Comparative Bioavailability of Two Extemporaneous Solid Formulations of Carbamazepine against the Innovator in Mexican Healthy Subjects

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Abstract

Extemporaneous or off-label prescribing is not illegal and may sometimes be clinically and economically appropriate. However it is associated with a number of clinical, safety and ethical issues. Bioequivalence of these products must be proven before they are used in place of patent medicines. In the present study, a single-center, open, randomized, single-dose, 2-period crossover, 2-sequences pilot assay (two subgroups with n=6) was carried out to evaluate the bioavailability of two extemporaneous capsule of carbamazepine (200 mg): A-Formula® (A); and Formule® (B) in comparison to a tablet of the innovator product Tegretol® (C). Twelve healthy volunteers were randomly assigned to one of two arms to receive one test/reference formulation and following a two week wash-out period they received the other compound. Blood sampling was performed over 72 hours after dosing and levels of carbamazepine were determined by HPLC. Main findings of the study include that peak of plasma carbamazepine was faster following the extemporaneous capsules as compared to reference (Tmax: A: 6.58 h; B: 4.83 h vs 8.25-10.00 h, respectively), although no changes was observed in Cmax (A: 3.32 µg/mL; B: 3.10 µg/mL vs C: 3.14-2.85 µg/mL) neither in AUC0-t (A: 116.34 µg*h/mL; B: 145.66 µg*h/mL vs C: 123.18-138.37 µg*h/mL). The elimination half-life that ranged between 38.64-61.29 h but not difference were observed between all formulations. By using bioequivalence statistics it appears that A-Formula® or Formule® is bioequivalent to Tegretol® in terms of AUC0-t but not regarding Cmax. In conclusion, we demonstrated that two extemporaneous capsules of carbamazepine, A-Formula® and Formule® show similar concentration-time profiles to the reference tablet of immediate Tegretol®, however further studies with longer sample size are needed to confirm its bioequivalence and interchangeability.

Keywords: Carbamazepine; Pharmacokinetics; Anti-epileptics; HPLC; Bioavailability; Bioequivalence

Introduction

Suitable dosage forms are not always available for specific patient (e.g. patients with epilepsy) populations and must be extemporaneously compounded. Extemporaneous preparation refers to the manipulation of drugs and excipients for a particular patient using traditional compounding techniques; these are referred to as ‘off-label’ and ‘unlicensed’ [1,2]. Off-label use can include altered doses, dosage forms or indications for use. Registered medicines are produced to internationally recognized standards of Good Manufacturing Practices. Within the pharmaceutical manufacturing industry, quality, safety and efficacy are enforced by regulatory legislations. In contrast, the responsibility for acceptable standards for the compounding of ‘off-label’ medicines falls on the prescriber, pharmacist or hospital nurse [1]. Among other requisites, extemporaneous solid formulations should comply with bioavailability properties as compared to the innovator product [3].

Currently in Brazil several forms of carbamazepine are commercially available but due to the economic-limited access to medicines from abroad of population in this country, community pharmacy practitioners have been authorized to dispense a number of extemporaneous compounding products including carbamazepine capsules [4]. However, several reports have raised concerns about clinically meaningful differences in serum level, therapeutic effect, and adverse effects between generic and brand-name anticonvulsants, including carbamazepine [5-8]. Thus, extemporaneous products should be advised to demonstrate that are bioequivalent to the reference in order to guarantee efficacy and safety in patients treated with the drug in question.

Currently, it is not known whether compounding from bulk carbamazepine and packaging as capsules would alter the drug’s pharmacokinetic profile as compared to commercially available tablets. For these reasons, we aimed to determine the bioavailability of two extemporaneous gelatin capsule formulations and compare it versus that from an identical dose (200 mg. p.o) of the marketed carbamazepine tablet following a pharmacokinetic sampling schedule of 72 hours.

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Received June 14, 2013; Accepted February 24, 2014; Published March 04, 2014

Citation: de Araujo Ferreira AA, Coelho Guerra GB, da Silva Solon LG, Dibildox E, Perez-Urizar J, et al. (2014) Comparative Bioavailability of Two Extemporaneous Solid Formulations of Carbamazepine against the Innovator in Mexican Healthy Subjects. J Bioequiv Availab 6: 033-037. doi:10.4172/10.1000177

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Subjects and Methods

Study population

Twelve normal healthy mestizo volunteers (7 males and 5 females) aged from 19 to 43 years, weighing from 51 to 91 kg, and measuring from 159 to 184 cm in height were included. All the volunteers gave written informed consent after they had received detailed instructions about the aims, restrictions and possible adverse effect which could be experienced as a result of taking the drugs. The study was approved by the Ethics Committee of the Instituto de Investigación Clínica de Occidente S. A. de C.V. in Guadalajara, Jal, México. All volunteers were found to be in good physical health, this is the subjects' medical histories were documented and a physical examination was conducted.

Supine systolic and diastolic blood pressures were recorded. Inclusion eligibility was also based on the successful completion of a clinical health evaluation that consisted of a personal interview, a complete physical examination (blood pressure, pulse, weight, height, temperature, respiratory rate), and diagnostic testing that included a 12-lead electrocardiogram and chest radiograph. Laboratory testing included a complete blood cell count, metabolic and hepatic tests (alanine aminotransferase [reference range, 8-44 U/L] and aspartate aminotransferase [13-34 U/L]), urinalysis, pregnancy test (for female subjects), serologic tests for glucose (72-100 mg/dl), blood urea nitrogen (5.7-23.1 mg/dl), creatinine (0.6-1.3 mg/dl), as well as hepatitis B and HIV antibodies. Volunteers were excluded if laboratory values were significantly above or below the reference range and/or if all tests had not been performed. The demographic characteristics of the study population are summarized in Table 1. Subjects did not take any other medications for at least 2 weeks prior to and throughout the entire study. Each subject was fasted overnight (12 hours) prior to the experiment. All medicines were administered with 250 ml of tap water and a standard lunch was given to all subjects 4 h after dosing.

Study design

This was a collaborative single-center, open, randomized, single-dose, 2-period crossover, 2-sequences pilot study. Volunteers were randomized into three groups (A, B, C) to receive a single dose of carbamazepine 200-mg as an extemporaneous capsule formulation of:

A) A-Formula®;
B) Formule® (both from Farmacias de manipulación, Natal, Rio Grande del Norte, Brazil), or
C) a tablet of the innovator Tegretol® (Novartis Biosciencias S.A., Taboao da Serra, Brazil). Subjects were given in a crossover fashion (sequences AC-CA, BC-CB), one of the 3 treatments in each of the 2 periods with a two-weeks washout period. Blood samples were withdrawn prior to drug administration and at 0.50, 1.00, 1.50, 2, 3, 4, 6, 8, 10, 12, 24, 48, and 72 hours after drug intake. All study subjects were confirmed to be in good physical health based on medical history.

Sample treatment and drug analysis

Plasma from EDTA blood samples was obtained by centrifugation and kept at -70°C until drug analysis. Drug plasma levels were assessed by using adapted method of high performance liquid chromatography coupled to UV detection [9]. Briefly, 100 μl of plasma samples were added with 50 μg/ml of phenytoin as internal standard. Compounds were extracted with 0.5 ml of methylene chloride. The organic phase was transferred to a clean tube and evaporated to dryness under nitrogen and then redisolved with 600 μl of mobile phase. Aliquots of 10 μl were then injected into the chromatographic system which consisted of a 1525 solvent delivery system, 717 autosampler and 2487 UV detector (215 nm ) all of Waters Assoc. (Milford, MA, USA). The eluent was quantified using an Zorbax C18, 4.6x75 mm column (Agilent, CA, USA) and a mobile phase consisting of a 30:70 (v/v) acetonitrile/water mixture at a flow rate of 1.2 ml/minute. Prior to use, the assay was validated by following domestic and international guidelines [10,11]. Results include standard curves for analyses of subject samples encompass a concentration range of 0.1–50 μg/ml. The quantification and detection limits for carbamazepine are 0.1 μg/ml and 0.01 μg/ml, respectively. Recovery was determined to be 56%. Accuracy and precision for quality control samples were between 96-104% and 1.2-7.5% CV for intra- and inter-assay experiments respectively.

Pharmacokinetic and statistical analysis

To compare the rate and extent of absorption as well as the elimination properties of the study drugs, the following pharmacokinetic variables were calculated for each volunteer and product using the actual plasma sampling times. The area under the plasma concentration curves (AUC∞) were calculated with the linear trapezoidal rule [12]. The maximum plasma concentration (Cmax) and time to reach maximum plasma concentration (Tmax) were obtained directly from the plasma-concentration data. The AUC∞ was calculated by dividing the last measured concentration by the elimination rate constant and adding the result to the AUClim. The elimination rate constant was calculated by least-squares regression, using the last points of each curve. All pharmacokinetic parameters were obtained by using standard noncompartmental analysis implemented within WinNonLin 6.2 (Pharsight Co. Mountain View, CA). A crossover analysis of variance of the bioavailability indicators Cmax, AUC∞, and AUClim was used to evaluate the effects due to sequence, subject, period and formulation. The 90% CI of Cmax, AUC∞, and AUClim for the ratio of each drug into the new formulation (test) and the individual drug (reference) was determined to assess the bioequivalence between different products using the equivalence interval of 0.80 and 1.25. Tmax and T½ were analyzed by means the non-parametric Wilcoxon signed-rank test. All statistical analysis assumed that differences were significant if p<0.05.

Results

Treatment with either the testing capsules, A-Formula®, Formule® or the tablet Tegretol® was well tolerated. Assessment of clinical

| Subject No. | Sex | Age (y) | Height (cm) | Weight (kg) |
|-------------|-----|---------|-------------|-------------|
| 1           | Male| 19      | 184         | 91          |
| 2           | Female| 23     | 162         | 56          |
| 3           | Female| 25     | 167         | 63          |
| 4           | Male| 34      | 173         | 80          |
| 5           | Male| 20      | 176         | 80          |
| 6           | Female| 31     | 160         | 51          |
| 7           | Male| 19      | 173         | 66.2        |
| 8           | Female| 23     | 169         | 64.5        |
| 9           | Male| 37      | 160         | 67          |
| 10          | Female| 32     | 159         | 56          |
| 11          | Male| 43      | 178         | 85          |
| 12          | Male| 19      | 166         | 72          |
| Mean        |      | 27.08   | 168.9       | 69.0        |
| S.E.M.      |      | 1.1     | 1.14        | 3.9         |
| Range       |      | (19-43) | (159-184)   | (51-91)     |

Table 1: Demographic characteristics of the study population.
for the capsule. After peak plasma concentrations were reached, the formulations, although the rate of drug absorption was slightly greater 6 h. Table 1 summarizes the pharmacokinetic parameters for both tablet. Concentrations of carbamazepine were detectable since 0.5 was absorbed slowly after administration of the extemporaneous following the test formulation A-Formula® shown). biochemistry, vital signs, ECG and physical examination did not reveal 

The mean plasma carbamazepine concentrations over time following the test formulation A-Formula® or Formule® compared to the reference is shown in Figures 1 and 2. A-Formula® carbamazepine was absorbed slowly after administration of the extemporaneous tablet. Concentrations of carbamazepine were detectable since 0.5 hours post-administration and they reached a maximum in about 6 h. Table 1 summarizes the pharmacokinetic parameters for both carbamazepine formulations. $T_{\text{max}}$ values were comparable for the two formulations, although the rate of drug absorption was slightly greater for the capsule. After peak plasma concentrations were reached, the plasma concentration declined approximately in a monoeXponential fashion with wide interindividual variability. Carbamazepine appeared to be eliminated more rapidly following the test formulation (39 h) as compared to the reference formulation (65 h). At the end of the sampling time, none of the subjects showed plasma levels near or lower the limit of quantification, instead only about 70% of the AUC$_{0-\infty}$ had been sampled. Given the mentioned values for mean carbamazepine elimination half-life with both formulations, the AUC$_{0-\infty}$ rather than the AUC$_{0-t}$ was primarily used for statistical analysis between the formulations. Considerable inter-individual variability among the 6 subjects in C$_{\text{max}}$, $T_{\text{max}}$, AUC$_{0-\infty}$ or AUC$_{0-t}$ was seen, however there were no statistical differences. Moreover the mean concentration-time profiles for the two formulations were very similar. The mean relative bioavailability of the testing formulation versus the tablet (reference) formulation was 94%.

Regarding the second test capsule, Formule® the peak of plasma carbamazepine was also similar as compared to that of Tegretol®, although in contrast to the other A-Formula®, $T_{\text{max}}$ following Formule® was significantly reduced as compared to the reference formulation (10.0 ± 2.12 h vs 4.83± 2.01, respectively). When evaluated the entire drug exposition measured as AUC$_{0-\infty}$, the test formulation did not differ from the reference formulation. However, when the drug exposition was measured as AUC$_{0-t}$ the test formulation was lower than the reference (249.47 ± 50.84 vs 329.98 ± 62.26, probably due to the incomplete sampling period. The difference in $T_{\text{max}}$ between Formule® (58 h) and Tegretol® (89 h) was significant (p<0.05) and can be suggested as the basis of discrepancies seen with AUC$_{0-\infty}$.

In order to determine bioequivalence, C$_{\text{max}}$, AUC$_{0-t}$ and AUC$_{0-\infty}$ were compared by analysis of variance for a crossover design, followed by the 90% classic confidence (CI) interval and Schuirmann bilateral tests (Table 2). The 90%CI limits for both A-Formula® and Formule® ranged from 87 to 112% for AUC$_{0-t}$ however that ranged from 48 to 151% and 74 to 125% for C$_{\text{max}}$, as well as from 68 to 132% and 31 to 168% for AUC$_{0-\infty}$, respectively. Since the confidence interval and Shuirmann tests for AUC$_{0-t}$ was fully contained within the limits of acceptance but C$_{\text{max}}$ and AUC$_{0-\infty}$ did not, it can be thought that an increase in sample size is needed to confirm the bioequivalence between test formulation and reference.

Discussion

Carbamazepine formulated as compounding extemporaneous capsules is commonly found in locations so-called "farmacias de manipulacion" in Brazil. Although this kind of medicines have been long-time used, its bioavailability is unknown. Given the concerns on the efficacy and safety of these products, this pilot study was designed to evaluate the pharmacokinetic profile of two different compounding formulations, A-Formula® and Formule® (both from Farmacias de manipulacion, Natal, Rio Grande del Norte, Brazil), as well as to compare to the bioavailability of a tablet of the innovator product Tegretol®. Results of this study pointed out that from a pharmaceutical point of view, the extemporaneous carbamazepine 200 mg capsules are absorbed more rapidly than the reference tablet but its total bioavailability is not significantly different.

Compounding pharmacy refers to a facility that both makes and sells prescription drugs. A compounding pharmacy can often concoct drug formulas, also called compounding preparations that are specially tailored to patients: for example, liquid versions of medications normally available only in pill form, for patients who cannot swallow
On the other hand, in contrast to A-Formula® and Formule®, the test/reference ratio for C_{max} was higher than A-Formula® and Formule®, respectively. However, the test/reference ratio for Tmax was lower than A-Formula® and Formule®, respectively. The test/reference ratio for AUC_{0-∞} was higher than A-Formula® and Formule®, respectively. The test/reference ratio for AUC_{0-72h} was lower than A-Formula® and Formule®, respectively.

Table 2: Pharmacokinetic parameters of carbamazepine in healthy volunteers receiving a 200 mg single dose of the test extemporaneous capsules A-Formula®, Formule®, or the reference tablet Tegretol®.

| Parameter | Test Formulation A | Reference Formulation C | Ratio % | 90% CI | Schuirmann bilateral test (p<80; p>120) |
|-----------|--------------------|------------------------|---------|--------|-------------------------------------|
| C_{max} (µg/ml) | 3.32 (0.84) | 3.14 (1.48) | 111.81 | 73.16 - 170.87 | (0.0838 - 0.3701) |
| Tmax (h) | 4.83 (3.41) | 10.00 (6.02) | 107.99 | 86.25 - 135.22 | (0.0014 - 0.0136) |
| AUC_{0-∞} (µg·h/ml) | 145.66 (33.62) | 138.37 (33.79) | 104.34 | 95.58 - 113.89 | (0.0014 - 0.0136) |
| AUC_{0-72h} (µg·h/ml) | 244.93 (38.46) | 271.65 (39.79) | 92.71 | 80.16 - 107.22 | (0.0014 - 0.0136) |
| Tmax (h) | 55.84 (8.09) | 45.04 (8.41) | 123.18 | 80.16 - 107.22 | (0.0014 - 0.0136) |

C_{max}: peak plasma level; Tmax: time to peak; AUC_{0-∞}: area under the concentration-time curve from time zero to last point of sampling; AUC_{0-72h}: area under the concentration-time curve from time zero to 72 hours; T_{1/2}: half-life.

On the basis of results with both extemporaneous capsule products, it is evident that bioavailability studies are necessary to demonstrate that these kinds of products can be efficacious and safe. Indeed, although extemporaneous formulations are not intended to be part of the generic drug politics in any country, different efforts are being by health authorities to regulate this practice to protect the patient’s safety and to prevent risk of ineffectiveness or over consumption and side effects [2,14].

Latest is of particular importance for drugs with narrow therapeutic index such as anti-epileptic drugs [15]. For instance, carbamazepine, that is poorly soluble in water with erratic oral absorption and bioavailability less than 70%, has been reported to show a recurrence of convulsions after a shift to a generic antiepileptic agent [16]. Indeed, Hartley and collaborators [17] reported similar bioavailability for generic and brand-name carbamazepine in 12 epileptic children. While other groups reported [18,19] described 2 cases of clinical complications arising after a switch to a generic formulation, Meyer and collaborators [20] reported differences in bioavailability between generic and brand-name carbamazepine in 20 healthy volunteers. On the other hand, groups conducted previously randomized, double-blind studies in fairly large patient populations and reported no differences in bioavailability or clinical efficacy [21,22]. Thus two issues namely the demonstration of bioequivalence and thereafter the generic substitution are pending for a variety of extemporaneous formulation including those formulated with carbamazepine.

**Conclusion**

In the present pilot study in healthy, Mexican subjects, we report...
that two extemporaneous capsules of carbamazepine (200 mg), A-Formula® and Formule® showed similar concentration-time profiles to the reference tablet of immediate Tegretol® (200 mg), however further studies with longer sample size are necessary to confirm its bioequivalence and interchangeability.

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