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Research Article

IN VIVO COMPARATIVE BIOAVAILABILITY STUDY OF TWO VALPROIC ACID SYRUP FORMULATIONS DISPENSED IN GAZA STRIP

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ABSTRACT

Objective: This study was conducted to establish either two syrup formulations (Duvalep and valporal as test and reference products, respectively) of valproic acid (VA) dispensed usually in pediatric clinics of Gaza Strip were bioequivalent to each other or not.

Methods: A randomized, two crossover design study was conducted on six healthy male rabbits. Rabbits received a single oral dose (25 mg/kg) of valproic acid formulations with a washout period of one week. Serial blood samples were collected over a period of 48 hours. Chemiluminescent enzyme immunoassay (CLEIA) was used to measure valproic acid in serum. Pharmacokinetic (PK) parameters C_{max}, T_{max}, $t_{1/2}$, AUC_{0-t}, AUC $_{0-\infty}$, and k_e were determined for the two formulations. After log-transformation of the data obtained the pharmacokinetic parameters $C_{max,}$ AUC_{0-t} and AUC $_{0-\infty}$ of tested and reference products were used to test bioequivalence. The two formulations were to be considered bioequivalent if the log transformed ratios of $C_{max,}$ AUC_{0-t} and AUC $_{0-\infty}$ were within predetermined criteria for bioequivalence range 80 – 125 %.

Results: Six rabbits were enrolled in the study which exhibited good tolerability to VA formulations. No statistical differences were found based on analysis of variance. The mean values and 90 % confidence intervals ratios of test/reference of the corresponding parameters were as follows: C_{max}; 49.58 versus 49.30 µg/ml (86.80 % to 112.28 %), AUC_{0-t} ; 355.90 versus 381.0 µg.h/ml (81.24 % to 116.22 %) and AUC $_{0-\infty}$; 399.70 versus 409.70 µg.h/ml (83.35 % to118.50 %).

Conclusion: Pharmacokinetic analysis of VA test and reference formulations indicated that they were bioequivalent.

Keywords: Comparative, Bioequivalence, Valproic acid, Syrup formulations.

INTRODUCTION

Epilepsy is a common chronic disorder that requires long-term antiepileptic drug therapy. Valproic acid is used for epileptic patients of all ages with absence seizures, either alone or with other seizure types. It is also used for patients older than 10 years of age with partial seizures [1].VA is available in oral, rectal and parenteral dosage forms.

The bioavailability of VA is nearly complete with all formulations. Absorption phase of VA varied according to dissolution characteristics and absorption rate [2, 3]. Peak plasma concentrations are usually achieved within 2 hours for VA and valproate sodium oral formulations. VA is highly protein bound and is metabolized hepatically to produce active metabolite. Elimination $t_{1/2}$ ranges between 5 and 20 hours [4]. Differences in bioavailability BA of different brands of an antiepileptic were reported [5-12]. For a successful long term therapy, is required an appropriate antiepileptic regimen and optimal dosing [13].

Prescribing a generic instead of innovator antiepileptic drugs may lead to either loss of seizure control or intoxication [14, 15]. To introduce generic equivalents of innovator with advanced cost of medication, a proper assessment to compare BA of both products, as directed by the international regulatory authorities, should be performed [16-19].

In the present study we aimed to compare between the bioavailability of two VA syrups; duvalep (Test formulation, Birzeit Pharmaceutical Company) with valporal (Reference formulation, Teva Pharmaceutical Industries Ltd.), which are usually dispensed in pediatric clinics of Gaza Strip.

MATERIALS AND METHOD

The study was carried out at College of Pharmacy, Al-Azhar University-Gaza, Gaza, Palestine. An approval from the institutional ethic committee – Al-Azhar University-Gaza, was obtained to start the study. The study was conducted under supervision of a veterinary physician.

Animals and study design

Six healthy adult male rabbits (Weighed: 3.2 - 3.5 Kg, mean 3.3 ± 0.12 Kg, aged: 8-10 months) were enrolled in the study. The rabbits were obtained from Asdda for animal production and welfare centre, where follow up care and clinical examination were performed and rabbits' health state was certified (Khanunis, Palestine). Rabbits were fasted for 12 hours with free access to water by add libitum before the study started.

A single dose, two crossover design study was conducted on rabbits. There was a washout period of one week between the two doses. The rabbits were divided into two groups. The first one received a dose of (25 mg/Kg) of valporal syrup (Reference product), whereas the second group received the same dose of duvalep syrup (Test product). Syrup formulations were purchased from a local pharmacy (Gaza, Palestine). After one week, the first group received test drug and the second received reference drug to complete the cross-over design. The dose was given by means of a syringe connected to an oral gavage. The syringe was put in the corner of the mouth and the liquid was pushed down slowly, to avoid choking.

General clinical safety was assessed by physical examination during the study, during washout period and at the end of the study.

Blood sampling

Rabbits were placed in rabbit restraining box apparatus. The marginal ear vein was located and the hair was removed. Gentle stroking and tapping of the ear made the vein more visible. Local anesthetic was applied to prevent the jerking of the rabbit as a result of venipuncture 15 minutes before starting the study. Inserting a small needle (23 gauge) butterfly attached to a syringe in the marginal ear vein [20]. Serial venous blood samples were collected (0.5 ml) in vacutainer tubes according to the time schedule 0.0, 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 24 and 48 h after rabbits received the dose. Blood samples were centrifuged at 3,000 rpm for 5 minutes and serum was transferred into clean plastic tubes. Serum samples (ca. 200 µl) were kept in refrigerator until being analyzed within 24 h. Analysis was performed by valproic acid kit based on

chemiluminescent enzyme immunoassay (CLEIA) and Immulite 1000 immunoassay system (Siemens Healthcare Diagnostics).

Evaluation of pharmacokinetic parameters

The plasma pharmacokinetic parameters were estimated. It included the observed maximum plasma concentration C_{max}, the time to reach C_{max}, (T_{max}) and the area under the plasma concentration-time curve from 0 hour to last measurable concentration (AUC_{0-t}) and 0 hour to infinity ($_{AUC0-\infty}$). The C_{max} and T_{max} were directly determined by the serum concentration versus time curves. The area under the curve from 0 hour to t (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve from 0 hour to infinity ($AUC_{0-\infty}$) was estimated by summing the area from AUC_{0-t} and $AUC_{0-\infty}$, where $AUC_{0-\infty} = AUC_{0-t} + C_t / k_e$, with '$C_t$' defined as the last measured serum concentration at time t, and k_e the slope of the terminal portion of the plasma concentration versus time curve, obtained by linear regression. Logarithmic transformation was done before data analysis for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. Analysis of variance (ANOVA) was used to assess effects. Intra-subject variability in terms of the overall percentage coefficient of variation (%CV), were evaluated from the ANOVA results for log transformed data. For the pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ 90% confidence intervals for the ratios of Test and Reference product averages were calculated using the ANOVA of the ln-transformed data. The product was tested for bioequivalence using ratios of the Log-transformed pharmacokinetic parameters C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ and its 90% confidence interval. The formulations were to be considered bioequivalent if the log- transformed ratios (test/reference) of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were within the predetermined bioequivalence range of 80% to 125% [21]. Pharmacokinetic analysis was performed by means of model independent method (Non-Compartmental Approach) WinNonlin Professional Software (Version 6.3, Pharsight Corporation, Cary, NC).

RESULTS AND DISCUSSION

The mean serum concentration–time curves of the two syrup formulations of VA received as a single oral dose by rabbits are shown in figure 1. The concentration time profile obviously indicated that the two formulations were comparable. The primary PK parameters for both formulations are listed in Table 1. The mean C_{max} and T_{max} values of the test and reference formulations were 49.58, 49.30 µg/ml and 1.08, 1.12 hours, respectively. The extent of absorption, based on mean of AUC_{0-t} and $AUC_{0-\infty}$ values, were 355.9 and 399.7 µg.h/ml, respectively after administration of the test formulation and 381.2 and 409.7 µg.h/ml respectively after administration of the reference formulation. Mean plasma $t_{1/2}$ was 14.43 h for the test and 10.45 h for the reference formulation. ANOVA statistical analysis of PK showed no significant differences between the two formulations ($p > 0.05$) (Table 1). A crossover study design is recommended to minimize the between subject variability since each subject received the test and reference products [19].

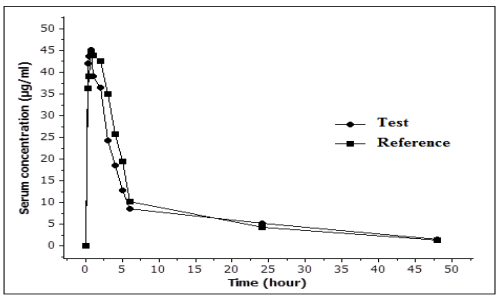

Fig. 1: The mean rabbit serum concentration-time profile for valproic acid test and reference formulations

The rate of absorption is characterized by maximum concentration C_{max} and T_{max}. A difference is predicted according to dose, route of administration and dosage form. The recorded mean T_{max} in the study was comparable with published value for syrup (0.9 h) applied on epileptic patients [22].

Table 1: Summary of mean pharmacokinetic parameters of valproic acid, following administration of single dose (25 mg/kg) test and reference formulations in rabbits (n=6)

Pharmacokinetic parameter[a]	Test[b] Mean ± SD[d]	Reference[c] Mean ± SD[d]	p-Value[e]
C_{max} (µg/ml)	49.58 ± 16.39	49.30 ± 5.54	0.973
T_{max} (h)	1.083 ± 0.75	1.125 ± 0.72	0.941
AUC $_{0-48}$ (µg.h/ml)	355.90 ± 90.0	381.20 ± 104	0.744
AUC $_{0-\infty}$ (µg.h/ml)	399.70 ± 96.2	409.70 ± 116	0.907
$t_{½}$ (h)	14.43 ± 5.89	10.45 ± 2.84	0.276
K_e (h^{-1})	0.057 ± 0.03	0.071 ± 0.02	0.480

[a]: Abbreviations are C_{max}: Maximum serum concentration, T_{max}: Time to C_{max}, AUC_{0-48}: Area under the curve from 0 h to 48 h, $AUC_{0-\infty}$: Area under the curve from 0 h to infinity, $t_{1/2}$: Terminal half-life and K_e: Terminal elimination constant, [b]: Rabbits received duvalep syrup, [c]: Rabbits received valporal syrup, [d]: SD: Standard deviation, [e]: Statistical significance $p \leq 0.05$.

Inter-individual variations among rabbits were statistically insignificant ($p > 0.05$), when received test and reference formulations. The results of one-way ANOVA test for homogeneity are listed in Table 2.

Table 2: One-way ANOVA test for homogeneity among rabbits (n=6)

Group	d.f.	F	p-Value[c]
Test[a]	5	0.063	0.997
Reference[b]	5	0.156	0.974

[a]: Rabbits received duvalep syrup, [b]: Rabbits received valporal syrup single dose (25 mg/kg), respectively, [c]: Statistical significance $p \leq 0.05$.

The utility of rabbit as a model in comparative bioavailability studies is well documented after local or systemic administration [23-28]. Study has been completed on all six rabbits. There were no rabbit death or replacement during the study. Clinical physical examination during and post study indicated no abnormalities. The present study showed good tolerability of both formulations.

The bioavailability of a drug is the quantum of the drug available in the systemic circulation for its action after absorption [5]. To assure the safety and efficacy of generic formulations the BA should be compared with a reference product. When two formulations of the same drug are equivalent in rate and extent to which the active drug ingredient is absorbed, and becomes equally available at the site of drug action, they are bioequivalent and thus are assumed to be therapeutically equivalent [19].

To demonstrate bioequivalence, certain limits should be set, depending on the nature of the drug, patient population and clinical end-points [26, 29]. It is generally accepted that the 90% confidence interval for the ratio of averages of logarithmically transformed AUC and C_{max} should lie within the range of 80 to 125 % [21].

The 90 percent confidence intervals of the log transformed mean values for the test/reference ratios were 86.80 to 112.28 for C_{max}, 81.24 to 116.22 for AUC_{0-t} and 83.35 to 118.50 for $AUC_{0-\infty}$, respectively (Table 3). The results of study were within the bioequivalence acceptance range.

Table 3: 90% Confidence interval for the ratio of log-transformed data comparing test to reference formulations

Parameter	Lower confidence limit	Upper confidence limit
C_{max}	86.80 %	112.28 %
AUC_{0-48}	81.24 %	116.22 %
$AUC_{0-\infty}$	83.35 %	118.50 %

CONCLUSION

It has been confirmed that both syrup formulations of VA dispensed in pediatric clinics of Gaza Strip are bioequivalent. Therefore, both are interchangeable.

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