

BIOEQUIVALENCE OF TWO TABLET FORMULATIONS OF CARBAMAZEPINE

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REZUMAT

Bioechivalența a două formulări de carbamazepină

Scopul studiului de bioechivalență a fost compararea a două formulări cu carbamazepina sub formă de comprimate (200 mg): Finlepsin (Asta Medica AG, formularea de testat) și Tegretol (Novartis, formularea de referință). Studiul s-a desfășurat pe 12 voluntari sănătoși care au primit o doză unică pe cale orală din cele două produse, pe stomacul gol, într-un studiu randomizat, încrucișat, cu două perioade, cu două tratamente. Concentrațiile plasmatice ale carbamazepinei și ale metabolitului său activ, carbamazepin 10, 11-epoxidul s-au determinat până la 120 ore de la administrare. Determinarea cantitativă a carbamazepinei și a metabolitului s-a făcut cu o metodă validată HPLC. Forma farmaceutică testată s-a considerat bioechivalentă cu cea de referință în privința mărimii absorbției dacă intervalul de încredere 90% al raportului valorilor medii logaritmice al ariei de sub curbă și al concentrației maxime a celor două produse au fost în domeniul de bioechivalență de 0,80 - 1,25 (testul t dublu unilateral Schuirman) precum și referitor la viteza de absorbție evaluată din diferența valorilor medii ale timpului concentrațiilor maxime ca fiind nesemnificativă, presupunând un model aditiv (testul nonparametric Friedman). Comprimatele cu carbamazepina ale formulării testate s-au dovedit bioechivalente cu cele ale produsului de referință în privința mărimii și vitezei de absorbție după administrarea unei doze unice pe cale orală.

Cuvinte cheie: carbamazepină, farmacocinetică, biodisponibilitate, bioechivalență.

ABSTRACT

The purpose of the present bioequivalence study was to compare two different formulations of carbamazepine tablet (200 mg): Finlepsin (Asta Medica AG, test formulation) and Tegretol (Novartis, reference formulation). The study was carried out on 12 healthy male volunteers who received a single dose of 200 mg of the test and reference products in the fasting state, in a randomized, two period, two treatment, two sequence crossover study. Plasma concentration time profiles of carbamazepine and its active metabolite carbamazepin-10, 11-epoxide were determined until 120 hours after administration. The quantitative drug and metabolite analysis was done with a validated HPLC method. Tested dosage form was considered bioequivalent to reference dosage form with regard to the extent of absorption, if the 90% confidence intervals of the area under the curve ratio and of the maximum concentration ratio (log mean values) were within the range of 0.80-1.25 (Schuirman's two one sided t test) and with regard to rate of absorption if the difference of the mean values of the time of maximum concentration was not significant assuming an additive model (Friedman nonparametric test). Carbamazepine test tablets were bioequivalent to reference with regard to extent and rate of absorption after single dose administration.

Key words: carbamazepine, pharmacokinetics, bioavailability, bioequivalence

Introduction

Carbamazepine (CBZ) is a dibenzazepine derivative structurally related to the tricyclic antidepressants, which has been used in the treatment of epileptic seizures. Following administration of conventional tablets it is slowly but completely absorbed, with peak plasma concentrations being reached within 2-8 hours. The drug is about 70-80% bound to plasma proteins. CBZ undergoes an intense hepatic metabolism to form at least 7 metabolites. One pharmacodynamically active metabolite, CBZ-10, 11-epoxide (epoxiCBZ) contributes to the anticonvulsant activity. The half-life of CBZ varies between 8-72 hours (average 36 hours). This large variability of the elimination is partially caused by the enzyme inducing properties of CBZ, which also accelerates its own metabolism. The active and safe plasma concentrations are between 4-14 mg/ml (1,4,9,13).

The following study in healthy volunteers was designed

to determine extent and rate of absorption of carbamazepine of a commercial test formulation Finlepsin (Asta Medica AG) relative to the reference formulation Tegretol (Novartis). Bioequivalence of the two drug products was assessed based on the plasma concentration data obtained following their administration to 12 healthy male volunteers in a balanced two-way crossover design.

Methods

Subjects

Twelve male healthy volunteers participated in the study. Their mean age (S.D.) was 22.5 (1.56) years, body weight of 75.6 (9.03) kg and height of 181.3 (6.27) cm. All volunteers were non smokers, HIV- and HbsAG- negative and free of drugs. No subject had a history or evidence of hepatic, renal,

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gastrointestinal or hematological deviations or any acute or chronic diseases or drug allergy (on the basis of medical history, clinical examination and laboratory investigation). Informed consent was obtained from the subjects after explaining the nature and purpose of the study. University Ethical Committee approved the study protocol.

Standard in vitro tests of carbamazepine tablets

Both the test formulation (Finlepsin tablets, 200 mg, lot No. 9009, expiry date 09.2003 and the reference formulation (Tegretol, tablets, 200 mg, lot No. 229600, expiry date 01.2004) were examined for conformation to compendial standards and manufacturer specifications for weight variation, disintegration, dissolution and assay. Both products met the specifications stipulated by the USP 23 as well as by the manufacturer.

Study design

The study design was a single dose, two treatments, two period, and two-sequence crossover with a one-week washout period between treatments. Each subject received a single dose of 200-mg carbamazepine of either brand with 150 ml of water after an overnight fast for at least 10 hours. Subjects were allowed to eat a standard meal at 4 h, lunch at 8 h and dinner at 12 h after drug administration. Multiple blood samples were before and at 0.5 - 1 - 1.5 - 2 - 3 - 4 - 6 - 8 - 12 - 24 - 48 - 72 - 96 - 120 hours after each drug administration. The blood samples were obtained at the level of the forearm via direct veinipuncture. Each sample (5 ml) was collected into heparin tubes correctly labeled. The samples were centrifuged at 4°C, 1500 rpm for 10 minutes immediately after collection. Afterwards, the plasma was collected into two dry tubes, frozen, and stored at -20°C.

Analysis of plasma samples

The concentrations of carbamazepine and carbamazepine -10,11 -epoxide in plasma were measured by modification of published HPLC methods (5-7). Chromatographic system: HP 1100 Series binary pump; HP 1100 Series autosampler; U.V. HP 1100 Series detector with variable wavelength. Chromatographic conditions: column: Chromosphere C18 250mm x 4.6 mm (5 mm); mobile phase methanol: KH_2PO_4 0.01M 65 : 35 ; flow rate 1 ml/min; retention time: 5.469 min for carbamazepine and 3.737 for carbamazepineepoxid; detection at 256 nm.

Standard samples were prepared in blank plasma at 0.02-0.05-0.1-0.25-0.5-0.75-1-2.5-5-7.5-10-15-20 mg/ml. The standard curves for the analytes in plasma were linear ($r > 0.995$). Linearity was obtained by analyzing blank plasma samples with CBZ and CBZ-E added at the concentration 0.02-0.05-0.1-0.25-0.5-0.75-1-2.5-5-7.5-10-15 and 20 mg/ml. The curves were linear at plasma concentration of 0.02-20 mg/ml for CBZ and CBZ-E ($r = 0.9993$ and 0.9978). Aliquots of serum (100 ml) were treated with 250 ml methanol for deproteinisation. After centrifugation at 4000 rpm for 6 min the organic phase was injected. The limit of quantitation of carbamazepine in plasma was 0.02 mg/ml for CBZ and epoxy-CBZ.

Intrarun coefficients of variation ranged from 0.27-13% and interrune CVs ranged from 2.73-17.3% for carbamazepine and intrarun CVs from 6.8-13.3 and interrune CVs from 2.2-

18.1 for epoxy-carbamazepine. The recoveries ranged from 97.1-119.1 and 95.3 - 109.7 for carbamazepine and from 96.3-109.6 and from 95.2 - 107.2 for epoxy-carbamazepine.

Pharmacokinetic analysis

The Pharmacokinetics characteristics for carbamazepine were determined from the plasma concentration-time data. The maximum plasma concentrations (Max) and time to reach maximum plasma concentrations (Max) were obtained directly by inspection of the individual drug plasma concentration-time data. The area under the plasma concentration-time curve (AUCt) up to the last time showing a measurable concentration (Ct) was determined by using the linear trapezoidal rule (3,6). A computer program (PharmK) calculated the apparent elimination rate constant Kel. The AUCi from zero to infinite values (express the magnitude of absorption) were determined by adding the quotient of Ct and Kel to the corresponding AUCt. The apparent elimination half-life (T_{1/2}) of carbamazepine in plasma was calculated by using the equation: $T_{1/2} = \ln 2 / \text{Kel}$. The sampling period covered more than 90% of total area under the curves for both brands.

Statistical analysis

The two-way analysis of variance (ANOVA) for crossover design was used to assess the effect of formulations, periods, sequences and subjects within sequence on logarithmically transformed data of AUCt, AUCi, and Cmax. The ANOVA for T_{max} was carried out on the untransformed data. Parametric 90% confidence intervals for the characteristic T_{max} was performed under the assumption of additive model and the equivalence range was expressed in absolute differences of the mean testreference. Nonparametric confidence interval was also performed (Friedman). In addition bioequivalence between the two formulations was also assessed by Schuirmann's two one sided t tests (8, 10-12, 14-16). All analyses of the data were performed with the statistical software package PharmStat (Simed, France).

Results and discussion

Table I shows the results of the mean plasma concentrations of carbamazepine following oral administration of the two brands to 12 healthy volunteers.

In **fig. 1** are shown the mean values of of plasma concentrations of carbamazepine following the administration of single oral doses of 200 mg carbamazepine as Finlepsin (Asta Medica) and Tegretol (Novartis) tablets to 12 volunteers

Table I The mean values of plasma concentrations of carbamazepine following the administration of single oral doses of 200 mg carbamazepine as Finlepsin (Asta Medica) and Tegretol (Novartis) tablets to 12 volunteers.

Time (hours)	Finlepsin (µg/ml)	± S.D.	Tegretol (µg/ml)	± S.D.
1	1.7459	0.9315	1.3167	0.9757
1.5	2.1267	0.9067	1.9300	0.3243
2	3.4608	1.2911	2.3300	1.2673
3	3.4608	1.0666	2.6816	0.9866
4	4.2250	0.9424	3.4450	0.9384
6	4.1750	0.8987	3.7025	0.4571
8	3.3608	0.7863	3.3833	1.1074
12	3.5767	1.4986	2.7200	0.7948
24	3.1917	0.8604	2.9425	0.8855
48	2.3433	0.9045	2.1233	0.6898
72	1.5450	0.4775	1.4467	0.4963
96	1.0150	0.4775	1.0067	0.3442
120	0.7042	0.1944	0.6275	0.4414

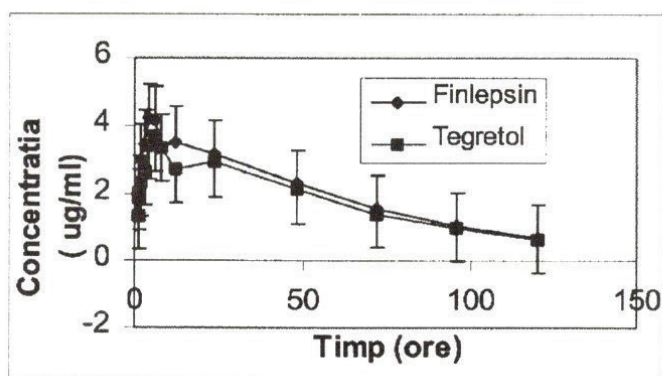


Fig. 1. Mean values of plasma concentrations of carbamazepine following the administration of single oral doses of 200 mg carbamazepine as Finlepsin (Asta Medica) and Tegretol (Novartis) tablets to 12 volunteers

As it is observable, the mean values of plasma concentrations of both products are similar.

Standard bioequivalence analysis for the extent and rate characteristics of carbamazepine from test product Finlepsin (Asta Medica) vs. reference product Tegretol (Novartis) is presented in **table II**.

Table II shows the parametric 90% confidence intervals of the mean values of the AUC and C_{max} as well as the point estimates for test/reference ratio assuming multiplicative model. Nonparametric (Friedman) confidence interval for T_{max} is also presented.

The biological half-life of carbamazepine (t_{1/2}) was calculated from the plasma concentrations of carbamazepine following the administration of the two studied products Finlepsin and Tegretol as single 200-mg doses, and the results are shown in **table III**.

The parameters of both Finlepsin and Tegretol tablets are similar and consistent with reported values.

The relative bioavailability (F) of carbamazepine from test product versus reference product was F% = (ASC test/ASC reference) · 100 = (244/241.5) × 100 = 101.4%. The difference

is not clinically significant.

The confidence limits for the mean AUC_i and C_{max} indicated that these values are entirely within the bioequivalence acceptable range of 80-125%. With regard to the characteristic T_{max} untransformed data were used and the bioequivalence range was expressed in absolute difference instead of proportions. The difference between the two formulations is non-significant.

In conclusion, based on the pharmacokinetic and statistical results of this study, the bioequivalence of the test product and the reference product was demonstrated as the amount and rate of absorption, and we can assume interchangeability of both preparations in clinical practice.

The biologically active biotransformation product of carbamazepine is carbamazepine -10,11-epoxide (CBZ-E). It may represent 10-50% of the intact drug. CBZ-E is almost fully biotransformed to the biologically inactive product carbamazepine-10,11-dihydroxide. In **table IV** are shown the plasma carbamazepine-epoxide following single oral doses of 200-mg carbamazepine to 12 volunteers as the two brands studied.

The biological half-life of carbamazepine-epoxide was calculated by using its plasma concentrations following single oral dose administration of 200 mg carbamazepine as Finlepsin and Tegretol tablets to 12 volunteers (**table V**).

The pharmacokinetic parameters which were used for the statistical evaluation were: AUC, C_{max}, T_{max}.

It must be stressed that the epoxy-metabolite of carbamazepine do not reflect the absorption rate and amount of the parent drug which is governed by formulation, but essentially by the enzymatic characteristics of each subject.

Table VI shows the mean values of the pharmacokinetic parameters of carbamazepine-epoxide, for the two products, Finlepsin and Tegretol tablets.

It can be seen that AUC_t has a confidence interval, which not complained with the bioequivalence range. However, in the case of AUC_i the C.I. is in the bioequivalence range. The

Table II Summary of statistical analysis of single dose pharmacokinetic characteristics of carbamazepine following oral administration of 200 mg to 12 volunteers as Finlepsin (Asta Medica) and Tegretol (Novartis) tablets

Pharmacokinetic parameters	Tested product Finlepsin (Asta Medica) Media \pm D.S.	Reference product Tegretol (Novartis) Media \pm D.S.	Confidence interval 90%
AUCt ($\mu\text{g}\cdot\text{h}/\text{ml}$) (ln)	2.385(0.041)	2.377(0.076)	0.91:1.15
AUCi($\mu\text{g}\cdot\text{h}\cdot\text{ml}^{-1}$) (ln)	2.423(0.046)	2.409(0.070)	0.93:1.15
Cmax ($\mu\text{g}/\text{ml}$)	0.641(0.093)	0.599(0.065)	1.01:1.20
Tmax (ore)	5.75(1.712)	6.50(1.508)	20.91% (ANOVA, Westlake)
Tmax (ore)	5.75(1.712)	6.50(1.508)	CHI ² =1.333 (N.S) (Friedman)
ANOVA	N.S.	N.S.	N.S.

Table III The individual and mean half-life values of carbamazepine following administration of single 200 mg doses of carbamazepine as Finlepsin and Tegretol tablets to volunteers

Subject	T1/2 (ore) Finlepsin	T1/2 (ore) Tegretol
Mean	42.7667	44.8083
SEM	1.7397	2.4093
S.D.	6.0265	8.3462
Median	43.5	45
Min	31.8	33.7
Max	51.8	59

Table V The biological half-life of carbamazepine-epoxide following single oral dose administration of 200-mg carbamazepine as Finlepsin and Tegretol tablets to 12 volunteers

Subject	T1/2 (ore) Finlepsin	T1/2 (ore) Tegretol
Mean	32.33	34.08
SEM	1.7767	1.7384
S.D.	6.1546	6.0221
Median	32	31.5
Mm	22	28
Max	47	45

Table IV Mean values of plasma carbamazepine-epoxide following single oral dose of 200 mg carbamazepine to 12 volunteers as Finlepsin (Asta Medica) and Tegretol (Novartis) drug products

Time (hours)	Finlepsin ($\mu\text{g}/\text{ml}$)	\pm S.D.	Tegretol ($\mu\text{g}/\text{ml}$)	\pm S.D.
1	<L.Q.		<L.Q.	
1.5	<L.Q.		<L.Q.	
2	<L.Q.		<L.Q.	
3	0.0434	0.0168	0.0445	0.0233
4	0.0458	0.0178	0.0631	0.0219
6	0.0702	0.0231	0.0837	0.0169
8	0.0947	0.0247	0.1035	0.0208
12	0.1077	0.0194	0.1124	0.0181
24	0.1060	0.0233	0.1030	0.0269
48	0.0993	0.0293	0.0845	0.0279
72	0.0677	0.0288	0.0694	0.0285
96	0.0392	0.0156	0.0490	0.0173
120	0.0228	0.0110	0.0220	0.0083

<L.Q. = under limit of quantitation

Table VI Statistical analysis of pharmacokinetic parameters of carbamazepine following administration of single oral doses of 200 mg carbamazepine as Finlepsin (Asta Medica) and Tegretol (Novartis) tablets.

Pharmacokinetic parameters	Tested product Finlepsin (Asta Medica) Media \pm D.S.	Reference product Tegretol (Novartis) Media \pm D.S.	Confidence interval 90%
ASCt ($\mu\text{g}\cdot\text{h}/\text{ml}$) (ln)	0.983(0.132)	0.961(0.106)	0.86:1.29
ASCI($\mu\text{g}\cdot\text{h}\cdot\text{m}^{-1}$) (ln)	1.024(0.119)	1.042(0.080)	0.83:1.12
Cmax ($\mu\text{g}/\text{ml}$)	-0.931(0.083)	-0.910(0.086)	0.78:1.16
Tmax (ore)	22.333(13.694)	21.667(19.256)	68.70% (ANOVA, Westlake)
Tmax (ore)	22.333(13.694)	21.667(19.256)	CHI ² =0.75 (N.S) (Friedman)
ANOVA	N.S.	N.S.	N.S.

explanation may be the great fluctuation of the enzymatic activity between subjects, which is more evident as consequences during the absorption step of the parent drug.

The confidence interval for the Cmax ratio is not in the bioequivalence range. This range is interpreted even by the specialized organisms devoted to bioequivalence studies, greater than for amount absorbed (0.7-1.43). Carbamazepine-epoxide being a metabolite of carbamazepine is strongly dependent by the rate of metabolism of the parent drug, with much more interindividual differences.

Tmax for the two products studied was analyzed as the difference between mean values of the 12 subjects. ANOVA test showed a confidence interval greater than 20%, but the Friedman nonparametric test showed that the difference is not statistically significant. The active metabolite is not taken into account for the bioequivalence trial.

In conclusion it may be stated that the two drug products studied, Finlepsin (Asta Medica) and Tegretol (Novartis) are bioequivalent as amount and rate of absorption of parent drug.

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