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Bioequivalence Evaluation of Two Brands of Furosemide 40 mg Tablets (Salurin and Lasix) in Healthy Human Volunteers

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ABSTRACT: A randomized, two-way, crossover, bioequivalence study was conducted in 24 fasting, healthy, male volunteers to compare two brands of furosemide 40 mg tablets, Salurin (Julphar, UAE) as test and Lasix (Hoechst AG, Germany) as reference product. The study was performed at the International Pharmaceutical Research Centre (IPRC), in a joint venture with Al-Mowasah Hospital, Amman, Jordan. One tablet of either formulation was administered with 240 ml of water after a 10 h overnight fast. After dosing, serial blood samples were collected for a period of 12 h. Plasma harvested from blood was analysed for furosemide by a validated HPLC method. Various pharmacokinetic parameters including AUC_{0-t} , $AUC_{0-\infty}$, C_{max} , T_{max} , $T_{1/2}$, and elimination rate constant were determined from plasma concentrations of both formulations. Statistical modules (ANOVA and 90% confidence intervals) were applied to AUC_{0-t} , $AUC_{0-\infty}$, and C_{max} to assess the bioequivalence of the two brands which revealed no significant difference between them, and 90% CI fell within the US FDA accepted bioequivalence range of 80%–125%. Based on these statistical inferences, Salurin was found to be bioequivalent to Lasix. Copyright © 2003 John Wiley & Sons, Ltd.

Key words: furosemide; bioequivalence; pharmacokinetics; HPLC; Julphar

Introduction

The bioequivalence of two formulations of the same drug comprises equivalence with respect to the rate and extent of their absorption. The area under concentration time curve (AUC) generally serves as the measure for the extent of absorption while the peak concentration (C_{max}) and the time of its occurrence (T_{max}), reflect the rate of absorption, especially in fast releasing drug formulations [1,2]. The present study was conducted to evaluate the bioequivalence of two

brands of furosemide 40 mg tablets in fasting, healthy human volunteers.

Furosemide is a short-acting sulfonamide loop diuretic. Chemically it is 4-chloro-N-furfuryl-5-sulphamoylanthranilic acid, having a molecular formula of $C_{12}H_{11}ClN_2O_5S$ and molecular weight of 330.75 [3].

The exact mechanism of action has not been fully elucidated, but furosemide is believed to reversibly bind to the sodium, potassium and chloride co-transport mechanism on the luminal side of the ascending loop of Henle thereby inhibiting the active reabsorption of these ions. Furosemide also inhibits reabsorption of sodium and chloride in the proximal and distal tubules [4]. The inability to reabsorb salts therefore

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results in a higher osmolality and decreases the kidney's ability to reabsorb water [5–8]. Furosemide enhances renal blood flow, without increasing filtration rate; this increase is short-term [9].

Oral administration leads to bioavailability of 60%–70% showing an incomplete but fairly rapid absorption. The rate and extent of absorption are complicated by a large degree of intersubject and intrasubject variabilities [5,6,8–11]. The rate of absorption may be decreased in patients with oedema but the total bioavailability remains unchanged [12]. The onset of diuresis following oral administration is within 1 h, and the peak effect occurs within the first or second hour with a duration that lasts 6–8 h [8,12,13]. Furosemide is extensively bound to plasma proteins, mainly to albumin, plasma concentrations ranging from 1 to 400 µg/ml are 91% to 99% bound in healthy individuals while the unbound fraction averages 2.3% to 4.1% at therapeutic concentrations [8,10,11,13]. Recent evidence suggests that furosemide glucuronide is the only or at least the major biotransformation product of furosemide in man [8]. It is excreted mainly (60%–90%) in urine [8,9], 7–9% in faeces [4]. In a reported study [14] in normal subjects approximately 58.8% of a dose was recovered in the urine as unchanged drug within 24 h while 17.8% in the form of the glucuronide metabolite. The reported elimination half-life is approximately 2 h [8,9].

The purpose of this study was to determine the pharmacokinetic parameters of two brands of furosemide 40 mg tablets and then compare these parameters statistically to evaluate the bioequivalence between the two brands. Salurin (Gulf Pharmaceutical Industries-Julphar, UAE) was used as a test while Lasix (Hoechst AG, Germany) was used as a reference product.

Material and Methods

Study products

The test product was Salurin 40 mg tablets, (batch no. 0009, expiry 01/2007). The manufacturer was Gulf Pharmaceutical Industries - Julphar, United Arab Emirates.

The reference product was Lasix 40 mg tablets, (batch no. 40W258, expiry 12/2005). The manufacturer was Hoechst AG, Germany.

Study subjects

Twenty four healthy adult male volunteers participated in this study at Al-Mowasah Hospital, Amman, Jordan. The mean age was 24.38 ± 4.8 years with a range of 18–33 years and the mean body weight was 70.63 ± 7.62 kg with a range of 57–90 kg. The medical history, clinical examination and laboratory investigation (haematology, blood biochemistry and urine analysis) indicated a lack of evidence of hepatic, renal, gastrointestinal or haematologic disorders or any acute or chronic disease or drug allergy to sulfonylureas. The consumption of alcohol and beverages or food containing methylxanthines was not permitted for 48 h prior to the study and after drug administration until the last blood sample was collected in the respective study phase. The subjects were instructed to abstain from taking any medication for at least 1 week prior to and during the study period. Informed consent was obtained from the subjects after explaining the nature and purpose of the study. The study protocols were approved by the Institutional Review Board (IRB) of Al-Mowasah, Hospital.

Drug administration and blood samples collection

This study was based on a single dose, randomized, two-treatment, two-period cross over design. In the morning of phase I, after an overnight fast (10 h) volunteers were given a single dose of either formulation (reference or test) of furosemide 40 mg with 240 ml of water. No food was allowed until 5 h after dose administration. Water intake was allowed after 2 h of dose; lunch and dinner were given to all volunteers according to a time schedule. The volunteers were continuously monitored by Al-Mowasah hospital staff throughout the confinement period of the study. They were not permitted to lie down or sleep for the first 5 h after the dose. Approximately 10 ml of blood samples for furosemide assay were drawn into heparinized tubes through indwelling canula

before (0 h) and at 0.25, 0.50, 0.75, 1.0, 1.25, 1.50, 1.75, 2.0, 2.50, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10 and 12 h after dosing. Blood samples were centrifuged at 3500 rpm for 10 min, plasma was separated and kept frozen at -20°C until assayed. After a washout period of 7 days the study was repeated in the same manner to complete the crossover design.

Chromatographic conditions

An HPLC method was developed and validated at IPRC Laboratory for furosemide analysis in plasma samples with fluorescence detection. All solvents used were of HPLC grade and were purchased from ACROS, USA; furosemide and naproxen (internal standard) reference standards were obtained from Julphar, UAE.

The HPLC system was from Shimadzu, Japan and consisted of a solvent delivery pump (LC-10AD_{VP}), fluorescence detector (RF-10A_{XL}), system controller (SCL-10A_{VP}) and a manual injector (Model 7725I Rheodyne injector, Rheodyne, USA); Class VP-5 software version 5.03 (Shimadzu, Japan) was used for data interpretation. Chromatographic separation was performed using μ -Bonda-pak C₁₈ HPLC column (5 μm , 3.9 mm \times 150 mm) (Waters, Ireland). The mobile phase consisted of 33.3% acetonitrile and 66.7% 0.02 M potassium dihydrogen phosphate buffer; the pH was adjusted to 3.0 using phosphoric acid. The mobile phase was eluted at a flow rate of 1.0 ml/min, and the effluent was monitored at an excitation wavelength of 230 nm and emission wavelength of 410 nm. Each analysis required not more than 10 min. Quantitation was achieved by measurement of the peak area ratio of the drug to the internal standard. The method was validated by following international guidelines [15].

Sample preparation for HPLC injection

A 100 μl internal standard (naproxen, 1.0 $\mu\text{g}/\text{ml}$) was added to 0.5 ml of plasma sample and vortexed for 30 s and then 100 μl of 1.0 M HCl was added and vortexed for 30 s; 7 ml of extraction solvent (tert-butyl methyl ether) was added and vortexed for 1 min and then centrifuged for 5 min at 3000 rpm. The supernatant (organic) layer was transferred to another 10 ml glass tube and evaporated to dryness in a water

bath at 57°C ; the residue was reconstituted with 300 μl of mobile phase, vortexed for 30 s and transferred to a microcentrifuge tube (1.5 ml) and centrifuged at 13 000 rpm for 2 min; 50 μl of the aliquot was injected to the column and the peak area was recorded.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed by means of a model independent method using a KineticaTM 2000 computer program [16]. The elimination rate constant (λ_Z) was obtained as the slope of the linear regression of the log-transformed concentration values versus time data in the terminal phase. The elimination half-life ($T_{1/2}$) was calculated as $0.693/\lambda_Z$. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + C_t/\lambda_Z$, where C_t is the last measurable concentration.

Statistical analysis

For the purpose of bioequivalence analysis AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} were considered as primary variables. The bioequivalence of the two products was assessed by means of an analysis of variance (ANOVA GLM procedure; KineticaTM 2000 Computer program) [16] for crossover design and calculating standard 90% confidence intervals [17] of the ratio test/reference (T/R) using log-transformed data. The products were considered bioequivalent if the difference between the two compared parameters was found statistically insignificant ($p \geq 0.05$) and 90% confidence intervals for these parameters fell within 80%–125% [17].

Results and Discussion

Furosemide was well tolerated by all volunteers and no side effects were reported. Under the conditions described, the lower limit of quantitation in 500 μl plasma was 20 ng/ml for furosemide. The relationship between concentration and peak area ratio was found to be linear within the range 20–1200 ng/ml. The intra-day accuracy

of the method ranged from 98.40% to 100.50%, while the intra-day precision ranged from 5.47% to 6.85%. The inter-day accuracy ranged from 98.50% to 99.60%, while the inter-day precision ranged from 5.92% to 7.58%. Absolute recovery was 86.90%; relative recovery ranged from 95.17% to 102.33%. Stability studies showed that furosemide was stable in plasma for 8 weeks when stored at -20°C .

Both formulations were readily absorbed from the gastrointestinal tract and furosemide was measurable at the first sampling time (0.25 h) in the majority of the volunteers. The mean concentration-time profiles of two formulations, shown in Figure 1, were closely similar and superimposable. ANOVA was applied on the concentration attained at individual time intervals for both formulations and indicated no significant difference. The peak concentration of 1163.25 ng/ml and 1109.71 ng/ml for furosemide was attained at 1.47 and 1.36 h after administration of test and reference products, respectively and then declined rapidly and remained detectable up until 12 h. Table 1 shows the pharmacokinetic parameters of furosemide for the two brands. The relative bioavailability of Salurin was 106.7% for AUC_{0-t} , 106.0% for $AUC_{0-\infty}$, and 109.0% for C_{\max} .

The most important objective of bioequivalence testing is to assure the safety and efficacy of generic formulations. When two formulations of the same drug are equivalent in the rate and extent to which the active drug becomes available to the site of drug action, they are bioequivalent and thus considered therapeutically equivalent [18]. To demonstrate bioequivalence certain limits should be set depending on the nature of drug, patient population and clinical end points. It is generally accepted that for basic pharmacokinetic characteristics, such as AUC_{0-t} , $AUC_{0-\infty}$ and C_{\max} the standard equiva-

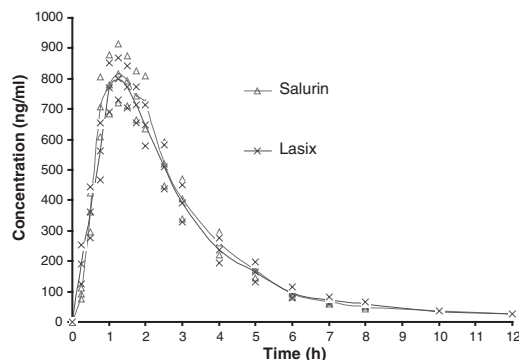


Figure 1. Mean plasma concentration of furosemide 40 mg tablets after oral administration of single dose of two brands to 24 healthy human volunteers

Table 1. Pharmacokinetic parameters of furosemide tablets (mean \pm standard deviation; $n = 24$)

Pharmacokinetic parameter	Salurin (test)	Lasix(reference)
AUC_{0-t} (ng/ml h)	2596 \pm 992.2	2516 \pm 959.0
$AUC_{0-\infty}$ (ng/ml h)	2690 \pm 989.0	2609 \pm 953.5
C_{\max} (ng/ml)	1163 \pm 334.6	1109 \pm 309.1
T_{\max} (h)	1.47 \pm 0.77	1.36 \pm 0.59
$T_{1/2}$ (h)	2.45 \pm 0.82	2.47 \pm 0.92
λ_z (/h)	0.319 \pm 0.115	0.322 \pm 0.124

lence range is 0.8–1.25 [17]. The results of statistical analysis are shown in Table 2.

The mean and standard deviation of AUC_{0-t} , $AUC_{0-\infty}$ and C_{\max} of the two products did not differ significantly, suggesting that the plasma profiles generated by Salurin are comparable to those produced by Lasix. Analysis of variance (ANOVA) for these parameters, after log-transformation of the data, showed no statistically significant difference between the two formulations, with p values greater than 0.05. 90% confidence intervals also demonstrated that the ratios of AUC_{0-t} , $AUC_{0-\infty}$, and C_{\max} of the two

Table 2. Statistical analysis of log-transformed data

Statistical analysis	AUC_{0-t}	$AUC_{0-\infty}$	C_{\max}
ANOVA GLM (p -value)	0.791 (0.943)	0.774 (0.985)	0.501 (0.767)
90% CI	90.71%–114.43% (86.15%–114.92%)	91.50%–113.46% (89.0%–111.81%)	93.90%–116.14% (88.0%–109.41%)

Parenthesis values indicate analysis for periods.

formulations lie within the FDA acceptable range of 80%–125% [17].

For T_{\max} the parametric point estimate of difference (test – reference) was 0.11 h, which is within the acceptance limits ($\pm 20\%$ of reference mean).

Conclusion

Statistical comparison of AUC_{0-t} , $AUC_{0-\infty}$ and C_{\max} clearly indicated no significant difference in the two brands of furosemide 40 mg tablets. 90% confidence intervals for the mean ratio (T/R) of AUC_{0-t} , $AUC_{0-\infty}$ and C_{\max} indicated that the reported values were entirely within the bioequivalence acceptance range of 80%–125% (using log-transformed data). Based on the pharmacokinetic and statistical results of this study, it is concluded that Salurin 40 mg tablets (Gulf Pharmaceutical Industries, U.A.E.) is bioequivalent to Lasix 40 mg tablets (Hoechst AG, Germany), and that the two products can be considered interchangeable in medical practice.

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