64	6.97	1.37
Mean	24.17	49.25			360.38	7.46	2.35
SD	3.10	11.17			80.26	1.67	1.52

Figure 1 shows how to determine the Vmax and Km values for subject number 5. At least two dose data and two steady-state levels of phenytoin data were needed to determine Vmax and Km values according to the Michaelis-Menten equation. This equation was described in the form of a relationship between R (y-axis) and R/Css (x-axis) and the curve line that intersects the y-axis as Vmax and the slope as Km. After the two-dose data and the steady-state level of phenytoin in subject number 5 were entered into the equation, the value obtained was Vmax = 347.59 mg/day (intercept on the y-axis) and Km = 8.9 mg/L (slope).



(aged 22 years, body weight 39 kg, who received doses of phenytoin 300 and 325 mg/day with steady levels of phenytoin 8.40 and 19.17 mg/L)

Figure 1: Calculation of Vmax and Km values of phenytoin in subject 5

The values of Vmax and Km obtained in this study have followed those found by other researchers (Ismail & Rahman, 1990; Suzuki *et al*., 1994; Chanruang *et al*., 2021). Like other researchers, this study also found large inter-subject variability in the values of Vmax and Km. The value of Km was more variable than the value of Vmax, or in other words, the coefficient of variation of Km was greater than Vmax (Ismail & Rahman, 1990; Suzuki et al., 1994; Edeki & Brase, 1995).

Discussion

By looking at the large inter-subject variability of the Michaelis-Menten pharmacokinetic parameters of phenytoin Vmax and Km, it was necessary to individualise the phenytoin dose in each epileptic patient using these parameters to obtain the desired steady-state level of phenytoin. Other researchers have tested dose adjustment using this method and demonstrated a very significant correlation between the calculated steady-state phenytoin levels (Css predicted) and the observed steady-state phenytoin levels (Css observed). Individual phenytoin dosage adjustments were based on the Vmax and Km values obtained from each patient. The dose of phenytoin given to the patient should not exceed the Vmax value for each patient, because if it exceeds the Vmax value, then phenytoin will follow non-linear kinetics due to enzyme saturation which causes a decrease in phenytoin metabolism. This can prolong the half-life of phenytoin and the accumulation of phenytoin in the blood so that it has the potential to cause toxicity. The Vmax and Km values for adult epileptic patients, respectively, ranged from 3.78 to 9.65 mg/kg/day (162.55 to 470.13 mg/day) and from 0.71 to 5.58 mg/L. In this study, no correlation was found between the Micahelis-Menten parameters (Vmax and Km) and age because this study was conducted in the same age group (Ismail & Rahman, 1990; Alqahtani *et al*., 2019 & Shargel & Yu, 2022).

Generally, variations of phenytoin levels were caused by many factors, including poor absorption in the gastrointestinal tract, poor dosages, or polymorphisms of the genes that metabolise the enzyme. In addition, phenytoin is a drug with significant individual variations in its pharmacokinetic parameters. Phenytoin has non-linear pharmacokinetics, whereas phenytoin was saturated (steady-state) with additional doses, and an increase in phenytoin levels was not proportional. This non-linear kinetics occurs due to metabolic saturation and protein bond saturation. It made the levels of the free drug that enter the membrane of hepatocyte cells and hepatic ratio decrease so that the blood levels of drugs remain high (Alqahtani *et al*., 2019; Shargel & Yu, 2022).

Phenytoin is a drug that has a steady state ranging from 5-7 days (6.65 x t1/2), where half-life (t1/2) phenytoin ranges from 8 - 36 hours. The saturation of metabolism in non-linear kinetics based on Michaelis-Menten causes a decrease in clearance and longer half-life (t1/2) of phenytoin. The clinical implication of this condition was increased blood concentration of phenytoin. Under these conditions, only the amount of phenytoin could be metabolised per day because the enzyme system was entirely saturated, and it can change phenytoin kinetic to non-linear kinetics (Bauer, 2008; Alqahtani *et al*., 2019; Shargel & Yu, 2022).

Polymorphism of Cytochrome P450 2C9 (CYP2C9) can influence phenytoin metabolic ability. Genetic polymorphisms from enzymes and transporters can affect phenytoin pharmacokinetics in patients (Dagenais *et al*., 2017). The existence of polymorphisms from the variants of CYP2C9*2 and *3 in patients can reduce the metabolism of phenytoin (Twardowschy *et al*., 2011; Ozkaynakci *et al*., 2015; Silvado *et al*., 2018). The presence of autoinduction by phenytoin or inducer will induce enzymes, thereby accelerating the metabolism of phenytoin itself. Therefore there are low levels of concentration even though given high phenytoin doses. Phenytoin autoinduction is also influenced by CYP2C9 polymorphisms, especially CYP2C9*1B and CYP2C9*2 (Chaudhry *et al*., 2010).

Genetic polymorphism of CYP2C9 may reduce the metabolism of phenytoin by 25 – 50% in patients who have the genetic variants *2 and *3, as compared to those with variant *1 (normal metabolizers), named the “wild types” or the “extensive metabolizers” (Silvado *et al*., 2018).

Conclusion

The optimal adjustment of phenytoin dose individually in adult epilepsy patients based on Vmax and Km parameters needs to be done to get the desired steady level according to the clinical response. The results from this study are beneficial for phenytoin dosage individualisation in Indonesian adults with epilepsy.

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Ethics and consent

This study has received an ethical approval/statement from the Health Research Ethics Committee of Dr. Soetomo Hospital Surabaya.

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