Enhanced Bioavailability of Cefoxitin Using Palmitoyl L-Carnitine. I. Enhancer Activity in Different Intestinal Regions

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The conditions under which the absorption enhancer palmitoyl L-carnitine chloride (PCC) improved the bioavailability of the poorly absorbed antibiotic cefoxitin throughout the rat intestine has been studied. Cefoxitin alone was appreciably absorbed only in the duodenum (31% vs <7% elsewhere). PCC solutions (3 mg/rat, pH 4.0) enhanced cefoxitin bioavailability (F) by 0-, 22-, 16-, and >32-fold in the duodenum, jejunum, ileum, and colon regions, respectively. The inability of PCC to improve F in the duodenum could not likely be attributed to enzymatic degradation of the enhancer, since coadministration with protease and esterase inhibitors produced similar results (F = 30%). Coadministration of PCC solution with cefoxitin in the unligated or ligated colon, increased F to 33 and 76%, respectively. Qualitatively similar results were seen with PCC suspensions (3 mg/rat, pH 6.0). Maintaining a high concentration of cefoxitin and PCC in a restricted region (i.e., by ligating a 2- to 3-cm section of the colon) afforded a two- to threefold advantage over an unligated colon section. The difference in cefoxitin bioavailability between ligated and unligated colon was probably due to sample spreading and subsequent/simultaneous dilution.

KEY WORDS: oral absorption enhancer; cefoxitin; colon; rat small intestine.

INTRODUCTION

In the rat, rectal (1) and nasal (2) administration of the absorption enhancer, palmitoyl L-carnitine chloride (PCC), with the poorly absorbed model compound, cefoxitin, has been shown previously to result in a substantial improvement in cefoxitin bioavailability. In contrast with rectal and nasal administration, peroral administration of PCC and cefoxitin provided very little improvement in cefoxitin bioavailability. Successful incorporation of an absorption enhancer into a peroral formulation has been lacking, and no examples have been reported in the literature. In general, the peroral route of administration is preferred over nasal and rectal administration. However, in light of the above findings, it is uncertain whether peroral administration of a poorly absorbed compound and an absorption promoter can be effective. In a recent review by Muranishi (3) the large rather than the small intestine was shown to be the preferred site for absorption enhancer effectiveness. The work reported here are the results of a systematic study of PCC effectiveness throughout the rat intestinal tract, with special emphasis on the colon.

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MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (250–300 g) were fasted for 18 hr with access to water prior to experimentation. Refer to the tables and figures for the number of animals in each experiment. Anesthesia was induced with intraperitoneal pentobarbital (50 mg/kg).

Chemicals. Sodium cefoxitin (Mefoxin) and palmitoyl L-carnitine chloride (PCC) were from Merck Sharp & Dohme Research Laboratories (Westpoint, PA), sodium cefmetazole was from Sankyo Co., Ltd. (Tokyo), and leupeptin, beef trypsin/chymotrypsin inhibitor (Bowman-Birk Inhibitor, soybean origin), phenylmethylsulfonyl fluoride (PMSF), and NaF were from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of reagent grade.

Drug–Adjuvant Administration. Cefoxitin (40 mg/kg) was administered as an i.v. bolus (1 ml/kg) in 0.9% saline directly into an externalized jugular vein either alone (i.v. control) or coadministered with PCC. PCC (2.5 or 5 mg/kg) was either coinjected i.v. with cefoxitin or rectally administered (28 mg/kg) simultaneously with i.v. cefoxitin. Blood was sampled from the contralateral jugular vein just before and 5, 10, 15, 30, 60, and 90 min after administration, immediately processed to isolate serum, stored at −80°C, and assayed by HPLC (see below). Cefoxitin (12 mg) and (when appropriate) PCC were administered in a total volume of either 250 μl (rectal) or 300 μl (intestinal). PCC is soluble in citrate–phosphate buffer (0.1 M citric acid, 0.2 M dibasic sodium phosphate) at pH ≈4. Above pH 4, first PCC completely dissolves, then precipitation of the zwitterion slowly occurs, reaching equilibrium at 25°C within 24 hr (data not shown). PCC was added to pH 4 citrate–phosphate buffer when administered as a solution and to pH 6 citrate–phosphate buffer (mixed overnight at room temperature) for PCC suspension studies. Cefoxitin was added to PCC solution or suspension immediately before dosing. Rectal and intestinal administration was completed as described previously (1). Aqueous microenemas were administered to anesthetized rats with a 1-ml syringe at an intrarectal depth of 2.5 cm, and anal tissue was clamped to prevent leakage. In experiments where the intestine was ligated, a 2- to 3-cm section was gently manipulated to clear the lumen of contents, carefully ligated with 4-0 silk suture without interruption of mesenteric blood flow, and returned to the abdominal cavity. In experiments designed to inhibit intestinal esterases, ligated sections of the duodenum were pretreated for 5 min with 150 μl citrate–phosphate buffer containing 0.5 mg NaF prior to coadministration of 150 μl buffer consisting of 0.5 mg NaF, 12 mg cefoxitin, and 3 mg PCC. In protease inhibition studies, 0.02% of a trypsin/chymotrypsin inhibitor and 0.2 mM leupeptin was coadministered with cefoxitin and PCC into ligated duodenum. These amounts of inhibitors were 10–80 times that found to be effective in crude intestinal homogenates (4).

Osmolarity. The osmolarity of dosing solutions was determined using a Wescor 5100 C vapor pressure osmometer (Wescor, Inc., Logan, UT).

Analytical. Major modifications of a published assay (5) were used. Sodium cefoxitin in serum samples was assayed by reverse-phase HPLC with sodium cefmetazole as the in-

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ternal standard. Using 2 Rheodyne 7001 valves, Skinner 12-V dc solenoids, a 12-V dc switching power supply, an FMI lab pump, a Perkin–Elmer ISS-100 autosampler, and timed events, the sample (25 μl) was injected onto a Brownlee RP C18 4.6 mm × 3.0-cm guard column before being introduced to the analytical column. This allowed a window to be introduced from the guard column to the analytical column (Brownlee RP C18, 4.6 mm × 10 cm, + RP C18, 4.6 mm × 3 cm), thus preventing contaminants from destroying the analytical column. The same mobile phase was used in both columns: 12% acetonitrile (ACN), 0.4% formic acid, 0.04% ammonium hydroxide in water. Once the valve switched the guard column in-line with the analytical column, internal standard and cefoxitin were eluted onto the analytical column. The mobile phase continued to separate and elute the two components on the analytical column, while the guard column was flushed with ACN from a 1-ml loop attached to the second valve. This flush removed retained contaminants from the guard column. The guard column was then reequilibrated with mobile phase in preparation for the next injection and the 1-ml loop was refilled with ACN from the FMI lab pump.

The pumps delivered the mobile phase at rates of 1.5 and 3.0 ml/min to the guard and analytical columns, respectively. Cefoxitin and cefmetazole were monitored at 254 nm. A refrigerated circulating bath was connected to the autosampler tray and maintained at 10°C throughout the run. Standards were injected twice, at the beginning of the run and again after all unknowns. Standards at or above the 3.12 μg/ml level showed less than 3% degradation over an 8-hr period. Standards at or below 1.56 μg/ml degraded by 10% over the same time period. Unknown sample concentrations were determined by a linear plot of the ratio of cefoxitin area over internal standard area versus cefoxitin concentration. Standards were linear over the range of 0.78 to 100 μg/ml (slope = 0.033; intercept = −0.005; r^2 = 0.99909). The limit of detection for cefoxitin was 0.75 μg/ml.

Pharmacokinetic Calculations and Statistical Tests. The area under the serum cefoxitin vs time curve (AUC) was determined by the trapezoidal method using curve stripping software (SIPHAR, SIMED, Creteil Cedex, France). In absorption studies, the residual area from the last time point (t^*) to infinity was calculated (6) by Eq (1):

$$AUC_{t^*-\infty} = C_{t^*}/\beta \quad (1)$$

where β is the terminal disposition rate constant after i.v. cefoxitin. Cefoxitin bioavailability (F) was calculated by Eq. (2):

$$F = \frac{D_{iv}}{D_{int}} * \frac{AUC_{int}}{AUC_{iv}} \quad (2)$$

where D_{iv}, D_{int} and AUC_{iv}, AUC_{int} are the dose and AUC for i.v. (with coadministration of PCC; see below) and rectal/intestinal absorption studies, respectively. Clearance (Cl) and steady-state volume of distribution (V_{ss}) were calculated (6) by SIPHAR. Comparisons were made with Student's t test or ANOVA and differences were considered significant at $P < 0.05$ (7). All data are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

i.v. Cefoxitin. Following i.v. cefoxitin, serum cefoxitin concentrations declined in a biexponential fashion. Coadministration of 2.5 or 5.0 mg/kg PCC solutions or simultaneous rectal administration of 28 mg/kg PCC solutions did not affect the shape of the cefoxitin concentration–time profile (Fig. 1). Although Cl was apparently decreased ($P < 0.05$), V_{ss} and β were unchanged by coadministration of PCC (Table I). Because of the apparent effect of PCC on Cl, the AUC_{iv} used in Eq. (2) was the mean of values obtained following PCC coadministration.

Rectal PCC Solution and Suspension Dose Response. As shown in Fig. 2, the rectal bioavailability of cefoxitin increased with increasing amounts of coadministered PCC (pH 4 buffered solution), with a maximum cefoxitin bioavailability (F) at a dose of 1.5 mg PCC. Although F following a dose of 3 mg PCC appeared greater than F at 1.5 mg PCC, this increase was not statistically significant. In a similar comparison, following the dose of 6 mg PCC, F appeared to be decreased, yet again, this decrease was not statistically significant. The 1.5-mg dose of PCC (solution, pH 4), F was nearly identical to the minimum enhancer dose previously reported (1) to yield the maximum improvement in F. When PCC was added to pH 6 buffer immediately prior to administration (and before significant precipitation had occurred), enhancement was identical to that of the pH 4 PCC solutions (data not shown). When PCC was equilibrated for 24 hr in pH 6 buffer, the soluble fraction was <0.1 mg/ml (data not shown). As expected, a dose of 1.5 mg/rat as PCC suspension did not appear to result in the degree of enhancement seen with the solution (although the difference was not statistically significant) (Fig. 2). The maximum enhancement of F by PCC suspension was observed at a dose of 7.5 mg PCC/rat. It can be speculated that a portion of the suspended PCC (zwitterion) becomes soluble *in vivo,* thereby increasing the soluble fraction available to enhance the absorption of cefoxitin. Since F was similar following a dose of 3 mg PCC whether solution or suspension, this dose was used in subsequent *in vivo* studies.

Intestinal Region Studies. The effects of PCC solution

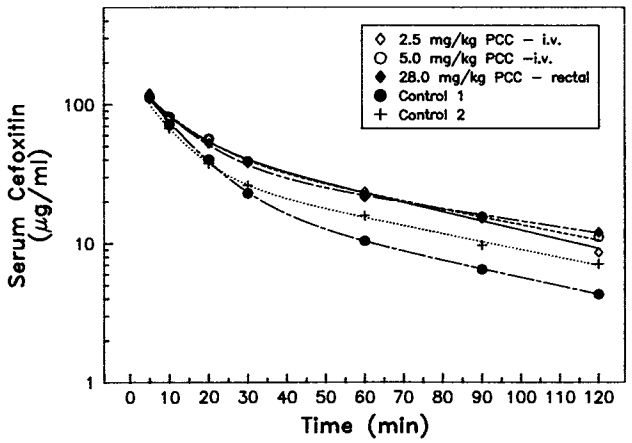

Fig. 1. Average serum cefoxitin concentrations (n = 3–6) following i.v. cefoxitin (40 mg/kg) administration alone and when coadministered with either i.v. PCC or rectal PCC. The relative SD at each point averaged ±20% relative to the average plasma concentration.

Table I. Selected Pharmacokinetic Parameters Following i.v. Cefoxitin: Effect of Palmitoyl L-Carnitine (PCC)

PCC treatment (mg/kg)	β (min^{-1})	Cl (ml/min/kg)	V_{ss} (L/kg)
2.5 i.v.	0.014 ± 0.007[a]	6.30 ± 2.18*	0.388 ± 0.059
5.0 i.v.	0.018 ± 0.007	6.61 ± 0.72*	0.316 ± 0.118
28.0 rectal	0.012 ± 0.005	6.77 ± 1.94*	0.466 ± 0.100
Control 1	0.020 ± 0.007	12.4 ± 2.06	0.518 ± 0.232
Control 2	0.019 ± 0.009	14.4 ± 2.32	0.529 ± 0.197

[a] Average ± SD; n = 3–6.
* Different from either control: ANOVA, $P < 0.005$.

on F were determined in ligated regions of the small intestine and colon. Compared to controls, there was a marked increase in cefoxitin absorption from the jejunum, ileum, and colon in the presence of PCC (Table II). Cefoxitin absorption from the duodenum, which was significant in the absence of PCC, was not increased in the presence of PCC. The poor performance of PCC in the duodenum was probably not due to enzymatic degradation of the enhancer, since coadministration with effective amounts of protease and esterase inhibitors [0.5 m*M* PMSF, 0.2 m*M* leupeptin, 0.02% beef trypsin/chymotrypsin inhibitor (2000 U/ml), 0.5 mg NaF] produced similar results (Table II). It is not clear why PCC did not produce the magnitude of enhancement in the duodenum that it produced in the distal intestine. It is possible that duodenal mucosa is uniquely protected against the effects of these enhancers since it is also subject to high concentrations of bile acids. Interestingly, although bile acids can improve the solubility of PCC, the resulting mixed micelles do not improve the cefoxitin rectal bioavailability beyond that seen with PCC solutions alone (data not shown).

When PCC solutions were coadministered with cefoxitin into the ligated colon, F was 76% (versus 0% in controls). These same PCC suspensions or solutions were coadministered with cefoxitin in the unligated colon and resulted in an F of only 11 and 33%, respectively (Table III). Thus, maintaining a high concentration of cefoxitin and PCC in a restricted region (i.e., by ligating a 2- to 3-cm section of the colon) afforded a two- to threefold advantage over an unligated colon section. Since the PCC solutions and suspensions were hyperosmotic (440 ± 17.2 and 507 ± 14.0 mOsmol, respectively; n = 3), lumenal flux of water could result in their substantial dilution. Therefore, the difference between ligated and unligated colon was probably due to sample spreading and subsequent/simultaneous dilution. von Hoogdalem and co-workers (8) invoked a similar explanation when 3-amino-1-hydroxypropylidene-1,1-diphosphonate (APD) was coadministered with cefoxitin in rats. A much greater enhancement effect was observed when APD was delivered as an infusion rather than as a rectal bolus. The authors stated that following rectal infusion, APD was delivered to a smaller mucosal area, the amount per unit area was higher and the effect was stronger. The infusion limited the area of spreading, as did colon ligation.

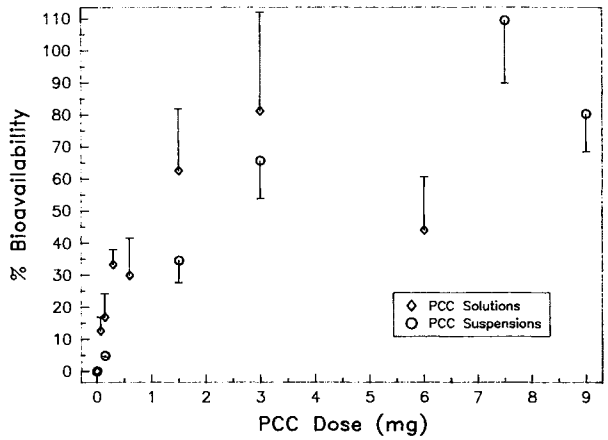

Fig. 2. Effect of rectally coadministered PCC (mg/rat) on cefoxitin (40 mg/kg) bioavailability, when administered as a PCC solution (pH 4) or PCC suspension (pH 6). Mean ± SD; n = 3–9; animals are shown.

Table II. Cefoxitin Bioavailability from Rat Small Intestine: Effect of Palmitoyl L-Carnitine (PCC)

Intestinal region	Percentage cefoxitin bioavailability[a]		
	− PCC	+ PCC[b]	+ Inhibitors[c]
Duodenum	30.7 ± 6.6[d]	21.2 ± 12.3	29.9 ± 7.3
Jejunum	3.6 ± 1.3	77.4 ± 13.0	ND[e]
Ileum	5.2 ± 1.1	80.6 ± 22.7	ND

[a] Bioavailability of a 12-mg dose compared to i.v. cefoxitin coadministered with PCC.
[b] Coadministered 3 mg PCC.
[c] See text for details.
[d] Mean ± SD; n = 3–6 animals.
[e] Not determined.

A major difference between the nasal and the rectal route of administration and the unligated intestinal (and presumably peroral) route is the degree of potential spreading of the formulation components. Although there is some degree of nasal clearance due to ciliary movements, and minor movement in the rectal region (e.g., defecation), compared to the continuous movement known to occur in the gastrointestinal tract, the former are insignificant. As suggested by Muranishi (9,10), both movements and fluid secretion in the colon are less than in the small intestine and, therefore, less

Table III. Effect of Segment Ligation on Cefoxitin Bioavailability from the Colon

Treatment	PCC[a]	F[b]	N
Control	None	0 ± 0	3
Unligated	Suspension	11.1 ± 10.5	4
Unligated	Solution	33.7 ± 16.4*	5
Ligated	Suspension	31.9 ± 9.95**	5
Ligated	Solution	75.6 ± 27.6*,**	5

[a] PCC was administered as a solution (pH 4.0) or suspension (pH 6.0) (see text for details).
[b] Percentage bioavailability compared to i.v. cefoxitin coadministered with PCC.
* Different from suspension treatment ($P < 0.01$).
** Different from unligated colon ($P < 0.01$).

likely to produce the spreading/dilution that may reduce the initial concentration of adjuvant and drug. Efforts are now directed at formulation optimization as a method to minimize apparent sample spreading in the colon.

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