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Enhancement of carbamazepine dissolution: in vitro and in vivo evaluation

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Abstract

The absorption of carbamazepine (CBZ) is characterized by being dissolution-rate limited. Thus improvement of its dissolution characteristics may increase its rate and extent of absorption, hence its oral bioavailability. The objective of the work is to enhance the dissolution of CBZ, through utilizing different carriers [polyethylene glycols (PEG), phospholipids, and hydroxypropyl-β-cylcodextrin (HPβCD)]. The prepared systems were evaluated in vitro through dissolution testing, X-ray diffraction, and differential thermal analysis (DTA). The screened lead systems were further evaluated in vivo using the rabbit as an animal model. In vitro results showed that the dissolution of CBZ was profoundly enhanced from (1:2) CBZ/PEG_{6000} solid dispersion, (10:1) CBZ/L-α-dimyristoyl phosphatidyl glycerol coprecipitate, and (1 M:1 M) CBZ/HPβCD complex. X-Ray diffraction and DTA were used to explain any interaction and/or complexation between CBZ and the different carriers. For in vivo evaluation, a parallel design with four groups was adopted, with each group receiving the equivalent of 100 mg CBZ either as one of the three lead prepared systems suspended in water or as Tegretol® suspension, the reference standard. Serial blood samples were drawn over 24 h from the marginal ear vein, and plasma samples were analyzed for CBZ and its active metabolite, carbamazepine-10,11-epoxide, CBZE, by HPLC. The in vivo results showed that the extent of absorption of CBZ from its HPβCD complex was the highest among the systems tested. For all prepared systems, areas under the CBZE concentration–time curve were significantly higher than that resulting after Tegretol® suspension. This may be explained by the fact that CBZ is being presented from the Tegretol® suspension at a slower rate, with absorption occurring further down the intestine, thus bypassing the liver.

Keywords: Carbamazepine; Solid dispersion; Coprecipitate; Complex; Dissolution; Bioavailability

1. Introduction

Carbamazepine (CBZ), a major antiepileptic, has become a best selling anticonvulsant, due to

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its favorable therapeutic profile. The conventional CBZ tablets yield peak plasma concentrations varying from 4 to 32 h. This irregular and delayed absorption of CBZ is attributed to slow dissolution (Bertilsson, 1978). Thus, the absorption of CBZ from the gastrointestinal tract (GIT) may be described as being dissolution-rate limited. Improvement of CBZ dissolution characteristics can result in an increase in the rate and/or extent of its absorption, after oral intake, thus increasing its bioavailability.

The main objective of this work is to investigate the possibility of improving the aqueous dissolution and oral bioavailability of CBZ via methods involving: (a) solid dispersion with polyethylene glycols (PEG); (b) coprecipitation with phospholipids (PL); and (c) complexation with hydroxypropyl-β-cylcodextrin (HPβCD). The prepared systems are evaluated in vitro through dissolution testing, X-ray diffraction studies, and differential thermal analysis (DTA). Systems giving the highest dissolution profiles are evaluated in vivo using the rabbit as an animal model, and Tegretol® suspension as the reference standard. Plasma levels are evaluated for CBZ and its active metabolite, carbamazepine-10,11-epoxide (CBZE) (Eichelbaum et al., 1985). Relevant pharmacokinetic parameters to assess the bioavailability of the systems relative to Tegretol® suspension are determined for CBZ and CBZE.

2. Materials and methods

2.1. Materials and equipment

The following materials were used in the study: Carbamazepine (CBZ, Ciba-Geigy, Basle, Switzerland); carbamazepine-10,11-epoxide (CBZE, Ciba-Geigy, Ardsley, NY); cyheptamide, internal standard (Supleco, Bellefonte, PA); PEG_{6000} and PEG_{4000} (Breox, Hythe, Southampton, UK); pure synthetic phospholipids (PL) with label claim of 98% purity (Sigma, St. Louis, MO) included L-α-dimyristoylphosphatidyl choline (DMPC), L-α-dimyristoyl phosphatidyl glycerol (DMPG), L-α-distearoylphosphatidyl choline (DSPC), and egg phosphatidyl choline (EPC); hydroxypropyl-β-cyclodextrin (HPβCD) (Pharmatec, USA); absolute ethanol, methanol, and chloroform, super purity solvents (Romil, UK). Tegretol® suspension, batch # 113 (Swisspharma, now Novartis Pharma). All other chemicals were AR grades and used as received.

For in vitro evaluation, USP dissolution tester, Apparatus 1 (Pharma Test, Germany), UV/visible double beam spectrophotometer (Shimadzu UV-240, Kyoto, Japan), X-ray diffractometer (XRD-610 Shimadzu, Japan) operated by a DP-61 computer and a computerized analysis system (Perkin-Elmer, 7 Series Thermal Analysis System) for differential thermal analysis (DTA) were used. For plasma samples, the HPLC system consisted of an isocratic pump (LC-10 AS, Shimadzu, Japan), UV/visible detector (Model SPD 10A, Shimadzu, Japan), and an integrator (Chromatopac C-R6A, Shimadzu, Japan).

2.2. Preparation of carbamazepine systems

CBZ/PEG solid dispersions (Sd.) were prepared by the fusion method (Craig, 1990) involving heating a physical mixture of CBZ and either PEG_{6000} or PEG_{4000} in ratios 1:2, 1:4, 1:6 and 1:8 (drug/carrier) to the liquid state. The fluid mixtures were cooled to room temperature and stored in a desiccator over silica.

Coprecipitates (coppt.) in the ratio 10:1 (CBZ:PL) were prepared by the solvent method (Habib et al., 1992, 1993). Solutions of CBZ and PL in chloroform were mixed, and the mixture evaporated to dryness using a rotavapor at 65°C. DMPC, DMPG, DSPC, and EPC phospholipids were used to prepare the different CBZ/PL coppts. The resultant film was scratched off the flask, and stored in a desiccator over silica.

Complex (Cx.) of CBZ/HPβCD was prepared in a 1:1 molar ratio (Choudry and Nelson, 1992) by the solvent method using absolute ethyl alcohol (Badawy et al., 1994). CBZ and HPβCD equivalent to 236 and 1450 mg respectively were dissolved separately in ethanol and then mixed. The mixture was then evaporated using a rotavapor at 65°C, and the resultant film removed and stored over silica in a desiccator at room temperature.

2.3. In vitro evaluation of carbamazepine systems

Prior to any in vitro evaluation, CBZ systems were freshly prepared and CBZ content was determined in duplicate for each system. An amount equivalent to 10 mg CBZ was accurately weighed and placed in a 10-ml volumetric flask to prepare a 1 mg/ml solution in methanol. Fifty microliters of CBZ solution (1 mg/ml) were added to 5 ml distilled water to yield a theoretical concentration of 10 mg/ml. The sample was measured at λ_{max} of 285 nm, and CBZ concentration was calculated from the standard curve prepared simultaneously. Also, particle size was controlled for the prepared systems as well as plain CBZ powder by grinding using a mortar and pestle, and by passing the powders through sieves number 0.4 mm and 0.1 mm. The powder retained on a 0.1-mm sieve was subject to in vitro and in vivo evaluations.

2.3.1. Dissolution

For each system as well as plain CBZ, dissolution was run in triplicate. An accurately weighed amount of the prepared system equivalent to 200 mg CBZ was placed in each vessel in a modified USP dissolution basket covered with a stainless steel screen (mesh size 100 μm). Dissolution was carried out in 900 ml distilled water. The basket was rotated at 100 ± 5 rev./min, and the temperature of the dissolution medium maintained at 37°C. One-milliliter aliquot samples were withdrawn with replacement at 5, 10, 15, 30, 45, 60, 75, 105 and 120 min. Samples were filtered using 0.45-μm Millipore® filters and properly diluted prior to measuring their absorbance at 285 nm. The corresponding concentrations were determined from the linear regression equation of the standard curve run simultaneously. Standard curves of CBZ in either PEG or HPβCD aqueous solution were prepared and compared to that of plain CBZ in distilled water. For CBZ/PL coppt., the amount of phospholipid is assumed to be too small to affect the UV absorbance of CBZ.

2.3.2. Powder X-ray diffraction

Systems giving the best dissolution profiles were further evaluated with X-ray diffraction. The samples were exposed to Cu Kα radiation (40 kV × 30 mA) at a scan rate of 8 deg/min. A fixed slit system was employed with the following slit parameters: divergence, 1°; scatter, 1°; receiving, 0.3 mm. The output is given as intensity versus 2θ angle. For comparative purposes, the Hanawalt method (Cullity, 1990) was adopted, where the three highest values for relative intensity and their corresponding [d] spacings were compared for the drug, carrier and the corresponding systems. A difference of ± 0.02 for the [d] spacings suggests a change in the crystal lattice of drug and carrier.

2.3.3. Differential thermal analysis

Those systems evaluated by X-ray diffraction were also subject to differential thermal analysis (DTA) to examine any interaction between CBZ and carrier. DTA of each system was compared against that of plain CBZ and the corresponding drug-free carrier. The heating rate used was 3°C/min from 25 to 225°C.

2.4. In vivo studies

Only CBZ systems giving the highest dissolution profiles were subject to in vivo evaluation. A parallel design comprising four groups with five rabbits in each group was adopted using male New Zealand White rabbits. Prior to dose administration, rabbits were fasted overnight in restraining cages to prevent coprophagy. All groups received an equivalent of 100 mg CBZ. Group A received CBZ/HPβCD Cx.; Group B received the lead 10:1 CBZ/PL coppt.; Group C received the lead CBZ/PEG Sd.; and Group D received 5 ml of commercially available Tegretol® suspension, equivalent to 100 mg CBZ. Each of the CBZ systems was suspended in 5 ml water and given orally to the rabbit followed by 2 × 10-ml consecutive aliquots of distilled water used in rinsing the dose-containing beaker. Rabbits receiving the suspension were also given 20 ml of water, thus maintaining the same water intake among all groups. After dose administration, rabbits were kept in restraining cages, and access to food and water was allowed after 6 h. Serial blood samples (2-ml aliquots) were withdrawn from the marginal ear vein at 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, and 24 h post dose and placed in heparinized tubes.

Plasma was harvested by centrifugation, and stored at −20°C until analyzed.

2.5. Sample processing and analytical method

Plasma samples were analyzed for CBZ and CBZE using a published method (Sawchuk and Cartier, 1982) which was modified to render it adaptable to our laboratory conditions. These modifications comprised of: constructing standard curves ranging from 0.25 μg/ml to 12 μg/ml for CBZ and 0.05–2.5 μg/ml for CBZE; using a 300 × 3.9 mm, 10-μm particle size C_{18} reversed phase column (Bondclone, Phenomenex, Torrence, CA), and running the flow rate of 50:50 v/v methanol/water at 2 ml/min using the liquid chromatograph described under equipment. Peak area ratios of CBZ or CBZE to cyheptamide (IS) were plotted versus the corresponding concentrations of the standards. Weighted linear regression was adopted, using the variance at each concentration as the weighting function (Oppenheimer et al., 1983). Sample concentrations of CBZ and CBZE were calculated from the corresponding linear regression equation.

2.6. Data analysis

The plasma concentration–time data were evaluated for CBZ and its active metabolite, CBZE. The following pharmacokinetic parameters were calculated: C_{max}, taken as the highest observed concentration during the study period; T_{max}, taken as the time at which C_{max} occurred, and $AUC_{(0-\infty)}$, determined as the area under the plasma concentration–time curve up to the last measured time point calculated by the trapezoidal rule, plus the residual area calculated as the concentration of the last measured time point divided by the elimination rate constant.

2.7. Determination of relative bioavailability of CBZ and CBZE

For CBZ, $AUC_{(0-\infty)} = FD/CL$ where FD is the bioavailable dose of CBZ and CL is the total body clearance of CBZ. Thus, the relative bioavailability of CBZ for each system is calculated by dividing the mean $AUC_{(0-\infty)}$ of a particular system by that of Tegretol® suspension, the assumption being that CBZ clearance (CL) is the same among all rabbit groups.

For CBZE, according to metabolite kinetics (Houston, 1982), $AUCE_{(0-\infty)} = fm.FD/CL_e$, where FD is the bioavailable dose of CBZ; fm is the fraction of CBZ metabolized via the epoxide, CBZE, and CL_e is CBZE clearance. Thus, the $AUCE_{(0-\infty)}$ ratio of all systems relative to that of Tegretol® suspension gives the relative increase in the term, $F.fm$, assuming that clearance of CBZE (CL_e) is the same among rabbit groups.

Area ratios of CBZE, $AUCE_{(0-\infty)}$, relative to the $AUC_{(0-\infty)}$ of CBZ is: $AUCE_{(0-\infty)}/AUC_{(0-\infty)} = fm.CL/CL_e$. This represents a ratio of the formation clearance of CBZE ($fm.CL$) to its elimination clearance (CL_e). This ratio is calculated and compared for all rabbit groups.

3. Results and discussion

3.1. In vitro dissolution studies

The dissolution of plain CBZ and all prepared systems was carried out in distilled water. Aqueous dissolution of CBZ/PEG solid dispersions in the ratio of (1:2) and (1:8) drug/carrier, gave dissolution profiles that were not significantly different from each other for PEG_{4000} as well as PEG_{6000} at all sampling points during the 2-h dissolution period, $p > 0.05$, using 2-tailed unpaired Student t-test. This suggests that the dissolution of CBZ from the solid dispersion is neither related to the molecular weight nor the weight fraction of PEG. The 1:2 CBZ/ PEG_{6000} was chosen for further evaluation. To examine the effect of PEG_{6000} on the dissolution of CBZ from the Sd., a physical mixture of CBZ and PEG_{6000} in the ratio of 1:2 (drug to carrier) was prepared and its dissolution profile compared to that of the solid dispersion. Also, dissolution of plain CBZ was conducted in 0.444 mg/ml aqueous PEG_{6000} solution, which is equivalent to that concentration of PEG_{6000} resulting from the dissolution of 1:2 CBZ/PEG_{6000} Sd., equivalent to 200 mg CBZ in 900 ml distilled water (dissolution medium). The

different profiles are given in Fig. 1. A worthy point is that the presence of 0.444 mg PEG_{6000}/ml dissolution medium did not show significant enhancement of CBZ dissolution. The higher dissolution profile of the physical mixture relative to the plain drug may be explained by the wetting effect of PEG on plain CBZ. The increased drug release from the solid dispersion relative to the physical mixture demonstrates the interactive nature between the drug and carrier. Thus, the enhancement in dissolution of CBZ/PEG_{6000} solid dispersion may be attributed to complex formation between CBZ and PEG_{6000} during melting and may not have occurred in any other state (i.e. solution), with the solubility of the formed complex being higher than that of plain CBZ. Since CBZ exhibits different polymorphic forms (Umeda et al., 1984; Kaneniwa et al., 1984; Krahn and Mielck, 1987; Lowes et al., 1987), another possible explanation may be a polymorphic change during the preparation of the solid dispersion by fusion, with CBZ crystallizing in a metastable form of higher dissolution rate. PEG binding improves not only water solubility of bound molecules, but also their solubility in organic solvents, therefore, PEG could be used to move molecules across cell membranes. Besides, PEG is non toxic, familiar to living cells and approved by the FDA for internal consumption (Harris, 1992).

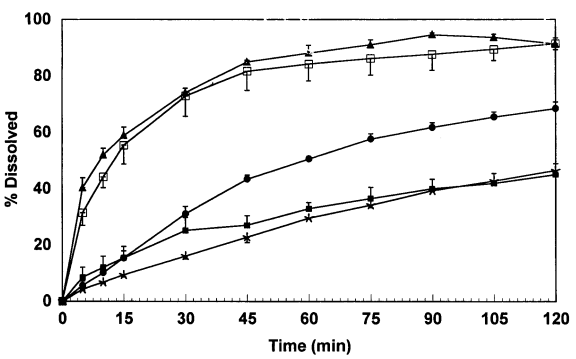

Fig. 1. Dissolution of carbamazepine from CBZ/PEG_{6000} solid dispersion: (■) CBZ in distilled water; (*) CBZ in 0.444 mg/ml aqueous PEG_{6000} solution; (●) 1:2 CBZ/PEG_{6000} physical mixture; (▲) 1:2 CBZ/PEG_{6000} Sd., and (□) 1:8 CBZ/PEG_{6000} Sd.

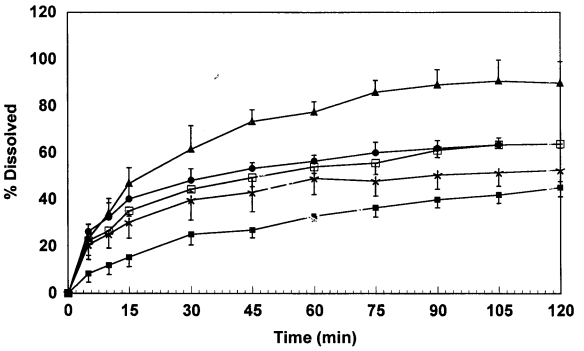

Fig. 2. Dissolution of carbamazepine from CBZ/phospholipid coprecipitates: (■) CBZ; (*) CBZ/DSPC; (□) CBZ/DMPC; (●) CBZ/EPC; and (▲) CBZ/DMPG.

The ratio of 10:1 CBZ to phospholipid was chosen, since higher phospholipid concentrations did not significantly improve dissolution (Biswas et al., 1993), besides, a ratio of 10:1 CBZ/PL was sufficient to disperse the drug in the coprecipitate (Habib et al., 1992). Fig. 2 represents the dissolution profiles of CBZ/DMPG, CBZ/DMPC, CBZ/EPC, and CBZ/DSPC coprecipitates as well as plain CBZ in distilled water. At each time point during the 2-h dissolution period, the four profiles were compared with each other and with those of plain CBZ using the 2-tailed unpaired Student *t*-test. At all points, CBZ/DMPG, CBZ/DMPC, and CBZ/EPC coprecipitates showed significantly higher percentages dissolved than plain CBZ, $p < 0.05$. For CBZ/DSPC coprecipitate, the mean percent dissolved was significantly higher than plain CBZ for the first hour, $p < 0.05$; while during the second hour, the mean percent dissolved from the CBZ/DSPC coppt. was not significantly different from plain CBZ, $p > 0.05$. CBZ/DMPG coppt. gave a higher profile than DMPC and EPC coprecipitates, $p < 0.05$; while CBZ/DMPC coppt. dissolution profile was not statistically different from that of CBZ/EPC at all points, $p > 0.05$. Phospholipids are known to improve the dissolution of a drug by their ability to form liposomes when in contact with water (Venkataram and Rogers, 1984). The bilayer structures formed entrap or sequester a solute, which during the dissolution process, is transported to the diffusion layer, then to the bulk solution then to the site of absorption to yield rapid delivery of a drug to the general

circulation. It is also observed that drug release decreases with the increase in the chain length of the fatty ester in the phospholipid. This explains the lower dissolution profile observed with DSPC relative to the tested phospholipids, as it has the longest chain length. The highest dissolution profile seen with DMPG may be attributed to an electrostatic complex between the net negative charge on the polar head group of DMPG and the amino group of CBZ, thus increasing CBZ concentration in the vicinity of DMPG liposomes and hence increasing its initial dissolution rate (Biswas et al., 1993).

The aqueous dissolution of the 1:1 molar ratio of CBZ/HPβCD Cx. was examined in comparison with plain CBZ in distilled water, as well as plain CBZ in 1.365 mg/ml aqueous solution of HPβCD, corresponding to that concentration of HPβCD resulting from the dissolution of the complex in water. The profiles are given in Fig. 3. At all examined points, the 1 M:1 M CBZ/HPβCD Cx. showed significantly higher percentages dissolved than plain CBZ in distilled water or in 1.365 mg/ml of aqueous HPβCD solution, $p < 0.05$. Thus, the concentration of 1.365 mg/ml HPβCD was too low to exhibit a significant solubilizing effect.

3.2. Physicochemical properties of carbamazepine systems

Fig. 4 summarizes the X-ray diffraction patterns of each system in comparison with plain CBZ and the corresponding carrier. The X-ray diffraction pattern of CBZ is similar to that of β-carbamazepine reported by Lowes et al. (1987). Only the CBZ/HPβCD Cx. exhibited an amorphous diffraction pattern similar to that of HPβCD, suggesting that CBZ was totally entrapped within the complex. The change in the X-ray diffraction pattern of the systems, suggests an interaction between CBZ and the carrier and/or a possible polymorphic change of CBZ, since for all systems, the difference in [d] spacing values was greater than ± 0.02 for the three peaks having the highest relative intensity, which suggests change in the crystal lattice.

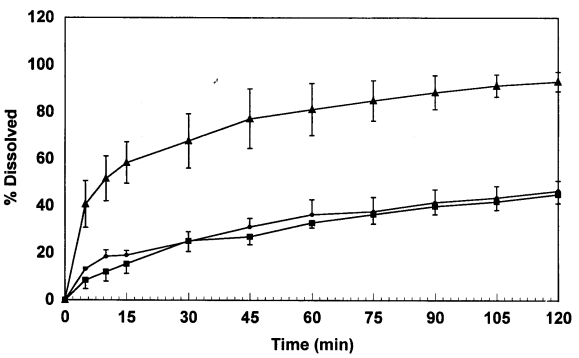

Fig. 3. Dissolution of carbamazepine from CBZ/HPβCD complex: (■) CBZ in distilled water; (●) CBZ in 1.365 mg/ml aqueous HPβCD solution, and (▲) CBZ/HPβCD Cx.

Fig. 5 summarizes the DTA for all systems examined versus plain CBZ and the carrier. The endotherms show disappearance of the characteristic CBZ melting peak or a shift in endotherms, suggesting an interaction. For the 1 M:1 M CBZ/HPβCD complex, the CBZ endotherm was totally absent suggesting that CBZ was totally entrapped inside HPβCD. The same was observed with the CBZ/PEG_{6000} Sd., where the endotherm due to CBZ was absent. For the CBZ/DMPG, endotherms characteristic to CBZ or DMPG were absent, with a new endotherm appearing for the system.

3.3. In vivo data analysis

Good linearity of standard curves was exhibited for CBZ ($r^2 > 0.999$ for all standard curves) and CBZE (r^2 ranged from 0.968 to 0.982) in the concentration range used. Run-to-run standard deviations were used in defining the observed sample variance for purposes of weighting data in calculating the line of best fit (Oppenheimer et al., 1983). The advantage of using weighted least squares is that it does not assume a constant variance over the entire concentration range of interest. Thus, observations with large variance are given less weight than observations with smaller variance, which is appropriate when non-constant variance exists.

A parallel study design was adopted since it was practically impossible to give each rabbit the four different treatments in crossover with long enough wash out periods to prevent any sequence

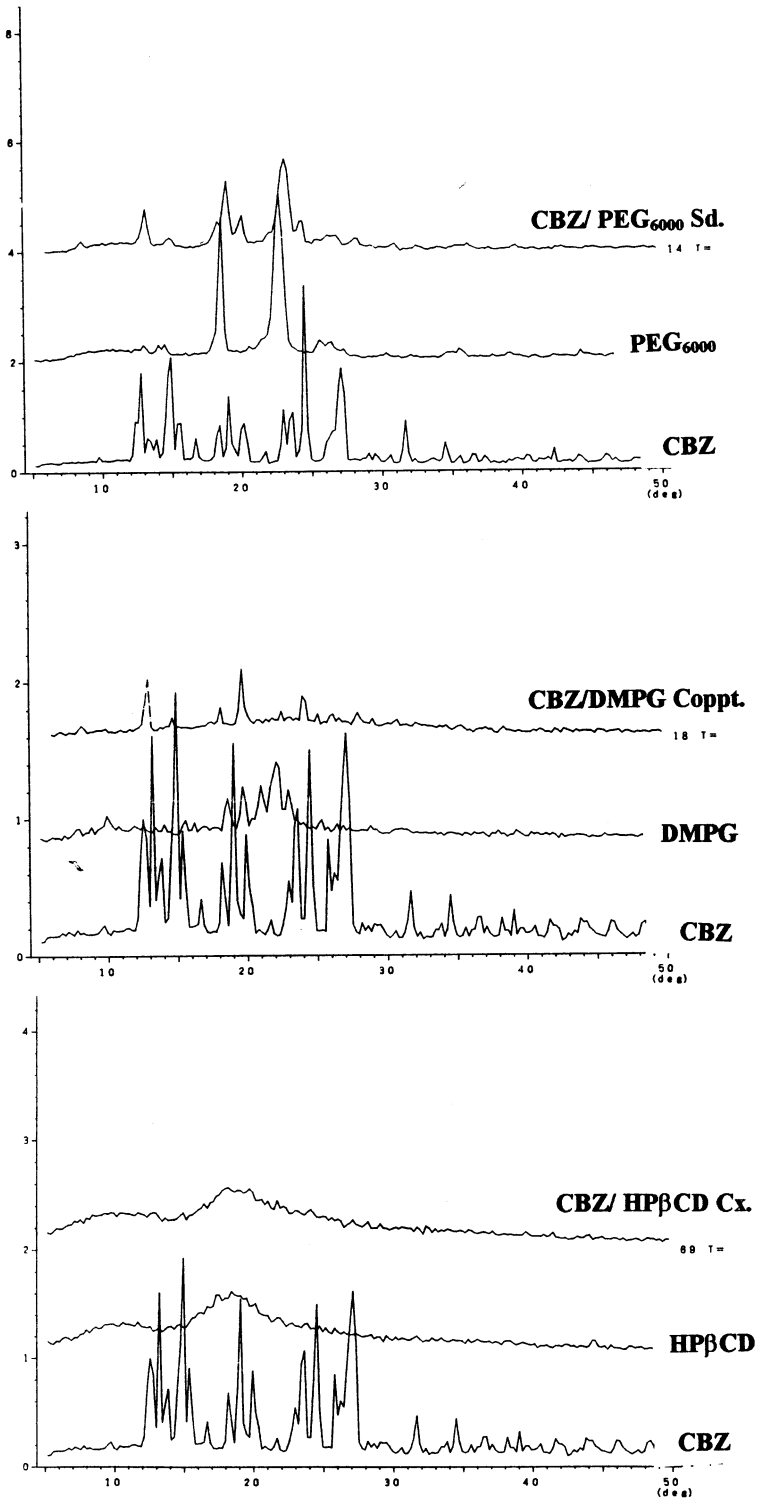

Fig. 4. X-Ray diffraction patterns of carbamazepine systems.

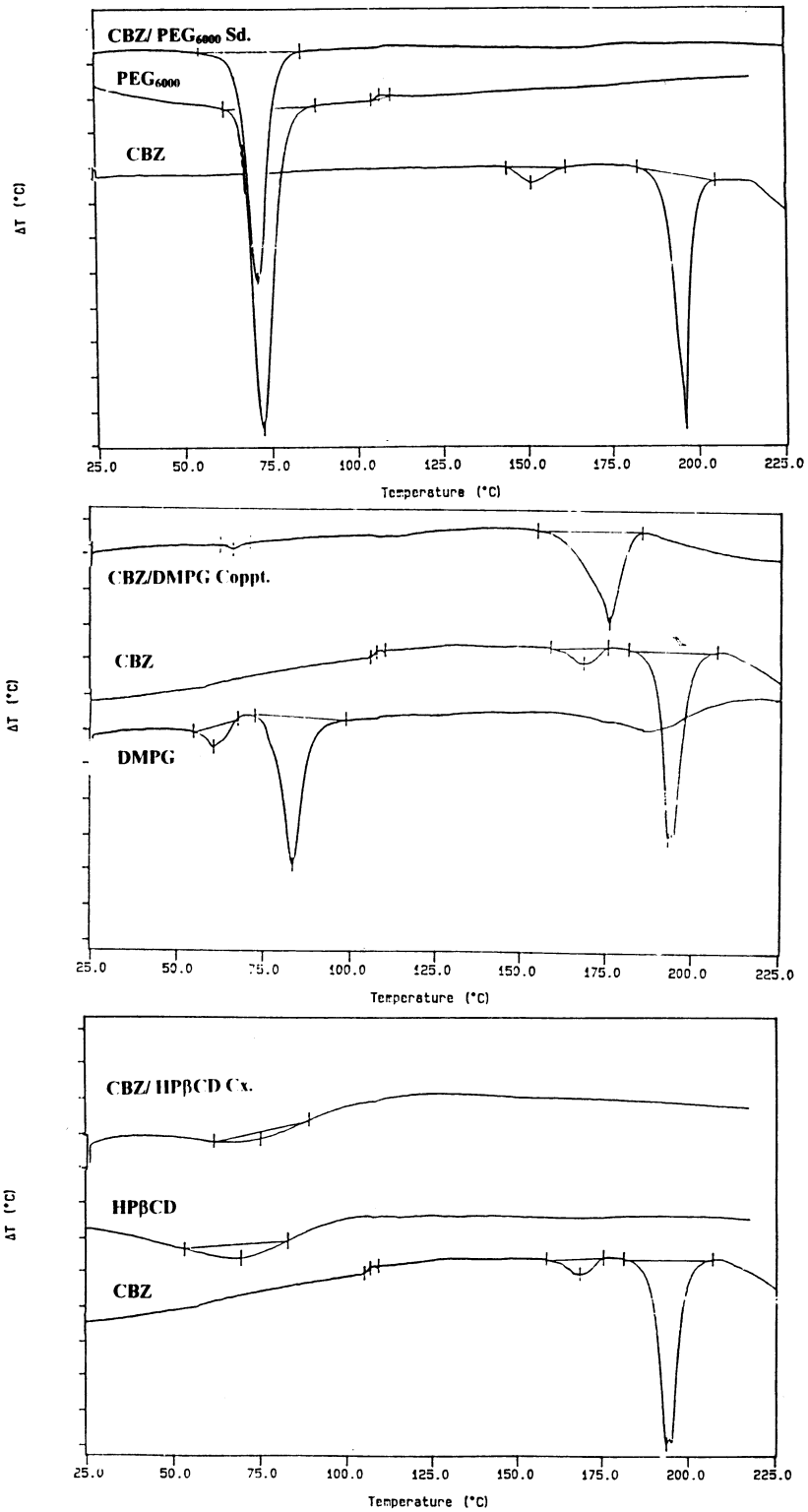

Fig. 5. Differential thermal analysis of carbamazepine systems.

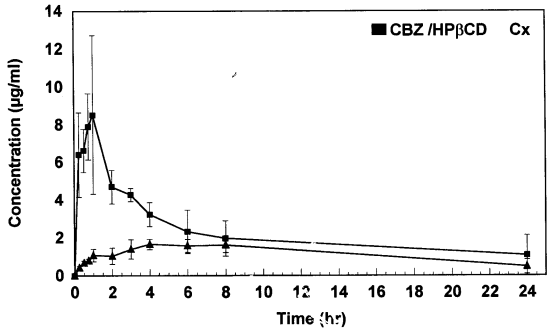

Fig. 6. Mean ± S.D. plasma concentration–time profiles of CBZ (■) and CBZE (▲) in five rabbits following the administration of CBZ/HPβCD Cx. equivalent to 100 mg CBZ.

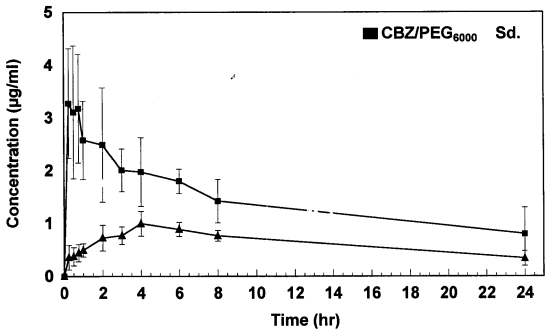

Fig. 8. Mean ± S.D. plasma concentration–time profiles of CBZ (■) and CBZE (▲) in five rabbits following the administration of CBZ/PEG_{6000} Sd. equivalent to 100 mg CBZ.

effect. Mean plasma concentration–time profiles for CBZ and CBZE for all treatments are presented graphically in Figs. 6–9. Pharmacokinetic parameters for CBZ and CBZE are presented in Table 1. This table summarizes C_{max}, T_{max}, $AUC_{(0-24)}$, and $AUC_{(0-\infty)}$ for CBZ and CBZE for the four treatments. Due to lack of enough data points in the terminal portion of the log concentrations between 8 and 24 h, it was not possible to estimate reliably the half life for CBZ and CBZE. Therefore, to extrapolate areas to infinity, literature values for elimination rate constants of 0.420 h^{-1} for CBZ and 0.192 h^{-1} for CBZE were used (Riad, 1989). The $AUC_{(0-24)}$ for CBZ after HPβCD Cx., DMPG Coppt., PEG_{6000} Sd., and Tegretol® suspension accounted for 96 ± 2.2, 96 ± 2.6, 95 ± 1.9 and 97 ± 1.3% respectively of the $AUC_{(0-\infty)}$. For CBZE, the $AUCE_{(0-24)}$ represented 92 ± 4.4, 87 ± 7.5, 88 ± 7.5, and 92 ± 4.4% of the $AUCE_{(0-\infty)}$, for HPβCD Cx., DMPG Coppt., PEG_{6000} Sd., and Tegretol® suspension respectively.

For CBZ, peak plasma concentrations, C_{max}, averaged 9.92 ± 3.48, 3.66 ± 0.702, 3.36 ± 1.09, and 5.49 ± 3.48 μg/ml for CBZ/HPβCD Cx., CBZ/DMPG coppt., CBZ/PEG_{6000} Sd., and Tegretol® suspension respectively. Median CBZ peak times, T_{max}, were 0.875 h (range: 0.75–1.0 h) for CBZ/HPβCD Cx., 1.0 h (range: 0.75–1.0 h) for CBZ/DMPG coppt., 0.25 h (range 0.25–0.75 h) for CBZ/PEG_{6000} Sd. and 0.50 h (range: 0.25–0.75 h) for Tegretol® suspension respectively. Mean $AUC_{(0-\infty)}$ values were 58.2 ± 19.1, 41.2 ± 8.89, 36.9 ± 9.59, and 34.9 ± 11.6 μg.h/ml for HPβCD Cx., CBZ/DMPG coppt., CBZ/PEG_{6000} Sd., and Tegretol® suspension respectively.

For CBZE, peak CBZE plasma concentrations, C_{max}, averaged 1.81 ± 0.232, 1.29 ± 0.426, $1.05 \pm$

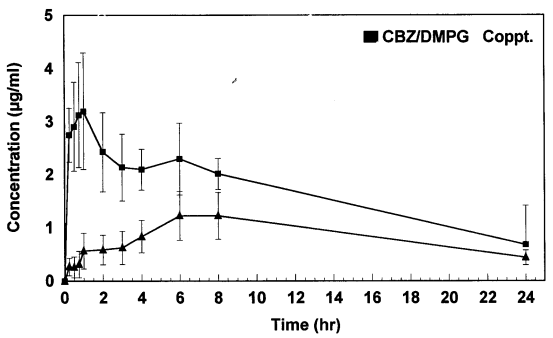

Fig. 7. Mean ± S.D. plasma concentration–time profiles of CBZ (■) and CBZE (▲) in five rabbits following the administration of CBZ/DMPG coppt. equivalent to 100 mg CBZ.

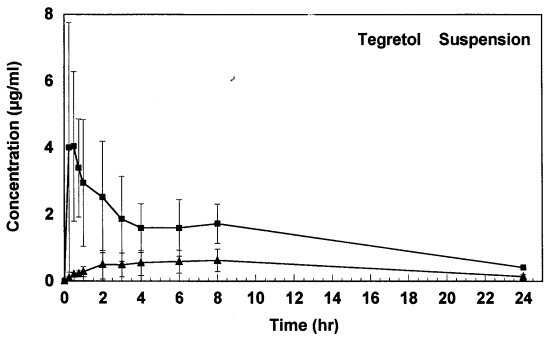

Fig. 9. Mean ± S.D. plasma concentration–time profiles of CBZ (■) and CBZE (▲) in five rabbits following the administration of Tegretol® suspension equivalent to 100 mg CBZ.

Table 1
Mean estimates of CBZ and CBZE pharmacokinetic parameters following oral administration of 100 mg CBZ as (1 M:1 M) CBZ/HPβCD Cx., (10:1) CBZ/DMPG coppt., (1:2) CBZ/PEG_{6000} Sd. and Tegretol® suspension

Parameter	CBZ/HPβCD Cx.	CBZ/DMPG Coppt.	CBZ/PEG_{6000} Sd.	Tegretol® suspension
Carbabamazepine				
T_{max} (h)				
Median	0.875	1.0	0.25	0.50
Range	0.75–1.0	0.75–1.0	0.25–0.75	0.25–0.75
C_{max} (μg/ml)	9.9 ± 3.48	3.67 ± 0.702	3.36 ± 1.09	5.49 ± 3.48
$AUC_{(0-24)}$ (μg.h/ml)	55.5 ± 16.9	39.6 ± 7.60	34.9 ± 8.38	33.9 ± 11.6
$AUC_{(0-\infty)}$ (μg.h/ml)	58.2 ± 19.1	41.2 ± 8.89	36.9 ± 9.58	34.9 ± 11.6
Carbamazepine-10,11-epoxide				
T_{max}				
Median	6	8	4	6
Range	4–8	4–8	4–8	4–8
C_{max} (μg/ml)	1.81 ± 0.232	1.29 ± 0.426	1.05 ± 1.09	0.662 ± 0.364
$AUCE_{(0-24)}$ (μg.h/ml)	27.6 ± 6.58	20.1 ± 6.33	15.9 ± 1.46	10.0 ± 5.07
$AUCE_{(0-\infty)}$ (μg.h/ml)	30.1 ± 8.00	23.0 ± 6.32	18.2 ± 2.09	10.8 ± 5.07

1.09, and 0.662 ± 0.364 μg/ml for CBZ/HPβCD Cx, CBZ/DMPG coppt., CBZ/PEG_{6000} S.D., and Tegretol® suspension respectively. Median CBZE peak times, T_{max}, were 6 h (range: 4–8 h) for CBZ/HPβCD Cx., 8 h (range: 4–8 h) for CBZ/DMPG coppt., 4 h (range 4–8 h) for CBZ/PEG_{6000} Sd. and 6 h (range: 4–8 h) for Tegretol® suspension. Mean $AUCE_{(0-\infty)}$ values were 30.1 ± 8.00, 23.0 ± 6.32, 18.2 ± 2.09, and 10.8 ± 5.07 μg.h/ml for HPβCD Cx., CBZ/DMPG coppt., CBZ/PEG_{6000} Sd., and Tegretol® suspension respectively.

One tailed Student t-test was used to compare pharmacokinetic parameters among the different systems in a pair wise manner with α set at 0.05. A statistical summary is given in Table 2. The $AUC_{(0-\infty)}$ values for CBZ were compared for the three systems and the Tegretol® suspension. It was found that after administration of the HPβCD complex, the resultant $AUC_{(0-\infty)}$ was significantly higher than the (1:2) CBZ/PEG_{6000} solid dispersion and the Tegretol® suspension, but not significantly different from that resulting after administration of CBZ/DMPG coprecipitate. All other $AUC_{(0-\infty)}$ pairs were found to be non-significantly different from each other, $p > 0.05$. On the other hand, it was found that the $AUCE_{(0-\infty)}$ values for CBZE for all prepared systems were significantly higher than that resulting after administration of Tegretol® suspension, $p < 0.05$. No significant difference was observed with areas under the epoxide concentration–time curves for CBZ/PEG_{6000} and CBZ/DMPG pairs as well as CBZ/HPβCD and CBZ/DMPG.

Regarding C_{max} for CBZ plasma levels, it was observed that C_{max} resulting from administration of the HPβCD complex was significantly higher from the other two prepared systems as well as the Tegretol® suspension. All other pairs compared showed no significant difference between the corresponding C_{max} values. C_{max} values for CBZE were compared pair wise, and it was found that all pairs compared were significantly different from each other except for CBZ/DMPG and CBZ/PEG_{6000}. A summary of the statistics is presented in Table 2.

Due to the lack of enough data in the absorptive phase, the absorption rate constant for CBZ could not be estimated accurately. Therefore, $C_{max}/AUC_{(0-24)}$, an effective metric in evaluating the absorption rate (Tothfalusi and Endrenyi, 1995), was calculated and compared for all systems as well as Tegretol® suspension. The mean $\pm$ S.D. estimates were 0.19 ± 0.064, 0.093 ± 0.013, 0.10 ± 0.042, and 0.16 ± 0.091 h^{-1} for CBZ/HPβCD Cx., CBZ/DMPG coppt., CBZ/PEG_{6000} Sd., and Tegretol® suspension respectively.

Table 2
Statistical summary of pharmacokinetic parameters

Groups compared	$AUC_{(0-\infty)}$	$AUCE_{(0-\infty)}$	C_{max} CBZ	C_{max} CBZE
Group A vs. B	Non Sig.	Non Sig.	Sig.	Sig.
Group A vs. C	Sig.	Sig.	Sig.	Sig.
Group A vs. D	Sig.	Sig.	Sig.	Sig.
Group B vs. C	Non Sig.	Non Sig.	Non Sig.	Non Sig.
Group B vs. D	Non Sig.	Sig.	Non Sig.	Sig.
Group C vs. D	Non Sig.	Sig.	Non Sig.	Sig.

Group A: Rabbits receiving (1 M:1 M) CBZ/HPβCD Cx.; Group B: Rabbits receiving (10:1) CBZ/DMPG coppt.; Group C: Rabbits receiving (1:2) CBZ/PEG_{6000} Sd.; Group D: Rabbits receiving Tegretol® suspension.

3.4. Determination of relative bioavailability of CBZ and CBZE

The relative bioavailability of each CBZ system to Tegretol® suspension was calculated. Estimates for CBZ/HPβCD Cx, CBZ/DMPG coppt., and CBZ/PEG_{6000} Sd. were 1.66, 1.18, and 1.06 respectively. Therefore, only the HPβCD complex is considered to have a higher bioavailability than the suspension.

Also, $AUCE_{(0-\infty)}$ ratios for all systems relative to Tegretol® suspension were calculated and were found to be 2.79, 2.14, and 1.68 for CBZ/HPβCD Cx., CBZ/DMPG coppt., and CBZ/PEG_{6000} Sd. respectively.

Mean $\pm$ S.D. area ratios of CBZE, $AUCE_{(0-\infty)}$, relative to the $AUC_{(0-\infty)}$ of CBZ calculated for all systems and Tegretol® were 0.52 ± 0.072, 0.57 ± 0.13, 0.52 ± 0.14, and 0.33 ± 0.091 for CBZ/HPβCD Cx., CBZ/DMPG coppt., CBZ/PEG_{6000} Sd., and Tegretol® suspension respectively. The area ratios for all prepared systems were not significantly different from each other, but were significantly higher than that after Tegretol® suspension, which may suggest an increase in *fm*, the fraction of CBZ metabolized via the epoxide, rather than an increase in *CL* or a decrease in CL_e.

It is unlikely that CBZ is metabolized in the intestinal lumen to CBZE, since CBZ perfused through rabbit small and large intestine did not show detectable CBZE concentrations in the perfusate samples (Riad and Sawchuk, 1991). On the other hand, the absorption of CBZ after administration of Tegretol® suspension is dissolution-rate limited, thus absorption may occur further down in the gastrointestinal tract and as low as the rectum (Graves et al., 1985; Riad et al., 1986), bypassing the liver. CBZ in the prepared systems dissolves faster and is therefore absorbed earlier in the GI tract thus suffering higher first pass than the suspension.

Therefore, had the ratio of $AUCE_{(0-\infty)}$ to $AUC_{(0-\infty)}$ (ratio of relative formation and elimination clearances of CBZE) for the different systems been similar to that of Tegretol® suspension (i.e. 0.309), the theoretical mean $AUC_{(0-\infty)}$ would have been 94, 76, and 62 μg.h/ml for the HPβCD Cx., DMPG coppt., and the PEG_{6000} Sd. respectively. Thus, although a significantly higher bioavailability was expected for the prepared systems based upon their dissolution profiles, the reduced bioavailability observed may be explained by a higher ratio of the absorbed CBZ dose being metabolized via the epoxide pathway for all the tested CBZ systems.

4. Conclusions

It may therefore be concluded that the dissolution of CBZ from (1:2) CBZ/PEG_{6000} Sd., (10:1) CBZ/DMPG coppt., and (1 M:1 M) CBZ/HPβCD Cx. is significantly increased as shown from the dissolution profiles. Also, X-ray diffractometry and DTA revealed an interaction between CBZ and carrier, with the possibility of a polymorphic change in CBZ for some systems. In vivo evaluation showed that HPβCD gave the highest $AUC_{(0-\infty)}$ among the tested systems. All systems

showed significantly higher $AUCE_{(0-\infty)}$ in comparison with the Tegretol® suspension due to improved dissolution and faster absorption thus suffering significant first-pass by the liver.

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