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# The oral absorption of cefoxitin from oil and emulsion vehicles in rats

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## Summary

The oral absorption of cefoxitin in rats is increased by inclusion in Miglyol 812 but not arachis oil. Dispersion of drug in the oil was shown to be essential as pre-dosing with Miglyol did not increase absorption from an aqueous suspension. At a fixed oil volume the amount of cefoxitin absorbed was independent of dose. Neither lymphatic absorption nor an effect of oil on gastric emptying were mechanisms involved in the enhanced absorption. A study of the effect of chain length of the constituent saturated fatty acids of Miglyol showed that maximum absorption was obtained with the $C_{12}$ acid. Prior exposure of the intestine to lauric acid had no effect on the absorption of cefoxitin but incubation of intestinal tissue with the radiolabelled fatty acid did result in uptake of radioactivity into the membrane lipids. It is proposed that co-administered oil initially protects cefoxitin from acid degradation in the stomach and that digestion of medium chain triglyceride liberates fatty acids that have a transient effect on membrane fluidity and allow increased absorption.

## Introduction

The oral absorption of a variety of drugs has been shown to be altered in the presence of lipids and emulsions (Armstrong and James, 1980). Most of the reports have been related to water insoluble compounds but there are a few studies of the effects of oily vehicles on the absorption of water soluble drugs. The oral absorption of insulin in rats sufficient to cause reduction of blood glucose has been reported using a liposomal formulation (Patel and Ryman, 1976) and the absorption of ceftizoxime, sodium was shown to be increased by dispersion in oils, especially when ethyl cellulose was present (Ueda et al., 1983).

Various physiological mechanisms have been proposed to explain the effect of oils on the absorption of water insoluble compounds including altered gastrointestinal motility, increased bile flow and drug solubilization (Bates and Sequeira, 1975), increased mucosal permeability (Muranushi et al., 1980), enhanced mesenteric lymph flow (De Marco and Levine, 1969) and increased lymphatic absorption (Palin et al., 1982a). No satisfactory explanation has been given for the enhanced absorption of water soluble compounds.

The oral absorption of cefoxitin from arachis oil and Miglyol 812 has been investigated in rats. Cefoxitin is a broad spectrum antibiotic of the cephamycin family which is water soluble at phys-

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iological pH and typical of compounds of this class, is poorly absorbed orally (Schrogie et al., 1978).

## Materials and Methods

*Materials*

Cefoxitin acid hydrate, [$^{14}$C]cefoxitin acid hydrate (cefoxitin-thienylacetic-carboxyl-$^{14}$C) and cefoxitin sodium were supplied by Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey, U.S.A. and 6-dodecenoic acid ($C_{12:1}$) by MSDRL, Hoddesdon, Herts. The oils investigated were arachis oil BP (peanut oil, Evans Ltd., Liverpool), Miglyol 812 (fractionated coconut oil, Dynamit Nobel, Slough, Berks), lauric acid ($C_{12}$), caprylic acid ($C_8$), palmitic acid ($C_{16}$) and oleic acid ($C_{18:1}$) were obtained from Sigma Chemical Co. Ltd., Poole, Dorset, and [1-$^{14}$C]lauric acid from Amersham International, Slough, Berks. Silicic acid was obtained from Unisil Clarkson Chemical Co. Inc., Williamsport, U.S.A. The surfactants used were Pluronic L122 (Wyandotte, Belgium) and Span 85 (Koch Light, Haverhill) and the scintillant was Fisofluor "1" (Fisons, Loughborough). Modified Krebs buffer was prepared to give a solution isotonic with body tissue (NaCl 118 mM, KCl 4.7 mM, $MgSO_4 \cdot 7H_2O$ 1.2 mM, $NaH_2PO_4 \cdot 2H_2O$ 0.9 mM, $NaHCO_3$ 25 mM, glucose 11.1 mM, $CaCl_2$ 2.5 mM) and Dulbecco's solution (A + B) was obtained from Oxoid Ltd. (Basingstoke, Hants). All organic solvents were HPLC grade.

*Oral absorption*

Formulations were prepared containing different weights of cefoxitin acid hydrate suspended in water, oil or in the oil phase of an oil-in-water emulsion and in solution in phosphate buffer (see Table 1). Suspensions in oil or water were prepared by triturating cefoxitin with the vehicle in a mortar, diluting to volume in a flask and sonicating for 15 min in a chilled bath. Each dose contained 4 $\mu$Ci [$^{14}$C]cefoxitin acid hydrate mixed with unlabelled drug by cocrystallisation from methanol. The emulsions contained 1% w/v Span 85, 1% w/v Pluronic L122 and 10% v/v arachis oil or Miglyol 812 or 10% w/v lauric acid. Cefoxitin was dispersed in Span 85, diluted with oil and added to an aqueous solution of Pluronic L122 to form a crude emulsion. The beaker was surrounded by melting ice and a 1-in. working-face, stainless steel probe was introduced into the emulsion which was sonicated (Ultrasonics, Shipley, Yorkshire) at 12.5 kHz for 4 min with continual stirring. The emulsion was diluted to volume with distilled water and stored at 4°C until tested. Microscopic examination of the emulsions showed that cefoxitin was dispersed in both phases. Male Wistar rats weight range 190–210 g, were fasted overnight with water ad libitum prior to oral administration of the test dose. Plasma samples collected from the tail tip at intervals over 8 h were analyzed by scintillation counting. TLC analysis of extracted plasma samples taken at 20 and 40 min after administration of Miglyol emulsion (0.5 mg/ml) showed that at least 41 and 61% respectively, of the radioactivity was associated with cefoxitin.

The I.V. solution was administered via the tail vein.

*Lymphatic absorption*

The thoracic duct was cannulated as described by Palin et al. (1982a) and lymph and plasma samples were collected at intervals up to 3 h after dosing with either the arachis oil or the Miglyol 812 emulsion (2.8 mg/0.5 ml). Samples were analyzed by scintillation counting.

*Intraduodenal administration*

Rats were anaesthetized with sodium pentobarbitone (i.p. 90 mg/kg) and a ligature tied around the pyloric sphincter. Arachis oil or Miglyol emulsions (2.8 mg/0.5 ml) were injected directly into the duodenum and blood samples were collected via a cannula in the portal vein.

*In situ intestinal loop study*

Emulsions containing cefoxitin sodium (6 mg/ml), 10% w/v fatty acid, 10% v/v Span 85 and 10% w/v Pluronic L122 were prepared as described previously. Emulsion (0.5 ml) was injected into the lumen of a ligated in situ loop of jejunum (approximately 5 cm length) in anaesthetized rats. Blood samples were collected from the

tail tip at intervals over 2 h and the plasma samples assayed by HPLC (Charles and Ravenscroft, 1984). This procedure was repeated for an emulsion containing 1% w/v lauric acid, a control formulation containing surfactant but no fatty acid and a solution of cefoxitin in Krebs buffer after exposure of the loop to a 10% w/v lauric acid emulsion for 10 min.

In a separate experiment the intestinal loop was exposed to a solution containing [$^{14}$C]lauric acid (10%) and cefoxitin sodium (6 mg/ml), the tissue removed and the membrane lipids isolated by silicic acid chromatography (Ning, 1983). The neutral lipids were eluted with diethyl ether and the phospholipids with methanol. The radioactivity in the two fractions was determined by liquid scintillation counting.

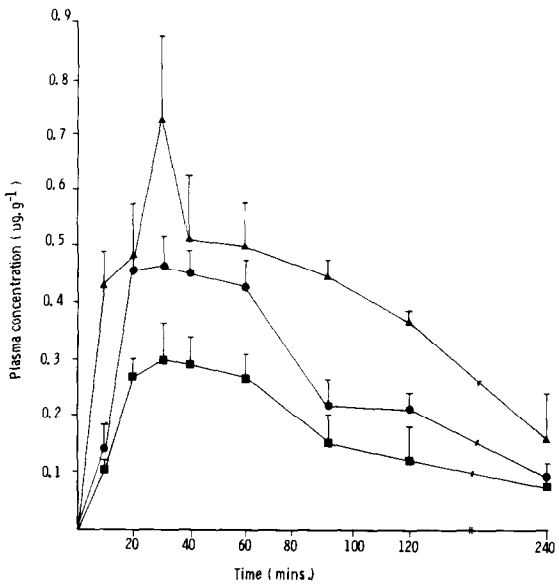


Fig. 1. The effect of different emulsion vehicles on the plasma concentration of [$^{14}$C]cefoxitin acid hydrate following oral administration to rats. ■, arachis oil; ●, Miglyol 812; ▲, lauric acid. Each value is the mean + S.E.M. of 6 animals.

## Results

The plasma concentration of cefoxitin acid hydrate ($\mu$g/g) at the different sampling times were calculated. Plasma–time curves were constructed for each formulation and the peak plasma concentration ($C_{p\,max}$) identified and the area under the curve between 0 and 4 h ($AUC_{0-4h}$) determined using the trapezoidal method, (Fig. 1 and Table 1). Statistical comparison of the data was made using the unpaired Student's *t*-test with a level of significance of 0.05.

Administration of [$^{14}$C]cefoxitin (0.44 mg) dispersed in the oil phase of a Miglyol oil-in-water emulsion significantly increased absorption compared with an aqueous solution. Drug absorption following administration of 2.8 mg cefoxitin was dependent on the formulation. A higher peak plasma level was produced by the Miglyol emulsion than the arachis oil emulsion, or aqueous suspension with predosing of Miglyol. Due to prolonged absorption from the aqueous suspension, the $AUC_{0-4h}$ was not significantly different to that obtained after administration of the Miglyol emulsion. The highest plasma levels and overall drug absorption were obtained following administration of cefoxitin as a suspension in Miglyol, and in lauric acid emulsion.

Administration of 3 different doses of cefoxitin in Miglyol emulsion (0.44 mg/ml, 1 mg/ml and 2.8 mg/0.5 ml) produced no significant difference in the peak plasma concentration or $AUC_{0-4h}$. This shows that as the dose is increased from 0.44 to 2.8 mg the total amount of drug absorbed remains essentially constant but the percentage absorption decreases significantly (Table 1). A dose of 2.8 mg is equivalent on a body weight basis to the human parenteral dose.

The ratio of the lymph to plasma $^{14}$C-concentration remained close to unity at all time points for both emulsion formulations, showing that there was no selective absorption into the lymph.

The plasma activity following intraduodenal administration of [$^{14}$C]cefoxitin in Miglyol emulsion was significantly higher (unpaired Student's *t*-test, $P < 0.05$) than following administration in arachis oil emulsion at 10, 20, 30 and 40 min after dosing (Fig. 2).

Oleic and 6-dodecenoic fatty acids had no effect on cefoxitin absorption from an in situ intestinal loop (Fig. 3). Palmitic, lauric and caprylic fatty acids increased the plasma drug levels at 40,

TABLE 1

THE EFFECT OF FORMULATION AND DOSE ON THE ORAL ABSORPTION OF $^{14}$C-CEFOXITIN IN RATS

| Vehicle | Cefoxitin dose (mg) | Dose-volume (ml) | Oil volume (ml) | $C_{p\max}$ $\mu g \cdot g^{-1}$ | $AUC_{0-4h}$ ($\mu g \cdot g^{-1} \cdot h$) | % equivalent IV dose absorbed |
|---|---|---|---|---|---|---|
| IV solution (in phosphate buffer) | 0.46 | 0.5 | – | – | 7.340 ± 1.425 | 100 |
| Aqueous solution (in phosphate buffer) | 0.45 | 1.0 | – | 0.09 ± 0.01 | 0.268 ± 0.04 | 3.7 ± 0.8 |
| Miglyol 812 emulsion | 0.44 | 1.0 | 0.1 | 0.67 ± 0.22 | 0.870 ± 0.214 | 11.9 ± 3.4 |
| | 1.0 | 1.0 | 0.1 | 0.74 ± 0.23 | 0.97 ± 0.19 | 6.1 ± 1.2 |
| | 2.8 | 0.5 | 0.05 | 0.52 ± 0.02 | 0.93 ± 0.04 | 2.1 ± 0.1 |
| Arachis oil emulsion | 2.8 | 0.5 | 0.05 | 0.30 ± 0.009 | 0.63 ± 0.18 | 1.4 ± 0.4 |
| Lauric acid emulsion | 2.8 | 0.5 | 0.05 | 0.81 ± 0.26 | 1.40 ± 0.20 | 3.1 ± 0.7 |
| Miglyol 812 suspension | 2.8 | 0.05 | 0.05 | 0.70 ± 0.14 | 1.69 ± 0.32 | 3.8 ± 0.7 |
| * Aqueous suspension | 2.8 | 0.45 | 0.05 | 0.28 ± 0.08 | 0.80 ± 0.22 | 1.8 ± 0.5 |

Mean ± S.D. $n = 5$ per group.

* Animals were predosed with 0.05 ml Miglyol 812 immediately prior to the administration of the aqueous suspension.

60 and 90 min after dosing and the total drug absorption over 2 h (Fig. 3) compared to the control cefoxitin surfactant solution. The presence of lauric acid significantly increased cefoxitin absorption compared to caprylic acid but not to palmitic acid. Following reduction of the percentage of lauric acid in the emulsion from 10 to 1%, cefoxitin absorption was reduced to control levels. Prior exposure of the loop to lauric acid had no effect on cefoxitin absorption from solution in Krebs buffer. Following incubation of the intestinal loop with a solution containing [$^{14}$C]-lauric acid and cefoxitin sodium, 10% of the total radioactivity that was incorporated into the tissue was detected in the phospholipid fraction. This indicates that exchange between the endogenous fatty acids of the membrane phospholipids and the exogenous fatty acids had occurred.

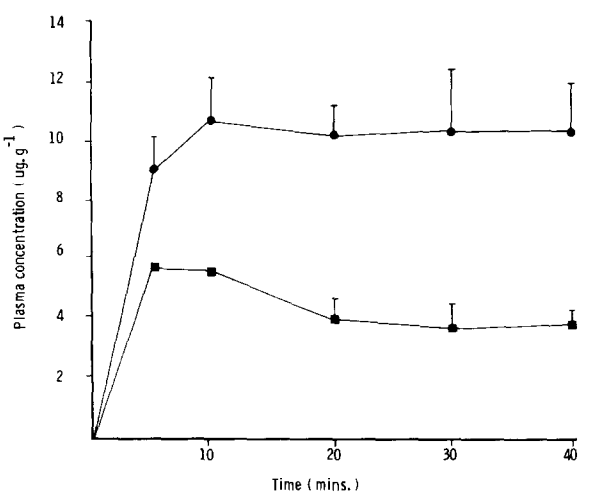


Fig. 2. The effect of different emulsion vehicles (0.5 ml volumes) on the portal plasma concentration of [$^{14}$C]cefoxitin acid hydrate following intraduodenal administration to anaesthetized rats. ■, arachis oil; ●, Miglyol 812. Each value is the mean + S.E.M. of 4 animals.

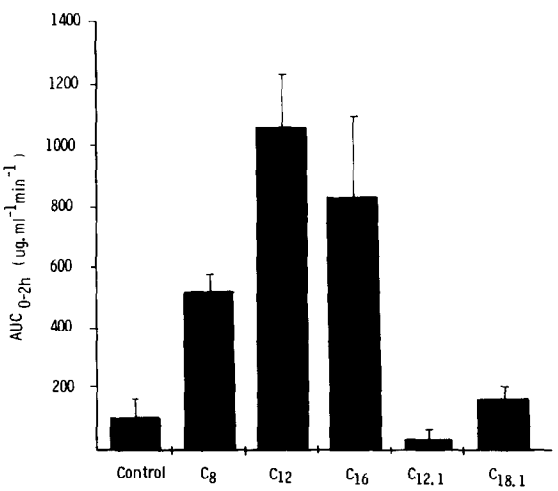


Fig. 3. The effect of different fatty acids on the absorption of sodium cefoxitin from in situ intestinal loops in anaesthetized rats. $C_8$, octanoic acid; $C_{12}$, lauric acid; $C_{16}$, palmitic acid; $C_{12:1}$, 6-dodecenoic acid; $C_{18:1}$, oleic acid. Each value is the mean + S.E.M. of 4 animals.

## Discussion

The data in Fig. 1 show that the absorption of [$^{14}$C]cefoxitin in rats was significantly increased by administration in a Miglyol emulsion compared with an aqueous solution. Similarly, greater drug absorption occurred from a Miglyol emulsion than from an arachis oil emulsion, and from a Miglyol suspension compared with the Miglyol emulsion and an aqueous suspension with immediate pre-dosing of the oil. These results show that oils can promote the absorption of cefoxitin but that the effect is dependent on the nature of the oil and on administration of the drug within the oil phase.

An investigation of the lymphatic absorption of the drug showed that neither Miglyol 812 nor arachis oil stimulated selective uptake into the lymph. The lymph : plasma ratio of $^{14}$C-concentration remained approximately one, indicating that cefoxitin is absorbed via the portal route and is then distributed into the whole body fluid.

Pre-dosing the animals with Miglyol 812 prior to administration of cefoxitin in an aqueous suspension did not enhance absorption (see Table 1). This suggests that increased drug absorption is not due to a slowing of gastric emptying. Enhanced absorption with the Miglyol emulsion compared to the arachis oil emulsion, was still obtained following intraduodenal administration which also indicates that gastric emptying is an unimportant factor.

The absence of an effect following pre-dosing with Miglyol 812 suggests that the drug has to be in the oil phase for absorption to be enhanced. This may in part explain why administration of an oily suspension, or a reduction in the dose promoted absorption. In each of these preparations there is the potential for a higher percentage of the dose to be associated with the oil phase. Cefoxitin degrades in acid conditions (Das Gupta, 1981) and is therefore unstable at gastric pH. It is possible that Miglyol 812 promotes cefoxitin absorption simply by protecting the molecule from the acid environment of the stomach. Drug partitioning from the oil phase will be minimal as at low pH cefoxitin is unionized and very poorly water soluble. However, it would be anticipated that cefoxitin incorporated into the arachis oil would be protected in the same way.

Digestion of Miglyol liberates saturated medium chain fatty acids ($C_8$–$C_{12}$) whereas arachis oil digestion produces mainly long chain unsaturated $C_{18}$ fatty acids, i.e. oleic and linoleic. Several reports have suggested that the presence of different fatty acids can have different effects on drug absorption from the gastrointestinal tract (Grisafe and Hayton, 1978; Muranushi et al., 1980). Increased intestinal and rectal absorption from Witepsol was attributed to free fatty acids liberated from the triglyceride base by lipases (Nishihata et al., 1986). The data produced from the in situ gut loops showed that regardless of chain length, the saturated fatty acids increased absorption whereas the unsaturated fatty acids had no significant effect. The adjuvant action of the saturated fatty acids depended on the carbon chain length, the $C_8$ caprylic acid had less effect than the $C_{12}$ lauric and $C_{16}$ palmitic acids. Although there was no significant difference between the effect of these two fatty acids, greater absorption was obtained in the presence of lauric acid.

The fatty acid concentration at the absorption site appears to be critical as cefoxitin absorption was not enhanced when the percentage of lauric acid in the test system was reduced by a factor of ten. Prior exposure of the intestinal tissue to lauric acid had no effect on cefoxitin absorption indicating that the oil has to be coadministered with the drug. This suggests that enhanced absorption occurs as the result of a transient effect on the membrane and not damage or by complex formation between the cefoxitin and the fatty acid. The latter is unlikely due to the chemical structure of cefoxitin. The results showed that following incubation, radiolabelled lauric acid was incorporated into the membrane and exchange with phospholipids may occur. The replacement of long chain fatty acids in the phospholipids with medium chain fatty acids could increase the permeability of the membrane by producing "spaces" for the drug to pass through. Lauric acid possesses surfactant properties and another possibility is that it solubilizes lipophilic constituents of the membrane such as cholesterol. The adjuvant action of lauric acid on cefoxitin uptake was con-

firmed when greater total drug absorption resulted from oral administration in the lauric acid emulsion compared to the Miglyol 812 emulsion.

In conclusion it has been shown that Miglyol 812 increases the oral absorption of cefoxitin in rats when the drug is administered in the oil phase whereas arachis oil has no effect. The mechanism by which Miglyol promotes cefoxitin uptake is not by increased lymphatic absorption of the drug, or a reduction in gastrointestinal motility. Bile salts would not appear to be implicated as Palin et al. (1982b) have shown that arachis oil and Miglyol do not increase their output. However, within the intestinal lumen oil emulsification and digestion releases the drug and fatty acids; saturated medium chain fatty acids from Miglyol 812 and predominantly unsaturated long chain fatty acids from arachis oil. In the presence of saturated fatty acids and in particular $C_{12}$ and $C_{16}$, cefoxitin absorption from the small intestinal lumen is increased possibly due to a transient effect of the fatty acid on membrane fluidity.

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