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Research Article

Comparative bioavailaibilty of two valproic acid delayed-release tablets in healthy volunteers with tighter acceptance criteria to anticipate breakthrough seizures

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ABSTRACT

The present study was performed to investigate if the generic of valproic acid delayed release is interchangeable on the basis bioequivalence to the reference product. Due to previous report of breakthrough seizures following a change from a brand name product to a generic product, this bioequivalence study was conducted using tightened acceptance criteria for assuring the therapeutic equivalence. Twenty eight healthy volunteers participated in an open-label, randomized and two-way crossover study under fasting conditions. Plasma samples were collected up 72 hours following drug administration and were determined by liquid chromatography-tandem mass spectrometry. Pharmacokinetic parameters used for bioequivalence assessment were AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}. The 90% CI bioequivalence limits was tightened from the traditional 80.00% -125.00% to 90.00%-111.11%. All volunteers completed the study. The 90% confidence intervals obtained by analysis of variance for AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were 98.38%-104.61%, 98.93%-105.31%, and 92.62%-102.93%, respectively. Both formulations were tolerated and no serious adverse events were reported. These results were all within the range of 90.00-111.11%. The low intra-subject variability observed in this study indicates that tightened acceptance criteria are still applicable for valproic acid BE study.

1. INTRODUCTION

Epilepsy is a chronic disorder of the brain that affects people worldwide. It is characterized by recurrent seizures, which are brief episodes of involuntary movement that may involve a part of the body (partial) or the entire body (generalized) and are sometimes accompanied by loss of consciousness and control of bowel or bladder function. Approximately 50 million people worldwide have epilepsy, making one of the most common neurological diseases globally. Nearly 80% of the people with epilepsy live in low- and middle-income countries[1].

Valproic acid, a branched chained fatty acid that is structurally unrelated to any other antiepileptic drugs (AEDs), is valuable for the treatment of primary generalized epilepsy, especially tonic-clonic fits, absence seizures, and myoclonus in

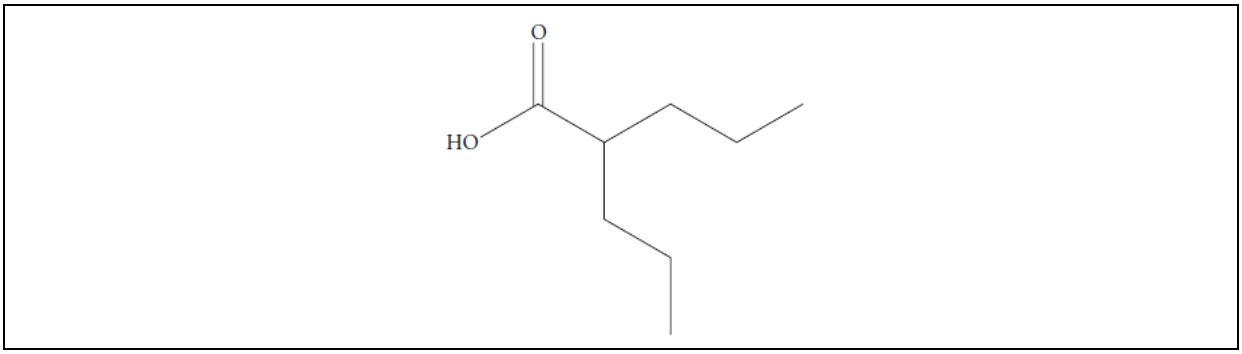

Figure 1. Chemical structure of valproic acid

adult patients[2]. The structure of valproic acid is shown in Figure 1. In pediatric patients with seizures disorders, valproic acid is effective treatment for generalized tonic-clonic seizures, generalized absences, and myoclonic epilepsy[3]. However, the drug's mechanism of action is not fully understood. It has been theorized that valproic acid acts by increasing the concentrations of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) within the central nervous system through inhibition of GABA degradation or enhancement of GABA synthesis and release. Other research has suggested that valproic acid acts via inhibition of excitatory neuro-transmitters or by action at sodium and calcium channels to reduce sustained neuronal firing[4-6].

Health care systems have been under increasing pressure to control the costs of prescription drugs and others services. In an effort to reduce the costs, many insurance companies strongly encourage or mandate the substitution of medication with generic preparation. The US Food and Drug Administration (FDA)'s position is that drug testing as bioequivalent, according to their requirements, are approved as therapeutically equivalent and substitution can occur without concern about efficacy or toxicity. The FDA's position does not make a distinction for drugs used to treat narrow therapeutic range conditions such as epilepsy[7,8].

Physicians and patients perceive that generic AEDs are not always equivalent to their branded counterparts, with about two-thirds of physicians reporting that they have cared for a patient who had a breakthrough seizure associated with switching to a generic drug. Breakthrough seizures can cause loss of driving license, job difficulties and sometime injury or death. Consequently, it is important to determine how accurately generic AEDs copy reference brand-name formulations. The European Medicine Agency (EMA) has developed tighter criteria for drugs with a narrow therapeutic index and recommends these drugs have AUC and C_{max} confidence intervals within 90.00 to 111.11%[8,9].

The present study was performed to investigate the pharmacokinetics and bioavailability of two valproic acid delayed-release tablet formulations in order to prove bioequivalence between two formulations with tighter acceptance criteria.

2. MATERIALS AND METHODS

2.1. Study drug

The test formulation of divalproex sodium delayed release tablet (equivalent to valproic acid 250 mg) was manufactured by PT. Novell Pharmaceutical Laboratories, Indonesia The reference formulation is Depakote® 250 mg enteric coated tablet manufactured by PT. Abbott Indonesia, under license of Abbot Laboratories, ILL, USA.

2.2. Subjects and study design

A single-dose, open-label, randomized, two-sequences, two-period crossover study with an overnight fasting and one-week wash out period was conducted in compliance with the ethical principles of the Declaration of Helsinki for biomedical research involving human subjects and Good Clinical Practice (GCP). The study protocol was reviewed by the Committee of The Medical Research Ethics of the Faculty of Medicine, University of Indonesia (Jakarta, Indonesia) and was approved by the National Agency of Drug and Food Control (Jakarta, Indonesia). All participants signed a written informed consent after they had been informed of the nature and details of the study.

The lack of data on intra-subject variability in published paper on bioavailability of divalproex sodium delayed release makes it difficult to establish the exact predetermination of sample size. The sample size n = 24 subjects is sufficient to ensure power of 80% for correctly concluding bioequivalence under the following assumption: $\alpha = 0.05$, $0.95<\mu T/\mu R<1.05$ and an intra-subject coefficient of variation of 25.0%[10].

Twenty eight volunteers were selected among Indonesia residents and participated in this study in order to have 24 evaluable volunteers at the end of the study. Additional 4 volunteers were added for possible dropouts and withdrawals. Volunteers were selected after passing a clinical screening procedure which included physical examination, ECG and clinical laboratory tests: hemoglobin, hematocrit, WBC, platelets, WBC differential, blood urea nitrogen, sGPT, sGOT, alkaline phosphatase, total bilirubin, total protein, fasting glucose, albumin, total cholesterol, creatinine, urine analysis, pregnancy test (for female subjects) and negative results of HBsAg, anti HBC and anti HIV. Volunteers were excluded if they had history of hepatic, gastrointestinal or renal disease, potentially sensitive or hypersensitive to valproic acid or other related drugs, consumed alcohol or drug abuse within 12 months, donate or lost >450 mL of blood within 3 months prior to the screening of the study. All volunteers were required not to use any drugs for at least two weeks prior to the study until completion of the study. They also refrained from ingesting alcohol, caffeine, chocolate, tea or coke-containing beverages at least 48 h before each dosing and until last blood sampling.

Volunteers were randomized to one of the two sequences to receive the formulations according to randomization scheme. Volunteers were confined to clinical unit of Clinisindo Laboratories one night before study to assure the fasting condition (10 h before drug administration). On the study day, each volunteers received one tablet of either product with 240 ml of water. Water intake was allowed 1 h before and after the dose. No food was allowed until 4 hours after dose administration. Standard meals were served at 4, 8 and 11 hours after dosing.

Volunteers were remained at the clinical unit of Clinisindo Laboratories for 24 h after drug administration and were not allowed to take strenuous exercise during the sampling days. Blood pressure, heart rate, body temperature and adverse events were monitored during blood sampling.

2.3. Bioanalytical method

Valproic acid plasma concentration was determined using LC-MS/MS (API 3200) method with TurboIonSpray mode and benzoic acid was used as an internal standard (IS).

Briefly, plasma samples (300 µL) were added with an internal standard. After mixing, 500 µL of methanol was added. The mixture was vortex-mixed for 1 minute and centrifuged at 3000 rpm for 10 minutes A volume of 5 µL of supernatant was injected and analyzed into LC-MS/MS.

The analytical separation was performed on a Synergi 4µ POLAR-RP-80A, 50 x 2.0 mm, 4 µm (Phenomenex®, Torrance, CA, USA) preceded by a guard column AQ C_{18}, 4 x 2.0 mm (Phenomenex®, Torrance, CA, USA). Mobile phase was 0.1% formic acid in methanol and 0.1% formic acid in water set as gradient. Flow rate used was 0.6 mL/min. Column temperature was maintained at 40°C. Multiple reaction monitoring (MRM) in positive ion mode was used to monitor transitions at *m/z* 143.04 → 143.04 and *m/z* 120.96 → 77.00 for valproic acid and the IS.

The assay had been validated in terms of selectivity, sensitivity, linearity, accuracy and precision, recovery, matrix effect and carry-over according to the Guideline on bioanalytical validation, EMA 2011[11]. This method also has been verified before being used in this study.

The best linear fit and least-squares residual for the calibration curve was achieved with $1/x^2$ weighing factor. The standard calibration curve for valproic acid was ranged from 0.5-50.4 µg/mL and was created at the following concentration 0.5, 1.0, 2.0, 5.0, 10.0, 25.2 and 50.4 µg/mL. The lower limit of quantification was 0.5 µg/mL and the precision obtained at LLOQ was 7.35% and the accuracy at LLOQ was (-19.40%) – (-4.13%).

The intra-batch precision and accuracy were determined by analyzing five sets of QC samples (LOQ, QCL, QCM, and QCH) in a batch. The inter-batch precision and accuracy were determined by analyzing five sets of QC samples on three batch runs. The precision and accuracy are shown in Table 1.

Table 1. Precision and accuracy of the method for determining valproic acid in plasma samples

Concentration (µg/mL)	Intra-batch			Inter-batch		
	Mean ± SD (µg/mL)	RSD (%)	Diff (%)	Mean ± SD (µg/mL)	RSD (%)	Diff (%)
0.5	0.49 ± 0.04	7.4	-3.2	0.51 ± 0.02	3.2	0.3
1.5	1.61 ± 0.05	3.4	5.5	1.51 ± 0.14	9.4	0.3
20	22.67 ± 0.63	2.8	11.7	20.80 ± 2.26	10.9	2.5
40	42.59 ± 1.89	4.4	4.9	41.35 ± 4.49	10.9	1.8

The mean recoveries of valproic acid and IS were 89.79% - 97.03% and 82.21%. The matrix effect was also investigated. Matrix effect of human plasma on ionization efficiency was assessed by comparing the peak response of six determinations in two concentration levels of low and high QCs spiked in extracted drug-free human plasma samples (six individual sources) with that of neat standards at corresponding concentration. The same evaluation was also performed for internal standard. The CV of the IS-normalized matrix factor calculated from the 6 lots of matrix of low and high concentrations were 3.95% and 1.41%. Carry over in the blank samples was found not more than 20% of LOQ and 5% for the internal standard, indicating there was no carry over effect during validation.

The stability study showed that valproic acid in plasma was stable at room temperature for 6 hours, at -20°C for 60 days and after three freeze-thaw cycles. The stability auto-sampler showed that valproic acid was stable after reconstitution for 24 hours.

2.4. Safety evaluation

To access tolerability, vital signs (temperature, heart rate and blood pressure) were measured during the study. Clinical tolerability was monitored by a clinical investigator using interview and an adverse drug reaction checklist throughout the study period, and the incidence of any adverse effects was recorded.

2.5. Pharmacokinetic and Statistical Analysis

For the assessment of PK parameters [i.e., maximum concentration (C_{max}), time to maximum concentration (t_{max}) and area-under-the plasma concentration curve (AUC) for valproic acid, venous blood samples (5 mL) were collected at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, 36, 48, and 72 hours after drug administration in the Li-heparin tubes.

The maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}) were obtained from observed data of the individual drug plasma concentration time data, and used as a measurement of absorption rate. The AUC_{0-t} was calculated using the trapezoidal rule. The elimination rate constants (K_{el}) were calculated by least-squares regression from the data of the last 4-6 points of each plasma concentration data curve. The $AUC_{0-\infty}$ values were determined by adding the quotient of C_t (estimated last plasma concentration) and the appropriate K_{el} to the corresponding AUC_{0-t},: $AUC_{0-\infty} = AUC_{0-t} + C_t/K_{el}$

The apparent elimination half-life ($t_{½}$) of valproic acid in plasma was also calculated by using the following equation: $t_{½} = (\ln 2)/ K_{el}$

A multiplicative model was assumed for the parameters of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}, and analysis of variance (ANOVA) was applied using the respective ln-transformed data. 90% CI of the geometric mean ratio test/reference (T/R) for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were calculated assuming a multiplicative model. The accepted bioequivalence range for these parameters was tightened to 90.00%-111.11%. All statistical analyses were performed using EquivTest version 2.0 software (Statistical Solution, Cork, Ireland).

3. RESULTS AND DISCUSSION

Generic drugs are required to be bioequivalent to the reference formulation. Bioequivalence tests are carried out in small samples of healthy volunteers. The European Medicine Agency (EMEA) criteria for bioequivalence requires the upper and lower limits of 90% confidence intervals (CI) for a generic drug's area under the curve (AUC) and maximum concentration (C_{max}) to be within 80 to 125% to the reference formulation[9]. Many bioequivalence studies of valproic acid were conducted using these criteria[12-14].

Table 2. Disposition of adverse events

	Test	Reference
Adverse events:		
Headache	13 (65)	15 (75)
Nausea	9 (45)	10 (50)
Drowsiness	4 (20)	6 (30)
Fatigue	4 (20)	4 (20)
Vomiting	3 (15)	2 (10)

Table 3. Pharmacokinetic results of valproic acid

Parameter	Test Formulation		Reference Formulation	
	Mean	SD	Mean	SD
$AUC_{0\text{-}72h}$ (µg.h/mL)	580.87	166.50	570.59	160.77
$AUC_{0\text{-}\infty}$(µg.h/mL)	626.49	205.92	611.09	196.66
C_{max} (µg/mL)	31.76	5.55	32.42	5.22
t_{max} (h)	3.21	1.29	2.96	1.07
$t_{1/2}$(h)	17.35	4.92	16.97	3.47

Valproate is considered to be treatments with a narrow therapeutic index (i.e. only a small relative difference in dose between therapeutic and toxic effects). A narrow therapeutic index implies that slight variations in drug absorption could result in significant negative health outcomes. Narrow therapeutic index has also been used to describe medications that practitioners consider may present difficulties with generic substitutions and many evidences of generic substitutions may lead to breakthrough seizures or adverse events. Consequently, it is important to determine how accurately generic AEDs copy reference brand-name formulations.

In Denmark, AEDs other than benzodiazepines are designated as "narrow therapeutic index" drugs, and generic formulations must meet a 90.00 to 111.11% BE acceptance standard, particularly modified release (MR) formulations will have to meet additional bioequivalence criteria[7,15]. The aim of this study was to demonstrate the comparable bioavailability of two valproic acid delayed release tablets using tightened bioequivalence limits.

A total of twenty eight volunteers, both sexes were enrolled and randomized in the study. All volunteers successfully completed the study according to the protocol. Both formulations were well-tolerated and no serious adverse events were observed. Eighteen out of 28 volunteers experience 36 adverse events during the study. The disposition of adverse events is shown in Table 2.

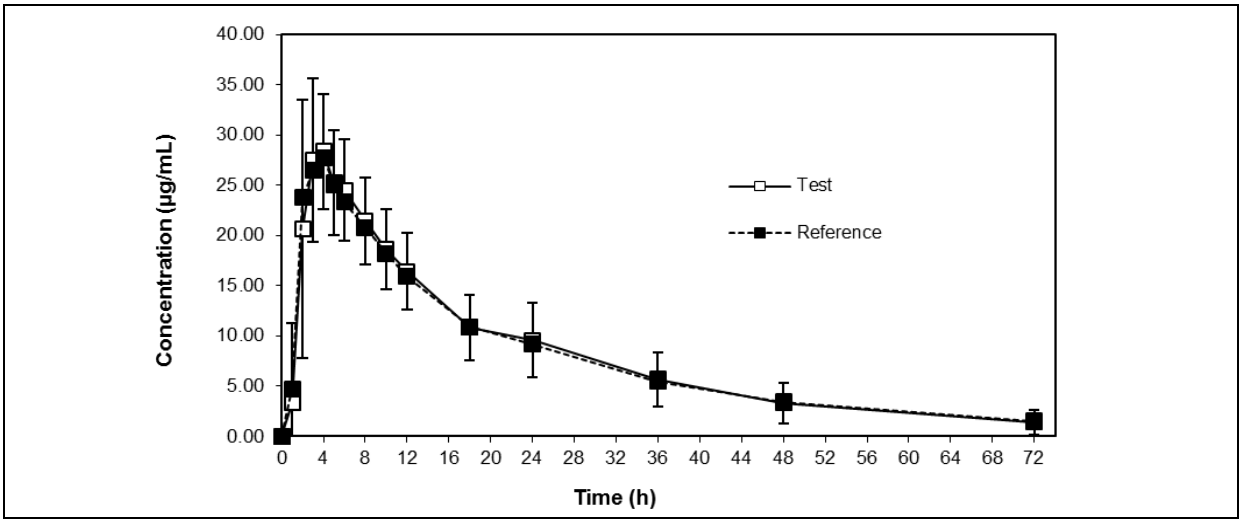

Figure 2. Mean plasma concentration-time profiles of valproic acid after a single dose of two 250 mg valproic acid delayed release tablet formulations

The mean of valproic acid concentration versus time profiles for both formulations are shown in Figure 2. Descriptive statistics of the pharmacokinetic parameters for valproic acid for test and reference products are summarized in Table 3, where the geometric mean values and the range for the $AUC_{0-t,}$ $AUC_{0-\infty,}$ and C_{max} values obtained for each formulation are shown. The pharmacokinetic characteristic t_{max} was presented as mean values. The mean obtained values for test and reference products were 31.76 µg/mL and 32.42 µg /mL for C_{max}, 580.87 µg.h/mL and 570.59 µg.h/mL for AUC_{0-72h}, 626.49 µg.h/mL and 611.09 µg.h/mL for $AUC_{0-\infty}$ The median t_{max} for test and reference formulations were 3 h. The parametric 90% confidence intervals for ratio T/R ranged from 92.62%-102.93% (point estimate 97.64%) for $C_{max,}$ 98.38%-104.61% (point estimate 101.45%) for AUC_{0-72h}, and 98.93%-105.31% (point estimate 102.07%) for $AUC_{0-\infty}$. The intra-subject variability for C_{max}, AUC_{0-72h}, and $AUC_{0-\infty}$ estimated from the coefficient of variables as determined by ANOVA (Table 4 and 5) were 11.58%, 6.71 and 6.78%, respectively. The results indicated that the pharmacokinetic parameters were entirely included within the tightened bioequivalence acceptance limits of 90.00-111.11%.

Table 4. Statistical results of valproic acid

	AUC_{0-72h} (%)	$AUC_{0-\infty}$((%)	C_{max} (%)
Ratio	101.45	102.07	97.64
90% Geometric CI	98.38 to 104.61	98.93 to 105.31	92.62 to 102.93
Intra-Subject CV	6.71	6.78	11.58

Table 5. ANOVA of pharmacokinetic parameters of valproic acid for ln-transformed data

AUC_{0-72h}					
	df	SS	MS	F	p-value
Inter-Subjects					
Carry-over	1	0.0839	0.0839	0.5026	0.4846
Residuals	26	4.3425	0.1670	36.7512	<.0001
Intra-Subjects					
Drug	1	0.0028	0.0028	0.6346	0.4328
Period	1	0.0279	0.0279	6.1420	0.0200
Residuals	26	0.1181	0.0045		
Total	55	4.5754			
$AUC_{0-\infty}$					
	df	SS	MS	F	p-value
Inter-Subjects					
Carry-over	1	0.0967	0.0967	0.4947	0.4880
Residuals	26	5.0838	0.1955	41.6476	<.0001
Intra-Subjects					
Drug	1	0.0058	0.0058	1.2562	0.2726
Period	1	0.0363	0.0363	7.7379	0.0099
Residuals	26	0.120	0.0046		
Total	55	5.3449			
C_{max}					
	df	SS	MS	F	p-value
Inter-Subjects					
Carry-over	1	0.0590	0.0590	1.3147	0.2619
Residuals	26	1.1680	0.0449	3.3516	0.0014
Intra-Subjects					
Drug	1	0.0080	0.0080	0.5969	0.4466
Period	1	0.1041	0.1041	7.7734	0.0097
Residuals	26	0.3485	0.0134		
Total	55	1.6878			

5. ACKNOWLEDGEMENTS

Conflict of interest
The authors declare no conflicts of interest.

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Ethical approval
The study protocol was reviewed by the Committee of The Medical Research Ethics of the Faculty of Medicine, University of Indonesia (Jakarta, Indonesia) and was approved by the National Agency of Drug and Food Control (Jakarta, Indonesia).

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