Carbamazepine (CBZ) (5H-dibenz[b,f]azepine-5-carboxamide) has been routinely used clinically in the treatment of trigeminal neuralgia and epilepsy since 1965. When CBZ is given in solution in ethanol or propylene glycol or in aqueous suspension, its absorption is rapid from GI tract with peak plasma levels of 1-7 h (1-3). However, absorption of drug from commercially available tablets formulations seems to be sluggish, as the peak plasma level varies from 6 to 24 h (4, 5). This is probably due to poor aqueous solubility of CBZ, which may be reflected in differences in the rates of dissolution and absorption of the drug from different tablet formulations. Not surprisingly, its oral bioavailability depends on dissolution (6), which is affected by the crystalline form used in the dosage form (7). The extremely low solubility is also responsible for the incomplete, slow and erratic gastrointestinal absorption (8). Owing to its narrow therapeutic index as well as relatively high variation in drug plasma concentration (9), a uniform distribution of CBZ in the solid dosage form and reproducible dissolution rate are essential for achieving the desired therapeutic effect without high risk of toxicity.

In recent years, pharmaceutical cocrystals have emerged as a potential strategy to boost the solubility concerns of weakly soluble drugs (10). CBZ is a BCS class II drug, thus shows dissolution limited bioavailability, and thus cocrystals with a number of soluble coformers have been reported and extensively studied in order to improve the CBZ solubility. About 40 different coformers have been accounted in the literature that formed cocrystals with CBZ (11). Hickey and co-workers studied the in vivo performance of carbamazepine-saccharine (CBZ-SAC) cocrystal in comparison to brand product Tegretol®. The cocrystal in powder form was found to be bioequivalent as it gave similar oral bioavailability in four dogs to that of marketed immediate release (IR) product (11). Jung et al. also observed similar results with indomethacin-saccharine (IND-SAC) cocrystal, outcomes of the study revealed that the bioavailability of cocrystal was above pure indomethacin powder but was found to be equivalent to that of the

**RELATIVE BIOAVAILABILITY STUDY OF SUCCINIC ACID COCRYSTAL TABLET AND MARKETED CONVENTIONAL IMMEDIATE RELEASE TABLET FORMULATION OF CARBAMAZEPINE 200 MG IN RABBITS**

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**Abstract:** A single-dose study was performed to observe the bioequivalence of the newly formulated carbamazepine-succinic cocrystal (CBZ-SUC) immediate release tablet (F1) with marketed immediate release formulation Epitol® 200 mg tablet (F0). In this study on albino rabbits, the plasma levels resulting from 250 mg cocrystal equivalent to 200 mg of carbamazepine and conventional tablets 200 mg immediate release tablets were compared. An open-label, randomized 2 × 2 crossover study design, with a 1-week washout period, was used. Carbamazepine (CBZ) plasma concentrations were determined by a high-performance liquid chromatography validated method using ultraviolet detection. CBZ plasma levels were measured at predose and various postdose time points up to 72 h and the following pharmacokinetic parameters were used for evaluation: area under the curve (AUC), maximum plasma drug concentration (Cmax), time to achieve Cmax (tmax), and elimination rate constant (Kₑ). By applying paired t-test to AUC₀₋₇₂ (calculated by linear trapezoidal rule), the experimental formulation F1 was found to have statistically significant (**p < 0.05**) improvement in bioavailability of CBZ. However, these statistical differences do not have practical implications and the two formulations (F₀ and F₁) were found to be bioequivalent as the relative bioavailability of both formulations (106.9%) falls within the acceptable FDA set range of two bioequivalent products 80-125%.

**Keywords:** carbamazepine, cocrystal, bioequivalence

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IND immediate release commercial formulation - Indomee® (12). Similarly, in our recent study on bioavailability of CBZ powder, carbamazepine-succinic (CBZ-SUC) cocrystal powder filled in ‘0’ sized capsule shells, the bioavailability of CBZ-SUC cocrystal powder was almost double to that of CBZ powder and was equivalent to marketed product Epitol® tablets 200 mg in four healthy rabbits n = 4 (12).

The widespread approach for in vivo evaluation of cocrystals is centred on deliberately excluding additional formulations so as to compare the ‘neat’ aqueous cocrystal suspension or filling ‘neat’ cocrystal into capsule shells of suitable sizes. But, studies on cocrystals conducted with the intention of using this solid in a proper drug product are odd. This work aims to use this solid form in tablet dosage form and compare its performance with marketed formulation of CBZ in order to facilitate and ease the use of this solid form as a podium in dosage form design. Tablet formulation development is usually time and resource intensive. We were encouraged by recent study on mefloquine (MFL) where significant improvement in the dissolution rate was observed in cocrystals tablets over pure MFL tablets (13). In this work, we used intrinsic dissolution rate (IDR) as a material-sparing tool to guide appropriate polymer for an efficient tablet formulation development of an inherently unstable cocrystal, and compared its in vivo performance to the marketed immediate release tablet formulation of carbamazepine i.e., Epitol® tablets 200 mg.

EXPERIMENTAL

Materials

Kollidon VA® 64 (Lot No. 46581856Po) was purchased from BASF SE, Germany. Carbamazepine (Lot No. SLBB3655V) was received from Sigma Aldrich, USA. Croscarmellose sodium (Lot TN08819630) and Avicel PH-102® (Lot No. XN06817380) were obtained from FMC Biopolymer, USA. Lactose monohydrate, spray dried (Lot No. 8508091061) was purchased from Foremost Farms, USA. Magnesium stearate (Lot No. J03970) was procured form Mallinkrodt, USA.

Formulations

Slurry crystallization and liquid assisted grinding methods were used for cocrystal synthesis, phase purity of cocrystal were confirmed by solid state characterization techniques powder x-ray crystallography (PXRD), Raman spectroscopy, FTIR, and thermogravimetric analysis (TGA). Final tablet formulation contained 5 : 1 cocrystal to polymer (Kollidon® VA/64) ratio and was finalized based on IDR and in vitro studies of prototype formulations in comparison to Epitol® tablets 200 mg in physiologically relevant 900 mL of modified simulated intestinal fluid (SIF containing 0.2% sodium lauryl sulfate) (12).

In vivo evaluation

Ethical approval

For in vivo studies, the protocols used were approved by Research Ethical Committee (Ref. No. PHM-0023/E.C/M-4), Department of Pharmacy, COMSATS Institute of Information and Technology, Abbottabad. These studies were conducted in agreement with Helsinki declaration and Animal Scientific Procedure Act (1986 UK).

Design of the study

This study was an open-label, single-dose, randomized, 2-period, 2-sequence, crossover design under fasting conditions.

Subjects and treatments

Albino rabbits (n = 4 for each treatment) aged between 1.5 to 2.5 years, having body mass indices 1.8-2 kg, were isolated for two weeks before initiation of the experiment. During the experimental period, the animals were maintained on fresh green fodder thrice a day and water was provided ad libitum. All animals were kept under the same experimental conditions with natural day and night cycle. After an overnight fasting period, these subjects were given single oral doses of the 2 treatments: F1 and F0 (eq. to 200 mg of CBZ). The treatments were taken apart by one-week washout periods.

Blood samples were collected in heparinized test tubes by jugular vein puncture prior to and after administration of the drug at predetermined time point i.e., 0, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 h. The collected samples were centrifuged at 3500 rpm for 5 min, 200 µL of serum was collected in Eppendorf tube and stored at -20°C for further studies. The extraction of drug was carried out with acetonitrile (1 : 1), vortexed for 90 s and centrifuged at 12000 rpm for 10 min and were analyzed by HPLC (14).

Analysis of drug in plasma samples

For chromatographic separation, Phenomenex Luna® 5 µm (particle size) C18 having pore size 100 Å, LC column (250 × 4.6 mm) was used. Separations of drug in plasma samples were carried out by slight adjustment of already reported method.
Relative bioavailability study of succinic acid cocrystal tablet and...

Mobile phase used was methanol : water (50 : 50, v/v). The temperature of the column oven was maintained at 50°C and flow rate was kept at 1 mL/min. For HPLC analysis, 25 µL of aliquot was injected into the column. Drug concentrations were detected at 285 nm. Under the prescribed chromatographic conditions CBZ eluted at 12.5 min (15).

**Statistical analyses**

Non-compartment model was utilized for analysis of serum drug concentration versus time data. Kinetica® was used for the calculation of various pharmacokinetic parameters, i.e., AUC, Cmax, tmax and K. Normal log of AUC and Cmax was taken for determination of relative bioavailability. Moreover,
significance of difference was demonstrated by using SPSS version 19.0. Paired ‘t’ test with p < 0.05 was applied.

RESULTS AND DISCUSSION

All solid state characterization techniques (PXRD) and thermal technique (TGA) authenticated the formation of carbamazepine-succinic acid CBZ-SUC cocrystal in accordance with all reported reference data. The solubility of this soluble cocrystal has been reported to be 4.5 times higher than carbamazepine dihydrate (CBZDH) at 25°C at pH 3 (15-18). Recently, the solubility behavior of CBZ-SUC cocrystal in four different buffers at 37°C i.e., simulated intestinal fluid (SIF), simulated gastric fluid (SGF), phosphate buffer pH 1.2 and pH 6.8 phosphate buffer for 24 h have been examined, and CBZ-SUC cocrystal demonstrated higher aqueous solubility in all the four buffers studied than the stable CBZ dihydrate form (13). Solid state analysis of solid residue confirmed CBZ dihydrate formation in three buffers while the cocrystal maintained its integrity in SGF only and gave maximum concentration of CBZ than all other buffers. Similarly, in our recent study on solution stability of CBZ-SUC cocrystal with and without added polymers, the cocrystal converted immediately to the stable carbamazepine dihydrate form in three buffers while the cocrystal maintained its integrity in SGF only and gave maximum concentration of CBZ than all other buffers. Similarly, in our recent study on solution stability of CBZ-SUC cocrystal with and without added polymers, the cocrystal converted immediately to the stable carbamazepine dihydrate form immediately as confirmed by in situ Raman spectroscopy and off line PXRD and FTIR techniques (12). Childs et al., have also reported similar results (1). Regardless of aforesaid solubility advantages presented by CBZ-SUC cocrystal, no study has been reported in literature that describes concrete formulation approach, that thermodynamically stabilizes the cocrystal to translate in vitro higher aqueous solubility of this cocrystal system, when used in a suitable medical application. Therefore, we opted for this soluble cocrystal system, and devised suitable tablet formulation with proper crystallization inhibition/solubility enhancing polymer Kollidon® VA/64 (5 : 1 cocrystal : polymer ratio with added excipients) and studied its bioequivalence study to carbamazepine tablets in rabbits.

Plasma concentration-time curves of Epitol® tablets and experimental formulation F1, after oral administration at equal doses of 200 mg of CBZ/rabbit (250 mg of CBZ-SUC is equivalent to 200 mg of CBZ) are demonstrated in Figure 1, and pharmacokinetic (PK) parameters are shown in Table 1. Comparison of PK parameters of F1 with F0 tablets demonstrated that values of AUC0-72 and Cmax of F1 were higher than marketed formulation F0. AUC0-72 of F0 and F1 were found to be 58 ± 2 µg.h/mL and 64.18 ± 1.9 µg.h/mL, while Cmax values were in the order of 4.9 ± 0.35 µg/mL and 5.3 ± 0.3 µg/mL, respectively. Similarly, Tmax of F0 reduced from 5.3 ± 0.31 of F0 tablets to 3.9 ± 0.43. By applying paired ‘t’ test (two tailed), the means of the four pairs of bioavailability of F1 and F1 were found to be significantly different statistically (***p < 0.05) as shown in Figure 2. This increase in AUC0-72 of F1 formulation is due to the stability of cocrystal caused by polymer in the formulation due to hydrogen bonding between the polymer and cocrystal (data not shown here), and as cocrystal is more soluble than the CBZDH, thus, resulted in statistically significant improvement in bioavailability. We attribute the improved drug release from the experimental tablets formulation F1 to the higher dissolution rate, which leads to improved in vivo performance of CBZ than F0. These findings were promising since cocrystal given as pure powder filled in capsule shells did not yield the required results and formulation with crystallization inhibitor polymer improved in vivo performance of cocrystal. More studies are needed to explore the functions of excipients on phase transition of cocrystals in order to maximize the benefits offered by cocrystals. The relative bioavailability calculated for F1, with the marketed product F0, was found to be 106.9%. Thus, the main end point was bioequivalence, as bioequivalence is believed to be established if 90% confidence intervals (CIs) for AUC and Cmax (ln-transformed ratios) fall within the 80-125% range (17-20).

Table 1 Pharmacokinetic parameters of F0 and F1 in albino rabbits, obtained after single oral administration of single dose (200 mg of CBZ/animal, n = 4).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>AUC0-72 (µg.h/mL)</th>
<th>Cmax (µg/mL)</th>
<th>Tmax (h)</th>
<th>Ke (/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0 (tablet)</td>
<td>58 ± 2.00</td>
<td>4.9 ± 0.35</td>
<td>5.3 ± 0.31</td>
<td>0.04</td>
</tr>
<tr>
<td>F1 (tablet)</td>
<td>64.18 ± 1.9</td>
<td>5.3 ± 0.3</td>
<td>3.9 ± 0.43</td>
<td>0.05</td>
</tr>
</tbody>
</table>

AUC0-72 = area under the plasma concentration vs. time curve from time 0 extrapolated to 72 h; Cmax = maximum plasma concentration; Ke = elimination rate constant; Tmax = time to reach Cmax.
CONCLUSION

There is a dire need for using cocrystals as an alternative solid form in pre-formulation studies; however, the selection of a cocrystal strategy at the pre-clinical formulation stage over other available formulation techniques for improving bioavailability has been relatively rare. Cocrystal tablet formulation F1 was bioequivalent to the marketed immediate formulation F0 under fasting conditions. Formulation of soluble cocrystal with crystallization inhibitor polymer improved in vivo performance of cocrystal. These findings are encouraging, to use more soluble cocrystal of otherwise unstable cocrystal system in tablet formulations without phase transition to stable polymorph or parent drug by judicious selection of appropriate polymers.

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