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Bioequivalence study of carbamazepine tablets: in vitro/in vivo correlation

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Abstract

After the in vitro dissolution profiles of three Mexican carbamazepine products and the innovator product (Tegretol) in four different dissolution media: HCl 0.1 N, simulated gastric fluid, simulated intestinal fluid, and water containing 1% sodium lauryl sulfate (USP method) were assesed, their bioequivalence was evaluated in 12 healthy volunteers in a randomized crossover study. Single oral doses of 400 mg of each product were administered at intervals of 2 weeks. The products tested in this study showed significant differences in the peak concentration and the area under the curve ($p < 0.05$). A faster dissolution was observed when USP method was used; however, no correlation was observed between the in vivo parameters and the in vitro dissolution results with this method. A linear relationship was found between the area under the curve and the percent dissolved at 45, 60 and 90 min when simulated intestinal fluid was used. © 1997 Elsevier Science B.V.

Keywords: Carbamazepine; Dissolution; Bioequivalence; Antiepileptics; In vitro/in vivo correlations

1. Introduction

Carbamazepine (CBZ) is a drug widely used in the treatment of epilepsy and trigeminal neuralgia. While much is known about its efficacy in adults and children (Blom, 1962; Callaghan et al., 1985), there are few reports about the release characteristics of the drug when used in oral dosage forms (Kahela et al., 1983; Kaneniwa et al., 1984; Neuvonen, 1985). The drug is poorly soluble in aqueous media (Clarke, 1986); besides the gastrointestinal absorption is characterized as slow, erratic and possibly incomplete (Riad et al., 1986); in addition, its rate of absorption can differ markedly with different pharmaceutical formulations (Martindale, 1982).

One objective of the dissolution tests and in vitro/in vivo correlations is to identify poorly

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bioavailable products from those considered therapeutically acceptable. Several studies have been conducted in order to determine the bioavailability of carbamazepine following rectal administration (Graves et al., 1985), chewable tablets (Chan et al., 1985), and the influence of meals on the bioavailability of this drug, however, there are few reports about the relationship between carbamazepine in vitro release characteristics and in vivo data (Meyer et al., 1992); therefore the purposes of the present study were to examine the bioquivalence of CBZ from products currently used in Mexico and to determine the relationship between the in vitro dissolution results by using four different methods and the area under the curve and C_{max} of the products.

2. Materials and methods

2.1. In vitro studies

The dissolution profiles of three Mexican CBZ products supplied to the Mexican Social Security Institute (IMSS) (Amstrong, Wayne and Precimex Laboratories) and the innovator product (Tegretol, Ciba Geigy) were carried out by using USP dissolution apparatus and four different methodologies:

Method 1: HCl 0.1 N, 900 ml, basket, 100 rpm pH 1.0.

Method 2: Simulated USP gastric fluid pH 1.2, 900 ml, paddles, 50 rpm.

Method 3: Simulated USP intestinal fluid, pH 7.5, 900 ml, paddles, 75 rpm.

Method 4: Water containing 1% sodium lauryl sulfate, 900 ml, paddles, 75 rpm (USP XXIII method).

The studies were performed by using a Hanson Research apparatus. Twelve tablets of each product were tested with each of the different methodologies.

Samples (3 ml) were removed at 5, 10, 15, 30, 45, 60, 90 and 120 min, filtered and assayed spectrophotometrically at 285 nm in a Beckman spectrophotometer. The calibration curves for the different media were constructed over the concentration range of 2–22 μg/ml. No interferences were found with the excipients of the products.

Quality control tests were performed according to USP XXIII (1994). Since Mexican Pharmacopeia (1988) includes disintegration test using the basket-rack assembly, this procedure was also included, using water as the immersion fluid.

2.2. In vivo study

Study was performed on 12 healthy male volunteers (ages 20–30 years; weight 60–80 kg) according to a latin-square design. The subjects were informed of the purpose, protocol and risks of the study. All the subjects had normal clinical chemistry laboratory values. The clinical study protocol was approved by the local ethics committee. Each subject gave his written consent to participate.

Subjects did not take any other medication or alcohol for at least 14 days prior to and during the entire study. Subjects were randomly divided into four groups and each group recieved a 400 mg oral dose (2 tablets) of each of the four products in a different sequence, with 200 ml of water after an overnight fast. Fasting was continued for the first 4 h after dosing, then a light meal was provided. A standard supper was given 4 h after lunch. Blood samples were collected just before the drug administration and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96 and 120 h after dosing. Plasma was separated by centrifugation and stored at −4°C until assayed. This procedure was repeated every 2 weeks until all the dosage units were administered.

3. Plasma assay

The analytical method of Chan (1985), reported briefly here, was used to assay CBZ in plasma: to 500 μl of plasma containing 0.8 μg of clonazepam as an internal standard, 200 μl of methanol and 1.5 ml of distilled water were added and mixed. The sample was alkalinized with 20 μl of NaOH (5 M) to adjust the pH to 12. The basified solution was extracted twice with 7 ml of dichloromethane/ether (1:3) by mixing for 15 min. After centrifugation for 10 min at 3000 rpm, the organic extracts were combined and evaporated to

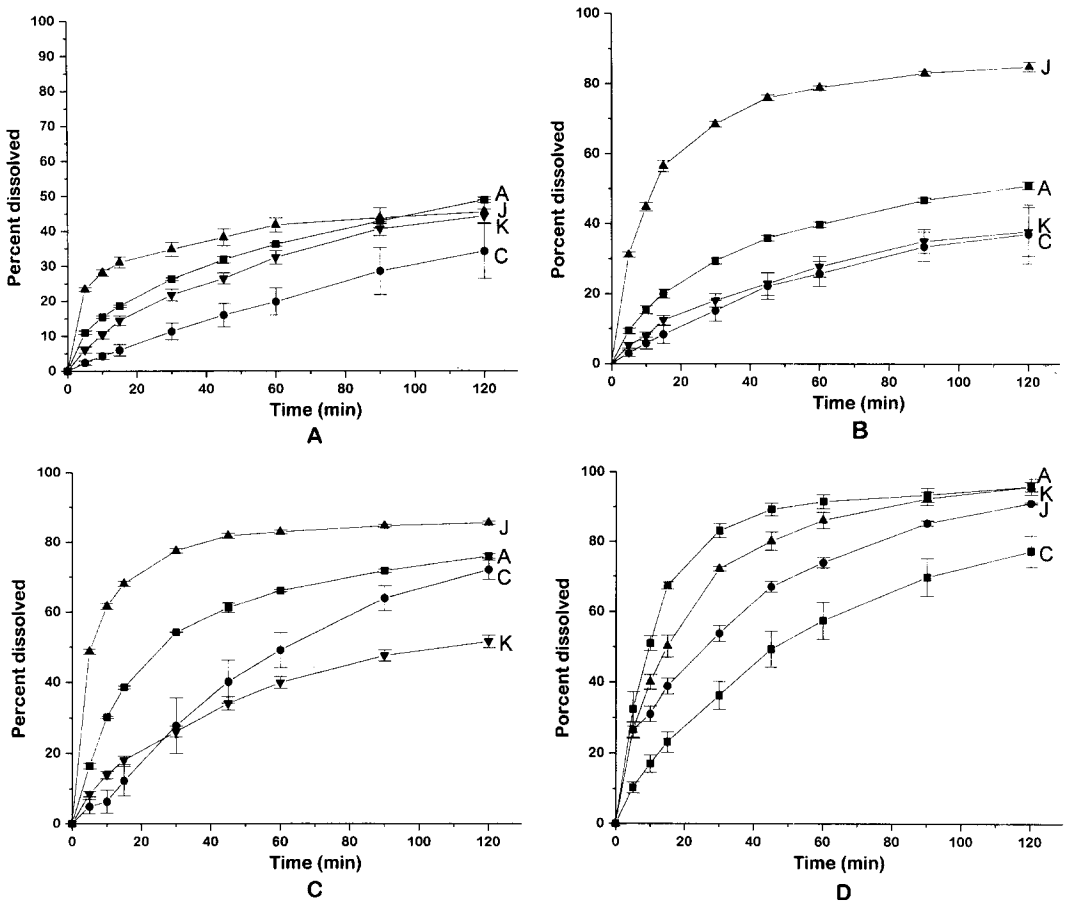

Fig. 1. Mean cumulative percent of carbamazepine dissolved using (A) Method 1: HCl 0.1N 900 ml, 100 rpm, basket pH 1.0; (B) Method 2: simulated gastric fluid (pH 1.2), paddles, 50 rpm; (C) Method 3: simulated intestinal fluid (pH 7.5), paddles, 75 rpm; (D) Method 4: sodium lauryl sulfhate 1%, paddles, 75 rpm. Each data point is the mean of 12 tablets; bars indicate the standard error.

dryness under stream nitrogen at 45°C in a water bath. The residue was dissolved in 50 μl of methanol and injected in a Waters HPLC apparatus by using a LiChrosorb RP 8 (10 μm, 30 cm) column and acetonitrile/water (40:60) as a mobile phase. Detection was made using an UV detector at 215 nm. Results showed that the assay was sensitive (0.1 μg/ml), linear between 0.2 and 10 μg/ml and precise (coefficient of variation 7%). Samples were stable for 1 month when stored at −4°C.

4. Statistical analysis

The area under the plasma concentration time curve to the last concentration was calculated using the linear trapezoidal rule. The terminal first order constant (k) was determined by least squares fit of the log-transformed terminal plasma concentrations in each data set. Peak concentration (C_{max}) and time of peak concentration (t_{max}), were obtained directly from the individual plasma concentration–time profiles. The fraction

absorbed was calculated by using Wagner Nelson method. The analysis of variance for a complete crossover design was utilized to determine whether there were differences in biovailability parameters. Plasma concentration at each time, area under the curve, C_{max}, slow rate constant, and peak time were statistically analyzed.

5. Results and discussion

5.1. In vitro studies

According to the Mexican Pharmacopeia (1988), the disintegration times of carbamazepine tablets should be less than 3 min. Products A, C and K failed this test; all 12 tablets from each product had disintegration times between 7 and 9 min.

Fig. 1 shows the dissolution profiles of the four products in HCl 0.1 N (method 1), simulated gastric fluid pH 1.2 (method 2), simulated intestinal fluid pH 7.5 (method 3), and water containing 1% sodium lauryl sulfate (method 4). Each data point represents the mean of 12 dosage forms. When method 1 was used, dissolution was extremely low (less than 50% at 120 min) for all formulations. Products A, C and K showed the same profile when methods 1 or 2 were used, however product J improved its dissolution when method 2 was used. When pH was changed from 1.2 (method 2) to 7.5 (method 3), dissolution improved only slightly. Carbamazepine may be considered as a neutral substance with no acidic or basic functions in a wide range of pH (Martindale, 1982), which could explain the results obtained. In water containing 1% sodium lauryl sulfate (USP XXIII method), the dissolution profiles of products A and K were higher than those with the other dissolution media, which could be due to the surfactant effect of lauryl sulfate. The USP specification for carbamazepine tablets (method 4) requires no less than 75% of the drug be dissolved within 60 min. Product C failed this requirement.

As seen in Fig. 1, product K (Innovator product) showed a great difference in behavior after using methods 3 and 4; thus with method 3 it dissolved less than 40% in 60 min, and with method 4 the dissolution improved to 86%.

Analysis of variance for the percentage of carbamazepine dissolved at 15, 45 and 60 min showed significant differences between products ($p < 0.05$) using methods 2, 3, 4; however, method 3 showed wider differences on dissolution profiles. Kinetic studies revealed that the products were released by a first order mechanism in all media. Significant correlations were found between disintegration time and percent dissolved at 45, 60 and 120 min ($r = 0.7703$, 0.8030 and 0.8085 respectively, $p < 0.05$) using method 2, however with the other dissolution media no correlations could be found.

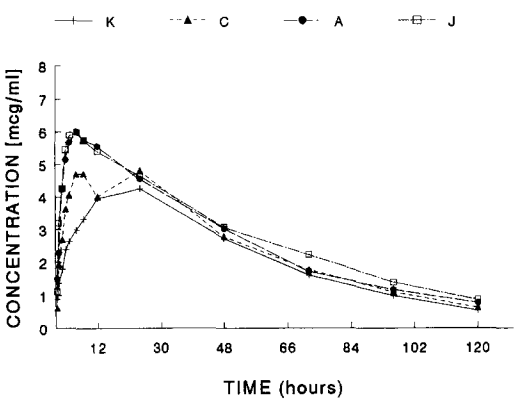

Fig. 2. Plasma concentrations of carbamazepine in 12 healthy volunteers after the ingestion of a single oral dose of 400 mg as four different preparations. Key: (+) product K; (▲) product C; (●) product A; (□) product J.

5.2. In vivo studies

Fig. 2 shows the mean plasma profiles after the administration of the four formulations. Analysis of variance showed that plasma concentrations of carbamazepine were significantly higher ($p < 0.05$) from 0.5 to 12 h after the administration of products A and J than after the other formulations. Also the peak concentration was about 30% higher ($p < 0.05$) when products A and J were given and the peak time was significantly shorter for these two brands. After 24 h, concentration

Table 1
Some pharmacokinetic parameters of carbamazepine following a single 400 mg oral dose as four different preparations[a]

Parameters	Product				Significance level among treatments
	A	C	J	K (innovator)	
Peak concentration (mg/l)	5.98 (0.83)	4.81 (1.04)	5.98 (0.57)	4.24 (0.92)	$p<0.05$
Peak time (h)	8.54 (5.87)	14.61 (9.06)	8.6 (6.1)	15.8 (7.3)	$p<0.05$
Elimination half life (h)	39.9 (9.7)	36.6 (8.82)	39.6 (10.8)	33.3 (10.56)	NS
$AUC_{0\rightarrow 120}$	331 (25.71)	316 (27.71)	368 (25.52)	296 (30.7)	$p<0.05$

[a] Results are expressed as average and (S.D.).

obtained with the different products did not differ significantly.

It can be seen that the lowest plasma concentrations were obtained with the innovator product (product K) characterized by a slow release of the drug as compared to others. The delayed absorption of the innovator product has been well documented by other investigators (Palmer et al., 1973; Morselli et al., 1975; Pynnönen et al., 1978; Neuvonen, 1985; Chan et al., 1985). The slow release may be a benefit in trying to maintain a continuous anticonvulsive level. Table 1 shows the bioavailability parameters of the four products studied. Significant differences were obtained in C_{max} ($p<0.05$) between products.

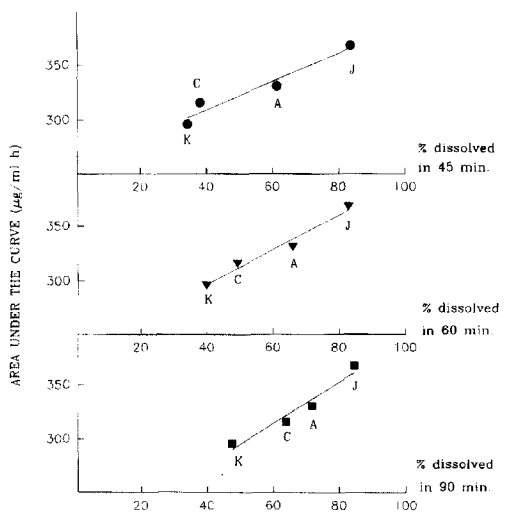

Fig. 3. Linear correlations between area under the curve ($AUC_{0\rightarrow 120}$) and percent drug dissolved in 45, 60 and 90 min using method 3.

After the administration of product C, a second peak in the plasma concentration time curve was found. This phenomenon has been suggested to be due to the solubilization of the drug by bile secreted after meals or to enterohepatic cycling (Levy et al., 1975). It is worthwhile notice that in the present study product C presented an incomplete dissolution in all media; thus the solubilization of the drug could be a plausible explanation.

5.3. In vitro-in vivo correlations

Few studies have been carried out in order to determine the in vitro in vivo correlation of CBZ products. Kaneniwa et al. (1984) found in vitro/in vivo correlations between the in vitro time required for 30, 50, 60 and 80% dissolved and in vivo absorption rate constant by using 2 l of gastric fluid as dissolution media and 100 rpm. Neuvonen (1985) studied the dissolution rates of CBZ tablets using USP basket method, 1 l of HCl 0.1 N and 100 rpm and found a good agreement between the rate of carbamazepine absorption in vivo and the dissolution rate of the tablets in vitro. Hartley et al. (1991)

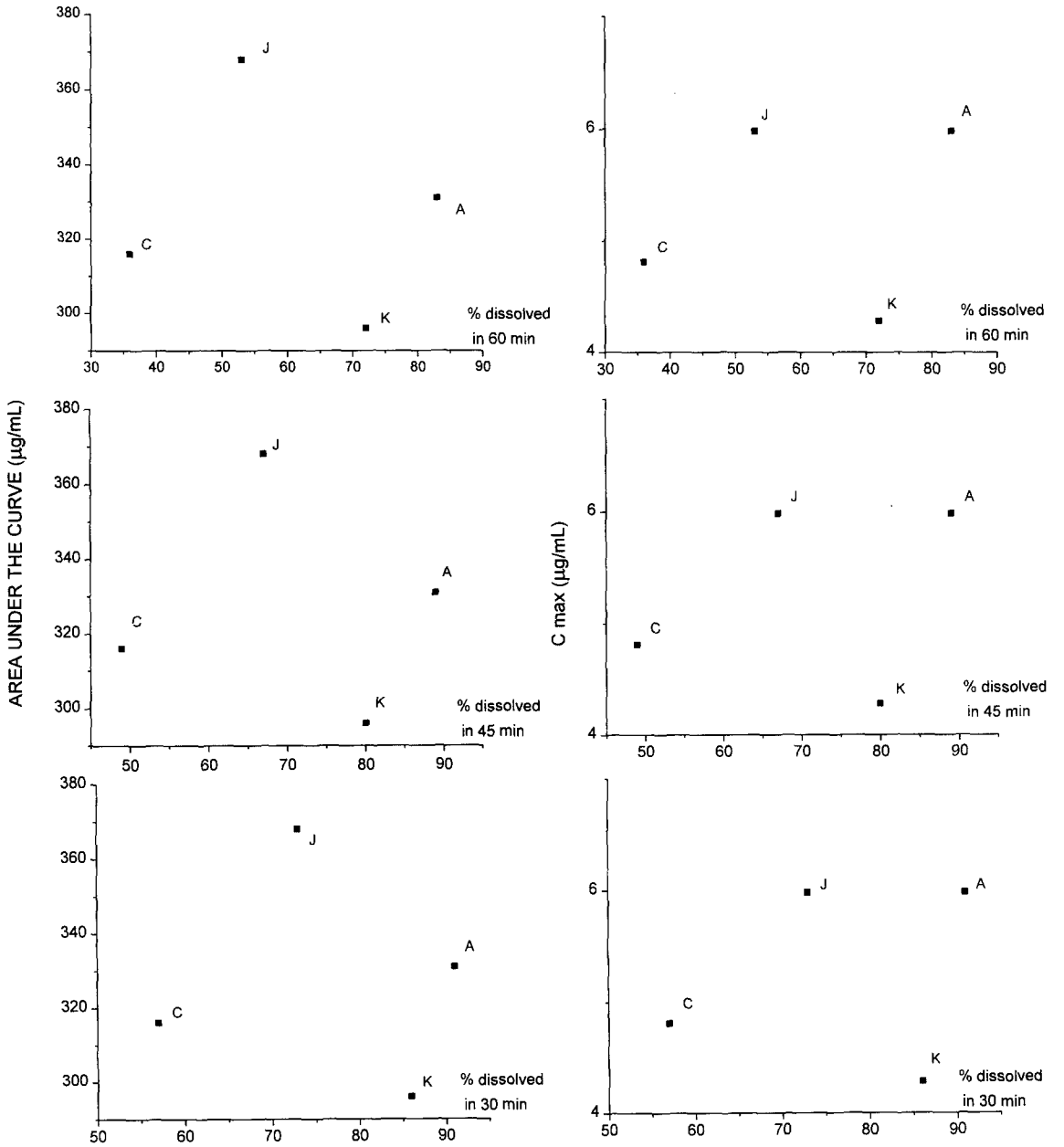

Fig. 4. Correlations between area under the curve ($AUC_{0\rightarrow 120}$) and C_{max}, and percent of drug dissolved in 45, 60 and 90 min using method 4 (USP).

determined the in vitro dissolution profiles of two carbamazepine formulations by using the USP paddle method at 100 rpm and HCl 0.1 N as dissolution medium and assessed the bioavailability in epileptic children at steady state. They found that the differences in the in vitro rate of drug dissolution between preparations had no detectable effect on the bioavailability when assessed at steady state.

Until 1985 USP required disintegration tests for carbamazepine tablets by using simulated gastric fluid as immersion media. Supplement I of USP XXI modified the disintegration test by requiring distilled water as immersion fluid. In 1990, USP XXII incorporated the dissolution requirement for this drug which utilizes the paddle method at 75 rpm and 900 ml of water containing 1% sodium lauryl sulfate. Meyer et al. (1992) evaluated the bioavailability of three lots of generic 200 mg tablets products and the innovator product and determined the relationship between the in vivo data and the in vitro dissolution results by using the USP XXII method. Even though a linear relationship between the percentage dissolved and the percentage absorbed was found, such relationship could not be employed to predict the bioavailability of the products. In contrast simpler relationships between the percentage dissolved during the first 45 min and the C_{max} and AUC values were useful in predicting the bioequivalence of the products.

From the results of the current study, it can be seen that the USP dissolution medium: water containing 1% sodium lauryl sulfate, may not reflect in vivo data, since product K and J showed similar dissolution profile, however product K (innovator product) presented the lowest plasma levels. Although there were no significant differences in the area under the curve beween product C and innovator product, the last failed to meet dissolution specifications (not less than 75% in 60 min).

Attempts to correlating the bioavailability data with the dissolution rate indicated that linear correlations were obtained when relating area under the curve and percent drug dissolved in 45, 60 and 90 min using intestinal fluid as dissolution medium. Fig. 3 shows the results obtained. No correlations were found between USP method and area under the curve or C_{max} (Fig. 4).

Additional work is needed before a dissolution rate method can be used to predict carbamazepine bioavailability. The results of the in vitro test performed according to the USP XXII methods can not be used to accurately predict the bioavailability of a carbamazepine formulation.

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