Enhanced Bioavailability of Cefoxitin Using Palmitoylcarnitine. II. Use of Directly Compressed Tablet Formulations in the Rat and Dog

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The performance of tablets containing the absorption enhancer palmitoylcarnitine chloride (PCC) and the antibiotic cefoxitin (CEF) was determined by direct placement of tablets in the rat stomach, small intestine, and colon. While the bioavailability (F) of tablets containing 12 mg CEF without PCC ranged from 0.6 to 3.9%, the addition of 24 mg PCC resulted in an enhanced CEF bioavailability in the rat colon (mean ± SD: F = 57 ± 19%) and rat jejunum (F = 71 ± 16%) but not in the rat stomach. Following oral administration to dogs, tablets of 200 mg CEF without or with 600 mg PCC resulted in the same low bioavailabilities (7.0 ± 10.3 and 7.0 ± 3.6%, respectively). However, when these tablets were enteric coated, PCC improved CEF bioavailability from 2.44 ± 1.84 to 29.0 ± 13.4%. Therefore, the use of enteric-coated direct compressed tablets containing PCC and direct compression excipients improved the peroral bioavailability of a poorly absorbed compound.

KEY WORDS: absorption enhancer; colon; enteric-coated tablet; dog; rabbit; stomach; small intestine.

INTRODUCTION

Many approaches to achieve bioavailable peroral products with poorly absorbed compounds and peptide-like drugs are currently under study (e.g., exploitation of peptide carriers, use of enzyme inhibitors; see Ref. 1 for review). However, for a compound that is enzymatically stable and/or not suitable for structural modification necessary to use a carrier transport system, oral absorption might be improved by absorption enhancing agents (2).

In the rat, direct intestinal administration of the absorption enhancer palmitoylcarnitine chloride (PCC) with the poorly absorbed compound cefoxitin (CEF) significantly improved CEF bioavailability (3). A previous study (2) demonstrated that in the rat, PCC maximally enhanced the rectal bioavailability of CEF only when the two compounds were administered together. If the administration of the two compounds was separated by 60 min, the enhancement was halved. Further, CEF administered together with PCC into a restricted region (e.g., by ligating a 2- to 3-cm section of the intestine) afforded a two- to threefold advantage in CEF bioavailability over administration to an unligated section. The difference in CEF bioavailability observed between ligated and unligated intestine may be due to spreading, separation, and/or subsequent dilution of the administered components.

There is a previous report (4) of the successful incorporation of an absorption enhancer into a peroral formulation, using enteric-coated capsules containing glycerol caprylate. A successful peroral formulation must deliver sufficient amounts of the compound of interest and the enhancer to the appropriate absorption site(s). The optimal site(s) of absorption may be identified by maximal compound absorption and/or maximal enhancer activity. Other factors, such as compound/enhancer interaction, both within the formulation and during dissolution, must also be considered (5). This work attempts to define the requirements of a standard, solid, peroral formulation affording enhanced bioavailability of a poorly absorbed compound.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (250–300 g) and beagle dogs (12–15 kg) were fasted for 18 hr with access to water before experimentation. For the rat experiments, anesthesia was induced with intraperitoneal sodium pentobarbital (50 mg/kg).

Chemicals

Sodium cefoxitin and palmitoylcarnitine chloride (PCC) were from Merck Research Laboratories, Westpoint, PA; Avicel PH 101 was from FMC Corp., Newark, DE; lactose from Foremost Whey Products, Baraboo, WI; corn starch from Staley, Decatur, IL; and magnesium stearate from Spectrum Mfg Corp., Gardena, CA. All other chemicals were of reagent grade.

Tableting

Tablets contained varying amounts of "placebo mix" [62.5:31:6:0.5 Avicel PH 101:lactose hydrous (Fast-Flo No. 316):STA-Rx 1500:magnesium stearate). The descriptions of the various formulations are shown in Table I. The tablets tested in rats were compressed on a Stokes tablet press Model F (Pennsalt Chemical Corp., Warminster, PA) into 0.1 × 0.33-in. extra deep concave capsule-shaped tablets. A compression force of 600–700 psi was maintained, resulting in tablet weights ranging from 44.5 to 45.5 mg. The tablets tested in dogs were compressed on a Carver laboratory press Model C (Fred S. Carver, Inc., Menomonee Falls, WI) into 8 × 20-mm deep concave caplet-shaped tablets. Tablets were individually compressed at 5000 lb, resulting in tablet weights of 970 ± 3 mg. An enteric coat (6) was applied to some tablets administered to dogs. The tablets were dip-coated with an enteric coating solution (cellulose acetate phthalate:propylene glycol:sorbitan monooleate:ethyl alcohol:acetone 120:30:10:450:540). Tablet disintegration in 900-mL vol of HCl/KCl buffer (pH 1.2) and in phosphate buffer (pH 7.4; 0.07 M phosphate) was determined by visual inspection using USP dissolution apparatus 2 (50-rpm paddle speed/37C; Vankel Industries, Edison, NJ). The acceptance

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Table I. Tableted Formulations of Cefoxitin Without and with Palmitoylcarnitine Chloride (PCC)

Intended species	Placebo mix (mg)[a]	Cefoxitin (mg)[b]	PCC (mg)
Rat	33	12	0
Rat	30	12	3
Rat	21	12	12
Rat	9	12	24
Dog	800	200	0
Dog	200	200	600

[a] 65.5:31:6:0.5, Avicel PH 101:lactose hydrous (Fast-Flo No. 316):STA-Rx 1500:magnesium stearate.
[b] Lot No. 7097901, assayed 93% of label.

criteria for the coat and core were (a) intact after 1 hr at pH 1.2 and (b) fully disintegrated within 1 hr at pH 7.4.

Animal Studies

All animal studies were approved by the institution's Animal Care and Use Committee according to regulations proposed by the USDA.

Rats. Solutions of CEF and PCC were injected into the unligated stomach and jejunum as described previously (3). Compressed tablets were also directly inserted into the stomach or intestine of anesthetized rats. A small incision was made into the region of interest and the tablet was inserted approximately 1 cm distal to the incision. Tablet insertion was followed by 0.5-mL phosphate-buffered (pH 7.4) saline. The intestine was then carefully ligated with 4-0 silk suture between the incision and the tablet. Care was taken not to interrupt mesenteric blood flow as determined by visual inspection of vascular bed and tissue appearance. For the stomach-dosing experiments, surgical adhesive was placed on the incision. Retrieval of tablet residuals was attempted at the end of each experiment. Following euthanasia, the intestinal lumen distal to the site of tablet insertion was exposed. In some cases, the residual mass had traversed more than 20 cm from the insertion site. Blood was sampled from the jugular vein just before and 15, 30, 60, 90, 120, 180, and 240 min after tablet administration, serum was harvested and stored at −80°C until assayed by HPLC (3).

Dogs. The peroral and i.v. dose of cefoxitin were selected based on earlier studies (2). For i.v. administration, 1 mL of a solution of CEF (50 mg/mL) was injected into each dog through the foreleg vein. Blood samples (1 mL) were taken from either the contralateral foreleg or the jugular vein immediately before dosing and at 0.25, 0.5, 1, 2, 3, and 4 hr after administration, processed and assayed as above. Tablets were orally administered to dogs with up to 5 mL of water. Blood samples were collected immediately before tablet dosing and at 0.5, 1, 2, 4, 6, 8, 10, 12, 14, 16, and 24 hr after tablet administration, processed, and assayed as above.

Tablets were also placed in the dog colon through the use of an endoscope (7). Dogs were lightly tranquilized with intravenous acepromazine. Once the anal sphincter was relaxed, a lubricated veterinary endoscope (American Optical, Rochester, NY) was passed 30 cm into the colon. Tablet placement was accomplished by wire grapplers extending from the endoscope. Blood sampling and sample preparation was similar to the other dog studies.

Pharmacokinetic Calculations and Statistical Tests

Individual sets of i.v. CEF concentrations (C) vs time data from each dog were fitted to a one-compartment model using SIPHAR (SIMED, Créteil Cedex, France) and a weight of 1 (8). Area under the serum CEF vs time curve (AUC) was determined by the trapezoidal method. Extrapolated area from the last sampling time point (t^*) to infinity was calculated (8) by Eq. (1):

$$\mathrm{AUC}_{t^*-\infty} = \frac{C_{t^*}}{k_{el}} \quad (1)$$

where k_{el} is the elimination rate constant after i.v. cefoxitin. For rat experiments, k_{el} after i.v. CEF coadministered with PCC was used (3). It is not known whether PCC alters CEF i.v. clearance in the dog [as reported (3) for rat]. Since the dog model was used to compare formulations (not study the mechanism of enhancers), the k_{el} from individual dogs following i.v. CEF alone was used to estimate the extrapolated AUC following oral CEF. A relative bioavailability (F) parameter was calculated by Eq. (2):

$$F = \frac{D_{iv}}{D_{test}} * \frac{\mathrm{AUC}_{test}}{\mathrm{AUC}_{iv}} * 100 \quad (2)$$

where D_{iv}, D_{test} and AUC_{iv}, AUC_{test} are dose and AUC following i.v. and test formulation (e.g., solution or tablet) administration, respectively. Comparisons were made with Student's t test or ANOVA and differences were considered significant at $P < 0.05$ (9). All data are presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Absorption of Cefoxitin from Solutions Injected into the Rat Stomach and Jejunum

As shown in Table II, PCC had little effect on CEF absorption from the rat stomach (F ~80%). This is similar (F ~4%) to the F reported (10) following peroral administration of CEF alone. Since the stomach was not ligated in the present experiment, this F was probably due to absorption in both the stomach and the small intestine. Regardless, this low bioavailability is comparable to that observed in the jejunum (F = 3.6 ± 1.3%) and ileum (F = 5.2 ± 1.1%) when CEF was administered alone (3). In contrast, PCC enhanced CEF bioavailability in the unligated jejunum approximately fivefold (F ~18%). Similar to that seen previously (3) for colon, ligating the jejunal segment containing the enhancer and compound resulted in a further enhancement of CEF bioavailability (F = 77.4 ± 13.0%, $P < 0.05$). Attempts were made to minimize contact of drug and enhancer with luminal contents in each study by fasting the animals, and selecting intestinal segments relatively clear of bulky contents. However, any effects of luminal contents binding drug and/or enhancer were more likely in the unligated experiment where (i) intestinal contents were free to move into the in-

Table II. Effect of Palmitoylcarnitine Chloride (PCC)/Cefoxitin Formulations on the Cefoxitin Bioavailability from the Rat Stomach, Jejunum, and Colon

Formulation & methods[a]	PCC content (mg)[b]	Cefoxitin bioavailability (%)[c]		
		Stomach	Jejunum	Colon
Solution				
Unligated	3.0	8.3 ± 5.4	17.7 ± 11.8	33.7 ± 16.4[d]
Ligated	3.0	—	77.4 ± 13.0[d]	75.6 ± 27.6[d]
Tablet	3.0	—	11.5 ± 12.2*,[e]	22.1 ± 9.6#
Tablet	12	—	41.3 ± 15.5*	41.8 ± 21.2
Tablet	24	13.8 ± 29.3	71.8 ± 15.7*	57.0 ± 19.0#

[a] Ligated and unligated refer to intestinal procedure; see text for details.
[b] Amount of palmitoyl carnitine chloride in formulation.
[c] Mean ± SD of 6–11 animals.
[d] From Ref. 3.
[e] (*,#) Tablet groups with like symbols are different from each other (ANOVA, $P < 0.05$).

jected region and (ii) drug and enhancer were free to move out of the initially clear region into one where luminal content could be greater. Therefore, factors that adversely affect enhancer activity in the jejunum, such as drug-enhancer separation, dilution, and potential loss due to luminal contents effects, were partially overcome by segment ligation.

Absorption of Cefoxitin from Tablets Inserted into the Rat Stomach and Intestine

Rigorous tablet disintegration measurements of these tablets were not completed in acidic and neutral pH media; however, it was observed that the tablets disintegrated in water within minutes. Visual inspection of tablet residuals (at the end of *in vivo* experiments) always indicated complete wetting and disintegration of tablet ingredients. The bioavailabilities of CEF following insertion of CEF tables into the rat stomach and intestine are shown in Table II. Although control tablets (no PCC) yielded bioavailabilities in the 0.6–3.9% range (data not shown), coadministration of PCC resulted in a significant ($P < 0.05$) improvement of CEF bioavailability in certain regions. For example, in the jejunum and colon, tablets containing 3 mg PCC afforded CEF bioavailabilities of $F = 11.5 \pm 12.2$ and $F = 22.1 \pm 9.6\%$, respectively. Tripling the tablet content of PCC to 12 mg tripled the CEF bioavailability in the jejunum (41.3 ± 15.5%) and doubled the CEF bioavailability in the colon (41.8 ± 21.2%). Increasing the amount of PCC in the tablet from 12 to 24 mg further improved CEF bioavailability (jejunum, 71.8 ± 15.7%; colon, 57.0 ± 19.0%).

In the jejunum the maximum value of F (71.8 ± 15.7%) using tablets with 24 mg PCC was similar to the maximum value of F achieved following administration of 3 mg PCC solution in the ligated jejunum (77.4 ± 13.0%) (3) (see Fig. 1). Tablets with 24 mg PCC administered to the colon resulted in a maximum F (57.0 ± 19.0%) that was between that reported for ligated (75.6 ± 27.6%) and unligated (33.7 ± 16.4%) colon experiments with solutions containing 3 mg PCC. Thus, an eightfold excess in PCC was needed for the tablet formulation to simulate the experiment with ligated intestinal segments. C_{max} was similar following solution (30.5 ± 24.3 μg/mL) and tablet (26.5 ± 7.9 μg/mL) administration. T_{max} was 2.4-fold larger after tablet administration (108 ± 26.8 min) than after solution administration (45.0 ± 16.4 min). The larger T_{max} following tablet insertion was probably due to slow (albeit complete) tablet wetting and disintegration. One might speculate that a formulation designed to limit spreading and/or physical separation of enhancer and compound may reduce the amount of PCC required to afford the enhancement observed with ligated rat intestine.

Tablet insertion into the stomach resulted in an F value (13.8 ± 29.3%) similar to that seen with solutions (8.3 ± 5.4%). This observation suggests that besides an inherent low enhancement in the stomach, sufficient separation, dilution, and/or degradation of tablet components occurred before reaching the jejunum. PCC has been shown to have no effect in the duodenum (3). In that study, it was demonstrated that the lack of effect in the duodenum was not due to enzyme degradation or bile acids. While model intestinal fluids have recently been shown to reduce the efficiency of some adjuvants (11), it is not known whether this is the case with PCC. These observations suggest that following peroral administration, the tablet must be kept intact (i.e., enhancer and compound together) until it reaches the jejunum.

At the end of the experiment, visual examination of the

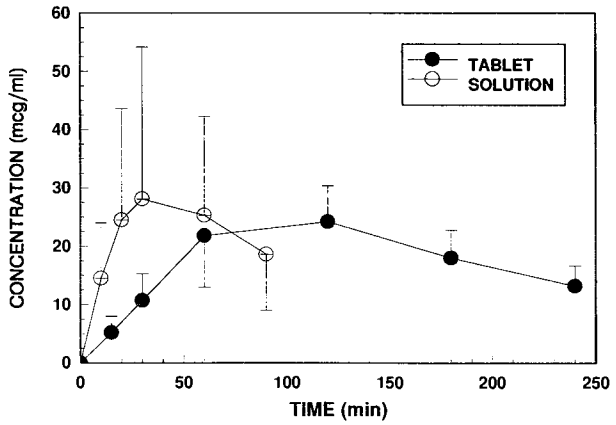

Fig. 1. Serum cefoxitin concentrations in the rat following administration of 12 mg cefoxitin (CEF) and 3 mg palmitoylcarnitine chloride (PCC) as a solution by injection into a ligated section of the jejunum (○) or 12 mg CEF and 24 mg PCC as a direct compressed tablet by placement into an unligated section of the jejunum (●).

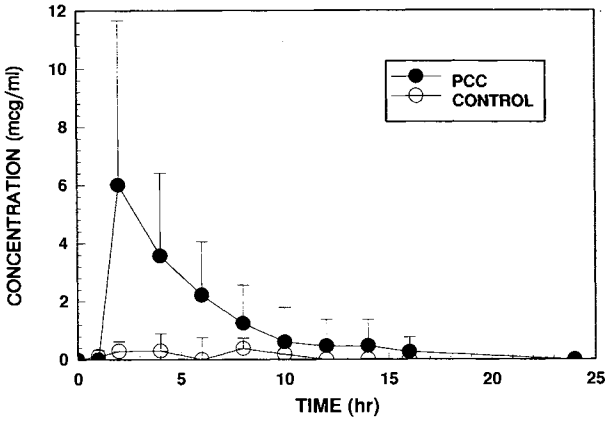

Fig. 2. Serum cefoxitin concentrations in the dog following peroral administration of a directly compressed enteric coated 200-mg cefoxitin tablet as a control [no palmitoylcarnitine chloride (PCC); ○] or an "active" (●) tablet containing 600 mg PCC.

intestines revealed normal morphology, devoid of inflammation and other overt signs of irritation.

Absorption and Bioavailability of Cefoxitin in Dogs

To study realistic dosage forms, orally administered tablets were examined in dogs. Following i.v. administration of CEF to dogs, serum CEF concentrations declined in a monoexponential fashion. Table III summarizes the CEF bioavailability findings in dogs following oral administration of uncoated and enteric coated tablets containing 200 mg CEF without (control) or with 600 mg PCC. The amount of PCC administered (600 mg) was selected based on known enhancement activity (2). Consistent with the results of experiments performed in rats, PCC had little or no effect when the tablet was not enteric coated and presumably allowed to dissolve in the stomach (Fig. 2). The enteric coat was designed to keep the coat and tablet core intact until it had passed out of the stomach. In fact, there was an apparent lag of at least 1 hr, during which serum CEF concentrations were not detected. T_{max} was similar following peroral administration of either the enteric-coated control or the PCC tablet (4.7 ± 3.0 and 3.5 ± 1.9 hr, respectively). C_{max} following peroral administration of the enteric-coated PCC tablet (6.8 ± 4.8 μg/mL) was 8.5-fold greater ($P < 0.05$) than after control tablets (0.8 ± 0.7 μg/mL). The CEF bioavailability (29.0 ± 13.4%) observed following the oral administration of enteric-coated tablets containing 600 mg PCC was 12 times ($P < 0.05$) that observed (2.44 ± 1.84%) with enteric-coated control tablets (no PCC).

Table III. Oral Palmitoylcarnitine Chloride (PCC)/ Cefoxitin Formulations in the Dog: Effect of an Enteric Coat on Cefoxitin Bioavailability

Coating[a]	PCC[a]	Route	F (%)[b]
−	−	Peroral	7.0 ± 10.3
−	+	Peroral	7.0 ± 3.6
+	−	Peroral	2.44 ± 1.84
+	+	Peroral	29.0 ± 13.4*
−	−	Colon	0.0 ± 0.0
−	+	Colon	36.1 ± 19.3*

[a] Refer to whether an enteric coat or palmitoylcarnitine chloride (PCC) is present (+) or absent (−) in the formulation.
[b] Mean ± SD, n = 3–4.
* Different from tablets without PCC, $P < 0.05$.

Following colonic placement of uncoated tablets, 600 mg PCC improved the CEF bioavailability from essentially zero to 36.1 ± 19.3%. Thus, the degree of improvement in peroral CEF bioavailability achieved with enteric-coated tablets was similar to that achieved following colonic placement. Possible explanations for this similarity are that (i) the enteric coat kept the tablet intact until it reached the colon or (ii) there are no major site differences with PCC activity once the formulation reaches the small intestine. The large T_{max} (~4 hr) and the fact that no serum CEF concentrations were detected at the 1-hr sampling point support this hypothesis. Unfortunately, no visual confirmation of the gastrointestinal location of tablet disintegration was possible.

Progression from the demonstration of safe and effective enhancer formulations in animal models to commercially successful products remains a significant challenge. Although Sekine *et al.* (4) and others (12,13) demonstrated the principle years ago, peroral formulations incorporating an absorption enhancer have not achieved clinical importance. Concerns that absorption enhancers might compromise the intestinal barrier to endotoxins or antigens, causing systemic sepsis, have not been confirmed. Recently, several studies have clearly shown that these enhancers operate by reversibly increasing paracellular transport (14), possibly by loosening tight junctions (15). The tight junctions, even in their "loosened" state, still maintain a formidable barrier to such macroscopic molecules. In contrast to effects of detergents, which solubilize whole cells in the epithelium (16), microscopic examination of rat colonic mucosa treated *in vitro* with similar concentrations of PCC revealed no such damage (17). Moreover in this study, following visual inspection, the intestine of rats administered tablets containing PCC was indistinguishable from the intestine of control rats.

In conclusion, the use of enteric-coated direct compressed tablets containing PCC and direct compression excipients improved peroral bioavailability of a poorly absorbed compound. Although the F reported here for PCC tablets includes a significant coefficient of variation (CV ~50%), such error is common with prototype formulations in pilot studies (n = 3–4). Formulation optimization may reduce the high variability reported in these studies and the total amount of PCC required to afford maximum enhancement, further increasing CEF bioavailability beyond that observed here. However, it is not known how these findings might be applicable to other compounds of commercial interest.

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