Comparison between Original and Generic Versions of Ceftriaxone Sodium Preparation for Injection: Compatibility with Calcium-Containing Product

Mio Tange, Miyako Yoshida, Yuka Nakai, and Takahiro Uchida*

School of Pharmaceutical Sciences, Mukogawa Women’s University; 11–68 Koshien 9-Bancho, Nishinomiya 663–8179, Japan; and Department of Pharmacy, Bell Land General Hospital; 500–3 Higashiyama, Naka-ku, Sakai 599–8247, Japan. Received July 26, 2011; accepted January 12, 2012; published online January 23, 2012

The purpose of this study was to compare the compatibility of ROCEPHINE® Intravenous, the original manufacturer’s ceftriaxone sodium preparation for injection, and seven generic versions thereof, with various calcium chloride injection 2%. The influence of calcium ion concentration, storage time and shaking strength on the appearance and quantity of insoluble microparticles in mixed solutions was examined using a light obscuration particle counter. In all products, the observed number of insoluble microparticles was proportional to the calcium ion concentration, storage time and shaking strength after the addition of calcium chloride solution. In several of the generic products, the number of insoluble microparticles was significantly higher than those of the original product, while in others it was lower. We evaluated the quality of the original and 7 generic preparations, measured the content of impurity and pH of the various ceftriaxone solutions, as impurity content and pH of solution are possible factor affecting compatibility. Three impurities were found in all products. The impurity content of several generic products, as estimated from their peak area on high performance liquid chromatography (HPLC), was significantly lower than that of the original product. pH of solution was difference between products. Although it was difficult that impurity and pH of solution verify critical factor affecting compatibility. The results show that there are differences in the appearance of insoluble microparticles between the original product and seven generic products, when calcium chloride injection 2% solution is added to the product.

Key words: ceftriaxone; generic version; calcium; compatibility; insoluble microparticle; light obscuration particle counter

In Japan, as elsewhere, the increase in medical costs in line with the aging of the population is becoming a major issue, with the result that the Japanese government now actively promotes the use of generic drugs in order to reduce medical expenditure. Preparations for intravenous injections of the generic products are exempted the bioequivalence test in application, since the preparation for intravenous injections was administered to the direct body, and drug disposition after administration was equal to the original product. Therefore the evaluation of physiochemical quality is important due to the evaluation of equivalence between the original product and generic products was only physicochemical quality. Therefore, the in vitro quality comparison of the original and generic products seems essential.

Ceftriaxone is a third-generation cephalosporin antibiotic, which is reported to form a complex with calcium, and serious adverse effects have been reported overseas when this has occurred. In Japan, as described on the drug package insert under Precautions, ceftriaxone and calcium-containing products should not be administered simultaneously. However, in clinical practice, ceftriaxone mixed in calcium-containing products with secondary additives at Y-injection site intravenous line for purposes of extracellular fluid supplementation. There has also been a case report of biliary pseudo-lithiasis caused by precipitation of ceftriaxone and in vivo calcium, even though only ceftriaxone was administered. Ceftriaxone has a prolonged biological half-life and is suitable for once-daily dosing; moreover, it has a wide spectrum of antimicrobial activity and superior tissue penetration, making it a useful and commonly prescribed antibiotic. We have previously examined the effect of calcium ion concentration, storage temperature and shaking strength on the compatibility of the original ceftriaxone sodium product with calcium-containing products, but there have been no reports on the compatibility of generic versions of ceftriaxone sodium with calcium-containing products.

In the present study, the compatibility of original ceftriaxone sodium and seven generic versions with calcium chloride injection 2% was evaluated by measuring the number of insoluble microparticles using a light obscuration particle counter. It was assumed that, under clinical conditions, the mixing of an intravenous fluid in an administration set with a secondary additive through a Y-injection site occurs in a 1:1 ratio. In case of this inline mixing, 100mL of 2.5mmol/L calcium-containing solution were mixed 100mL of 10mg/mL ceftriaxone isotonic sodium chloride solution, so that the final calcium concentration became 1.25mmol/L and ceftriaxone concentration became 5mg/mL. In the present study, we also evaluate compatibility by visual observation and its extent by microparticles weight. To clarify differences in the appearance of insoluble microparticles among all products, the content of impurity and pH of the various ceftriaxone solutions were examined. It was difficult that impurity and pH of solution verify critical factor affecting compatibility.

Experimental

Materials The original product (product A) and seven generic ceftriaxone sodium products (B to H) used in the study are summarized in Table 1. Commercially available isotonic sodium chloride solution (Terumo Co., Ltd., Tokyo, Japan) and calcium chloride injection 2% (Otsuka Pharmaceutical Factory, Inc., Tokyo, Japan) were used in this study.
Preparation of Sample Solutions Ceftriaxone solutions were prepared by dissolving the eight ceftriaxone injection products in isotonic sodium chloride solution to a final concentration of 5 mg/mL.

The Effect of Calcium Concentration on the Weight of Insoluble Microparticles Various amounts of 2% (w/v) calcium chloride solution were added to the eight ceftriaxone solutions (5 mg/mL) to make final calcium ion concentrations of 1, 1.5, 2.0 and 2.5 mmol/L. The solutions were gently agitated and stored at 25°C for 6 h. The weights of the ceftriaxone–calcium precipitates were measured after natural filtration and drying under reduced pressure.

The Influence of Storage Time on the Number of Insoluble Microparticles Appropriate volumes of 2% (w/v) calcium chloride solution were added to the eight ceftriaxone solutions (5 mg/mL) to make a final calcium ion concentration of 1.25 mmol/L. The solutions were gently agitated and stored at 25°C for 6 h. The weights of the ceftriaxone–calcium precipitates were measured after natural filtration and drying under reduced pressure.

The Influence of Shaking Strength on Number of Insoluble Microparticles Appropriate volumes of 2% (w/v) calcium chloride solution were added to the eight ceftriaxone solutions (5 mg/mL) to make a final calcium ion concentration of 1.25 mmol/L. The solutions were gently agitated and stored at 25°C. The pH was determined using a pH meter (F-21, HORIBA Co., Ltd., Kyoto, Japan) equipped with an integrator and reverse-phase column (CAPCELL PAK C18 UGI120 SS: 4.6 mm i.d. x 250 mm, Shiseido Co., Ltd., Tokyo, Japan). The column was kept at a constant temperature of about 25°C. The mobile phase consisted of acetonitrile containing tetra-n-heptylammonium bromide 4.00 g, 450 mL; water 490 mL; 40.9 mmol/L NaH₂PO₄·2H₂O, 25.9 mmol/L KH₂PO₄, 55 mL; 96.4 mmol/L citric acid monohydrate+196.3 mmol/L NaOH 5 mL, at a flow rate of 1.5 mL/min. The wavelength was set at 254 nm.

Reagents used were special-grade chemicals or suitable for liquid chromatography. When two batches of a product were available, samples were taken from both batches.

Measurement of pH Appropriate volumes of 2% (w/v) calcium chloride solution were added to each of the ceftriaxone solutions (5 mg/mL) to a final calcium ion concentration of 1.25 mmol/L. The pH was determined using a pH meter (F-21, HORIBA Co., Ltd., Kyoto, Japan), with or without the addition of calcium.

Statistical Analysis The comparison of the number of insoluble microparticles of the original and generic products were analysed by the Tukey test; the comparison of the proportion of pH of the original product and generic products were by Dunnet’s test; statistical significance was accepted at the p<0.05 or p<0.01 level.

Results The Influence of Calcium Ion Concentration The weight of precipitate 6 h after mixing calcium chloride solution with ceftriaxone solution stored at 25°C, is shown in Fig. 1. The weight of precipitate was proportional to the calcium ion concentration eight products, and there were different among

Table 1. Preparations Used in This Study

<table>
<thead>
<tr>
<th>Preparations</th>
<th>Company</th>
<th>Lot No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. ROCEPHIN® Intravenous 1 g</td>
<td>Chugai Pharmaceutical Co., Ltd., Tokyo, Japan</td>
<td>K336031</td>
</tr>
<tr>
<td>B. SEFIXOM®</td>
<td>Nichi-iko Pharmaceutical Co., Ltd., Toyma, Japan</td>
<td>9F177</td>
</tr>
<tr>
<td>C. Ceftriaxone Na for Intravenous Injection 1 g</td>
<td>Sandoz Co., Ltd., Yamagata, Japan</td>
<td>01331</td>
</tr>
<tr>
<td>D. LIASOPIHI for Intravenous Injection 1 g</td>
<td>Chemix Inc., Kanagawa, Japan</td>
<td>LV0808</td>
</tr>
<tr>
<td>E. CEFXONE</td>
<td>Shiono Chemical Co., Ltd., Tokyo, Japan</td>
<td>LV0302</td>
</tr>
<tr>
<td>F. ROZECLART</td>
<td>TAIYO Pharmaceutical Industry Co., Ltd., Aichi, Japan</td>
<td>A21503</td>
</tr>
<tr>
<td>G. Ceftriaxone Na for Intravenous Injection 1 g “Mylan”</td>
<td>Mylan Inc., Osaka, Japan</td>
<td>0420RO</td>
</tr>
<tr>
<td>H. CERONEED®</td>
<td>Sawai Pharmaceutical Co., Ltd., Osaka, Japan</td>
<td>10502</td>
</tr>
</tbody>
</table>

The In...
all products. In this experiment we evaluated critical point of
0.00173 g, the weight of precipitate when insoluble microparti-
cles were visible to the human eye. The precipitate weights of
products B, D and G (0.001902, 0.002023 and 0.001793 g, re-
respectively) were above the critical point, while the precipitates
of products A, C, E, F and H (0.001348, 0.000625, 0.001440,
0.001572 and 0.001673 g, respectively) were below at calcium
ion concentration of 1.0 mmol/L. In the products with heavier
precipitates, white insoluble microparticles were easily visible
to the human eye after 6h.

The Influence of Storage Time  Temporal changes in the
number of insoluble microparticles formed when 2% (w/v) calcium chloride solution was added to the ceftriaxone solu-
tions, and stored at 25°C, are shown in Table 2. The number
of insoluble microparticles, as measured using the light obscu-
tration particle counter, was proportional to storage time in all
products. Immediately after sample preparation, the number
of insoluble microparticles was under the permissible limit for
injection preparations administered at a volume of ≥100mL
in all samples. (The tolerated number of insoluble microparti-
cles was 0.00173 g, the weight of precipitate when insoluble microparti-
cles were visible to the human eye after 6h.)

Measurement of Impurities and pH for Products. The
Measurement of Impurities by HPLC  Representative liq-
uid chromatograms of ceftriaxone sodium preparations for
injection, are shown in Fig. 3. Three peak areas of impurity
were observed in all products. The content of impurity was
low, but the peak area was different between products. In
generic products, the proportion of total impurities to ceftri-
axone was higher than in the original product. There was no
difference in impurity content between two different batches
of a particular product. Peak 3 seemed to be an impurity de-
derived from a ceftriaxone sodium related substance,10 but peaks
1 and 2 seemed to have a different derivation.

The Influence of pH  The pHs of the ceftriaxone solutions
at 25°C, with or without calcium chloride solution, are shown in
Fig. 4. In product G, the difference in pH in the presence or
absence of calcium chloride solution was the greatest; it was
lower than in product H.

![Fig. 1. Weight of Precipitate after 6h](image)

The weight of the precipitate (mean of six observations) in each sample of cef-
triaxone solution (5mg/mL) to which 2% (w/v) calcium chloride solution has been
added, after storage at 25°C for 6h after preparation.

<table>
<thead>
<tr>
<th>Preparations</th>
<th>Particle size</th>
<th>Number of insoluble microparticles per mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After preparation immediately</td>
<td>5 min after preparation</td>
</tr>
<tr>
<td>A</td>
<td>10μm or greater</td>
<td>1.27±0.12</td>
</tr>
<tr>
<td>B</td>
<td>25μm or greater</td>
<td>0.13±0.12</td>
</tr>
<tr>
<td>C</td>
<td>10μm or greater</td>
<td>1.00±0.87</td>
</tr>
<tr>
<td>D</td>
<td>25μm or greater</td>
<td>0.13±0.23</td>
</tr>
<tr>
<td>E</td>
<td>10μm or greater</td>
<td>0.73±0.42</td>
</tr>
<tr>
<td>F</td>
<td>25μm or greater</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>G</td>
<td>10μm or greater</td>
<td>1.20±1.04</td>
</tr>
<tr>
<td>H</td>
<td>25μm or greater</td>
<td>0.20±0.20</td>
</tr>
<tr>
<td>I</td>
<td>10μm or greater</td>
<td>2.27±1.47</td>
</tr>
<tr>
<td>J</td>
<td>25μm or greater</td>
<td>0.53±0.31</td>
</tr>
<tr>
<td>K</td>
<td>10μm or greater</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>L</td>
<td>25μm or greater</td>
<td>0.80±0.53</td>
</tr>
<tr>
<td>M</td>
<td>10μm or greater</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>N</td>
<td>25μm or greater</td>
<td>0.87±0.42</td>
</tr>
<tr>
<td>O</td>
<td>10μm or greater</td>
<td>0.13±0.12</td>
</tr>
<tr>
<td>P</td>
<td>25μm or greater</td>
<td>0.87±0.42</td>
</tr>
</tbody>
</table>

Table 2. Number of Insoluble Microparticles with a Diameter 10μm or Greater and 25μm or Greater

The number of insoluble microparticles with diameter 10μm or greater and 25μm or greater (mean of three observations) formed when 2% (w/v) calcium chloride solution
calcium ion concentration 1.25mmol/L) was added to ceftriaxone solution (5mg/mL), and stored at 25°C, measured using a light obscuration particle counter.
Insoluble microparticles were formed when the original manufacturer’s ceftriaxone product, and seven generic versions thereof, were mixed with calcium chloride solution, simulating the mixture of the two solutions in an intravenous line. For all products, the observed number of insoluble microparticles formed was proportional to the calcium ion concentration, storage time and shaking strength. In generic products B, D, E, F and G, the insoluble microparticles were much easier to visualise than in the original product when mixed with cal-

![Graphs showing the influence of shaking strength on the number of insoluble microparticles in ceftriaxone solutions mixed with calcium chloride solution.](image)

**Fig. 2. Influence of Shaking on the Number of Insoluble Microparticles in Ceftriaxone Solutions Mixed with Calcium Chloride Solution**

The influence of shaking strength (120 cycles/min) on the number of insoluble microparticles with diameter ≥10 μm (A) and ≥25 μm (B) (mean of three observations) formed when 2% (w/v) calcium chloride solution (calcium ion concentration 1.25 mmol/L) was added to ceftriaxone solutions (5 mg/mL), and stored at 25°C, measured using a light obscuration particle counter. Tukey test; **p < 0.01.

**Discussion**

Insoluble microparticles were formed when the original manufacturer’s ceftriaxone product, and seven generic versions thereof, were mixed with calcium chloride solution, simulating the mixture of the two solutions in an intravenous line.
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cium chloride solution, while they were equally or more diffi-
cult to visualise in generic products C and H. In generic
products B, D, E, F and G, the insoluble microparticles had a
larger particle size. This may be due to the accelerated growth
of smaller crystals on the surface of the particles as described
in a previous article.\(^{11}\) Therefore the total number of insoluble
microparti-
cles detected by the light obscuration particle coun-
ter decreased as proportion of insoluble microparticles with a
large particle diameter precipitate increased. However when
insoluble microparticles were visible to the human eye, the
number of microparticles might exceed far the permissible
range.

In our experiments, the number of microparticles in the
mixed solutions was higher with than without shaking.
Shaking was intended to represent the physical stimulation
within the infusion pump that occurs when a mixture of
two solutions is added to the intravenous line, as it has been
suggested that this stimulation may cause particle collision,
which in turn promotes the appearance of insoluble micropar-
ticles.\(^{12}\) It is possible that differences in the appearance of
the insoluble microparticles in the various products are due
to different additives, pH, and/or impurities in the products
themselves. As there was no mention of additives on the
package inserts of the products, differences in the content of
impurities and pH of the ceftriaxone solutions were examined.
The impurities were different in the different products, which
was attributed to differences in the source of the active phar-
aceutical ingredient and in pharmaceutical processing. It is
probable that the impurity content affects the appearance of
the insoluble microparticles. For example, product H, in which
insoluble microparticles were barely visible, had a high im-
purity content while products B, D and G, in which insoluble
microparticles were easily visible, had a low impurity content.
In a previously report, it has shown that impurities attach to
crystal surfaces and interrupt crystal growth.\(^{13}\) It was there-
fore suggested that the presence of impurities may delay the
appearance of insoluble microparticles formed between ceftri-
axone and calcium. Generally, many impurity would becomes
the reason for avoid the use of generic product, but it is sug-
gest that many impurity have no in fl uence a choice of gene-
ric product about compatibility with calcium-containing products.
However, in this study, it was dif fi cult show a causal relation-
ship the appearance of the insoluble microparticles, because
there were no examined identification of impurity and confir-
mation of reaction of the ceftriaxone and impurity. Therefore
further examination is necessary. The various products dif-
fered in the pH of their solutions, these differences did not
 correspond with the visibility of insoluble microparticles, pH
is therefore unlikely to influence incompatibility with calcium
chloride solution.

Multiple factors affect incompatibility, and it does not seem
to be possible to predict the appearance of insoluble micropa-
ticles on the basis of any single factor (shaking strength, im-

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Fig. 3. Liquid Chromatogram of Ceftriaxone Sodium Preparations for Injection

Representative liquid chromatogram of original manufacturer’s and generic products of ceftriaxone sodium preparations for injection (A–H). a: Peak 1, b: Peak 2, c:
Peak 3, d: Ceftriaxone.

Fig. 4. pH of Ceftriaxone Solution with or without the Addition of Calcium Chloride Solution

The pH of ceftriaxone solution (5 mg/mL) diluted in sodium chloride (mean of three observations) with or without the addition of calcium chloride solution (calcium ion concentration 1.25 mmol/L) at 25°C. Dunnet test; *<p<0.05, **<p<0.01, versus A.
Conclusions
There were differences in both the quality and quantity of insoluble microparticles formed when original manufacturer’s ceftriaxone sodium preparation for injection and seven generic versions thereof were mixed with calcium chloride solution. In some of the generic products, the number of insoluble microparticles was significantly higher than in the original product. Whereas other products, the appearance of insoluble microparticles was harder than in the original product.

These results suggest that care should be taken when switching from the original product to a generic version of ceftriaxone sodium preparation for injection.

References
2) Chugai Pharmaceuticals, Drug Package Insert ‘ROCEPHINE® FOR INJECTION 1 g.’ 2009.
6) Chugai Pharmaceuticals, Drug information sheet ‘ROCEPHINE® FOR INJECTION 1 g.’ 2009.