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Research Article

BIOEQUIVALENCE AND PHARMACOKINETIC STUDY OF FORMULATIONS OF CLOBAZAM TABLETS IN HEALTHY VOLUNTEERS

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ABSTRACT

The bioavailability of Clobazam with Frisium tablet formulations (ovation Pharmaceuticals Inc, U.S.A) was compared in 12 healthy male volunteers who received a single dose of 10 mg of the test (T) and the reference (R) products in a randomized balanced 2- way crossover design. Plasma samples were obtained over a 16 h interval and Clobazam concentrations determined by HPLC with ultraviolet detection. The maximum plasma concentration (Cmax), area under the plasma concentration time curve up to the last measurable concentration (AUC0-t), as well as infinity (AUC0-∞), and the absorption rate (Cmax/AUC0-∞) were analyzed statistically under the assumption of a multiplicative model. The time to maximum concentration (Tmax) was analyzed assuming an additive model. The parametric confidence intervals (90%) of the mean values of the pharmacokinetic characteristics for T/R ratio were in each case well within the bioequivalence acceptable range of 80-125%. Therefore, the formulation was considered to be equivalent.

KEYWORDS: Clobazam, AUC, RP-HPLC, Bioequivalence, Frisium, Bioavailability.

INTRODUCTION

Clobazam (CLB) is a 1, 5-benzodiazepine (fig1) with anxiolytic and anticonvulsant properties and is used for sedation and as an antiepileptic drug, presenting some advantages over 1,4-benzodiazepines [1]. In other applications Clobazam is used as a covering drug when there is a change in therapy. The drug's action is very quick usually effective within a couple of hours but no longer than a few days [2] and the monitoring of the drug's haematic levels is of great clinical interest in order to determine its correct use. Clobazam (CLB) has been administered to patients before cardiopulmonary bypass surgery [3] and we were interested in the effect of such a procedure on CLB pharmacokinetics. Thus a sensitive method for the determined CLB in plasma was necessary. Several techniques have been reported For CLB quantification, using gas chromatography (GC) [4-6] and High-performance liquid chromatography (HPLC) [7-9]. These techniques have been reviewed. HPLC methods for CLB and other benzodiazepines can be sensitive determination in urine has been reported. This paper presents a simple and sensitive procedure for CLB determination in rat plasma suitable for therapeutic drug monitoring, pharmacokinetic and bioavailability studies as well as for toxicological purposes. . The objective of this study was to compare bioavailability of a new commercial Clobazam tablet formulation (Sanofy-aventis, newzealand) Ltd relative to the reference formulation of Frisium (Ovation pharmaceuticals, inc, U.S.A) following a single dose administration to healthy adult male volunteers.

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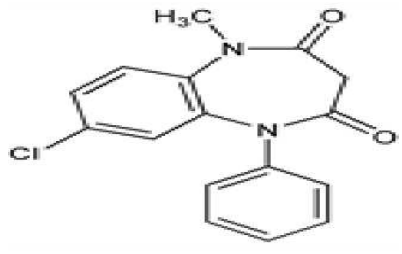

Fig.1. CLOBAZAM

EXPERIMENTAL

In vitro analysis

The Clobazam tablets were found to be similar in weight variation, disintegration time, dissolution, and assay as stipulated by the USP XXIII, as well as by the manufacturer.

SUBJECTS

Twelve healthy male adult volunteers participated in this study. Their mean age (±SD) was 35.5±6.7 years with a range of 18-45 years. (Appendix-II, Height-Weight chart for non-Medical cases for men and women, Life insurance Corporation of India). The volunteers were free from significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal and any acute or chronic disease or drug allergy as determined from their medical history, clinical examination, and laboratory investigation (hematology, blood chemistry, and urine analysis). The volunteers were asked to abstain from taking any medicine, including OTC drugs, for at least two weeks prior to and during the study. All subjects gave their written informed consent prior to participation in the study and after ex-planning the nature and purpose of this study.

STUDY DESIGN AND BLOOD SAMPLING

Administration of the two products (test and reference) to the subjects was carried out by means of a two-way crossover design with a one-week washout period. Subjects were randomly divided into two equal groups and assigned to one of the two sequences of administration. Each subject received a single dose of 10 mg tablet of either brand with 240 ml of water after overnight fasting for at least 10 h. Subjects were allowed to eat a standard breakfast at 4 h, lunch at 8 h, and dinner at 12 h after drug administration. Beverages and food containing caffeine were not permitted over the entire course of the study. Volunteers were ambulatory during the study, but strenuous activity was prohibited. Blood samples (7 ml) from an antecubital vein were collected into citrate containing evacuated glass tubes before and at 0.33, 0.67, 1.33, 1.67, 2, 3, 4, 5, 6, 8, 10, 12, 14 and 16 hours post dosing. The plasma was then separated after centrifugation and stored frozen at -20°C until quantitative analysis.

QUANTITATIVE DRUG ANALYSIS

In each period, 17 (1x5 ml) blood samples will be collected. The pre-dose blood sample (1x5 ml) will be collected within 1 hour prior to dosing. The post-dose blood samples (1x5ml each) will be collected at 0.25, 0.50, 075, 1.0, 1.50, 2.0, 2.50, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.00, 48.00 and 72.00 hours after dosing. 48.0 & 72.0 hours sample time pints should be collected on ambulatory basis. The pre-dose and post dose samples will be collected by indwelling cannula placed on the forearm vein. The pre-dose blood sample will be collected within a period of one hour before dosing and post dose samples will generally collected within 2 minutes of the schedule time. The actual mid-night time of collection of each blood sample (to the nearest time) will be recorded on the appropriate data sheet. For each subject the total number of blood samples drawn during the study will be 34 and the total volume of blood drawn including 15-ml for screening and 17-ml to be discarded prior to venous cannula collection will not exceed 202-ml for the entire study.

The blood samples will be collected in K3 EDTA vacutainers. The blood samples will be centrifuged at 3000 RPM at $10+2^0$C for 10 minutes. The plasma will be separated and transferred to labeled storage vials and frozen at -20^0C till the last sample is collected and later transferred to deep freezer at $-75+5^0$C until analysis.

RESULTS AND DISCUSSION

The test formulation of clobazam 10 mg compared with reference product (Frisium 10mg) of ovation Pharmaceuticals Inc U.S.A. The test formulation showed a Tmax (hr) of for Clobazam 1.813 $\pm$ 1.045 as compared to 1.833 $\pm$ 1.905 of reference formulation. The least square mean ratios for Cmax, AUCo- t and AUCo-a for Clobazam were 98,31,101.11 and 101.8 for untransformed data and 97.14, 101,62 and 101.54 for log-transformed data respectively indicating a comparable Bioequivalence of test formulation to the reference formulation. Their 90% confidence intervals for Clobazam were 86.93-108.55, 93.71-110.18 and 93.69-110.04 respectively for log-transformed data. The 90% confidence intervals Cmax, AUCo- t and AUCo-a were within the bioequivalence acceptance range of 80-1254 for the log-transformed data. As per the above results test product Clobazam 10 mg Tablets with batch No.515-08-6 Manufactured by sanofi-aventis, Newzealand Limited, was Bioequivalent with Frisium 10mg of ovation Pharmaceuticals Inc.U.S.A. This study was carried with Twelve (+2 Standby) healthy, adult, male, human subjects under fasting conditions.

CONCLUSION

Assessment of bioequivalence of local product to reference product is required to exclude any clinically important differences in the rate or extent at which the active entity of the drugs becomes available at the site of action. The drugs are considered to be bioequivalent if they are pharmaceutically equivalent and their bioavailability is so similar that they are unlikely to produce clinically relevant differences in regard to safety and efficacy. The aim of this study was to compare the bioavailability and Bioequivalence of the formulations of Clopazam 10-mg tablets, a locally manufactured (test) formulation, Frisium, and a reference formulation. From log-transformed data, and all values are within the bioequivalence accepted range of 80%-125%.Moreover, a further evaluation of the rate of absorption was performed by analyzing the Cmax/AUC0.since this parameter has been proposed to better reflect the absorption rate. The 90% confidence intervals for this parameter also indicated bioequivalence. In conclusion, the two formulations can be considered bioequivalent in regard to the extent and rate of absorption and therefore interchangeable.

Figure: 1.Mean Plasma Concentrations of Clobazam Vs Time Profile For 12 Subjects

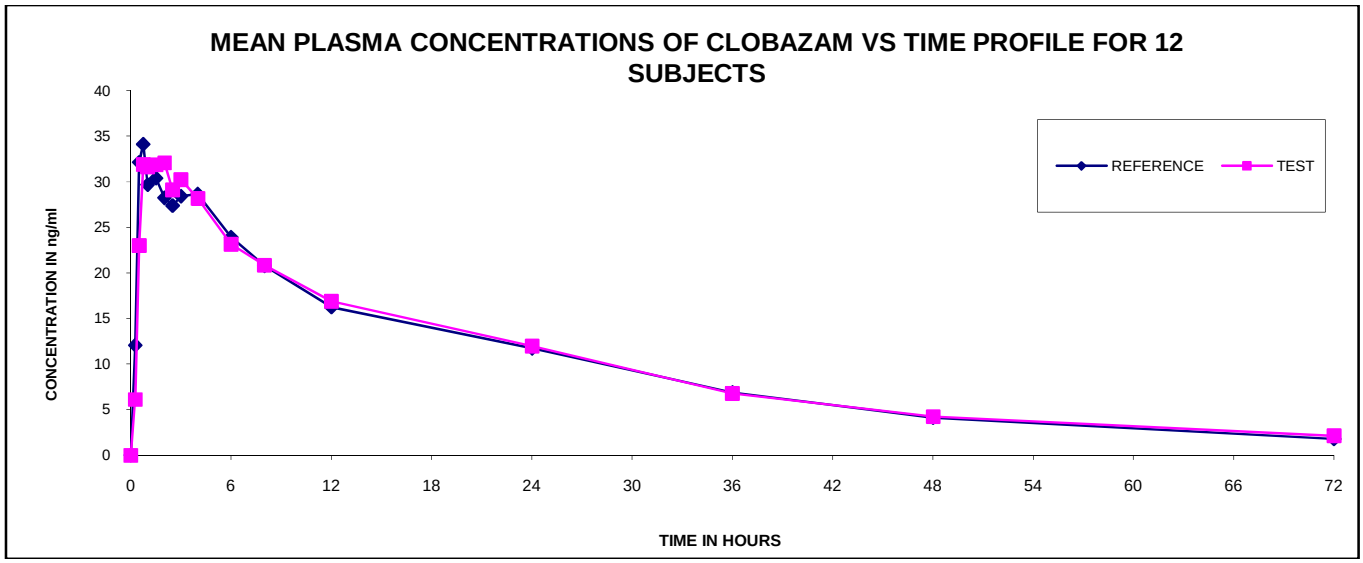

Table: 1 SUMMARY STATISTICS OF UNTRANSFORMED PHARMACOKINETIC PARAMETERS FOR CLOBAZAM IN 12 HEALTHY MALE SUBJECTS

PRODUCTS / STATISTICS	Cmax (ng/ml)	AUC o→t (ng.h/ml)	AUC o→∞ (ng.h/ml)	Tmax (h)
Test Product (T)				
Arithmetic Mean	37.572	704.225	751.798	1.813
SD	11.594	214.636	232.833	1.045
CV%	30.86	30.48	30.97	57.66
N	12	12	12	12
Ref Product (R)				
Arithmetic Mean	38.218	696.503	743.733	1.833
SD	9.745	221.545	240.039	1.905
CV%	25.50	31.81	32.27	103.91
N	12	12	12	12
Least Square Mean				
T	37.572	704.225	751.798	1.813
R	38.218	696.503	743.733	1.833

PRODUCTS / STATISTICS	Cmax (ng/ml)	AUC o→t (ng.h/ml)	AUC o→∞ (ng.h/ml)	Tmax (h)
Least Square Mean Ratio T/R (%)	98.31	101.11	101.08	---
90% Confidence Interval T vs R Lower Limit Upper Limit	88.17 108.45	91.41 110.81	91.73 110.44	--- ---
p-value (ANOVA) Sequence Sub(Seq.) Form Period	0.398 0.000 0.769 0.932	0.827 0.000 0.840 0.037	0.873 0.000 0.838 0.022	--- --- --- ---
Power	0.946	0.958	0.967	---

Table:2. Comparison of Tmax (hr) for Products 'T' and 'R'

SUBJECT NO	FORM	
	TEST	REFERENCE
Mean	1.81	1.833
SD (+/-)	1.045	1.905
CV %	57.66	103.91
Maximum	4.0	[6.00
Minimum	0.75	0.50
N	12	12

Table:3 COMPARATIVE EVALUATION OF AUC o→t (ng.h/ml) FOR Products T & R

SUBJECT NO	UN-TRANSFORMED		LOG-TRANSFORMED	
	TEST	REFERENCE	TEST	REFERENCE
Mean	704.225	696.503	6.518	6.502
SD (+/-)	214.634	221.545	214.634	221.545
CV %	30.48	31.81	31.697	33.247
Maximum	1116.183	1098.455	7.018	7.002
Minimum	437.792	433.945	6.082	6.073
N	12	12	12	12

Table :4 Comparative Evaluation of AUC O→∞ (Ng.H/Ml) For Products T And R

SUBJECT NO	UN-TRANSFORMED		LOG-TRANSFORMED	
	TEST	REFERENCE	TEST	REFERENCE
Mean	751.798	743.733	6.580	6.565
SD (+/-)	232.833	240.039	232.833	240.039
CV %	30.97	32.27	32.311	33.824
Maximum	1176.146	1182.732	7.070	7.076
Minimum	455.408	445.753	6.121	6.100
N	12	12	12	12

Table :5 COMPARISON OF Kel (1/h) FOR PRODUCTS 'T' AND 'R'

SUBJECT NO	FORM	
	TEST	REFERENCE
Mean	0.0458	0.0446
SD (+/-)	0.0162	0.0103
CV %	35.40	23.03
Maximum	0.0864	0.0685
Minimum	0.0279	0.0312
N	12	12

Table – 6:Comparison of t1/2 (hr) for products 't' and 'r'

SUBJECT NO	FORM	
	TEST	REFERENCE
Mean	16.583	16.271
SD (+/-)	4.849	3.542
CV %	29.24	21.77
Maximum	24.823	22.182
Minimum	8.020	10.124
N	12	12

Table :7 Percentage Ratio Of AUCo→T /AUCo→∞ For Clonazepam

SUBJECT NO	FORM	
	TEST	REFERENCE
Mean	94.09	94.04
SD (+/-)	4.94	5.21
CV %	5.25	5.54
Maximum	99.9	99.42
Minimum	81.34	80.38
N	12	12

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