―Regular Articles―

Gastro-retentive Dosage Form for Improving Bioavailability of Cefpodoxime Proxetil in Rats

Vasu Kumar KAKUMANU,[a] Vinod Kumar ARORA,[b] and Arvind Kumar BANSAL*,[a]

[a]Department of Pharmaceutical Technology (Formulations), National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, Phase X, SAS Nagar, Punjab 160 062, India, and [b]Ranbaxy Research Laboratories, Plot-20, Sector-18, Udyog Vihar Industrial Area, Gurgaon, Haryana, India

(Received April 27, 2007; Accepted September 3, 2007)

Cefpodoxime proxetil (CP) is a prodrug with poor oral bioavailability because of its metabolism to Cefpodoxime acid (CA) in luminal contents and intestinal epithelial cells. In the present investigation, regional variability in different segments of the gastrointestinal tract vis-à-vis solubility and metabolism were investigated, and the results indicated potential for a gastro retentive (GR) dosage form. Suitability of a GR dosage from for CP and finally *in vivo* efficacy were investigated. Thereafter, an effervescent floating GR dosage form was developed for CP and evaluated in rats. The GR dosage form improved the oral bioavailability of CP significantly by about 75%, hence providing a proof-of-concept. The T_{max} value increased to 1.43±0.24 h from 0.91±0.23 h of pure drug, while C_{max} values of 4735±802 ng/ml and 3094±567 ng/ml were obtained for the GR dosage form and pure drug respectively.

Key words――cefpodoxime proxetil; cefpodoxime acid; metabolism; stability; gastro retentive; pharmacokinetics

INTRODUCTION

Prodrugs are conventionally used for improving absorption and therapeutic performance of a drug. Specifically, prodrugs can be designed to improve either solubility or permeability of a drug. Esterification is a common way of synthesizing prodrugs, wherein the weak ester bond ensures easy reversal of the prodrug to the active parent moiety in the intestinal epithelial cell or blood. However, ester prodrugs are susceptible to metabolism in luminal contents, resulting in their pre-absorption degradation, thus defeating the purpose for which the prodrug was designed.

Cefpodoxime proxetil (CP) is a prodrug, third generation cephem type broad spectrum oral antibacterial (Fig. 1). CP is absorbed from the intestinal tract after oral administration and hydrolyzed to its parent moiety cefpodoxime acid (CA) by nonspecific esterases in the intestinal wall/plasma.[1–3] The drug is absorbed throughout the gastrointestinal tract (GIT), and shows relatively higher bioavailability in fed conditions than fasted conditions.[4] The bioavailability of CP administered as a tablet relative to cefpodoxime sodium intravenous infusion is about 50%.[5] Although CP was developed to improve the permeability and thus bioavailability of the parent molecule CA, only 50% of oral bioavailability is achieved. Some of the early reported studies have identified possible reasons for low bioavailability, as low solubility and a typical gelation behavior of CP, particularly in acidic environments,[6–8] and pre-absorption luminal metabolism into CA by the action of digestive enzymes.[1] However, a more recent descriptive study showed that oral bioavailability is limited because of typical efflux of CA, which is formed in the intestinal epithelial cells by metabolism of CP.[9]

However, the high solubility, chemical and enzymatic stability and absorption profiles of CP in acidic pH values (of stomach), points to the potential of a gastroretentive (GR) dosage form in altering the absorption profile of CP. The objective of the present study was to evaluate the suitability and advantage of a GR dosage form of CP to improve its oral bioavailability. The proposed advantage was evaluated by conducting various *in vitro* and *in vivo* experiments.

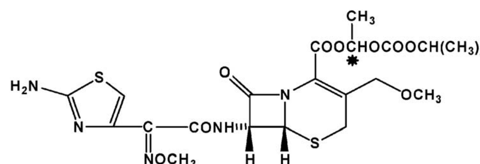

Fig. 1. Structure of Cefpodoxime Proxetil

*e-mail: akbansal@niper.ac.in

MATERIALS AND METHODS

Materials Reference standards of CP and CA were obtained from Ranbaxy Research Laboratories Ltd., Gurgaon, India. Eudragit S100 (EUD) was a gift of Rohm GmbH, Darmstadt, Germany. Sodium bicarbonate (SB) was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. All materials used in preparation of buffers, microsomal fractions and incubation studies were of analytical grade. All solvents and materials used in HPLC analysis were of chromatographic grade.

Analytical Method Two HPLC methods were developed and validated for detecting quantities of CP in the formulations and CA in the *in vivo* samples. An HPLC system (Shimadzu Corporation, Japan) equipped with a UV-Vis spectrophotometric detector and data acquisition software (CLASS-VP, version 6.14 SP1) was utilized for the purpose. The HPLC method used for detection of CP in formulations employed acetonitrile: ammonium acetate buffer (pH 5.0) as mobile phase at 38 : 62, pumped at a flow rate of 1 ml/m, and analysis was carried at a temperature of 30°C with detection at 235 nm.[10] Similarly, the HPLC method employed for detection of CA in the *in vivo* samples involved pumping of acetonitrile and phosphate buffer (pH 3.0) in 10 : 90 ratio at a flow rate of 1 ml/min, and the detection was carried out at 269nm. The method employed for quantifying CP had a calibration range of 5–150 μg/ml with a LOQ of 900 ng/ml, an accuracy of 98.45–101.63% and intra and inter day precision values of % RSD of 0.95–4.29. Similarly, the analytical method employed for quantification of CA from the *in vivo* samples had a LOQ of 50 ng/ml and operated in a concentration range of 100–5000 ng/ml with an accuracy of 93.68–107.09% and intra and inter day precision values with a % RSD in the range of 1.42–3.61.

Animal Studies All animal studies were done according to the guidelines of the Institutional Animal Ethics Committee (IAEC) of the National Institute of Pharmaceutical Education and Research (NIPER), Punjab, India. Male Sprague-Dawely (SD) rats in the weight range 250–275 g were used for the purpose. The rats were housed under standard laboratory conditions and fasted overnight with water allowed *ad libitum* before conducting the experiment.

Solubility and Enzyme Incubation Studies The shake flask method was used to determine the aqueous solubility of CP in various buffers. CP was added in excess to flat-bottomed 15 ml glass vials, each containing 5 ml of buffer (pH 1.2, 4.5, 5.4 or 6.8), pre-equilibrated to 37±0.1°C, and mechanically rotated in a shaker water bath (Julabo SW 23, Seelbach, Germany), at 175 rpm. At specified time periods, a vial was withdrawn ($n=3$), solution was filtered through a 0.45 μm membrane filter and drug content was analyzed by HPLC.

Preparation of microsomal fractions and conduction of *in vitro* enzyme metabolic studies was done as explained by Kakumanu *et al.*[9] The metabolism of prodrug was investigated at 37°C in 0.05 M phosphate buffer saline (pH 7.4). The reaction was initiated by adding 200 μl of drug solution to 100 μl of pre-equilibrated enzyme solution (100 μmol of drug and 0.05 mg of protein in total) obtained from respective segments of GIT. At a specified time point, the reaction was stopped by adding 100 μl of 10% trichloroacetic acid solution. The mixture was vortexed, and centrifuged at 16000 g for 10 m. The clear supernatant was separated and analyzed by HPLC. Preliminarily, esterase activity of the biological media was determined spectrophotometrically at 37°C by following the initial formation (0–60 s) of *p*-nitrophenol from a 100 mM *p*-nitrophenyl acetate solution. Concentration profiles were plotted and conversion rate constants of CA from CP were calculated from the results of HPLC analyses.

Surgical Method The duodenum portion was exposed after a minor incision in the abdominal region of an anesthetized rat, and the duodenal region after the stomach was ligated with a sterile surgical suture. The incision was sutured back, with due care being taken to avoid any loss of blood, and an experiment was performed within 10–15 m, under a horizontal laminar flow bench unit (Sonar Ltd., New Delhi, India). The ligation in the mid duodenal portion helped in the retention of contents of stomach. Thus, the orally administered drug was expected to be absorbed only from the stomach. After completion of the surgical procedure, CP was orally administered to the rats, and the blood samples were collected at specified time points, and analyzed by HPLC after suitable extraction.

Preparation and Evaluation of Floating Gastroretentive Dosage Form CP was mixed with specified amounts of Eudragit (EUD) and sodium bicar-

bonate (SB), and dry granulated. They were size reduced in a mortar and pestle and screened through sieves, and those which passed through sieve (BSS # 18～850 μ) and were retained on a sieve (BSS #28～550 μ) were used for further *in vitro* and *in vivo* evaluation. *In vitro* dissolution and buoyancy studies for the granules were carried out using 0.1 M HCl as medium.

***In Vivo* Evaluation of GR Dosage Form** Pure drug and GR dosage form were orally administered to six rats at 10 mg/kg (equivalent to CA) of dose. Blood samples (about 0.4 ml) were collected in heparinized micro-centrifuge tubes at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0 and 12.0 h after dosing. The blood was centrifuged at 6000×g for 5 m and the plasma was separated and stored at −20℃ until analysis. The collected samples were treated according to a validated procedure and drug content was estimated. Data acquisition and processing was done on Shimadzu Class-VP software. The data was further processed and the various pharmacokinetic parameters were calculated using PC-NONLIN™ (v.2.0, SCI, Lexington, USA). The computations and non-linear fit were performed with PC-NONLIN using the Neldar-Mead algorithm, and fitted to the model with first order input and first order output with no lag time. The pharmacokinetic values obtained after treatment of PC-NONLIN were further analyzed statistically by one- way ANOVA test for determining the significant difference.

RESULTS AND DISCUSSION

Proof of Concept for Suitability and Efficiency of GR Systems for CP

Solubility Studies The solubility of CP as observed in buffers of various pH values 1.2, 4.5, 5.4 and 6.8 are presented in Fig. 2. CP exhibited a pH dependent solubility phenomenon in various aqueous buffers.[6] Very high solubility of CP was observed in acidic pH values (5.56 mg/ml at pH 1.2), while the solubility dropped rapidly as the pH increased (0.38 mg/ml at pH 6.8).

The role of solubility in the low absorption of CP was investigated by calculating the dose number (D_0) for CP.[11–14] D_0 is calculated using Eq. (1). A drug is considered as highly soluble if the value of D_0 is less than one, and will not have solubility limited absorption.

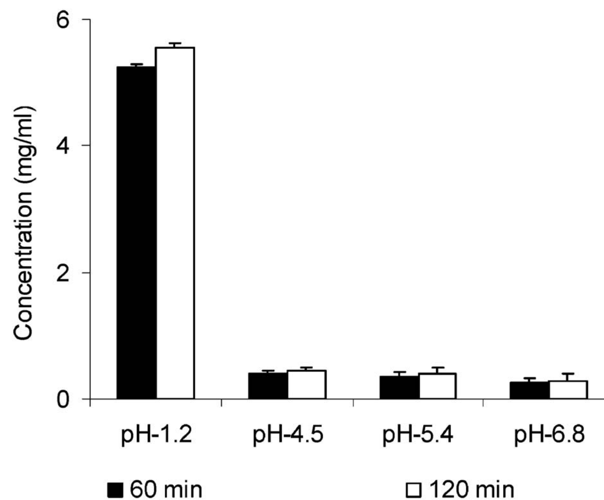

Fig. 2. Solubility of CP in Buffers of Various pH

$$D_0=\frac{\text{Dose}}{\text{C}\times\text{SIV}} \qquad (1)$$

where C is solubility of the drug and SIV is small intestinal volume, which is taken as 250 ml. The D_0 values calculated for CP at a dose of 100 mg are 0.072, 0.89, 0.99, and 1.38 for pH value of 1.2, 4.5, 5.4 and 6.8 respectively. The results show that the computed values of D_0 for CP were less than one at pH 1.2, 4.5 and 5.4. However, at pH 6.8, the D_0 value was slightly more than one, and is expected to have solubility-limited absorption at pH 6.8. Hence, from the results of solubility study and D_0 values for CP, it can be inferred that stomach and duodenum provide a suitable environment for delivery of CP where solubility will not be a contributing factor for low oral absorption of CP.

Enzyme Metabolism Studies Different regions of GIT (stomach, upper, mid, and lower small intestine) have unique role in digestion and the biological immune process, and produce secretions, according to their constitution, stimulations and purpose. The environment at each segmental region varies in terms of pH and digestive enzymes. Hence, enterocytes or epithelial cells in each segment can be regarded as having varying construction and constitution designed to meet the biological requirements. The effect of pH and enzymatic conditions on metabolism of CP was evaluated by incubating the drug with enzyme fractions obtained from various segments.

The pH dependent effect on enzymatic metabolism of CP was explained in our previous work.[9] The stability of the drug was assessed in buffers of pH 1.2,

4.5, 5.4 and 6.8 at 37°C. These pH values represent the local environments of stomach, duodenum, jejunum and ileum, respectively. The incubation of CP with the microsomal enzyme fraction clearly showed that the lower pH values (pH-1.2 and 4.5), which exist in stomach and duodenum showed less degradation of CP, possibly because of the inactivation of the metabolizing enzymes. In buffers of pH-5.4 and 6.8, about 50% of the drug is metabolized within one h of incubation. A similar situation of CP metabolism can be anticipated in the GIT corresponding to the contents with various pH distribution.

The enzyme mediated metabolism of CP was further evaluated by performing incubation studies with enzyme fractions collected from various regions of GIT namely-stomach, duodenum, jejunum and ileum. Species dependent and site-specific difference in metabolism of drugs has been studied previously. Narawane *et al.* reported segmental differences in drug permeability, esterase activity and ketone reductase activity in the albino rabbit intestine.[15] Few studies explained the actions of esterase enzymes particularly on ester type drugs, where esterase enzyme fractions were isolated from various sources like human intestine, Caco-2 cell lines, rabbit, rat and pig intestinal mucosa.[16–19] Van Gelder *et al.* reported species dependent esterase activity where rat intestinal homogenate showed the highest activity as compared to human and pig intestinal homogenates.[19] Metabolism profiles of CP after incubation with site specific enzyme fractions obtained are presented in Fig. 3. Enzyme fractions collected from the lower portions of GIT (jejunum and ileum) showed higher conversion of CP to CA, compared to those of upper portions of GIT (stomach and duodenum). The rate of conversion was in the order of jejunum>ileum>duodenum>stomach. The drug was relatively stable in the presence of enzyme fractions collected from stomach, whereas nearly 50% of the drug was metabolized within 30 m when incubated with jejunal homogenate.

The results of both enzyme metabolism studies (pH specific and site specific) indicate that CP is stable to the pH and enzymatic environment of both lumen and epithelial cell regions of stomach and duodenum. The drug is rapidly metabolized in the conditions of lower parts of small intestine, *i.e.*, jejunum and ileum, due to the combined effect of pH and enzymes. Hence, considering the drug release location in the lumen, and advantages offered by a GR system, it can confidently be said that a GR system can reduce preabsorption metabolism and thus improve the oral bioavailability of CP.

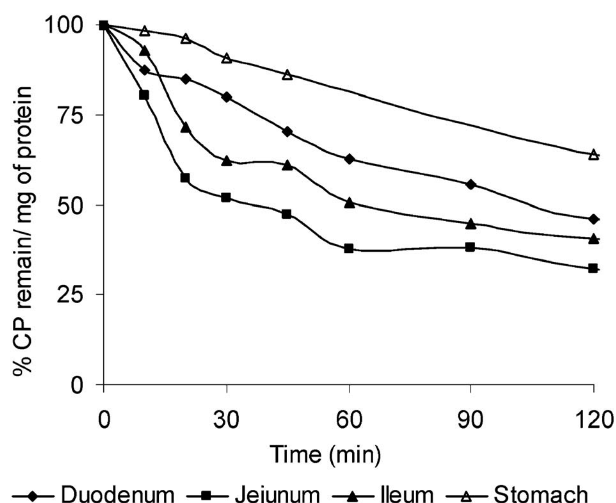

Fig. 3. Percentage of Remaining CP after Incubation of the Enzyme Fractions Isolated from Various Regions of GIT

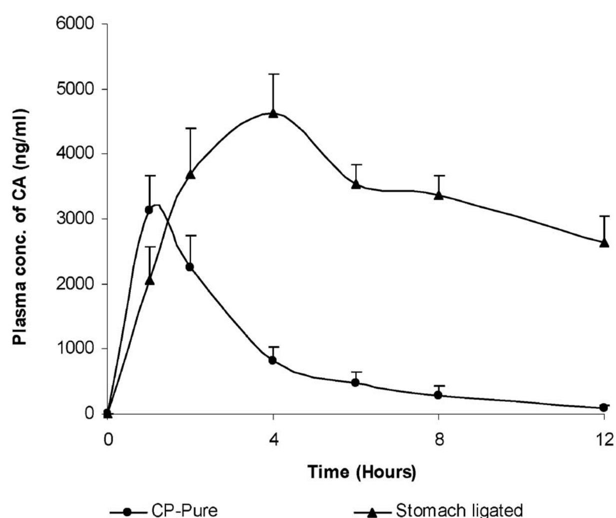

Fig. 4. Plasma Concentration Profiles of CA Obtained after Stomach Ligation and from Conscious Rats

SEM is shown as error bars.

Surgical Method To further confirm the advantage offered by a GR system for CP, a method involving a minor surgical procedure conducted in the abdominal region of the rat was adopted to ensure absorption of drug in the stomach region only. The results showed higher plasma concentration profiles of CA compared to those obtained when pure drug was administered to normal conscious rats (Fig. 4). The peak plasma concentration was obtained at nearly 4 h, and plasma concentrations were maintained at

higher levels even after 4 h. Such high levels indicate the potential of the GR dosage form for CP, although some variation is expected in the plasma concentrations due to restraining of regular physical/metabolic activities of the anesthetized rats. The observations in these experiments clearly indicated slow, sustained and improved systemic absorption of CP through stomach and proximal duodenal regions, thus necessitating further exploration of a GR system.

Development of GR Dosage Form The prominent issues and challenges to be considered for formulating a GR dosage form of CP are: (i) use of rats as an animal model limits the dimensions of formulation and (ii) pH dependent solubility behavior of CP demands precise control of drug release in gastric region. A multi unit floating GR dosage form was conceptualized to match the above mentioned requirements. Although such a system has disadvantages like need of high amounts of water in the stomach for its performance, it offers good control over drug release rate, avoidance of burst release and ensures evacuation as a bolus.

Preparation and *In Vitro* Evaluation To prove the concept of advantage of GR dosage form for CP in rats, only 2 h of floating time was targeted for this dosage form to be developed. A gas generating floating system, apart from the drug, essentially contains a gas generating agent and a release controlling agent. Sodium bicarbonate (SB) is a widely used gas generating agent in GR dosage forms,[20–22] and the same was selected to develop GR dosage form. Many release controlling polymers such as xanthan gum, PVP, HPMC, and HPC were commonly reported as suitable for this purpose. But the selected polymer should control the release of the drug in acidic pH where a drug is highly soluble, while it should rapidly release the drug once it enters the lower intestinal regions (*i.e.*, alkaline pH conditions), because it is no longer a gastro retentive system, releasing the drug in stomach. Eudragit S100 (EUD), which is soluble at pH values of more than 5.0 was selected to optimize the drug release in the gastric region. Optimization of SB concentration was done by preparing various compositions containing CP, EUD and SB (CP and EUD at 1 : 1 to make up to 100%), and the buoyancy effect (floatation time) was evaluated. Granules were added to a beaker containing 0.1 M HCl pre-equilibrated to 37°C. SB induced generation of carbon dioxide in the presence of 0.1 M HCl, and thus initiated the floating of granules. The total time of floating was measured as that during which most of the granules (>90%) floated on the surface. Granules containing SB at less than 10% w/w failed to float for the specified time. At concentrations of more than 15% w/w, the floating was maintained for 2 h. The concentration of SB at 20% w/w was fixed for preparing granules, which are to be evaluated in *in vitro* dissolution and *in vivo* absorption studies (Table 1).

Various batches of granules were prepared with compositions as listed in Table 1, and dissolution studies were performed in 0.1 M HCl. The composition of the GR dosage form for the *in vivo* evaluation was optimized based on the release profile of various formulations. Drug release could be controlled in 0.1 M

Table 1. List of Various Compositions Prepared for Dissolution Analysis in 0.1 M HCl

Formulation	% w/w of composition			% of drug released[#]	
	CP	EUD	SB	At 1 Hour	At 2 hour
G1	10	70	20	8.7	20.1
G2	20	60	20	19.2	35.2
G3	30	50	20	31.6	46.6
G4	40	40	20	39.2	53.8
G5	50	30	20	55.4	70.8
G6	60	20	20	69.6	88.1
G7	65	15	20	76.3	89.7
G8	70	10	20	83.7	98.4
G9	75	5	20	96.5	99.7

[#] $n=3$, and standard deviation was less than 4% in all cases.

HCl by use of EUD, and increase in the concentration of EUD retarded the release of the drug from the granules. In the formulations listed in Table 1, G7 containing 15% of EUD gave an optimized release profile, but the drug release was only up to 90% within 2 h (Fig. 5). Hence, the composition (G8) with a concentration of 10% EUD has been selected for *in vivo* evaluation, which released the total drug fraction within 2 h.

***In Vivo* Performance** The plasma concentration profiles of CA after oral administration of pure drug and the GR dosage form are presented in Fig. 6 and Table 2. Administration of the GR formulation to the rats resulted in improvement in the absorption and bioavailability of CP. The T_{max} value increased to 1.43±0.24 h from 0.91±0.23 h of pure drug, while maintaining C_{max} values of 4735±802 and 3094±567, respectively. The elevation of T_{max} represents delayed absorption of the drug due to its slow release from the GR dosage form, which was also confirmed by a decrease in the absorption rate constant (K01) to 1.01 ±0.27 h^{-1} compared to that of 1.50±0.40 h^{-1} for pure drug. The AUC obtained when GR dosage form administered was 17687±2693 (ng/ml) h in comparison to 10461±1687 (ng/ml) h, which was significantly higher ($p=0.007$). The GR dosage form with slow release of drug probably ensured absorption of the drug through stomach (and proximal duodenal region) and minimized pre-absorption degradation in the lower intestinal regions. The results provided a 'proof-of-concept' regarding the potential of the GR dosage form in improving the oral bioavailability of CP in humans. Optimizing the GR system for releasing the drug for a more extended time period at a controlled rate can provide even better therapeutic plasma levels of drug.

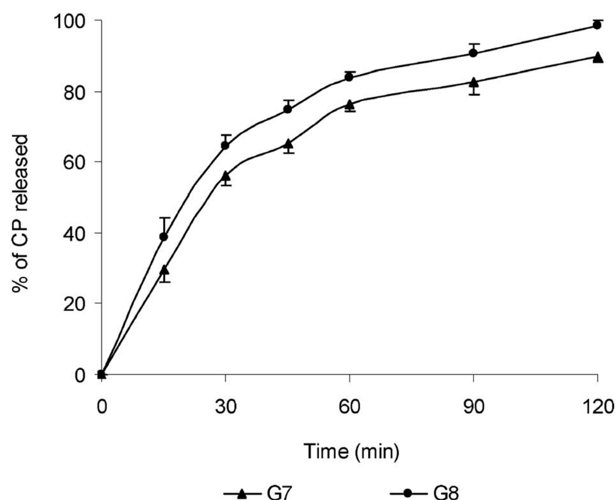

Fig. 5. Dissolution Profiles of Selected Granule Formulations in 0.1 M HCl

CONCLUSIONS

The results showed that CP is extensively metabolized by enzyme fractions in a site specific manner. The order of prodrug metabolizing activity is jejunum >ileum>duodenum>stomach. The metabolism of CP is arrested in acidic pH (1.2 and 4.5) *i.e.*, which simulates pH of the stomach and duodenum. Thus, it can be proposed that the enzymes responsible for metabolizing CP have varying distribution at different

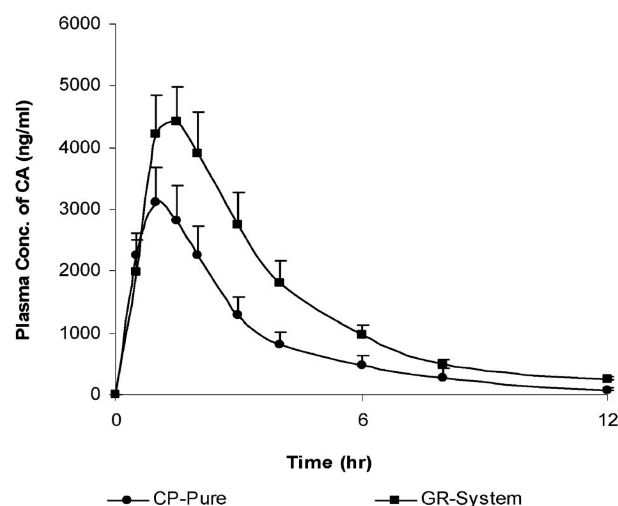

Fig. 6. Plasma Concentration Profiles of CA Obtained after Oral Administration of CP and GR Dosage Form in Rats
SEM is shown as error bars.

Table 2. The Pharmacokinetic Evaluation Parameters Obtained after Oral Administration of CP and GR System (data as obtained after submitting to PC-NONLIN™ algorithm)

	CP-Pure drug	GR System
C_{max} (ng/ml)	3094±567	4735±802
T_{max} (h)	0.91±0.23	1.43±0.24
$AUC_{0-\infty}$ (ng/ml) h)[#]	10461±1687	17687±2693
Absorption rate constant, K_{01} (h^{-1})	1.50±0.40	1.01±0.27
Elimination rate constant, K_{10} (h^{-1})	0.78±0.33	0.64±0.26
Volume (ml)	496.6±309.7	346.7±319.7
Improvement	—	0.75

[#] Significant difference at $p=0.007$.

locations of GIT. The solubility and metabolism studies showed that stomach and proximal duodenal conditions offered the most protection and provided an appropriate environment for CP absorption.

The rational selection of the GR system for CP was based on the results of various mechanistic experiments, which had pointed toward the benefits of delivery in the gastric region. Nicholas *et al.*[23] and Kakumanu *et al.*[9] suggested emulsions as an alternative means of dosage form to improve the oral bioavailability of CP, principally by avoiding the luminal metabolism. However, the present study established the efficiency of a GR system, which is convenient in terms of commercial manufacturing and administration to patients. From the salient findings of the study it can also be proposed that the GR systems may also be applied to improve oral bioavailability of cephalosporin prodrugs such as cefetamet pivoxil,[24] cefditoren pivoxil,[25] and cefuroxime axetil[26,27] and other non-cephalosporin drugs, which have low oral bioavailability and are predominantly metabolized in lower parts of the intestine.

Acknowledgement Mr. Vasu Kumar Kakumanu thanks Ranbaxy Research Laboratories, Gurgaon, India for providing the research fellowship.

REFERENCES

1) Crauste-Manciet S., Huneau J. F., Decroix M. O., Tome D., Chaumeil J. C, *Int. J. Pharm.*, **149**, 241–249 (1997).
2) Kobayashi S., Oguchi K., Uchida E., Yasuhara H., Sakamoto K., Sekine M., Sasahara K., *Chemotherapy*, **36**, 200–214 (1988).
3) Komai T., Kawai K., Tsubaki H., Tokui T., Kinoshita T., Tanaka M., *Chemotherapy*, **36**, 229–240 (1988).
4) Hughes G. S., Heald D. L., Barker K. B., Patel R. K., Euler A. R., *Clin. Pharmacol. Thera.*, **46**, 674–685 (1989).
5) Borin M. T., *Drugs*, **42**, 13–21 (1991).
6) Hamamura T., Kusai A., Nishimura K., *S.T.P. Pharm. Sci.*, **5**, 324–331 (1995).
7) Hamamura T., Ohtani T., Kusai A., Nishimura K., *S.T.P. Pharm. Sci.*, **5**, 332–338 (1995).
8) Hamamura T., Terashima H., Ohtani T., Mori Y., Seta Y., Kusai A., Sasahara K., Nishimura K., *Yakuzaigaku*, **55**, 175–182 (1995).
9) Kakumanu V. K., Arora V. K. Bansal A. K., *Int. J. Pharm.*, **317**, 155–160 (2006).
10) Kakumanu V. K., Arora V. A., Bansal A. B, *J. Chrom. B.*, **835**, 16–20 (2006).
11) Kasim N. A., Whitehouse M., Ramachandran C., Bermejo M., Lennernas H., Hussain A. S., Junginger H. E., Stavchansky S. A., Midha K. K., Shah V. P., Amidon G. L., *Mole. Pharm.*, **1**, 85–96 (2004).
12) Lindenberg M., Kopp S., Dressman J., *Eur. J. Pharm. Biopharm.*, **58**, 265–278 (2004).
13) Rinaki E., Valsami G., Macheras P., *Pharm. Res.*, **20**, 1917–1925 (2004).
14) Yu L. X., *Pharm. Res.*, **16**, 1883–1887 (1999).
15) Narawane M., Podder S. K., Bundgaard H., Lee V. H., *J. Drug. Target.*, **1**, 29–39 (1993).
16) Augustijns P., Annaert P., Heylen P., Van den Mooter G., Kinget R., *Int. J. Pharm.*, **166**, 45–53 (1998).
17) Inoue M., Morikawa M., Tsuboi M., Yamada T., Sugiura M., *Jpn. J. Pharmacol.*, **29**, 17–25 (1979).
18) Inoue M., Morikawa M., Tsuboi M., Ito Y., Sugiura M., *Jpn. J. Pharmacol.*, **30**, 529–535 (1980).
19) Van Gelder J., Shafiee M., De Clercq E., Pennickx F., Van den Mooter G., Kinget R., Augustijns P., *Int. J. Pharm.*, **205**, 93–100 (2000).
20) Ichikawa M., Watanable S., Miyake Y., *J. Pharm. Sci.*, **80**, 1062–1066 (1991).
21) Ichikawa M., Kato T., Kawahara M., Watanabe S., Kayano M., *J. Pharm. Sci.*, **80**, 1153–1156 (1991).
22) Singh B. N., Kim K. H., *J. Control. Rel.*, **63**, 235–259 (2000).
23) Nicholas G., Crauste-Manciet S., Farinotti R., Brossard D., *Int. J. Pharm.*, **263**, 165–171 (2003).
24) Koup J. R., Dubach U. C., Brandt R., Wyss R., Stoeckel K., *Antimicrob. Agents Chemother.*, **32**, 573–579 (1988).
25) Darkes M. J. Plosker G. L., *Drugs*, **62**, 319–336 (2002).
26) Finn A., Straugun A., Meyer M., Chubb J., *Biopharm. Drug Dispos.*, **8**, 519–526 (1987).
27) Williams P. Harding S. M., *J. Antimicrob. Chem.*, **13**, 191–196 (1984).