IMPROVEMENT OF BIOAVAILABILITY OF POORLY ABSORBED DRUGS. II. EFFECT OF MEDIUM CHAIN GLYCERIDE BASE ON THE INTESTINAL ABSORPTION OF CEFMETAZOLE SODIUM IN RATS AND DOGS

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The effect of medium chain glyceride (MCG) on the intestinal absorption of cefmetazole sodium (CMZ) was investigated in rats and dogs.

In rats, MCG containing glyceryl mono-, di- and tri-caprylate enhanced the intestinal absorption of CMZ after intraduodenal administration, though the promoting effect of MCG was less than that after rectal administration. The promoting effect of MCG was found to be mainly due to glycerylmonocaprylate and dependent on the dosage of MCG. The plasma CMZ levels after intraduodenal administration as MCG solution tended to be slightly higher than those observed after administration as MCG emulsion, though the differences were found to be statistically insignificant. Moreover, the intestinal absorption of CMZ after intraduodenal administration as MCG emulsion was decreased significantly by increasing the amount of water in the emulsion. The promoting effect of MCG in dogs was more clearly demonstrated in the lower intestine than in the upper intestine.

Furthermore, the oral bioavailability of pharmaceutical formulations of CMZ was also investigated in dogs. When enteric coated capsules filled with MCG solution were administered to dogs, the bioavailability of CMZ was enhanced significantly.

**Keywords** — medium chain glyceride; cefmetazole sodium; intestinal absorption; oral administration; enteric coated capsule; bioavailability; rat; dog

INTRODUCTION

Although many investigators have reported on the intestinal absorption of β-lactam antibiotics, factors affecting the absorption of these antibiotics across biological barrier are not yet clearly understood. However, as many injectable β-lactam antibiotics are not absorbed satisfactorily after oral administration, many attempts to improve their intestinal absorption either by using promoting agents such as surfactants or non-surfactant agents, or by devising differential formulations have been investigated.

Similarly, we have reported that rectal absorption in rats of cefmetazole sodium (CMZ), a semisynthetic cephemycin antibiotic, was enhanced significantly after administration with medium chain glyceride (MCG), which is a mixture of glyceryl mono-, di- and tri-caprylate.

The purpose of the present paper is to report the intestinal absorption of CMZ after intraduodenal administration with MCG and the new pharmaceutical formulations which improve the bioavailability of CMZ after oral administration.

MATERIALS AND METHODS

**Materials** — CMZ was synthesized in the Central Research Laboratories of Sankyo Co. Ltd. Sunsoft 700P-2® (Taiyo Kagaku), Sunsoft 707® (Taiyo Kagaku), Nikkol MGK® (MGK®, Nikko Chemicals), Sunfat GDC® (Taiyo...
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Kagaku) and Panasate 800® (Nippon Oil & Fats) employed as MCG containing various concentrations of glyceryl mono-, di- and tri-caprylate were obtained commercially. Sunsoft 760® and Sunsoft 767® employed as MCG containing various concentrations of glyceryl mono-, di- and tri-caprate were Taiyo Kagaku.

**Analysis of Glyceride Content** — Analysis of the glyceride content was performed by the gas chromatographic (GC) method described in a previous paper. The glyceride content of MCG used in this experiment is summarized in Table I.

**Test Preparations** — Aqueous solution of CMZ was prepared at concentrations of 60 mg/ml of distilled water.

Different kinds of oily solution of CMZ were prepared using monoglycerides at respective concentrations of 30 mg/ml for absorption experiments in rats and 125 mg/ml for those in dogs.

Different kinds of oily suspensions of CMZ were prepared using glyceryldicaprylate and glyceryltributyrinate at concentrations of 125 mg/ml for absorption experiments in dogs.

Emulsion of CMZ was prepared using MGK®. CMZ (60 mg) was dissolved in 1 ml water and the solution was added to 2 ml MGK®. The mixture was emulsified by sonication (No. 72 type, Branson) for 20 min.

**Enteric Coated Capsules** — Five gelatin capsules (No. 1, Parke-Davis) were filled with 300 mg CMZ dissolved in 2 ml MGK®. As a control, a gelatin capsule was filled with 300 mg CMZ just before the experiment. Enteric coated capsules were prepared following the method of Nishimura. The gelatin capsules filled with CMZ dissolved in MGK® were coated with hydroxypropyl methylcellulose (5% w/w) and then with hydroxypropyl methylcellulose phthalate (10% w/w) in methylene dichloride–ethanol (1:1 w/v) solution to obtain enteric coated capsules.

**Release of CMZ from MGK® into Water** — Phosphate buffer (pH 7.4, 1/15 M) was used as the aqueous phase. The volumes of the aqueous phase and of the MGK® phase containing 1% (w/v) of CMZ were 30 ml each. All solutions were shaken at 250 rpm at room temperature. A 0.1 ml sample was taken from each phase at given times. The concentrations of CMZ in the aqueous phase and MGK® phase were determined by the high performance liquid chromatographic (HPLC) method described in a previous paper.

**In Situ Experiments in Rats** — Male Wistar-Imamichi rats weighing 290–320 g with free access to water were fasted for about 18 h prior to the experiments. These rats were anesthetized with 4.0 mg/100 g of sodium pentobarbital by

<table>
<thead>
<tr>
<th>Preparations</th>
<th>Monoglyceride (%)</th>
<th>Diglyceride (%)</th>
<th>Triglyceride (%)</th>
<th>Fatty acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A b) I</td>
<td>88.0</td>
<td>6.5</td>
<td>5.5</td>
<td>N.D. d)</td>
</tr>
<tr>
<td>II</td>
<td>55.4</td>
<td>32.6</td>
<td>12.0</td>
<td>N.D.</td>
</tr>
<tr>
<td>III</td>
<td>54.3</td>
<td>37.0</td>
<td>6.0</td>
<td>2.7</td>
</tr>
<tr>
<td>IV</td>
<td>3.8</td>
<td>81.2</td>
<td>15.0</td>
<td>N.D.</td>
</tr>
<tr>
<td>V</td>
<td>N.D.</td>
<td>N.D.</td>
<td>100.0</td>
<td>N.D.</td>
</tr>
<tr>
<td>B c) VI</td>
<td>98.4</td>
<td>1.6</td>
<td>0.0</td>
<td>N.D.</td>
</tr>
<tr>
<td>VII</td>
<td>56.4</td>
<td>32.6</td>
<td>3.5</td>
<td>7.5</td>
</tr>
</tbody>
</table>

a) Commercial preparations: I; Sunsoft 700P-2®, II; Sunsoft 707®, III; Nikkol MGK®, IV; Sunfat GDC®, V; Panasate 800®, VI; Sunsoft 760®, VII; Sunsoft 767®.
b) Glycerides of caprylic acid, c) Glycerides of capric acid, d) Not detected.
intraperitoneal injection. The small intestine was exposed by a midline abdominal incision and was ligated at the end of the ileum. At 30 min after the operation, 30 mg/kg of drug preparation was administered into the duodenum and the pylorus was immediately ligated.

In the case of pre- and post-administration of MGK®, twelve rats anesthetized with sodium pentobarbital were used and divided into three groups of four rats each. The small intestine was exposed by a midline abdominal incision and was ligated at the end of the ileum. A small incision was made in the stomach to insert a polyethylene tube (i.d. 0.58 mm, o.d. 0.965 mm, Clay Adams), toward the middle duodenum, which was closed at the exposed end by a stopcock to prevent drainage of drug solution and/or MGK® from the duodenum.

In the pre-administration experiments, MGK® was administered into the duodenum through the polyethylene tube 10 min before dosing CMZ aqueous solution. In the post-administration experiments, MGK® was administered into the duodenum through the polyethylene tube 10 min after dosing CMZ aqueous solution. As a control experiment, co-administration of MGK® and CMZ aqueous solution was performed. Blood samples were taken from the jugular vein via a heparinized tube.

In Situ Experiments in Dogs — Twelve healthy male Beagle dogs weighing 9.5—12.0 kg with free access to water were fasted for about 18 h prior to the experiments and anesthetized with 25 mg/kg of sodium pentobarbital by intravenous administration. They were divided into four groups according to the segment to be ligated. After each dog was fixed on its back, the small intestine was exposed by a midline abdominal incision and a 20 cm segment of the duodenum, the upper jejunum, the middle jejunum, or the ileum was ligated. CMZ, 25 mg/kg, was injected as an MGK® solution into the ligated segment of each dog. Blood samples were taken with a heparinized syringe from the femoral vein. Furthermore, the effect of the composition of glyceride on the intestinal absorption of CMZ was investigated using the middle jejunum of dogs.

In Vivo Experiments in Dogs — Healthy male Beagle dogs weighing 9.0—11.5 kg with free access to water were fasted for about 18 h prior to the experiments. CMZ in capsule form was administered orally at 300 mg/body with 20 ml of water. Venous blood samples were taken after administration.

Analytical Procedure — At given times, blood samples were taken and plasma samples obtained after centrifugation were frozen until analysis at −15 °C. CMZ in plasma was determined by the HPLC method described in a previous paper.14) Residual CMZ in the intestinal segment was washed out with cold saline and determined by HPLC.

Bioavailability of the drug was calculated as the ratio of the area under the plasma concentration–time curve (AUC) of intestinal to intravenous administration.

RESULTS AND DISCUSSION

The effects of glycerides of mono-, di- and tricaprylate on the intestinal absorption of CMZ in rats are shown in Fig. 1. The highest plasma CMZ levels after intraduodenal administration

![Graph](image)

**FIG. 1. Effect of Glycerides of Caprylic Acid (C₈) on the Intestinal Absorption of CMZ in Rats**

_Dose: (30 mg CMZ/0.5 ml water + 0.5 ml glyceride)/kg. Each value is the mean ± S.E. for at least 4 animals. ●: preparation I, ○: preparation IV, ▲: preparation V. Roman numerals are explained in Table I._
with preparation I, containing 88.0% monocaprylate, were observed; peak concentrations were $2.84 \pm 0.84 \, \mu g/ml$ at 10 min. The mean AUC of plasma CMZ levels after intraduodenal administration with preparation IV, containing 81.2% dicaprylate, was one third of that obtained with preparation I. On the other hand, in the control experiment, when aqueous solution of CMZ was administered into the duodenum, CMZ was not detected in the plasma at any time. CMZ was also not detected in the plasma after administration with preparation V contain-

![Graphs showing plasma concentration of CMZ over time for different preparations](image)

**FIG. 2. Effect of the Number of Carbon Atoms in the Fatty Side Chain of Glycerides on the Intestinal Absorption of CMZ in Rats**

Dose: (30 mg CMZ/0.5 ml water + 1 ml glyceride)/kg. Each value is the mean ± S.E. for at least 4 animals.

(A) ○; preparation I. ●; preparation III. (B) △; preparation VI, ▲; preparation VII. Roman numerals are explained in Table I.

**TABLE II. Effect of Composition of Glyceride (C₈) a) on the Intestinal Absorption of CMZ in Dogs**

<table>
<thead>
<tr>
<th>Preparation b)</th>
<th>$AUC_{0-2} , (\mu g \cdot h/ml)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>12.7 ± 1.5</td>
</tr>
<tr>
<td>I + II c)</td>
<td>18.6 ± 3.2</td>
</tr>
<tr>
<td>II</td>
<td>24.8 ± 3.5</td>
</tr>
<tr>
<td>III</td>
<td>26.0 ± 2.6</td>
</tr>
<tr>
<td>IV</td>
<td>16.3 ± 2.2</td>
</tr>
<tr>
<td>V</td>
<td>0.9 ± 0.1</td>
</tr>
</tbody>
</table>

*Dose: CMZ; 25 mg/kg. Glyceride; 0.2 ml/kg. Loop site; Middle jejunum (20 cm). a) Glyceride of caprylic acid. b) Roman numerals are explained in Table I. c) 1:1.*
ing 100% tricaprylate. The order of intensity of the promoting effect after intraduodenal administration was found to be in good accord with the results obtained from those in the rectum. From these results, it was concluded that the promoting effect of monoglyceride on the intestinal absorption of CMZ was the highest among MCG components.

The promoting effects of various monoglycerides on the intestinal absorption of CMZ are shown in Fig. 2. In both glycerides C₈ (caprylic acid) and C₁₀ (capric acid), mean plasma CMZ levels after administration with glyceride base containing 55—56% monoglyceride were observed to be higher than those observed after administration with glyceride base containing more than 80% monoglyceride. Furthermore, the promoting effect of glycerides of caprylic acid on the intestinal absorption of CMZ was compared with that of glycerides of capric acid. No differences were observed between the plasma CMZ levels after administration with preparation I and those observed after administration with preparation VII containing 56.4% monocaprate. Mean plasma CMZ levels after administration with preparation III containing 54.3% monocaprylate were observed to be much higher than those observed after administration with other preparations. These results, shown in Figs. 1 and 2, suggested that glyceride base containing glyceryl mono-, di- and tri-caprylate enhanced the intestinal absorption of CMZ and the promoting effect was mainly due to glycercy1monocaprylate.

On the other hand, we have reported that the component ratio of glyceryl mono-, di- and tri-caprylate played an important role in the promoting effect of MCG in the rat rectum. So, commercially available preparations containing various component ratios of glyceryl mono-, di- and tri-caprylate were used to examine the effect on intestinal absorption of CMZ. The relationship between components and AUC obtained up to 2 h after administration with each preparation are summarized in Table II. As shown in Table II, AUC of CMZ after administration with preparation III was observed to be much larger than those after administration with other preparations. Therefore, these results supported the conclusion that there is an optimum range of monoglyceride concentration in promoting CMZ absorption, though the bioa-

**FIG. 3.** Effect of Dose of MGK® on the Intestinal Absorption of CMZ in Rats

Dose: 30 mg CMZ/ (0.125—1.0 ml) MGK®/kg. Each value is the mean ± S.E. for at least 4 animals.

(A) Relationship between C_max and dose. (B) Relationship between AUC and dose. As a control experiment, CMZ aqueous solution was administered into the duodenum; CMZ was not detected in the plasma.
availability was still found to be not enough. Furthermore, in addition to glyceride components, preparation III also contains 2.7% caprylic acid.

The effect of caprylic acid on the promoting effect of MCG was therefore investigated. The results, however, showed its effect to be negligible compared to the promoting effect of preparation III and preparation II containing glyceride components only.

From the facts described above, it may be concluded that MCG, which is a mixture of glyceryl mono-, di- and tri-caprylate, enhanced the absorption of CMZ not only in the rectum but also in the small intestine. In particular, the promoting effect of MCG containing about 50% glycerclylmonocaprylate was found to be most suitable as a promoting agent in improving the bioavailability of CMZ. Although many points of the mechanism of the promoting effect of MCG are still uncertain, these results indicate the possibility of application of MCG for promoting absorption of CMZ through the intestinal membrane. In order to clarify the promoting effect of MCG in more detail, subsequent experiments were performed using

**FIG. 4. Plasma Concentrations of CMZ after Administration as MGK® Solution and MGK® Emulsion in Rats**

Each value is the mean ± S.E. for at least 4 animals. ●: MGK® solution (30 mg/1 ml MGK®/kg) ○: MGK® emulsion (30 mg CMZ/0.5 ml water + 1 ml MGK®)/kg.

**FIG. 5. Release of CMZ from MGK® Phase into Water Phase**

Water phase; pH 7.4 phosphate buffer (1/15 M).

**FIG. 6. Effect of the Amount of Water in Emulsion on the Intestinal Absorption of CMZ in Rats**

Dose: (30 mg CMZ/(0—2 ml water + 1 ml MGK®)/kg. Each value is the mean ± S.E. for at least 4 animals.
MGK® containing 54.3% monopropionate.

The effect of dosage of MGK® on CMZ absorption from rat small intestine was investigated using in situ experiments. There results are shown in Fig. 3. Both the mean maximum plasma concentration (Cmax) (Fig. 3A) and AUC (Fig. 3B) rose with increasing dose of MGK®, and reached a plateau at 0.5 ml/kg of MGK®. These results suggested that the promoting effect of MGK® on the intestinal absorption of CMZ is found to be saturable.

The effect of dosage form on promoting absorption of CMZ after administration with MGK® was investigated in rats. The mean plasma CMZ levels after administration as MGK® solution and MGK® emulsion are shown in Fig. 4. The plasma CMZ levels after administration as MGK® solution were found to be slightly higher than those observed after administration as MGK® emulsion, though no statistically significant differences were found. When compared with the intravenous administration of CMZ aqueous solution, the absolute bioavailability calculated as the ratio of the AUC for intestinal administration to that for intravenous administration were 32.7 ± 2.7% for MGK® emulsion and 39.8 ± 7.8% for MGK® solution, respectively.

The results of CMZ release from the MGK® phase into the water phase at room temperature are shown in Fig. 5. As shown in Fig. 5, CMZ was released easily from the MGK® phase into the water phase and reached an equilibrium within about 5 min. From the results shown in Fig. 5, it was considered that CMZ was released immediately from MGK® into the intestinal fluid on the surface of the membrane when the drug was administered as MGK® solution. From this standpoint, it was concluded that there was no statistically significant difference between the

![Graphs](A) and (B)

**FIG. 7.** Effect of Pre- and Post-administration of MGK® on the Intestinal Absorption of CMZ in Rats

Dose: 30 mg CMZ/0.5 ml water and 1 ml MGK®/kg. Each value is the mean ± S.E. for at least 4 animals. —10: Pre-administration of MGK®, 10 min before dosing CMZ aqueous solution. 0: Co-administration of MGK® and CMZ aqueous solution. +10: Post-administration of MGK®, 10 min after dosing CMZ aqueous solution.
plasma CMZ levels after administration as MGK® solution and those after administration as MGK® emulsion. However, the promoting effect of MGK® decreased significantly with increasing amount of water in the emulsion. The effect of the amount of water in the emulsion on the promoting effect of MGK® for the intestinal absorption of CMZ is shown in Fig. 6. This phenomenon suggested that the promoting effect of MGK® is markedly affected by the large quantity of intestinal fluid which is secreted at the small intestine. Therefore, it was considered that co-existence of large quantities of MGK® and CMZ on the absorption membrane are an important factor affecting the promoting effect of MGK®.

To design the pharmaceutical formulation for CMZ/MGK® preparation, the effect of pre- and post-administered MGK® on the absorption of CMZ from the intestine was investigated. These results are shown in Fig. 7. As shown in Fig. 7, the mean $C_{\text{max}}$ of CMZ was obviously high for co-administration of CMZ aqueous solution and MGK®, but when $AUC$s were compared, the differences among respective bioavailabilities were small. These results suggested that the formulation which released both MGK® and CMZ into the intestinal lumen at the same time was most suitable for the promoting effect of MGK®.

To clarify the absorption site of CMZ as well as the promoting effect of MGK®, bioavailability studies were performed using in situ experiments in dogs. The mean plasma CMZ levels after administration as MGK® solution into each different segment of the intestine are shown in Fig. 8. High plasma CMZ levels were observed after administration into the middle jejunum and ileum; peak concentrations were 14.1 ± 1.7 and 13.9 ± 1.3 μg/ml at 60 min, respectively. Although the plasma CMZ plateau levels after administration into the duodenum and upper jejunum were observed to be maintained as in other segments, the peak concentrations were approximately one half those observed after administration into the middle jejunum and ileum. The disappearance percent of CMZ from these segments and the $AUC$ of CMZ up to 2 h after administration are shown in Fig. 9. Although statistically significant dif-

**Fig. 8.** Plasma Concentrations of CMZ after Administration as MGK® Solution to Various Intestinal Segments in Dogs

Dose; 25 mg CMZ/0.2 ml MGK®/kg. Each value is the mean ± S.E. for 3 animals. ●; duodenum, ○; upper jejunum, ▲; middle jejunum, △; ileum.

**Fig. 9.** Disappearances of CMZ from Various Intestinal Segments and $AUC$

Each value is the mean ± S.E. for 3 animals.
ferences were not found among the disappearance of CMZ from the upper jejunum, middle jejunum and ileum, the AUC of CMZ after administration into the duodenum and upper jejunum were one half those observed after administration into the middle jejunum and ileum. These results suggested that the promoting effect of MGK® on the intestinal absorption of CMZ was much larger in the lower intestine than in the upper intestine.

From the data described above, it was concluded as follows: (1) MGK® might be useful as a promoting agent for improvement of CMZ absorption through the intestinal membrane. (2) It is necessary that not only MGK® but also CMZ are present in large quantities on the surface of the absorptive membrane of the intestine.

It was, therefore, considered that the absorption of CMZ should be improved by an enteric coated preparation which is designed to have MGK® and CMZ released in great quantities at the absorption site. Therefore, enteric coated capsules filled with MGK® solution were prepared as a formulation to approximately satisfy these conditions. The mean plasma CMZ levels after oral administration of various formulations to dogs are shown in Fig. 10. Mean plasma CMZ levels after administration of enteric coated capsules filled with MGK® solution were observed to be much higher than those observed after administration of other formulations. The pharmacokinetic parameters of CMZ in dogs after dosing with these formulations are summarized in Table III. After oral administration of formulation (A), the plasma concentration peaked at 1.8 ± 0.8 h and then declined. The \(C_{max}\) was 18.9 ± 4.2 µg/ml and the absolute bioavailability calculated as the ratio of the AUC of oral to intravenous administration was 64.8 ± 11.0%. The plasma CMZ levels after administration of formulation (A) was found to exceed the therapeutically active level against many gram-positive and gram-negative bacteria.\(^{15,16}\)

### Table III. Pharmacokinetic Parameters Following Oral Administration of Each Preparation to Dogs

<table>
<thead>
<tr>
<th>Preparations</th>
<th>(C_{max}) (µg/ml)</th>
<th>(T_{max}) (h)</th>
<th>(AUC_{0-\infty}) (µg·h/ml)</th>
<th>Absolute bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>18.9 ± 4.2</td>
<td>1.8 ± 0.8</td>
<td>40.8 ± 6.9</td>
<td>64.8 ± 11.0</td>
</tr>
<tr>
<td>B</td>
<td>2.4 ± 0.4</td>
<td>3.3 ± 0.3</td>
<td>13.1 ± 2.4</td>
<td>20.8 ± 3.8</td>
</tr>
<tr>
<td>C</td>
<td>1.1 ± 0.3</td>
<td>2.0 ± 0.0</td>
<td>3.5 ± 1.1</td>
<td>5.6 ± 1.7</td>
</tr>
</tbody>
</table>

A: Enteric coated capsules filled with MGK® solution.
B: Uncoated capsules filled with MGK® solution.
C: Enteric coated capsules filled with CMZ powder.
Consequently, the improvement of the bioavailability of CMZ from enteric coated capsules is probably attributable to a transit near the absorption site and release of a great quantity of MGK® and CMZ on the surface of the intestinal membrane. In contrast, in the case of conventional capsules, MGK® released from the capsules was diluted by fluid secreted at the stomach and upper small intestine, resulting in a decrease of the promoting effect of MGK®.

Furthermore, microscopic observation of mucosa was made after treatment with MGK® in dogs. No apparent damage by MGK® to the intestinal mucosa was observed. Accordingly, the promoting effect of MGK® does not seem to be accompanied by mucosal damage.

In conclusion, enteric coated capsules were found to be suitable for inducing a strong promoting effect of MGK® after oral administration.

REFERENCES