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# Effect of triglyceride on small intestinal absorption of cefoxitin in rats

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Coadministration of trilaurin or monolaurin with sodium cefoxitin increased its absorption from the small intestine of the rat. Its absorption from the rectum was effected to a lesser extent except when lipase was present. Lipase, a natural constituent of the small intestine fluid, may therefore be essential for the adjuvant action of trilaurin on cefoxitin absorption across the intestinal membrane. Among the triglycerides used, trilaurin and tricaprin were the most effective enhancers of cefoxitin absorption. Both the rate of degradation of triglyceride to its fatty acid component and subsequently the rate of fatty acid absorption were factors influencing the enhancing action. Maintenance of fatty acid concentration at the small intestinal absorption site was shown to be necessary to obtain a cefoxitin bioavailability of up to 70%.

Triglycerides are widely used as fatty bases in suppositories. Recently, Nishihata et al (1986) reported that absorption of sodium cefoxitin, a low lipophilic antibiotic agent, from the small intestine was greater when a triglyceride base in a suppository vehicle was administered than when either an aqueous solution or powdered formulation was used. Rectal absorption of cefoxitin was not similarly affected by the presence of triglyceride. Since triglyceride is degraded into a fatty acid, monoglyceride and diglyceride by lipase (Börgstrom 1964), which is present in the small intestine, we proposed (Nishihata et al 1986) that the degradation product(s) may alter small intestinal membrane permeability to cefoxitin and thus triglyceride may have a use as a tablet matrix for the oral administration of low lipophilic drugs.

To clarify the enhancing action of triglyceride on cefoxitin absorption by the rat small intestine, the effect of trilaurin, monolaurin and lauric acid on the antibiotic's absorption from rat small intestine and rectum has been examined. Since commercially available triglyceride suppository bases contain predominantly lauric acid as the fatty acid component, trilaurin was selected as a model triglyceride. We also examined the effect of various other triglycerides on cefoxitin absorption by the small intestine with a view to their use as enhancers of drug absorption.

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## MATERIALS AND METHODS

### *Materials*

Sodium cefoxitin was supplied by Merck Sharp & Dohme (Rahway, NJ, USA). Fatty acids, their sodium salts, their triglycerides, 1-monolaurin and lipase (type II, 54 unit $mg^{-1}$) were obtained from Sigma Chemical Co. (St Louis, MO, USA). The carbon numbers of fatty acids used were caproic acid ($C_6$), caprylic acid ($C_8$), capric acid ($C_{10}$), lauric acid ($C_{12}$), myristic acid ($C_{14}$), palmitic acid ($C_{16}$) and stearic acid ($C_{18}$). Other reagents used were of analytical grade.

### *Animals*

Male Sprague-Dawley rats, 220–270 g, were fasted for 18 h before use. During the experiments, rats were anaesthetized with sodium pentobarbitone (40 mg $kg^{-1}$, i.p.) and kept on a warm surface at 38 °C.

### *Preparation and administration of samples*

Solutions were prepared by addition of sodium cefoxitin, 30 mg $mL^{-1}$, to distilled water. 0·5 mL $kg^{-1}$ of the solution as control was administered into the small intestine or the rectum through polyethylene tubing (PE 50). Sodium cefoxitin, 15 mg $kg^{-1}$, with a suitable dose of fatty acids or their derivatives as a powdered mixture, was introduced into either one of the administration sites through a Polytef tube (3 mm × 100 mm) with the aid of a

stainless steel rod to propel the powder through the tube.

In the intestinal loop and the rectum absorption studies, 0·2 mL of pure water, or rat bile previously collected from non-experimental rats, with or without lipase (100 mg $mL^{-1}$), was given following the powdered mixture through a polyethylene tube inserted into the sites via a drug administration tube.

An additional series of bile solutions containing 250 mg $mL^{-1}$ triglyceride was also prepared. Bile and the triglyceride were incubated with lipase (100 mg $mL^{-1}$) for 1 h at 37 °C. Then sodium cefoxitin (15 mg $mL^{-1}$) was dissolved in the medium, 1·0 mL $kg^{-1}$ of which was placed in the small intestine through a syringe without a needle.

*In-vivo small intestinal and rectal absorption study*

Both of the studies were carried out according to the method described by Nishihata et al (1986).

*In-situ small intestinal loop study*

After the loop had been washed via two cannulae with a 0·9% sodium chloride solution, a small intestinal segment (6 cm long) was isolated by ligation at a point just below the bile duct. A powdered mixture of 15 mg $kg^{-1}$ of sodium cefoxitin and 250 mg $kg^{-1}$ of trilaurin was administered into the loop. Blood samples were taken at designated times from the internal jugular vein. Some of rats were killed at 1·5 h, the lumen in the loop washed with isotonic saline, and remaining cefoxitin determined.

*Cefoxitin assay*

Cefoxitin in rat plasma was assayed by an HPLC method previously described (Nishihata et al 1984).

## RESULTS

Administration of cefoxitin alone in aqueous solution resulted in poor drug absorption from both the rectum and the small intestine (Fig. 1) as reported earlier (Nishihata et al 1983, 1984). Trilaurin coadministration in powdered form significantly increased the amount of cefoxitin passing from the intestine into the plasma but after rectal administration plasma cefoxitin increased only slightly (Fig. 1).

With the prewashed in-situ jejunal loop, cefoxitin given with trilaurin also did not produce an increase in plasma cefoxitin concentration (Fig. 2, cf. Fig. 1). However, lipase-bile solutions coadministered with a powdered mixture of sodium cefoxitin and trilaurin into the loop, significantly enhanced plasma cefoxitin (Fig. 2); the cefoxitin remaining in the loop 1·5 h later was about half the dose applied. Recovery when pure water was used was 96·1 ± 6·5% (n = 3) while recovery when lipase and bile were present was 55·9 ± 10·3% (n = 4).

In rectal studies, increased absorption of cefoxitin also occurred when trilaurin was coadministered with the lipase-bile solution, but in the absence of lipase, rectal drug uptake did not take place to the same extent (Fig. 1).

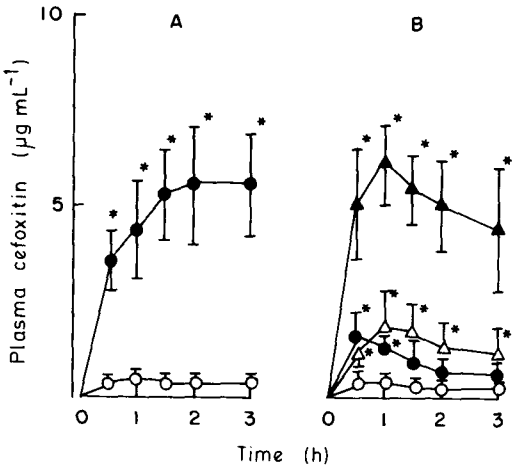


FIG. 1. Plasma cefoxitin concentration in rats after administration of sodium cefoxitin, 15 mg $kg^{-1}$, in an aqueous solution (○) and in a powder mixture with trilaurin, 500 mg $kg^{-1}$ (●) at (A) small intestine and (B) rectum. The effect of lipase on rectal absorption of cefoxitin with trilaurin was also studied. The symbols ▲ and △ represent plasma cefoxitin concentrations after administration of a bile solution with or without lipase, respectively. Each value represents the mean ± s.d. (n > 5). *$P < 0.001$ versus ○.

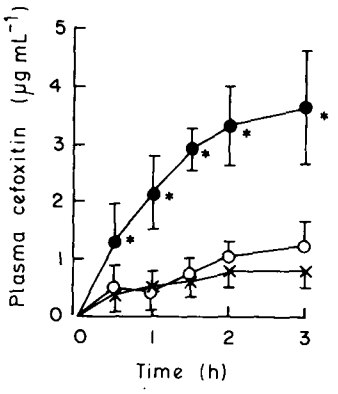


FIG. 2. Plasma cefoxitin concentration in rats after coadministration of sodium cefoxitin, 15 mg $kg^{-1}$, with trilaurin, 250 mg $kg^{-1}$, into washed jejunal loops. The effect of trilaurin with three different solutions was examined: aqueous solution (×); bile solution (○); bile-lipase solution (●). Each value represents the mean ± s.d. (n > 4). *$P <$ 0·001 versus ×.

The plasma cefoxitin profile after the administration of the antibiotic with monolaurin was similar to that after administration with trilaurin (Fig. 1).

Coadministration of lauric acid with sodium cefoxitin into the rectum and small intestine also significantly increased plasma cefoxitin concentrations (Fig. 3) compared with administration of cefoxitin alone. However, this enhancing effect was not augmented when lipase and bile were present.

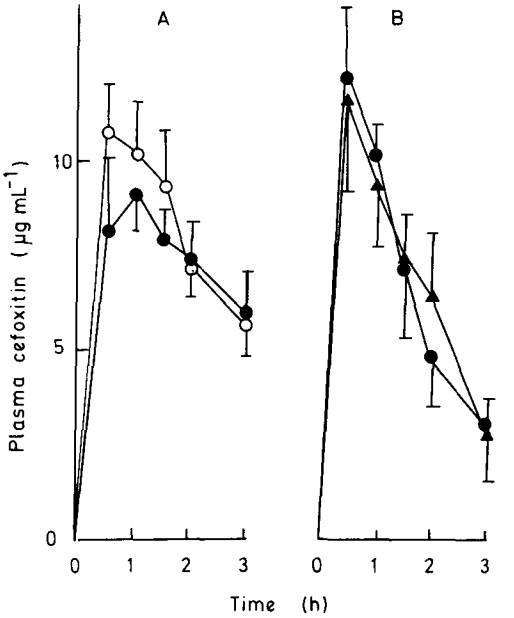


FIG. 3. Plasma concentration of cefoxitin in rats after coadministration of sodium cefoxitin, 15 mg kg$^{-1}$, in a powder mixture with lauric acid (●) and lauric acid sodium salt (○) (500 mg kg$^{-1}$) at (A) small intestine and (B) rectum. Effect of lipase (▲) in a bile solution coadministered with lauric acid was also studied. Each value represents the mean + s.d. (n > 5).

The effect of various other triglycerides, fatty acids and fatty acid sodium salts at the small intestine were also examined. The area under the curve (AUC) of plasma cefoxitin concentration over the 3 h following administration is shown in Fig. 4. It is difficult to calculate the 'true' bioavailability by the AUC method since plasma cefoxitin profiles varied depending on the triglyceride administered. About 50% bioavailability of cefoxitin was obtained when it was given with trilaurin. When the various fatty acids in free acid form were administered with sodium cefoxitin, plasma cefoxitin increased significantly above that obtained after sodium cefoxitin had been given with a triglyceride; an apparent cefoxitin bioavailability of more than 70% was obtained with either lauric or capric acid (Fig. 4).

When the fatty acids were given as their sodium salts, there was a further increase in plasma cefoxitin (Fig. 3). The rate of its absorption was significantly more rapid with the sodium salts than with free acid forms, especially with sodium myristate, palmitate and stearate. Moreover, the AUC of plasma cefoxitin tended to be greater with the sodium salts than with the free acid forms, particularly when the longer chain fatty acids were used (Fig. 4).

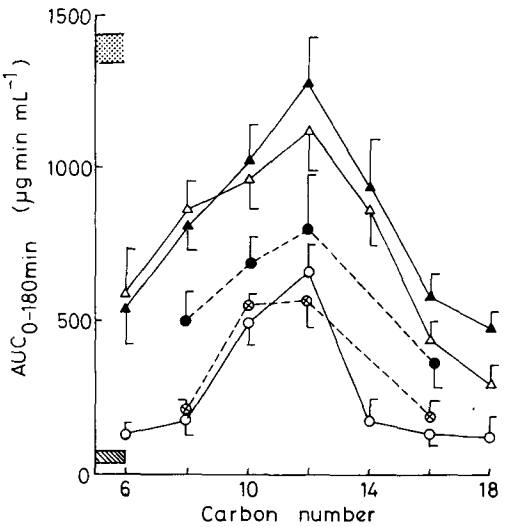


FIG. 4. Area under the curve [AUC] of plasma cefoxitin concentration for 180 min following administration of sodium cefoxitin, 15 mg kg$^{-1}$, into rat duodenum with fatty acids in the form of triglyceride (○), free acid (△) and sodium salt (▲) at a dose of 250 mg kg$^{-1}$. Administration of cefoxitin in preincubated medium of triglyceride with (●) or without (⊗) lipase in bile is also shown. The hatched area and dotted area represent the AUC of cefoxitin after administration of cefoxitin alone into rat duodenum and after cefoxitin intravenous administration, respectively. Each value represents the mean ± s.d. (n > 4).

The enhancing action of sodium caprylate, laurate and palmitate was dose-dependent (Fig. 5). Caprylate, the shorter chain fatty acid ion, showed the strongest dose-dependency of the three fatty acids within the dose range of 50–500 mg kg$^{-1}$.

When tricaprin, tricaprylin, trilaurin and tripalmitin were incubated with lipase in bile before administration with cefoxitin, their enhancing action on the small intestinal absorption of cefoxitin was greater than that of their untreated triglyceride forms (Fig. 4), and in the case of tricaprylin and tripalmitin resulted in AUCs of cefoxitin double those obtained without pretreatment.

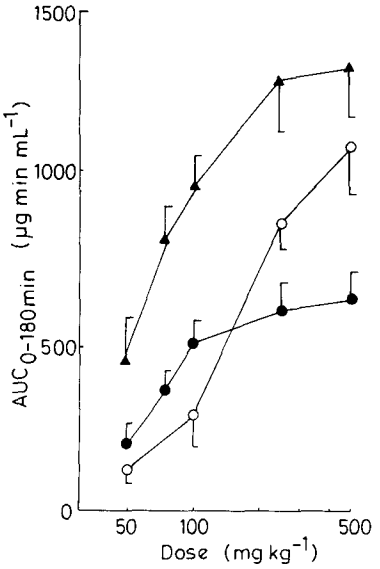


FIG. 5. Effect of a dose of sodium caprylate (○), sodium laurate (▲) and sodium palmitate (●) on the AUC of cefoxitin in plasma for 180 min following administration of sodium cefoxitin, 15 mg kg$^{-1}$. Each value represents the mean ± s.d. ($n > 4$).

## DISCUSSION

The disappearance of sodium cefoxitin from the intestinal loop preparation and its plasma concentration seemed to be related (Fig. 2), therefore it has been assumed that its intestinal absorption could be evaluated by measurement of its concentration in plasma from blood collected from the jugular vein.

The results suggest that lipase is needed for the enhancing action of trilaurin on cefoxitin uptake across both the intestine and rectal membranes. Improved rectal cefoxitin uptake when trilaurin was coadministered with lipase and bile, with only slight improvement when cefoxitin and bile alone were rectally administered, supports our premise. In the jejunal loop studies, prior removal of lipase by washing and its subsequent addition to the mixture resulted in a large increase in plasma cefoxitin concentration over that when the antibiotic was given with the triglyceride but without the lipase; this supports the evidence that trilaurin's adjuvant activity depends on the presence of lipase.

Lipase causes a rapid degradation of triglyceride into monoglyceride and a fatty acid (Börgstrom 1964), which in the case of trilaurin is lauric acid. Börgstrom reported that 1-monoglycerides are rapidly hydrolysed by lipase (hydrolysis of 2-monoglyceride is slower). The monoglyceride we used was 1-monolaurin and its main degradation product is also lauric acid. Since the enhancing action of trilaurin and monolaurin in the rectum occurred only if lipase was present, their degradation to lauric acid may be critical for the enhancement of drug uptake. Bile did not appear to contribute to the enhancement of drug absorption.

Triglycerides composed of $C_6$, $C_8$, $C_{14}$, $C_{16}$ and $C_{18}$ fatty acids did not enhance small intestinal absorption of cefoxitin as effectively as the $C_{10}$ and $C_{12}$ triglycerides, tricaprin and trilaurin, even though their free fatty acids and their sodium salts significantly enhanced cefoxitin absorption from the small intestine.

The absorption route of fatty acids differs depending on their carbon numbers. Short chain (-$C_{12}$) and long chain ($C_{14}$-) are mainly transported in portal blood and in the lymphatics, respectively (Isselbacher 1968). It was reported that the intestinal absorption of some lipophilic drugs, such as DDT (Palin et al 1982), was enhanced by coadministration of lipid, due to an increasing amount being taken into lymphatics. In our experiments, absorption rates of cefoxitin after coadministration of trilaurin and lauric acid (see Figs 1, 3) were greater than expected in accordance with lymphatic flow rate (25 mL/24 h, Bollman et al 1948) and also lag times were not observed in the plasma profiles. In addition, long chain fatty acids have a weak effect on absorption of cefoxitin compared with $C_{10}$ and $C_{12}$ acids. Therefore cefoxitin could be transported into portal blood after passing the intestinal membrane. This estimation is reasonable from the general rule that water-soluble drugs are transported into portal blood following intestinal absorption.

Isselbacher (1968) reported that the rate of triglyceride hydrolysis by lipase decreased as the carbon number of the fatty acid component of the triglyceride increased. Therefore, it is possible that degradation of long chain triglycerides in the lumen occurs too slowly to produce a concentration of fatty acid sufficient for enhancement of cefoxitin absorption. The observed enhancement of its absorption from an incubated medium of long chain triglycerides with lipase in bile, supports this hypothesis. Also, the weak adjuvant effect of long chain fatty acids compared with that of capric or lauric acids may be related to their low solubility (Ralston & Hoerr 1942).

Maintenance of an effective fatty acid level may also have been a problem with the shorter chain fatty acids since their triglycerides are known to be rapidly hydrolysed by luminal lipase, but the resulting fatty

acids are then readily absorbed from the intestine and so could not reach an effective concentration.

From both the preincubation and dose-dependent studies with the long and short chain triglycerides, we suggest that the concentration of fatty acids arising from triglyceride degradation in the small intestinal lumen is an important factor in triglyceride adjuvant action. The site of adjuvant action may therefore be primarily on the intestinal mucosal surface and this action of fatty acids, such as lauric acid, may be due to their surfactant activity as already reported (Nadai et al 1972; Nishihata et al 1981).

The problem of maintaining the necessary fatty acid concentration in the intestinal lumen when short or long chain triglycerides are used is as yet unresolved. Consequently, in considering the potential use of triglycerides as excipients in pharmaceutical products for oral administration of low lipophilic drugs such as cefoxitin, the medium chain triglycerides, trilaurin and tricaprin may be the most effective.

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