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Pharmacokinetics and Bioequivalence Study of Clobazam 10 mg Tablet

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Abstract: The bioequivalence of two brands of clobazam 10 mg tablets was demonstrated in this study. A single dose was carried out in 14 healthy volunteers with a two-sequence, crossover block-randomized design. Blood samples were taken prior to each administration and at 18 points within 72 h after the dose administration. Plasma concentrations of clobazam and N-desmethyloclobazam were determined by HPLC method. The pharmacokinetic parameters, C_{max} and T_{max} were obtained directly from plasma concentration-time profiles. k_e was estimated by log-linear regression and AUC was calculated by the linear trapezoidal rule for both clobazam and N-desmethyloclobazam. The pharmacokinetic parameters, AUC(0-t), AUC(0-∞) and C_{max} were tested for equivalence after logtransformation of data. Differences of T_{max} were evaluated by a non-parametric test. The 90% confidence intervals of the mean values for the test/reference ratios were 94-103% for AUC(0-t), 90-110% for AUC(0-∞) and 87-109% for C_{max} , which were within the acceptable bioequivalence limits of 80-125% for clobazam. Based on desmethyloclobazam data, the 90% confidence interval for AUC(0-t) and C_{max} were calculated to be 82-106% and 81-117% respectively. Therefore two formulations were considered bioequivalent.

Key words: Clobazam, N-desmethyloclobazam, bioequivalence, pharmacokinetics, HPLC

INTRODUCTION

Clobazam is a 1, 5-benzodiazepine with anxiolytic and anticonvulsant properties and is used as a second drug in the treatment of various forms of epilepsy. In other application, clobazam is used as a covering drug when there is a change in therapy (Brogden *et al.*, 1980; Ashto, 1994). Clobazam is rapidly and extensively absorbed following oral administration, with bioavailability close to 87%. Concomitant administration with alcohol increases its bioavailability by 50%. Food may slow the rate of absorption but dose not alter its extent and it is not influenced by age or sex. Clobazam binds in approximately large amount with plasma proteins (85%). Peak plasma concentration occurs 1 to 4 h after ingestion (Brogden *et al.*, 1980). The two most important chemical changes that clobazam undergoes during metabolism are dealkylation and hydroxylation and the main metabolites being N-desmethyloclobazam, 4-hydroxyclobazam and 4-hydroxy-N-desmethyloclobazam (Guberman *et al.*, 1990). Among all these metabolites, only N-desmethyloclobazam is pharmacologically active showing pharmacological

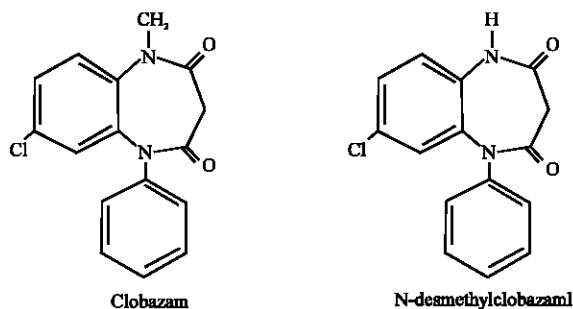


Fig. 1: Chemical structures of clobazam and N-desmethyloclobazam

profile similar to the parent drug (Fig. 1) (Brogden *et al.*, 1980). While the half life of clobazam has been reported to be about 48 hr, N-desmethyloclobazam possesses a longer elimination half-life of about 77 h (Brogden *et al.*, 1980). Moreover, N-desmethyloclobazam is accumulated during long-term treatment achieving concentration levels up to 10-times greater than clobazam and therefore it may be an important factor in both therapeutic and toxic responses.

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It is excreted mainly in urine (81-97%) and accumulation is expected in impaired renal function (Guberman *et al.*, 1990; Bun *et al.*, 1990).

The aim of the present study was to compare the bioequivalence of two different oral formulations (tablets) of clobazam for both clobazam and its major metabolite, N-desmethyloclobazam in a single dose, two-sequence and crossover randomized study in 14 healthy volunteers.

MATERIALS AND METHODS

***In vitro* dissolution test:** The *in-vitro* dissolution tests were carried out for both formulations according to the British Pharmacopoeia (BP) (BP, 2003), using a paddle apparatus method at 75 rpm in 500 mL hydrochloric acid (0.1 N) at 37.5°C. The samples were collected at 5, 10, 15, 20, 30 and 45 min after starting the dissolution test. The cumulative amount of clobazam dissolved, measured with HPLC method according to the monograph of clobazam in BP.

Participants: Fourteen healthy Iranian volunteers of both sexes were selected for this study. All participants were non-smoking, aged between 22 and 48 years (mean±SD, 26.7±8.1), weighing between 63 and 97 kg (79.5±9.1). The mean demographic data for subjects is shown in Table 1. The volunteers did not have any significant diseases, as determined by their medical history, physical examination and routine laboratory tests and they were negative for hepatitis B antigen. Subjects were not allowed to take any other medication for 2 weeks before and throughout the study and they were informed about the aim and risks of the study by the clinical investigator, based on a written informed consent. The protocol was approved by the Ethics Committee of Tehran University of Medical Sciences.

Drug administration and sample collection: The study was designed as a single dose, randomized, two-treatment and two-period cross over. Volunteers were not allowed to take any other medication for 2 weeks before and throughout the study. After an overnight fasting (10 h), the subjects received a 10 mg clobazam tablet as test formulation (formulated by Dr Abidi Pharmaceutical Co. Tehran, Iran, Batch No. CLB-T-01-10-81) or a 10 mg Frisium tablet as reference product (Aventis, UK, Batch No: D675) with 250 mL of water. The intake of food was delayed for 3 h after medication and all of the participants were continuously monitored throughout the period of study and clinical adverse events were recorded during that time.

Table 1: Mean demographic data for subjects (n=14)

	Age (year)	Weight (kg)	Height (cm)
Mean	29.7	79.5	177.30
SD	8.1	9.1	7.60
Min	22.0	63.0	167.00
Max	48.0	97.0	190.00

Approximately 3 mL of peripheral venous blood samples were collected in heparinized tubes, immediately before (0 h) and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.5, 8.0, 10.0, 24.0, 48.0 and 72.0 h after drug administration. After blood separation, plasma was immediately frozen at -20°C until drug analysis.

After a washout period of 14 days the study was repeated in the same manner to complete the cross over design.

Analysis of clobazam and N-desmethyloclobazam

Sample preparation: The preparation of plasma samples was by Liquid-liquid Extraction (LLE). The conditions consisted of mixing 0.5 mL of plasma with 20 µL alprazolam as internal standard (4 µg mL⁻¹) in a 2 mL Eppendorf polypropylene tube and then extracting with 1.5 mL of toluene. After vertical agitation (1 min) and centrifugation (10,000×g, 1 min), the upper organic layer was transferred into a conical tube and evaporated under a gentle stream of air. The dried extract was reconstituted in 150 µL of a mobile phase and a 100 µL aliquot was injected on to the HPLC system.

Apparatus and chromatographic condition: The chromatographic apparatus consisted of a low-pressure gradient HPLC pump, a UV variable-wavelength detector and an online degasser, all from Knauer (Berlin, Germany). A Rheodyne model 7725i injector with a 100 µL loop was used. The data was acquired and processed by means of ChromGate chromatography software (Knauer, Berlin, Germany). Chromatographic separation was achieved by a Chromolith™ Performance RP-18e 100×4.6mm column (Merck, Darmstadt, Germany) protected by a Chromolith™ Guard Cartridge RP-18e 5×4.6 mm. A mobile phase of phosphate buffer (pH 3.5; 10 mM)-acetonitrile (70:30, v/v), was delivered in isocratic mode at 2 mL min⁻¹. The eluents were monitored at wave-length of 228 nm (Rouini *et al.*, 2005).

Pharmacokinetic analysis: Plasma concentration-time curves of clobazam and its metabolite were evaluated by non-compartmental analysis. Maximum plasma concentration C_{max} and the time to C_{max} (T_{max}) were obtained directly from the individual plasma concentrations. The terminal half-life, ke was obtained from log-linear regression analysis of the plasma concentration time curves in the terminal phase. The area

under plasma concentration-time curve up to last quantifiable plasma concentration AUC(0-t) was determined according to the linear trapezoidal method and the AUC(0-∞) was calculated as AUC(0-t) + C_{last}/k_e, with C_{last} being the last measured concentration and k_e, the slope obtained from least-square regression of the terminal elimination phase. Clobazam half-life was calculated as Ln2/k_e.

Statistical analysis: For the purpose of bioequivalence analysis, AUC and C_{max} of clobazam were considered as the primary variables. Bioequivalence was assessed by means of an analysis of the variance (ANOVA). The difference between two related parameters was considered to be statistically significant for a p value of less than 0.05. Parametric 90% confidence intervals based on the ANOVA of the mean test/reference (T/R) ratios of AUCs and C_{max} were computed.

RESULTS AND DISCUSSION

The cumulative amount (%) of clobazam dissolved *in-vitro* from both formulations is plotted as a function of time in Fig. 2. Both formulations met the BP requirements with not less than 75% of the labeled amount of clobazam dissolved within 30 min (Fig. 2). Earlier described analytical method was proven to be sensitive and accurate for the determination of clobazam and its metabolite in plasma (Rouini *et al.*, 2005). In this study, following administration of a 10 mg single dose of clobazam, no serious or unexpected adverse events occurred and all volunteers were healthy and could continue the study. Plot of clobazam and N-desmethyloclobazam mean plasma concentrations as a function of time, is shown in Fig. 3.

According to this plot, both formulations were readily absorbed from the gastrointestinal tract and

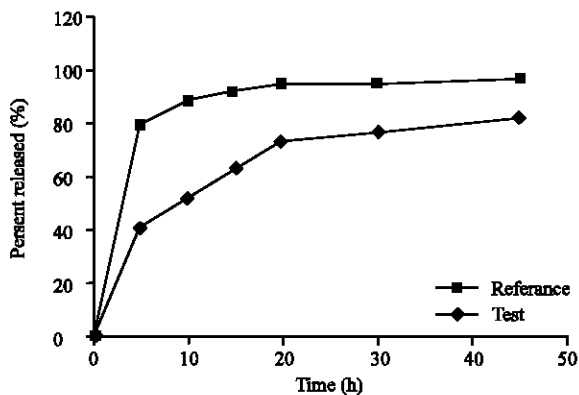


Fig. 2: *In vitro* dissolution profile for both formulations

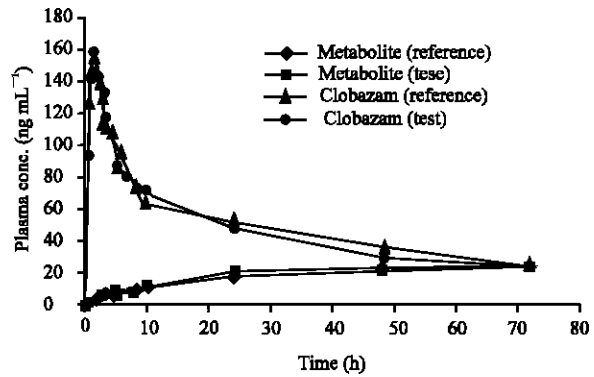


Fig. 3: Mean plasma concentration of clobazam and N-desmethyloclobazam after administration of 10 gm single oral dose of two brands to 14 healthy human volunteers

clobazam was measurable at the first sampling time (0.5 h) in almost all of the volunteers, while its metabolite was detectable after 1.5 h in most of the volunteers.

The plot indicates that the mean plasma concentration profiles of the two formulations were closely similar and super imposable on the basis of parent drug as well as active metabolite.

Peak plasma concentrations of 173.0±33.4 ng mL⁻¹ and 184.2±46.6 ng mL⁻¹ for clobazam were attained at 1.4 and 1.5 h for test and reference formulation, respectively. Clobazam was detectable in volunteers' plasma up to 72 h after drug administration of both formulations. Peak concentrations of 24.9±7.9 and 27.2±4.6 ng mL⁻¹ for metabolite were attained at 58.0 and 55.2 h, for test and reference formulation, respectively. The decline in metabolite plasma concentration was more slowly than parent drug such that the elimination phase could not be achieved until 72 h after drug administration.

There was also no significant difference in the bioavailability of the two products based on the statistical analysis. Pharmacokinetic parameters for clobazam and N-desmethyloclobazamL from the 14 participants are shown in Table 2.

The relative bioavailability of clobazam on the basis of the parent drug was 96.4% for AUC(0-t), 96.4% for AUC(0-∞) and 93.9% for C_{max}. These values were 94.1% for AUC(0-t) and 91.5% for C_{max} on the base of active metabolite, respectively. As no concentration was available at metabolite elimination phase, we could not calculate k_e, t_{1/2} and AUC(0-∞) as well.

Safety and efficacy of a generic formulation is the most important object in a bioequivalence study. Two formulations of the same drug are equivalent, when the rate and extent, to which the active drug becomes available in the site of action, are the same and thus they

Table 2: Pharmacokinetic parameters of clobazam and N-desmethylclobazam for two formulations (mean±standard deviation, n = 14)

Pharmacokinetic parameters	Test formulation		Reference formulation	
	Clobazam	Metabolite	Clobazam	Metabolite
AUC (0-t) (ng mL ⁻¹ h)	3420.7±984.9	1419.3±423.6	3564.9±1011.8	1532.1±372.9
AUC (0-∞) (ng mL ⁻¹ h)	5056.9±2252.5	NC	5244.0±2407.4	NC
C _{max} (ng mL ⁻¹)	173±33.4	24.9±7.9	184.2±46.6	27.21±4.6
T _{max} (h)	1.4±0.5	55.2±16.2	1.3±0.5	55.2±19.8
T1/2 (h)	48.7±12.5	NC	46.0±6.3	NC
Ke (h ⁻¹)	0.015±0.004	NC	0.015±0.002	NC

NC: Not Calculated

Table 3: Statistical analysis of Log-transformed data of clobazam and N-desmethylclobazam

Statistical analysis	AUC(0-t) (ng mL ⁻¹ h)		AUC(0-∞) (ng mL ⁻¹ h)		C _{max} (ng mL ⁻¹)	
	Clobazam	Metabolite	Clobazam	Metabolite	Clobazam	Metabolite
ANOVA (p-value)	0.3	0.4	0.6	NC	0.4	0.4
CI 90% Lower	94	82	90	NC	87	81
CI 90% Upper	103	106	110	NC	109	117

NC: Not Calculated

also considered to be therapeutically equivalent (Chow and Liu, 1992). To demonstrate bioequivalence, certain limits should be set depending on the nature of the drug, volunteers and clinical end points. It is generally accepted that for basic pharmacokinetic characteristics, such as AUC and C_{max}, the standard equivalence range is 0.8-1.25 (Shah *et al.*, 1991; FDA Guidelines, 1992). The results of statistical analysis are shown in Table 3. For parent drug and active metabolite mean and standard deviation of AUC(0-t), AUC(0-∞) and C_{max} of the two products did not differ significantly, suggesting that the plasma concentration-time profiles of test formulation are comparable to those of reference formulation. 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. The 90% confidence intervals also demonstrated that the ratios of AUC or C_{max} of the two formulations lie within the FDA acceptable range of 80-125%. For clobazam, the absolute difference in T_{max} (test-reference) was 0.06 h, within the acceptance limits, while for the metabolite there was no difference for two formulations.

CONCLUSIONS

Based on the statistical analysis (ANOVA and 90% CI) for AUC(0-t), AUC(0-∞) and C_{max} it is concluded that clobazam 10 mg tablet, manufactured by Abidi Pharmaceutical Co., is bioequivalent to Frisium,

manufactured by Aventis and both products can be considered effective and safe in medical practice.

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