Pharmacokinetic Study of Hydroxypropylmethylcellulose Microparticles Loaded with Cimetidine

Abstract

Objectives. The objective of this study was to assess the pharmacokinetic behavior of floating hydroxypropylmethylcellulose microparticles loaded with cimetidine (FMC) prepared using the non-solvent addition coacervation technique.

Material and Methods. Based on the physico-chemical characteristics of three formulations (FMC1, FMC2 and FMC3), FMC2 having a 1:3 ratio of cimetidine:HPMC was found optimum. For in vivo analysis, a new HPLC analytical method was developed and validated. The optimized formulations were subjected to in vivo studies to calculate the various pharmacokinetic parameters for developed optimized microparticulate formulation FMC3. The developed floating microparticles of cimetidine were further evaluated by in vivo experimentation.

Results. The bioavailability parameters were found as: $C_{\text{max}}$ 1508.79 ± 37.95 ng/ml, $T_{\text{max}}$ 3.67 ± 0.17 h and AUC 14366.19 ± 377.64 ng h /mL.

Conclusions. For prolonged drug release in the stomach, developed floating microparticles of cimetidine (FMC3) may be used, thereby improving the bioavailability and patient compliance (Adv Clin Exp Med 2013, 22, 1, 41–45).

Key words: microparticles, hydroxypropylmethylcellulose (HPMC), cimetidine, pharmacokinetics, human plasma, HPLC method.
This study is the second part of the microencapsulated formulation development of cimetidine and its pharmacokinetic assessment in humans. A previously published study involved formulation development and in vitro characterization [1]. For in vivo study, the RP-HPLC procedure for the quantitation of cimetidine from human plasma was developed and applied to pharmacokinetic studies. Cimetidine is a histamine H2 receptor antagonist (H2 blocker) that is extensively used for the management of gastro-esophageal reflux disorders, ulcers and heartburn [2, 3].

Such a study would be important since it should provide useful information on drug blood levels [4], which could influence the use of this developed formulation in humans. Furthermore, some dosage modifications may be required based on the pharmacokinetic data for patients [5].

The first phase of this study was comprised of the preparation of floating microparticles loaded with cimetidine (FMC) for oral delivery followed by their in vitro evaluation. The FMC were prepared using the non-solvent addition coacervation method and hydroxypropylmethylcellulose (HPMC) as the rate controlling polymer. Consequently, this study was conducted to assess the pharmacokinetic behavior of cimetidine in a healthy male human following a single oral dose administration, compare the data obtained with those reported in a couple of other studies, and propose a more coherent dosage regimen, if needed.

Material and Methods

Development and Physicochemical Analysis of Formulations

To prepare floating microparticles loaded with cimetidine (FMC), hydroxypropylmethylcellulose (HPMC, 40–60 cP, supplied by Fluka-Switzerland) was dissolved in dichloromethane (1 g of polymer per 20 ml, Merck-Germany) at room temperature and cimetidine (1 g, Stand Pharma, Lahore, Pakistan) was added to the polymer solution. For phase separation, liquid paraffin (two times the volume of dichloromethane, (BDH, UK)) was added gradually into the drug polymer solution with continuous stirring for 2 h using a magnetic stirrer set at 700 rpm, resulting in the formation of microparticles. The microparticles were filtered, washed with n-hexane (Merck-Germany) and dried in air and an oven at 40°C to ensure complete removal of all solvents. The microparticles (FMC1, FMC2 and FMC3) with different drug to polymer ratios (1:1, 1:2 and 1:3) were fabricated and tested using in vitro approaches for the selection of an optimum formulation. The dried microparticles were tested for percentage yield, percentage buoyancy, drug content, size distribution, in vitro drug release behavior, polymer-drug interaction, micromeritics, and stability studies. In-detail methodologies of these tests have already been presented [1].

Preparation of Mobile Phase, Stock/Working Solution and Plasma Extraction Process for HPLC Analysis

The mobile phase consisted of a phosphate buffer (K2HPO4, 0.05 M) By using ortho-phosphoric acid, the pH of the mobile phase was adjusted to 3.0. Then 0.2% triethylamine and 11% acetonitrile were added to the mobile phase. After sonication (Elma D78224, Germany), the mobile phase was filtered through a vacuum filter assembly (Sartorius Goettingen, Germany) by using a cellulose acetate filter (0.45 μm).

To prepare a stock solution, 100 mg of cimetidine was dissolved in 100 mL of methanol. Working solutions were prepared in methanol by appropriate dilutions of stock solution to 39, 78, 156, 312, 625, 1250, 2500 and 5000 ng/mL. All the solutions were kept at –20°C and protected from light.

To 500 μL plasma, 500 ng of cimetidine / 0.5 mL distilled water was added and vortexed for 2 min. Twenty μL perchloric acid was added to 500 μL plasma and vortexed for 1 min followed by centrifugation at 40 rpm for 10 min. The vial was briefly shaken and the content was filtered using a sample filtration assembly. Then 20 μL samples were injected into a HPLC port (Agilent 1100 Series, Germany). To construct a standard curve, working plasma samples were also prepared using serial dilutions. The samples were introduced into a rheodyne 20 μL fixed loop injector with a 50 μL glass syringe. The chromatographic separation conditions were: ambient temperature, Guard Column (Uniguard TEC) and ODS hypersil C18 stainless steel analytical column (5 μm pore size, 4.6 mm × 250 mm (Thermo Electron Corporation, UK).

Validation of HPLC Method

HPLC method validation guidelines [6] were trailed to validate this newly developed reverse phase HPLC method by determining the following parameters: linearity, intra-day and inter-day, precision and stability. Freeze–thaw stability testing of plasma samples (39 and 5000 ng/mL samples) was conducted at −20°C for two months. During this period of stability testing, these samples were left to thaw absolutely and after melting they were put back into the freezer. This cycle was replicated thrice at different time intervals in two month.
Experimental Design and Procedure for *in vivo* Studies

After a brief presentation about experimental procedures, eighteen healthy (according to their medical history and complete medical examination) male human volunteers [weight = 61 ± 3.7 kg, age = 22 ± 4.2 years] gave written consent to participate in this pharmacokinetic study as study subjects. A single dose of the optimum formulation (FMC3) (equivalent to 400 mg cimetidine) was administered orally on an empty stomach (after an over-night fast). All the subjects were retained at the study center from one hour prior to 24 h after the dosing. A standardized breakfast (4 pieces of toast with 3 teaspoons of butter, 2 scrambled eggs and two cups of milk) and lunch [7] was served to all subjects.

For the collection of blood samples, a 20-gauge venous catheter was inserted into a forearm of each subject and 3 mL blood samples were taken at predetermined time points as: 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 18 and 24 h after administration. Blood samples were centrifuged at 45 rpm for 10 min to and separate plasma which was stored at −20°C until further analysis. The plasma extraction and chromatographic separation conditions are mentioned above. Ethical approval of this study (M.Phil. 28:UB/2010) was obtained by the Board of Advance Studies and Research, the Islamia University of Bahawalpur, Pakistan.

Pharmacokinetic and Statistical Analysis

The sample data was normally distributed having homogeneity of variance. The results are presented as mean ± standard deviation. Pharmacokinetic analysis of plasma drug concentration versus time data was conducted by using the software Kinetica. One-way ANOVA (parametric analysis of variance) for significance at p < 0.05 was used for variation analysis using SPSS version 12.0.

Results

Development and Physicochemical Analysis of Formulations

The microparticles obtained exhibited the round and smooth surface of microparticles. A significant increase (p < 0.05) in size and encapsulation yield was observed with the increase in polymer quantity, which could be attributed to an increase in the thickness of the polymer covering around the core drug resulting in the increased capture of drug molecules. There was a non-significant (p > 0.05) variation in percentage yield for the formulations developed. The microparticles floated for an extended time on the surface of the dissolution medium showing a buoyancy of 63.40 to 67.70% for all three formulations [1].

An *in vitro* dissolution test was conducted by following sink conditions and making the microparticles sink in the dissolution medium using stainless steel sieves. The cumulative percentage drug release after dissolution of 24 h was greater than 80%. The rate of cimetidine release decelerated with the increase in polymer quantity employed, due to an increase in hydrophobic encapsulating wall thickness around the drug molecules. The drug release initiated with HPMC swelling, the creation of channels acting as the route for the drug and then in due course the cimetidine leached out into dissolution medium [1]. These results were in accordance with the previous presentations [8, 9]. Among all kinetic models, Higuchi’s model was best fit to the dissolution data as evident from the highest values of $R^2$ elaborating diffusion controlled release of the drug from the formulations. The diffusion controlled release of cimetidine from the microparticles was also corroborated from the values of “n” (n < 0.45) calculated using the Korsemeyer-Peppas model [1].

X-ray diffraction patterns and DSC thermograms of FMC, cimetidine and HPMC confirmed the absence of any chemical interaction between drug and polymer [1].

Results of the micromeritic studies i.e. tapped density, Hausner’s ratio, compressibility index and angle of repose, elaborated that the microparticles developed had excellent flowability and compressibility [1].

The stability studies showed that the microparticles remained stable for 3 months at 40 ± 1°C (HT) and 25 ± 1°C (RT), showing non-significant (p > 0.05) changes in percent residual drug content [1].

HPLC Method Validation

The linear calibration curve for cimetidine in human plasma (n = 3) was obtained at various concentrations: 39 ng/mL, 78 ng/mL, 156 ng/mL, 312 ng/mL, 625 ng/mL, 1250 ng/mL, 2500 ng/mL, and 5000 ng/mL. This curve exhibited a good linearity [regression coefficient ($R^2$) = 0.9973] regarding regression analysis of the peak-area of the drug in plasma versus its concentration (Fig. 1). The regression equation was: $y = 0.4245x + 61.6$.

To analyze intra-day and inter-day reproducibility/precision (relative standard deviation = 100×SD/mean), three cimetidine concentrations (low, intermediate and high i.e. 39, 625 and 5000 ng/mL) were injected into the HPLC in triplicate. Intra-day and inter-day relative standard deviation in plasma were
below 4%, exhibiting high repeatability and reproducibility of the currently developed method. Extraction efficiency was 82.10 ± 3.51%.

No significant variation (p > 0.05) was observed in the peak area and concentration of cimetidine in plasma, exhibiting that cimetidine was stable in plasma under the mentioned storage conditions.

**Pharmacokinetic Evaluation**

For sharp separation of cimetidine, various mobile phases with various proportions of their components were evaluated. The optimized mobile phase mixture at 228 nm with a retention time of 6.70±0.50 min was a phosphate buffer (K2HPO4, 0.05 M) By using ortho-phosphoric acid, the pH of the mobile phase was adjusted to 3.0. Then 0.2% triethylamine and 11% acetonitrile were added to the mobile phase. The optimized flow rate was 0.9 mL/min. A typical chromatogram is presented in Fig. 2.

A plasma drug concentration versus time curve is given in Fig. 3. The important pharmacokinetic parameters were calculated for the optimized formulation FMC3 and the results are shown in Table 1.

**Discussion**

In this study, the maximum plasma concentration (Cmax, Mean ± SD) for cimetidine was found to be 1508.79 ± 37.95 ng/ml. In a previous study conducted in healthy male volunteers by Moreno et al. [10], the value of Cmax was 2814.50 ng/ml with an oral dose of 400 mg of a cimetidine tablet. The higher value for Cmax could be due to the difference in the dosage forms, as Moreno et al. [10] used an immediate release tablet while in the present study, sustained release microparticles have been employed. In another study conducted by Kanto et al. [11], the value of Cmax was 1120.00 ng/ml after an oral dose of cimetidine 200 mg. The lower value of Cmax compared to that of the present study may be due to the difference in the dose administered i.e. Kanto et al. [11] gave 200 mg cimetidine, while 400 mg cimetidine was given in the present study.

In the present study, the time needed to reach maximum plasma concentration (Tmax, Mean ± SD) for cimetidine was found to be 3.67 ± 0.17 h. In a previous study conducted in healthy male vol-

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**Table 1.** Bioparameters of cimetidine 400 mg microparticles (n = 18)

<table>
<thead>
<tr>
<th>Bioparameters (Biowskaźniki)</th>
<th>Values (Mean ± SD)</th>
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<tbody>
<tr>
<td>Area under plasma drug concentration curve (AUC0-inf, ng.h/mL)</td>
<td>14366.19 ± 377.64</td>
</tr>
<tr>
<td>Maximum plasma concentration (Cmax, ng/mL)</td>
<td>1508.79 ± 37.95</td>
</tr>
<tr>
<td>Time needed to reach maximum plasma concentration (Tmax, h)</td>
<td>3.67 ± 0.17</td>
</tr>
<tr>
<td>Mean residence time (MRT, h)</td>
<td>8.85 ± 0.17</td>
</tr>
<tr>
<td>Volume of distribution (Vd, L)</td>
<td>214.48 ± 7.19</td>
</tr>
<tr>
<td>Half life (T1/2, h)</td>
<td>5.30 ± 0.18</td>
</tr>
<tr>
<td>Elimination rate constant (Ke, 1/h)</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>Clearance (Cl, mL/min)</td>
<td>28171.41 ± 641.47</td>
</tr>
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unters by Moreno et al. [10], the value of $T_{\text{max}}$ was 1.6 h with an oral dose of a 400 mg cimetidine tablet. The higher value for $T_{\text{max}}$ could again be due to the difference in the dosage forms, as Moreno et al. [10] used an immediate release tablet while in the present study, sustained release microparticles were employed. In another study conducted by Kanto et al. [11], the value of $T_{\text{max}}$ was 0.97 h after an oral dose of cimetidine 200 mg. The lower value of $T_{\text{max}}$ compared to that of the present study may again be due to the difference in the dosage forms, i.e. Kanto et al. [11] gave 200 mg cimetidine, while 400 mg cimetidine was given in the present study.

In this study, the area under the curve (AUC, Mean ± SD) for cimetidine was found to be $14366.19 \pm 377.64$ ng.h/mL. In a previous study conducted in healthy male volunteers by Moreno et al. [10], the value of AUC was $10464.60 \pm 2633.10$ ng.h/mL with an oral dose of a 400 mg cimetidine tablet. The higher value for AUC could again be due to the difference in the dosage forms, as Moreno et al. [10] used an immediate release tablet while in the present study, sustained release microparticles were employed. In another study conducted by Kanto et al. [11], the value of AUC was 3520.00 ng.h/mL after an oral dose of cimetidine 200 mg. The lower value of AUC compared to that of the present study may again be due to the difference in the dosage forms, i.e. Kanto et al. [11] gave 200 mg cimetidine, while 400 mg cimetidine was given in the present study. The same reasons can be attributed to the higher value of the plasma half life (5.30 ± 0.18 h, Mean ± SD) of cimetidine microparticles as compared the value of the half life (3.3 h) calculated by Moreno et al., [10] and other pharmacokinetic parameters.

The authors concluded that this study elaborates that the developed optimized floating microparticles of hydroxypropylmethylcellulose loaded with cimetidine can maintain a therapeutic level of cimetidine for about 24 h. Thus, it may improve safety, efficacy and patient compliance with reduced problems due to the high dose absorption at once in a conventional tablet and diminished dosing frequency.

References

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