Pharmacokinetics of Loxoprofen and Cefcapene following a Single Combined Dose in Healthy Volunteers before, during or Immediately after 120-min Enteral Tube Feeding of 400 mL Ensure Liquid®

Mikiko UEDA¹, Kazuo HARADA², Kenji IKEDA³, Minako OHISHI⁴, Maria KITAMURA⁴, Hiroko UEDA⁴, Katsumi AZENISHI⁵, Setsu INATSUKI⁶, Kazunori NIKI⁷, Chisato OKAJIMA⁸, Mamoru TEPAKU², Yuki FUKUDA², Saki YOSHIDA², Kazumasa Hirata², Shinichiro SUNA⁸, Kazusaburo KATAOKA⁹, Mikihiiko KOGO¹⁰ and Etsuko UEJIMA⁷

Abstract
Enteral feeding is commonly performed in medical and nursing care facilities. Enteral feeding takes a long time; therefore, the dosing of drugs indicated for postprandial administration is always challenging clinics that lack knowledge of the potential effects of enteral tube feeding on clinical pharmacokinetics. In this study, the effects of enteral tube feeding on the pharmacokinetics of loxoprofen and cefcapene were investigated in a parallel group study design, in which Group I (N = 7) received loxoprofen (fine granules, 120 mg) and cefcapene pivoxil hydrochloride (fine granules, 100 mg) co-administered 15 min before the start of 120 min tube feeding with 400 mL of Ensure Liquid®. Group II (N = 7) received test drugs during (i.e., 60 min after the start of) 120 min tube feeding, and Group III (N = 7) received the test drugs after (i.e., at the end of) 120 min tube feeding. The Cmax of loxoprofen and cefcapene were statistically higher in Group I when the drugs were administered 15 min before tube feeding. The Cmax of loxoprofen in Group I was 58.7% and 82.8% higher than that in Group II and Group III, respectively. The Cmax of cefcapene in Group I was 43.3% and 32.2% higher than that in Group II and Group III, respectively. Although the AUC∞ appeared to be similarly higher in Group I, no significant difference was observed between the 3 groups. However, these changes did not appear to be critically meaningful in the clinic. Administration of loxoprofen and cefcapene in patients under enteral tube feeding should be clinically manageable.

Key words: enteral tube feeding, pharmacokinetic profiles, loxoprofen, cefcapene, healthy volunteers

Introduction
Many patients in medical and nursing care facilities cannot ingest food orally. The reasons for the difficulty in ingesting food vary and may include decreased swallowing function, decreased cognitive function, and decreased gastrointestinal tract function, which may be age- or disease-related or associated with surgical management. In such patients, the enteral tube feeding method is commonly used for nutritional management, and oral drugs are often administered through a nasogastric tube for enteral tube feeding. Generally, tablets and capsules are administered either in the pulverized form or through a simple suspension method. The Osaka University Dental Hospital (the hospital at which this study took place) manages patients with diseases affecting the oral cavity in particular. Approximately 40% of the inpatients receive nutrition through an enteral tube feeding over the treatment period or as treatment progresses after surgery. In particular, antibiotics and analgesics are administered as part of the surgical management. The administration of many pharmaceutical products, with or without regard to food, is usually documented in package inserts. The pharmacokinetics of investigational drugs are to be investigated in clinical pharmacology studies, with concomitant ingestion of regular food as a prerequisite. However, the pharmacokinetics of drugs administered in patients receiving enteral tube feeding have not been investigated thus far for many drugs. Therefore, this study investigated the pharmacokinetic profiles of drugs commonly used for surgical management when a pharmaceutical product is administered before, during, and after tube feeding (meals) under enteral tube feeding management, using loxoprofen and cefcapene pivoxil hydrochloride fine granules as the test drugs. Loxoprofen is a representative nonsteroidal anti-inflammatory.

¹ Division of Pharmacy, Osaka University Dental Hospital, Japan ² Experimental Institute for Medicinal Plants Graduate School of Pharmaceutical Sciences, Osaka University, Japan ³ Laboratory of Clinical Pharmaceutics, Faculty of Pharmacy, Osaka Ohtani University, Japan ⁴ Department of Pharmacy, Mie University Hospital, Japan ⁵ Nutrition Support Service, Osaka University Dental Hospital, Japan ⁶ Clinical Pharmacy Research and Education Unit, Graduate School of Pharmaceutical Sciences, Osaka University, Japan ⁷ Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Japan ⁸ School of Pharmaceutical Sciences, Mukogawa Women’s University, Japan ⁹ First Department of Oral and Maxillofacial Surgery, Osaka University Graduate School of Dentistry, Japan

Address for correspondence: UEDA M., Division of Pharmacy, Osaka University Dental Hospital, 1-8 Yamada-Oka, Suita, Osaka 565-0871, Japan TEL: +81-6-6879-2379 FAX: +81-6-6879-2381 E-mail: uedakk@dent.osaka-u.ac.jp

Fast track publication: Manuscript received May 26, 2017; revised June 21 and August 20, 2017; accepted October 13, 2017

ISSN 0388–1601 Copyright: ©2017 the Japanese Society of Clinical Pharmacology and Therapeutics (JSCPT)
drug (NSAIDs), and its long-term administration is associated with a risk of gastric ulcers. It is frequently used for post-surgical pain. Cefcapene pivoxil hydrochloride is a third-generation cepham antibiotic that is often prescribed to prevent post-surgical infection in clinical settings. During absorption, cefcapene pivoxil hydrochloride is hydrolyzed by esterases in the wall of the gastrointestinal tract and converted into its active form, cefcapene, an antibacterial agent.

This study co-administered fine granules of loxoprofen and cefcapene pivoxil hydrochloride before, during, and after enteral tube feeding with Ensure Liquid®, which is the most frequently used nutritional supplement in medical practice. The drug pharmacokinetics were investigated by measuring serum drug concentrations to verify the validity of the administration methods that are actually used in clinical settings.

Methods

1. Subjects

The study subjects were 21 healthy adult male volunteers (20 years of age and older but younger than 40 years of age) who gave prior informed consent to participate in the study. Any individual with allergies to the test drugs, food allergies, liver or renal dysfunction, gastrointestinal disease or such a history, indications of abnormal fasting blood sugar, those showing gastrointestinal injury from the day prior to the study, and those taking a pharmaceutical product within 1 week prior to the start of the study were excluded from this study.

2. Ethical considerations

This study was conducted in compliance with the "Ethical guidelines on medical research involving human subjects" and approved (approval number: H25-E42) by the Institutional Review Board of the Graduate School of Dentistry Osaka University and the Dental Hospital. This open-label, single-dose administration study was registered at the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) (ID: UMIN 000015281).

3. Study design and drug administration

This investigation was an open-labeled, single-dose administration study. This study was invasive; therefore, we did not choose to perform a crossover study. The subjects were randomly allocated to three groups: Group I (administration 15 min before the start of tube feeding), Group II (administration at 60 min after the start of tube feeding), and Group III (administration immediately after the end of tube feeding), with seven subjects in each group. The subjects were fasted beginning at 10 p.m. the day before the study. On the day of the study, beverage intake was prohibited from 6 a.m. to lunchtime.

The drugs were administered in the morning, and 45 min after the completion of the Ensure Liquid® administration, the feeding tube was removed. Lunch, which included a specific meal of bread, vegetable salad, and milk, was given 6 h after initiating the tube feeding. Beverage intake was allowed after lunch. Dinner comprised a regular meal administered 8 h after the blood sampling. The subjects were placed in a sitting position throughout the administration of the Ensure Liquid®. Except during the time of the blood sampling, the subjects were allowed to move within the facility. The subjects were observed by the physicians from the beginning of the study until the study completion.

4. Sample collection, processing, and pharmacokinetic analysis

An enteral feeding tube (10 Fr, 120 cm) (Top Co., Ltd., Japan) was inserted into the nostril of each subject, and passed into the stomach 45 min prior to the administration of the meal. For breakfast, 400 mL of Ensure Liquid® (Abbott Japan Co., Ltd., Tokyo Japan) was administered over 120 min. The test drugs were two sachets of cefcapene pivoxil hydrochloride fine granules (100 mg) and loxoprofen fine granules (120 mg), which were dissolved in 20 mL of purified water and administered through a bolus injection. Blood sampling, at a volume of 7 mL at each time point, was performed at 15 and 30 min and 1, 2, 3, 4, 6, and 8 h after administering the test drugs in each group. After the blood sampling, the serum was separated through centrifugation (3,000 rpm, 10 min) and stored frozen below −20°C.

5. Materials and analysis method

5.1. Loxoprofen

The serum levels of loxoprofen were assayed using high-performance liquid chromatography (HPLC) analysis, according to a previously published method (with modifications)1. For the pharmacological test, loxoprofen sodium dihydrate was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). For HPLC, serum (500 μL) was mixed with an equal volume of acetonitrile (Wako Pure Chemical Industries Ltd. Osaka, Japan) and centrifuged at 5000 rpm for 10 min. The supernatant was filtered through a 0.45 μm filter (Kurabo Industries Ltd. Osaka Japan). The filtrate (10 μL) was injected into an HPLC system (LC–20A; Shimadzu Co., Kyoto, Japan) and separated on a C18 reverse-phase column (COMSMOSIL 5C18–AR–II, 4.6 mm I. D. × 150 mm, 5 μm). The flow rate was 1.0 mL/min, and the effluent was monitored at 220 nm with UV detector (Shimadzu Co.). A mobile phase consisting of acetonitrile, 10 mM KH₂PO₄ (pH 3.2), and methanol in a 160: 280: 60 vol/vol was used in the isocratic mode. The lower limit of quantification (LLOQ) was 0.4 μg/mL. Linearity (r) was obtained in the range of 0.4–100 μg/mL. The inter-day precision was less than 4.3% over the 10 days, and the intra-day precision was 8.6% (N = 10).

5.2. Cefcapene

In this study, we established a new method to measure the blood concentration of cefcapene.

Reagent

LC/MS-grade distilled water, methanol, acetonitrile, formic acid, and cephalonium were purchased from Wako Pure Chemical Industries Ltd. Phosphoric acid was purchased from Nacalai Tesque, Inc. (Kyoto, Japan) and diluted to 8.5% (w/v) with distilled water. Cefcapene standard used for calibration and method validation was provided by Sawai Co., Ltd. (Osaka, Japan).

Sample preparation

Serum (225 μL) was transferred to a 1.5-mL microtube, to which 12.5 μL of 20 μg/mL cephalonium, which was used as the internal standard, was added. Next, 25 μL of 8.5% (w/w) phosphoric acid and 762.5 μL of distilled water were added, and
the mixture was vortex-mixed. The sample was then applied to an Oasis PRIME HLB cartridge (3 cc, 60 mg, Waters, Milford, MA, USA). The cartridge was conditioned prior to sample loading with 3 mL of methanol, followed by 3 mL distilled water. Subsequently, the cartridge was rinsed with 3 mL distilled water. The water remaining in the cartridge was flushed out using a plastic syringe. The analytes absorbed on the cartridge were eluted with 1 mL of distilled water/acetonitrile (v/v, 50/50). The eluent was dried using a centrifugal concentrator, after which it was reconstituted with 250 μL of distilled water.

**LC/MS/MS conditions**

LC/MS/MS analysis was performed using an ACQUITY Ultra-Performance Liquid Chromatography (UPLC) system (Waters) and a Quattro Premier XE mass spectrometer (Waters). Chromatographic separation was achieved using the ACQUITY UPLC BEH C18 Column (2.1 mm × 50 mm I.D., 1.7 μm, Waters). The column temperature was maintained at 40°C. The mobile phase comprised 0.1% formic acid in water (A) and acetonitrile (B). The linear gradient program for B was as follows: 5% (0–1 min), increasing from 5% to 12.5% (1–2.5 min), 95% (2.6–3.5 min), and 5% (3.6–5.0 min). The flow rate of the mobile phase was set at 0.5 mL/min. The sample injection volume was 5 μL by partial loop, with overfill.

The mass spectrometer was operated in positive mode electrospray ionization. The operating conditions of the tandem mass spectrometer (MS/MS) were as follows: capillary voltage, 4.5 kV; source temperature, 120°C; desolvation temperature, 350°C; cone gas flow, 50 L/h; and desolvation gas flow rate, 800 L/h. MS/MS detection was performed in the selected reaction monitoring (SRM) mode. The SRM transitions were set at 454.2→393.1 and 459.0→152.0 for cefcapene and cephalonium, respectively. The dwell time and inter-scan delay were set at 0.1 and 0.05 s, respectively.

The abovementioned method was validated using the bioanalysis guidelines set by the Ministry of Health, Labour, and Welfare of Japan (MHLW). LLOQ was 10 ng/mL. Linearity (r) was obtained in the range of 10–3,000 ng/mL. The inter-day accuracy of quality control samples was 2.0–7.8%, and the inter-day precision was 6.4–14.2%. The detailed protocol and validation data are provided in the supplementary file (p. 192–3).

6. Pharmacokinetic parameters

The primary endpoints were the area under serum concentration-time curve up to the last measurable concentration (AUC<sub>∞</sub>), area under the curve to infinity (AUC<sub>∞</sub>), and the maximum serum concentration (C<sub>max</sub>) of loxoprofen and cefcapene. The secondary endpoints were the terminal half-life (t<sub>1/2</sub>), apparent volume of distribution (V/F), apparent clearance (CL/F), and mean residence time (MRT).

Pharmacokinetic parameters were calculated using the non-compartmental analysis by the moment analysis program available on Microsoft Excel. The AUC<sub>∞</sub> was calculated as the sum of the AUC<sub>∞</sub>, which was calculated using the linear trapezoidal rule, and the last quantifiable concentration (C<sub>last</sub>) divided by the terminal elimination rate constant (λ<sub>z</sub>), which was calculated as the negative of the slope of the log-linear regression of the natural logarithm concentration curve during the terminal phase (AUC<sub>∞</sub> = AUC<sub>∞</sub> + C<sub>last</sub>/λ<sub>z</sub>). The other pharmacokinetic parameters included the maximum observed concentration (C<sub>max</sub>), time to reach C<sub>max</sub> (t<sub>max</sub>), terminal elimination half-life (t<sub>1/2</sub>), calculated as t<sub>1/2</sub> = ln(2)/λ<sub>z</sub>, apparent clearance after extravascular administration (CL/F), calculated as CL/F = Dose/AUC<sub>∞</sub> after a single dose, and apparent Vd/F, calculated as Vd/F = CL/F/λ<sub>z</sub>.

7. Statistical analysis

Tukey’s multiple comparison test was performed to calculate the statistical significance of the differences between the sample groups. The data indicated in the figures denote the mean ± SD values.

The sample size was calculated using G*Power 3.1.9.2 Software. A sample size of 21 (3 groups) was required to attain 80% power (86% at the final analysis) and two-sided alpha = 0.05. The analysis of this sample size detected a rate of change (GMRs) of the natural log-transformed C<sub>max</sub>, AUC<sub>∞</sub> and AUC<sub>∞</sub> for loxoprofen and cefcapene were calculated to evaluate the magnitude of changes in pharmacokinetics between the groups, along with estimated 90% confidence intervals (90% CIs).

**Results**

1. Subjects

The details and background of the subjects participating in this study are presented in Table 1. There were no notable between-group differences in age, height, weight, and BMI.

2. Adverse experiences

One subject in Group II complained of discomfort caused by the
The changes in serum loxoprofen concentration are shown in subjects during the study period. Loxoprofen administered before (Ⅰ), during (Ⅱ), and after (Ⅲ) administration through a nasogastric tube of enteral nutrition. The serum concentration of loxoprofen was monitored. Values are mean ± standard deviation (SD). (n = 7).

![Graph 1](image1.png)  
**Figure 1** Serum concentration of loxoprofen following administration through a nasogastric tube. Loxoprofen administered before (Ⅰ), during (Ⅱ), and after (Ⅲ) administration through a nasogastric tube of enteral nutrition. The serum concentration of loxoprofen was monitored. Values are mean ± standard deviation (SD). (n = 7).

![Graph 2](image2.png)  
**Figure 2** Serum concentration of cefcapene following administration through a nasogastric tube. Cefcapene pivoxil administered before (Ⅰ), during (Ⅱ), and after (Ⅲ) administration through a nasogastric tube of enteral nutrition. The serum concentration of cefcapene was monitored. Values are mean ± standard deviation (SD). (n = 7).

### Table 2  Pharmacokinetic parameters after loxoprofen administration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (μg/mL)</td>
<td>10.0 ± 2.57</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>0.286 ± 0.095</td>
</tr>
<tr>
<td>AUC$_{\text{max}}$ (μg·h/mL)</td>
<td>15.7 ± 4.52</td>
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<tr>
<td>AUC$_{\infty}$ (μg·h/mL)</td>
<td>21.0 ± 9.80</td>
</tr>
<tr>
<td>$t_{1/2}$ (hr)</td>
<td>2.37 ± 1.48</td>
</tr>
<tr>
<td>Vd/F (L)</td>
<td>19.3 ± 5.18</td>
</tr>
<tr>
<td>CL/F (mL/h)</td>
<td>6.59 ± 2.41</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>3.38 ± 1.81</td>
</tr>
</tbody>
</table>

Groups indicate loxoprofen administration before (Ⅰ), during (Ⅱ), and after (Ⅲ) administration through a nasogastric tube of enteral nutrition. $C_{\text{max}}$: maximum concentration; $t_{\text{max}}$: maximum concentration time; AUC$_{\text{max}}$: area under the serum concentration curve up to the last measurable concentration; AUC$_{\infty}$: area under the curve to infinity; $t_{1/2}$: half-life; Vd/F: apparent volume of distribution; CL/F: apparent clearance; MRT: mean residence time. Values are mean ± standard deviation (SD). *p<0.05, **p<0.01 (vs. Group I, tukey’s multiple comparison test, n=7).

enteral feeding tube. Therefore, the tube was immediately removed after the nutritional supplement was administered. No other adverse events due to the administration through a nasogastric tube or the study drugs were observed in the 21 subjects during the study period.

### 3. Pharmacokinetics

#### 3.1. Loxoprofen

The changes in serum loxoprofen concentration are shown in **Figure 1**, and the pharmacokinetic parameters are shown in **Table 2**. The $C_{\text{max}}$ values were 10.0, 6.32, and 5.49 μg/mL in groups I, II, and III, respectively, and a significant difference was observed (group II vs. group I, p<0.05; group III vs. group I, p<0.01). The $C_{\text{max}}$ values in Group I were 58.7% and 82.8% higher than in Group II and Group III, respectively. The GMRs of $C_{\text{max}}$ and the 90% CIs were 0.64 (0.50-0.82) in II / I, 0.54 (0.41-0.71) in III / I and 0.84 (0.66-1.08) in III / II. There was no significant between-group difference in the AUC$_{\text{max}}$ and AUC$_{\infty}$ values.

The AUC$_{\infty}$ values in Group I were 39.7% and 21.7% higher than in Group II and Group III, respectively. The GMRs of the AUC$_{\infty}$ and the 90% CIs were 0.75 (0.53-1.05) in II / I, 0.76 (0.49-1.18) in III / I and 1.02 (0.69-1.52) in III / II. In addition, no significant between-group differences in $t_{1/2}$ (h), Vd/F(L), CL/F(mL/h), and MRT(h) were observed.
3.2. Cefcapene

The changes in cefcapene serum concentrations are shown in Figure 2, and the pharmacokinetic parameters are presented in Table 3. The $t_{\text{max}}$ in Group I was 0.929 h, while those in Groups II and III were 2.57 and 2.00 h, respectively (Group II vs. Group I, $p<0.001$, and Group III vs. Group I, $p<0.01$). The $C_{\text{max}}$ in Group I was 1300 ng/mL, while those in Groups II and III were 907 and 983 ng/mL, respectively (Group II and III vs. Group I, $p<0.05$).

The $C_{\text{max}}$ in Group I was 43.3% and 32.2% higher than in Group II and Group III, respectively. The GMRs of $C_{\text{max}}$ and the 90% CIs were 0.70 (0.59–0.84) in II/ I, 0.75 (0.62–0.92) in III/I and 1.07 (0.90–1.27) in III/II.

No significant between-group differences in the AUC$_{\text{int}}$ and AUC$_{\infty}$ values were observed.

The AUC$_{\infty}$ values in Group I were 10.4% and 17.7% higher than those in Group II and Group III, respectively. The GMRs of the AUC$_{\infty}$ and the 90% CIs were 0.91 (0.78–1.06) in II/ I, 0.83 (0.69–1.01) in III/I and 0.92 (0.76–1.11) in III/II.

In Group II, the $t_{1/2}$ (h) was significantly shorter, and MRT (h) was significantly greater compared to Group I (Group II vs. Group I, $p<0.05$). There was no significant between-group difference in Vd/F (L) and CL/F (mL/h).

The GMRs of $C_{\text{max}}$, AUC$_{\text{int}}$, and AUC$_{\infty}$ and the 90% CIs in groups are shown in Table 4.

### Discussion

In this study, we investigated the pharmacokinetic profiles of loxoprofen and cefcapene administered before, during, and after tube feeding to identify pharmacokinetic differences, and we found that the time of administration appeared to affect some pharmacokinetic parameters, although the clinical impact is expected to be manageable.

In this study, 21 healthy adult male volunteers were randomly assigned to three groups. The pharmacokinetic profiles of loxoprofen fine granules (120 mg) and cefcapene pivoxil hydrochloride fine granules (100 mg) administered through enteral tube feeding for 2 h before, during, and after the administration of 400 mL Ensure Liquid® were observed.

The $C_{\text{max}}$ of loxoprofen and cefcapene significantly increased in group I. In the case of cefcapene, $t_{\text{max}}$ and MRT significantly increased in Group II, whereas $t_{1/2}$ significantly decreased compared with the values in Group I. However, no significant between-group difference in AUC$_{\text{int}}$ and AUC$_{\infty}$ was observed for both drugs.

Previous studies have reported that switching from oral administration to administration in the presence of enteral tube feeding affects the pharmacokinetics of drugs. Compared to carbamazepine administered orally in fasting, carbamazepine suspension administered in the presence of enteral nutrition took longer to reach peak serum concentration ($t_{\text{max}}$) and exhibited a decline in the AUC to 90%.

In addition, the absorption rate constant of sodium valproate decreased when administered concomitantly with Ensure Liquid® compared to that observed when administered with pure water. The absorption rate was not affected by concomitant administration of Ensure Liquid®. However, there are no reports on the differences in the pharmacokinetic parameters associated with the time of administration.

According to the package insert, loxoprofen is rapidly absorbed when administered orally. When a single 60 mg dose of loxoprofen was administered orally to healthy volunteers, the $C_{\text{max}}$ was 5.04 μg/mL, $t_{\text{max}}$ was 0.45 h, $t_{1/2}$ was 1.22 h, and the AUC was 6.70 μg·h/mL. Although the doses differed, these values were not so different between the values described in this study. Loxoprofen is a short-acting NSAID, with an early onset of effects, and it is a pro-drug that is converted to its active trans-alcohol metabolite (trans-OH metabolite) through multiple aldehyde-ketone reductases and carbonyl reductases after absorption by the gastrointestinal tract. In addition, approximately 60% of loxoprofen is excreted in urine as glucuronic acid-conjugated loxoprofen, and its trans-OH metabolites within 12 h of drug administration. $C_{\text{max}}$ of trans-OH metabolite is approximately 15% of loxoprofen. We evaluated only the pharmacokinetics of loxoprofen, which is a limitation of this study.
Cefcapene pivoxil hydrochloride is hydrolyzed to cefcapene, the active form against bacteria, through esterases in the gastrointestinal wall during absorption and is excreted by the kidney without being metabolized. According to the package insert, when a single dose of cefcapene pivoxil hydrochloride (100 mg) was orally administered to healthy volunteers, the $C_{\text{max}}$ of cefcapene was 1.28 μg/mL, $t_{\text{max}}$ was 1.3 h, $t_{1/2}$ was 1.01 h, and $\text{AUC}$ was 3.86 μg·h/mL. These values not so different between the values from this study. It is documented in the package insert of cefcapene that "The absorption of this drug is better when administered after meals than on an empty stomach." In a phase I study on cefcapene, it was reported that "Ingested food may delay absorption of this drug; however, $C_{\text{max}}$ is not affected; $\text{AUC}$ and urine recovery rate are increased significantly, resulting in an increased absorption rate as the final effect of food ingestion." It has been reported that the pharmacokinetics of a prodrug containing pivoxil hydrochloride base, including cefcapene pivoxil, are affected by intragastric pH, resulting in a decrease in the $C_{\text{max}}$ and $\text{AUC}$. This finding may suggest that if cefcapene pivoxil hydrochloride is administered immediately before (in the fasted state) the administration of a nutritional supplement, the absorption rate would be high, resulting in a high $C_{\text{max}}$ because of the low intragastric pH, which is consistent with the results of the present study. When the administration of the nutritional supplement was initiated 15 min after the administration of cefcapene pivoxil hydrochloride, the intragastric pH may have increased rapidly, resulting in a rapid decrease in the absorption rate. The 15-min window just prior to the initiation of enteral tube feeding may be critical for the small increase in $\text{AUC}$ observed in this study in Group I. If so, the AUCs of cefcapene administered immediately before feeding may be lower than those observed for drug administration at 15 min before the feeding was initiated. Because the administration of nutritional supplement takes time, the AUC might be hardly affected. However, the values of $C_{\text{max}}$ and AUCs could be affected, albeit minimally, at this absorption stage because the absorption is affected by the pH level in the digestive tract.

In this study, the $C_{\text{max}}$ of loxoprofen and cefcapene significantly increased when the drugs were administered 15 min before tube feeding. However, no significant difference was observed in the $\text{AUC}_{\text{max}}$ and $\text{AUC}$ of loxoprofen and cefcapene, likely because of the detection inadequacy of statistical multiple

<table>
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<tr>
<th>Pharmacokinetic parameter</th>
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<th>Geometric mean</th>
<th>Comparison</th>
<th>Estimate of ratio (90% confidence interval)</th>
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<td>III</td>
<td>14.90</td>
<td>II / II</td>
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### Cefcapene

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<td>II</td>
<td>3370</td>
<td>II / I</td>
<td>0.83 (0.69–1.01)</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>3080</td>
<td>III / II</td>
<td>0.91 (0.76–1.10)</td>
</tr>
<tr>
<td>$\text{AUC}_{\text{max}}$ (ng·h/mL)</td>
<td>I</td>
<td>3790</td>
<td>I / I</td>
<td>0.91 (0.78–1.06)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3430</td>
<td>II / I</td>
<td>0.83 (0.69–1.01)</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>3150</td>
<td>III / II</td>
<td>0.92 (0.76–1.11)</td>
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</tbody>
</table>

### Table 4 Ratio of geometric means

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Group</th>
<th>Geometric mean</th>
<th>Comparison</th>
<th>Estimate of ratio (90% confidence interval)</th>
</tr>
</thead>
</table>
comparisons. Therefore, we analyzed the geometric mean ratio (90% CI) for the $C_{\text{max}}$, AUC$_{\text{last}}$, and AUC$_{\infty}$ of these drugs between the groups. These analyses showed similar results. Although the 90% CIs did not fall within the 80–125% reference range (standard equivalence criterion), the degree of differences observed among the 3 groups did not seem to be critically meaningful in the clinic.

We conclude that the administration timings of loxoprofen and cefcapene through an enteral tube in clinical settings similar to the present study might result in only minimal to modest changes in some pharmacokinetic parameters, including $C_{\text{max}}$, although the degree of the changes should be clinically manageable.

The major limitation of this study is its small sample size, as well as multiple statistical comparisons, which likely result in statistical detection inadequacy. However, given that loxoprofen and cefcapene are essential drugs for post-surgical management, we must emphasize that the clinical benefits of loxoprofen and cefcapene are essential drugs for post-surgical management, as well as multiple statistical comparisons, which likely result in statistical power analysis program for the social, behavioral, and biomedical sciences. Behavi Res Methods, 2007; 39 (2): 175-91.


Daichi Sanky Co., Ltd. Package Insert LOXONIN® Tab. 60 (Loxoprofen Sodium Hydrate). 2013.

Sawai Co., Ltd. Interview Form LOXOPROFEN Na® Tab, Fine Granules (Loxoprofen Sodium Hydrate). 2016.

Shionogi Co., Ltd. Package Insert FLOMOX Tab. 75 · 100 (Cefcapene Pivoxil). 2015.


Supplemental manuscript

1. Method

1-1. Reagent

LC/MS-grade distilled water, methanol, acetonitrile, formic acid and cephapirin (CEL) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Phosphoric acid was purchased from Nacalai Tesque, Inc. (Kyoto, Japan) and diluted to 8.5% (w/v) with distilled water before use. CFPN was provided by Sawai Pharmaceutical Co., Ltd. (Osaka, Japan).

1-2. Sample preparation

A volume of 225 μL of serum was transferred into a 1.5-mL microtube, to which 12.5 μL of 20 μg/mL CFPN solution was added. Next, 25 μL of 8.5% (w/v) phosphoric acid and 762.5 μL of distilled water were added and the mixture was vortex-mixed. The sample was then applied to an Oasis PRIME HLB cartridge (3 cc, 60 mg; Waters, Milford, MA, USA). The cartridge was conditioned prior to sample loading with 3 mL of methanol followed by 3 mL of distilled water. Afterwards, the cartridge was rinsed with 3 mL of distilled water. The water remaining in the cartridge was flushed out using a plastic syringe. The analytes absorbed on the cartridge were eluted with 1 mL of distilled water/acetonitrile (v/v, 50/50). The eluent was dried using a centrifugal concentrator, after which it was reconstituted with 250 μL of distilled water.

1-3. LC/MS/MS conditions

LC/MS/MS analysis was performed using an ACQUITY Ultra-Performance Liquid Chromatography (UPLC) system (Waters) and a Quattor-Premier XE mass spectrometer (Waters). Chromatographic separation was achieved using an ACQUITY UPLC BEH C18 column (2.1 mm i.d. × 50 mm, 1.7 μm; Waters). The column temperature was maintained at 40°C. The mobile phase was composed of 0.1% formic acid in water (A), and acetonitrile (B). The linear gradient program for B was as follows: holding at 5% (0–1 min), increasing from 5% to 12.5% (1–2 min), from 12.5% to 95% (2.5–2.6 min) holding at 95% (2.6–3.5 min), decreasing from 95% to 5% (3.5–3.6 min), and holding at 5% (3.6–5.0 min). The flow rate of the mobile phase was set at 0.5 mL/min. The sample injection volume was 5 μL by partial loop with overlift mode.

The mass spectrometer was operated in positive electrospray ionization mode. The operating conditions of tandem mass spectrometry (MS/MS) were as follows: capillary voltage, 4.5 kV; source temperature, 120°C; desolvation temperature, 350°C; cone gas flow, 50 L/h; and desolvation gas flow rate, 800 L/h. MS/MS detection was done in selected reaction monitoring (SRM) mode. The SRM transitions have been presented in supplemental table 1. Dwell time and inter-scan delay were set at 0.1 sec and 0.05 sec, respectively.

2. Validation results

2-1. MS/MS spectrum of cefcapene

The MS/MS fragmentation pattern of CFPN was analyzed using a standard solution of CFPN. Supplemental figure 1 illustrates the MS/MS spectrum of CFPN. The ESI mass spectrum of CFPN showed a protonated molecular ion [M + H]+ at m/z 454.2. The collision-induced dissociation spectrum of m/z 454.2 revealed many product ions. The product ion at m/z 393.1 was formed from the loss of 61 Da, which is consistent with the loss of a carbamate group. The product ion at m/z 208.2 was suspected to be a partial structure containing aminothiazole substructure, which was generated by the opening of the β-lactam ring. Supplemental figure 1 shows the suspected cleavage sites, which have been indicated with broken lines, and the resulting ion and its m/z value, which have been indicated with arrows. In order to monitor these product ions more effectively, parameters such as cone voltage and collision energy were optimized for the analysis. The optimized parameters are listed in supplemental table 1.

2-2. LC separation

Acquity UPLC BEH C18 with 1.7 μm particle size (Waters) was selected for the analysis because of its high resolution and stability. Over 2 of k factor of analyte achieves separation of inorganic ions in serum to it in general. For this reason, we set the gradient program of the mobile phase using an initial acetonitrile concentration of 5% as explained in section 1-3. The flow rate was set at 0.5 mL/min to reduce run time and peak width. The total run time included the times for column washing and equilibration was set as 5 min. Under the above conditions, the retention time of CFPN was found to be 2.26 min.

An isotope-labeled standard of the analyte would have been the ideal internal standard for the analysis; however, we were unable to obtain any. Thus, we studied the retention times of other cephalosporins and compared them to the retention time of CFPN under the above-mentioned conditions. The results showed that the retention times of CEL (2.33 min) and CFPN (2.26 min) were similar. Furthermore, the SRM transitions of CEL did not indicate any crossover with those of CFPN. Therefore, CEL was used as the internal standard in this study.

2-3. Sample preparation

Deproteinization of serum is required when analyzing drug concentrations in serum. It prevents contamination of the column and mass spectrometer, and reduces matrix effects to allow for accurate quantification of the analyte. Typical deproteinization methods include addition of organic solvents (such as acetonitrile and acetone) or divalent cations (such as zinc) to the sample, ultrafiltration, and solid phase extraction (SPE). We did not investigate the effect of ultrafiltration in the present study because the process is laborious and expensive. Therefore, the recovery of CFPN from serum after the addition of acetonitrile, zinc sulfate to serum samples was investigated. In each instance, the recovery was <30%. The reasons for the low recoveries were ionization suppression by coelution of hydophoric components, such as phospholipids in serum, and/or coprecipitation of proteins. Actually, the plasma protein binding ratio of cefcapene pivoxil (CFPN-P) is about 45%, therefore, that of CFPN might be similar.

Furthermore, we investigated the method involving the addition of the CFPN from serum proteins, followed by performing SPE. The Oasis PRIME HLB cartridge was selected for the SPE in order to remove phospholipids from the samples, as they suppress ionization. Serum samples spiked with CFPN and CEL were treated with phosphoric acid, diluted with distilled water, and then applied to an Oasis PRIME HLB cartridge. CFPN and CEL were not detected in the resulting solutions.

Supplemental Table 1. SRM parameters for CFPN and CEL

<table>
<thead>
<tr>
<th>Compound</th>
<th>Q1 (m/z)</th>
<th>Q3 (m/z)</th>
<th>cone voltage (V)</th>
<th>collision energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFPN</td>
<td>454.2</td>
<td>393.1</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>confirmation</td>
<td>454.2</td>
<td>208.2</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>CEL</td>
<td>459.0</td>
<td>152.0</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>confirmation</td>
<td>459.0</td>
<td>337.0</td>
<td>15</td>
<td>16</td>
</tr>
</tbody>
</table>
the fractions of flow through and distilled water-washing. Afterwards, water/acetonitrile (v/v, 50/50) was applied to the cartridge and the eluent was evaporated. The residue was then reconstituted with distilled water. The recoveries of CFPN and CEL were 78.7 ± 1.3% and 92.9 ± 2.5% at 10 ng/mL, 75.1 ± 5.5% and 96.0 ± 2.0% at 100 ng/mL, 80.0 ± 5.2% and 89.4 ± 3.2% at 1000 ng/mL, respectively. Typical chromatograms of the samples are shown in supplemental figure 2 (b). Blank serum was treated according to the above-mentioned process, after which CFPN and CEL were added to the prepared solution. The final concentrations of CFPN and CEL in serum were 10, 25, 100, or 1000 ng/mL. The matrix factors affecting the assay of CFPN and CEL were measured by comparing the peaks of CFPN and CEL obtained from analyzing standard solutions without serum. The matrix effects for CFPN and CEL were 104.1 ± 5.6% and 101.6 ± 11.4%, respectively. These results indicate that our sample preparation method can overcome matrix effects.

2-4. Method validation

We validated the developed method according to the bioanalysis guidelines set by the Ministry of Health, Labour, and Welfare of Japan (MHLW). In this experiment, zero-blank serum, which is serum spiked with the internal standard but not the analyte, was analyzed. As shown in supplemental figure 2 (a), signals corresponding to CFPN were not detected, which indicate that the selectivity of the method was sufficient.

Next, linearity of the response of CFPN/CEL peak area ratio was checked using serum spiked with 10, 25, 50, 75, 100, 250, 500, 750, 1000, or 3000 ng/mL of CFPN and 1000 ng/mL of CEL. The correlation coefficient (r) of the response was obtained as 0.9996, which suggests good linearity over a concentration range of 10-3000 ng/mL. The calibration curve was analyzed by 1/x weighting, and the determination coefficient (R²) was 0.9992. The signal to noise ratio of 10 ng/mL of CFPN was 38. Therefore, the lower limit of quantification (LLOQ) was 10 ng/mL.

Accuracy and precision were investigated using sera spiked with 10, 25, 100, 1000, or 2500 ng/mL of CFPN. Five replicates of each concentration were analyzed daily for three days, which means that 15 samples at the same concentration level were evaluated for accuracy and precision. The accuracy, repeatability (intra-day precision), and intermediate repeatability (inter-day precision) of the method at all concentration levels were found to be <15% (supplemental table 2).

These results indicate that our method is sufficient for the assay of CFPN in serum, according to the guideline of bioanalysis established by MHLW.

Reference


Supplemental table 2. Validation data

<table>
<thead>
<tr>
<th>LLOQ</th>
<th>10 ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>10-3000 ng/mL</td>
</tr>
<tr>
<td>Linearity (r) *</td>
<td>0.9996</td>
</tr>
<tr>
<td>Accuracy (%)*</td>
<td>10 (6.2)</td>
</tr>
<tr>
<td></td>
<td>25 (4.3)</td>
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<tr>
<td></td>
<td>100 (2.0)</td>
</tr>
<tr>
<td></td>
<td>1000 (7.8)</td>
</tr>
<tr>
<td></td>
<td>2500 (2.6)</td>
</tr>
<tr>
<td>Repeatability (%)*</td>
<td>10 (10.2)</td>
</tr>
<tr>
<td>(Intra-day precision)</td>
<td>25 (7.7)</td>
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<tr>
<td></td>
<td>100 (7.0)</td>
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<tr>
<td></td>
<td>1000 (7.3)</td>
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<tr>
<td></td>
<td>2500 (7.2)</td>
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<tr>
<td>Intermediate repeatability (%)*</td>
<td>10 (9.7)</td>
</tr>
<tr>
<td>(Inter-day precision)</td>
<td>25 (11.8)</td>
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<td>100 (6.4)</td>
</tr>
<tr>
<td></td>
<td>1000 (14.2)</td>
</tr>
<tr>
<td></td>
<td>2500 (8.2)</td>
</tr>
</tbody>
</table>

*For each concentrations, 5 replicate samples were prepared and analyzed each day for 3 days.