—Regular Articles—

Incompatibility of Ceftriaxone Sodium with Calcium-containing Products

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The purpose of this study was to evaluate the incompatibility of ceftriaxone with calcium-containing products, which had been the subject of an ALERT issued by the FDA. The influence of calcium ion concentration, storage temperature and shaking on the appearance and quantity of insoluble microparticles in mixtures of the two was examined using a light obscuration particle counter and a stereomicroscope. Appropriate volumes of 2% (w/v) calcium chloride solution were added to ceftriaxone sodium for injection (10 mg/ml) to make solutions with final calcium ion concentrations of 0.5–2.5 mmol/l, and stored at 20°C, 25°C, or 30°C. The number of insoluble microparticles increased on storage when the calcium ion concentration of the sample was $\geq$2 mmol/l; it exceeded the permissible range at all temperatures by 1 h. The microparticles had a greater diameter at higher temperatures, although fewer microparticles were observed. The weight of precipitate increased as a function of both the calcium ion concentration and the temperature. The number of microparticles was also significantly increased by shaking. The number of microparticles in mixtures containing 1000 μg/ml ceftriaxone was significantly increased, even though concentrations of calcium ion was 1.25 mmol/l. Overall, not only calcium ion concentration, but storage temperature and shaking affected the extent of precipitation of ceftriaxone with calcium.

Key words——ceftriaxone; calcium; incompatibility; insoluble microparticle; light obscuration particle counter; stereomicroscope

INTRODUCTION

In the 1980s there were several case reports of central venous catheter occlusion or pulmonary deposition caused by precipitation derived from incompatibilities in total parenteral nutrient mixtures.[1,2] In 1994, the US FDA issued a safety alert[3] on this subject and in 2007 this was followed by an alert on the interaction of ceftriaxone (Fig. 1) with calcium-containing products, due to a number of neonatal deaths caused by ceftriaxone–calcium precipitates in lungs and kidneys.[4] The alert suggested the possibility of a serious interaction between ceftriaxone and calcium-containing solutions, based on physical incompatibility. It was recommended that ceftriaxone should not be mixed with calcium-containing products and not administered in the same or different infusion lines or sites in any patient within 48 h (given the long half-life of ceftriaxone). In Japan, this information was not incorporated into the ceftriaxone package insert, nor was the recommended administration interval mentioned. In 2009, FDA published the updated recommendations based on the results from two *in vitro* studies.[5] FDA now recommends that ceftriaxone and calcium-containing products may be used concomitantly in patients>28 days of age, using the precautionary step. FDA had previously recommended, but no longer recommends, that in all age groups ceftriaxone and calcium-containing products should not be administered within 48 h of one another. But, both of two *in vitro* studies examined ceftriaxone-calcium precipitation by reducing recovery of ceftriaxone from plasma. Precipitation was not determined directly and the influence of temperature was not examined.

Ceftriaxone has a prolonged biological half-life and a wide spectrum of antimicrobial activity, mak-

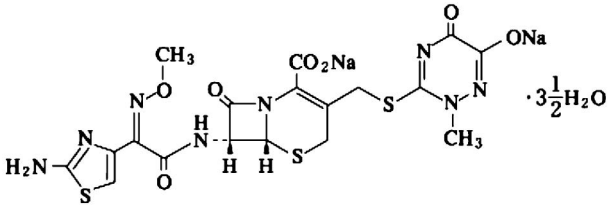

Fig. 1. Structural Formula of Ceftriaxone Sodium

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ing it a useful and commonly prescribed antibiotic.[6] Ceftriaxone is usually administered *via* the same cannula used for calcium-containing solutions. Koubo has previously reported the ceftriaxone-calcium precipitation increased with increasing calcium ion concentration and physical stimulation by infusion pump affected the precipitation,[7,8] but precipitation was observed with the naked eye and the influence of temperature was not examined. Precipitated particles of ceftriaxone and calcium, have a small diameter, and are difficult to see with the naked eye in mixed solutions, because the size of visible microparticles is 50 μm.[9] Therefore, there is a possibility that such precipitated particles could accidentally be administered into human veins. Shoji and Nakagawa suggested that the incompatibility of injection fluids should be considered from the standpoint of not only physical and chemical characteristics but also from a clinical viewpoint, and pointed out that insufficient drug dosages may be due to the adsorption of the drug on the surface of the infusion bag or line.[10,11] Recently, there have been some reports of contamination with insoluble microparticles at the time of ampoule opening, observed using a light obscuration particle counter.[12,13]

In this study, the influence of the temperature which was not demonstrated in the previous article or FDA Safety Alert, was examined. And, the previous studies related to incompatibility by measuring a number of insoluble microparticles were examined visually, a light obscuration particle counter was adopted to evaluate the incompatibility sensitively.

EXPERIMENTAL

Materials Rocephin® for injection containing 1 g ceftriaxone (Lot No.; K251831, Chugai Pharmaceutical Co., Ltd. Tokyo, Japan), isotonic sodium chloride solution 100 ml (Lot No.; 070625TA, Terumo Co., Ltd. Tokyo, Japan), and calcium chloride injection 2% (Lot No.; K6L70, Otsuka Pharmaceutical Co., Ltd. Tokyo, Japan) were purchased for use in this study.

Measurement of Insoluble Microparticles Using a Light Obscuration Particle Counter The employed method was essentially same as the 'Insoluble Particulate Matter Test for Injection by Method 1. Light Obscuration Particle Count Test' described in the 15th edition of the Japanese Pharmacopoeia (2006).[9]

All procedures were carried out on a clean bench. The number and size of microparticles were determined using a light obscuration particle counter KL-04 (RION Co., Ltd). The thresholds of microparticle size were 1.3, 2.0, 5.0, 10.0, 20.0, 25.0, 40.0, 50.0, and 100.0 μm. The volume of each sample was 5 ml, and the mean value of three samples was calculated. All instruments were washed with water for injection to eliminate insoluble microparticles derived from the devices. Gas bubbles in the sample solution were eliminated by standing for 2 min.

The results were evaluated according to the criteria for the maximum number of insoluble microparticles in the 15th edition of the Japanese Pharmacopoeia. For injection preparations administered at a volume of $\geq$100 ml, the tolerated number of insoluble microparticles with a diameter 10 μm or greater is 25 or less, and that of microparticles with a diameter 25 μm or greater is 3 or less, per ml.

Method of Preparation

The influence of calcium ion concentration 28, 56, 83, 111, 139 μl of 2% (w/v) calcium chloride solution were diluted up to 10 ml with ceftriaxone injection product (10 mg/ml) dissolved with isotonic sodium chloride solution, and the final calcium ion concentrations adjusted to 0.5, 1, 1.5, 2, and 2.5 mmol/l. The solutions were gently agitated and stored at 20°C, 25°C, or 30°C. The number of insoluble microparticles was measured according to the method described in the previons section, both immediately after mixing and 1, 3 and 6 h later. 139 μl of 2% (w/v) calcium chloride solution were diluted up to 10 ml with ceftriaxone injection product (10 mg/ml) dissolved with isotonic sodium chloride solution, and the final calcium ion concentrations adjusted to 2.5 mmol/l. The mixtures were observed visually and by stereomicroscope (SZX10, Olympus Co., Ltd.), both immediately after mixing and 3 and 6 h later.

The measurement of precipitate weight 83, 111, 139 μl of 2% (w/v) calcium chloride solution were diluted up to 10 ml with ceftriaxone injection product (10 mg/ml) dissolved with isotonic sodium chloride solution, and the final calcium ion concentrations adjusted to 1.5, 2, or 2.5 mmol/l. The solutions were gently agitated and stored at 20°C, 25°C, or 30°C for 6 h. Samples were weighed after filtration and drying under reduced pressure.

The influence of shaking 69 μl of 2% (w/v) calcium chloride solution were diluted up to 10 ml

with ceftriaxone injection product (10 mg/ml) dissolved with isotonic sodium chloride solution, and the final calcium ion concentration adjusted to 1.25 mmol/l. The solutions were gently agitated and shaken at 120 cycles/min throughout the experiment using a constant temperature shaking machine (Bio-Shaker VBR-36, TAITEC Co., Ltd.) with the temperature fixed at 25℃. The number of insoluble microparticles was measured according to the method described previously, both immediately after mixing and 3 and 6 h later. Samples stored without shaking after adding calcium, and shaken without adding calcium were prepared as controls.

Incompatibility of low concentrations of ceftriaxone sodium with calcium-containing products 69 μl of 2% (w/v) calcium chloride solution were diluted up to 10 ml with low concentrations of ceftriaxone isotonic sodium chloride solution (30, 300 and 1000 μg/ml), were shaken at 120 cycles/min at 30℃, and the number of insoluble microparticles was measured. The concentrations of ceftriaxone used were the postulated plasma concentrations in adults 12 h after administration of 1 g product (30 μg/ml), immediately after administration of 2 g product (300 μg/ml), and the postulated concentration in human gall bladder 1–3 h after administration of 1 g product (1000 μg/ml).[14] The plasma concentration of ionized calcium was postulated to be 1.25 mmol/l. The numbers of insoluble microparticles were measured according to the method described previously, both immediately after mixing and 3 and 6 h later.

Statistical Analysis The numbers of insoluble microparticles and the weight of the precipitate represent the mean of three values, plus or minus the standard deviation. The data were analyzed by two-way repeated ANOVA followed by Tukey's HSD test, or Tukey-Kramer test; statistical significance was accepted at the $p<0.05$ or $p<0.01$ level.

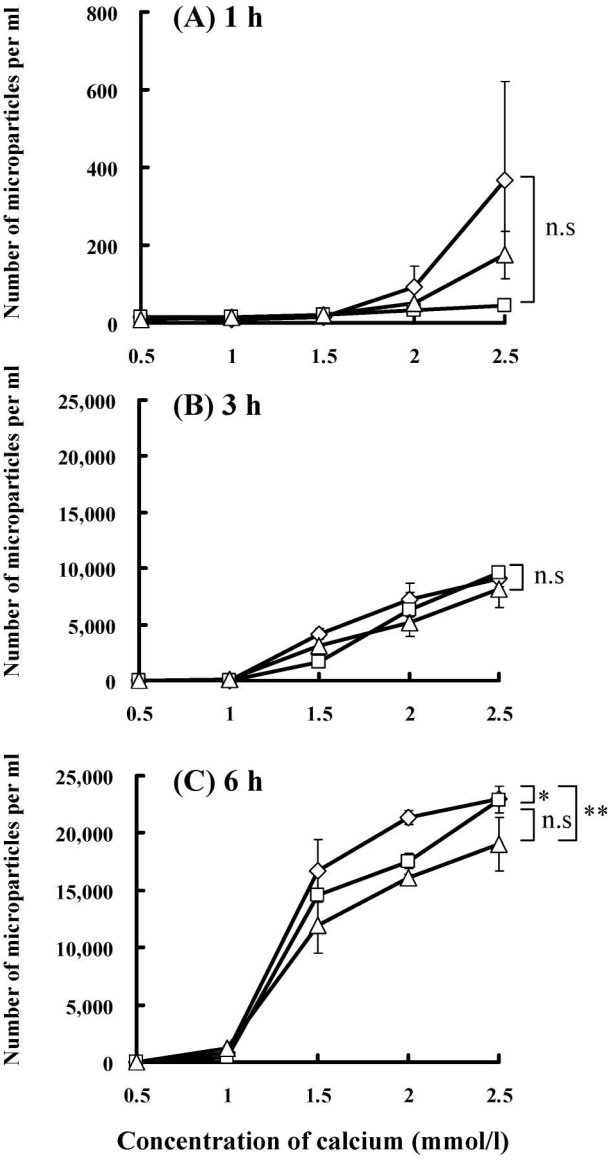

Fig. 2. Number of Insoluble Microparticles with a Diameter 10 μm or Greater in Ceftriaxone Solution Added Calcium Chloride Solution Using a Light Obscuration Particle Counter

The number of insoluble microparticles with a diameter 10 μm or greater ($n=3\pm$S.D.) formed when 2% (w/v) calcium chloride solution was added to ceftriaxone injection (10 mg/ml) made with isotonic sodium chloride solution, and stored at 20℃ (◇), 25℃ (□) and 30℃ (△) for 1 h (A), 3 h (B) and 6 h (C). Tukey's HSD test; $^{*}p<0.05$; $^{**}p<0.01$.

RESULTS

The Influence of Calcium Ion Concentration The numbers of insoluble microparticles with diameters 10 μm or greater and 25 μm or greater determined at 1, 3 and 6 h after mixing calcium chloride solution with ceftriaxone injection, are shown in Figs. 2 and 3, respectively. Immediately after sample preparation, the number of insoluble microparticles in all samples was under the permissible limit. The number of insoluble microparticles increased on storage, and by 1 h after sample preparation, exceeded the permissible range at all temperatures when the sample solution contained ≥2 mmol/l calcium ion. At 3 h after sample preparation, the number of insoluble microparticles exceeded the permissible range at all temperatures when the sample solution contained ≥1 mmol/l calcium ion. At 6 h after sample preparation, the number of insoluble microparticles exceeded the permissible range in a few samples when

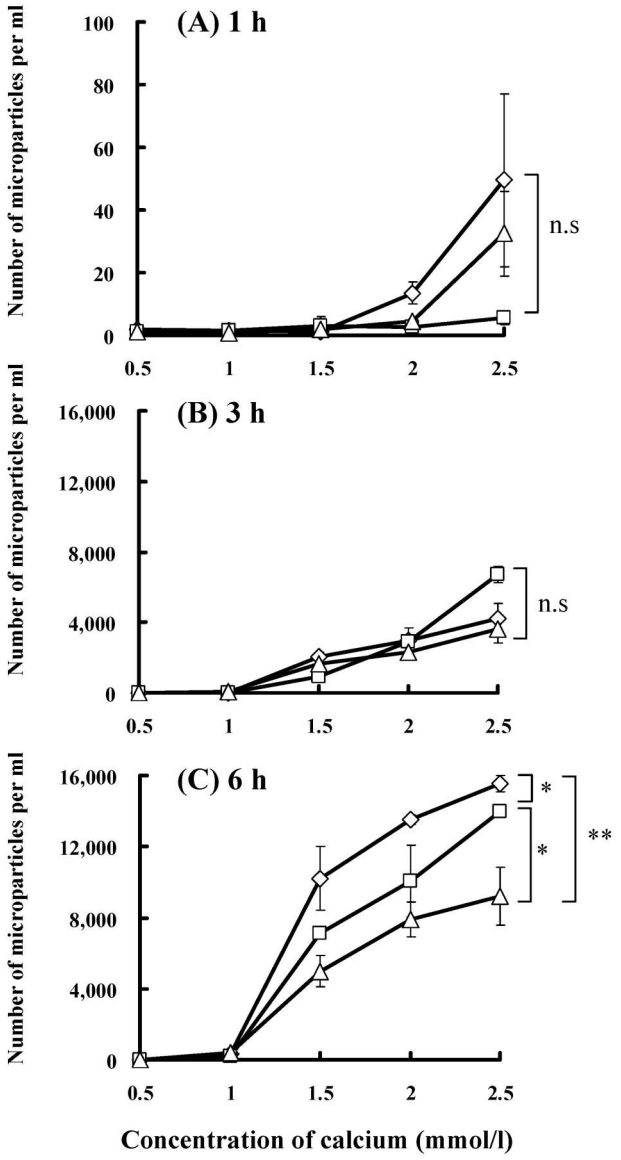

Fig. 3. Number of Insoluble Microparticles with a Diameter 25 μm or Greater in Ceftriaxone Solution Added Calcium Chloride Solution Using a Light Obscuration Particle Counter

The number of insoluble microparticles with a diameter 25 μm or greater ($n=3\pm$S.D.) formed when 2% (w/v) calcium chloride solution was added to ceftriaxone injection (10 mg/ml) made with isotonic sodium chloride solution, and stored at 20°C (◇), 25°C (□) or 30°C (△) for 1 h (A), 3 h (B) and 6 h (C). Tukey's HSD test; $^{*}p<0.05$; $^{**}p<0.01$.

the sample solutions contained $\geq$0.5 mmol/l calcium ion.

Storage temperature had no influence on incompatibility up to 3 h after sample preparation, as observed under the stereomicroscope. At 6 h after sample preparation, the microparticles were fewer but had a larger particle diameter (Figs. 2 and 3). The diameters of the insoluble microparticles increased with increasing temperature, as seen under the stereomicroscope (Fig. 4).

The Weight of Precipitate At 6 h after mixing, insoluble microparticles were just visible when the concentration of calcium ion was 1 mmol/l, but were quite obvious when the concentration was 1.5 mmol/l. A greater weight of insoluble microparticles was found at the bottom of the container at higher temperatures and in the presence of higher concentrations of calcium ion. The precipitated insoluble microparticles could not be redissolved in water, even after agitation. The weight of the precipitate increased as a function of both the calcium ion concentration and the temperature, as shown in Fig. 5.

The Influence of Shaking The number of insoluble microparticles with diameters 10 μm or greater and 25 μm or greater, was significantly higher after shaking at 120 cycles/min, than the number measured without shaking. The results are shown in Fig. 6.

Incompatibility of Low Concentrations of Ceftriaxone Sodium Solution with Calcium-containing Products Figure 7 shows the results of measuring the numbers of insoluble microparticles at lower concentrations of ceftriaxone. The numbers of insoluble microparticles with a diameter 10 μm or greater and/or less in mixtures containing 1000 μg/ml ceftriaxone were significantly higher than the numbers at lower concentrations of ceftriaxone, even though 1.25 mmol/l calcium isotonic sodium chloride solution was used throughout. When the incompatibility of

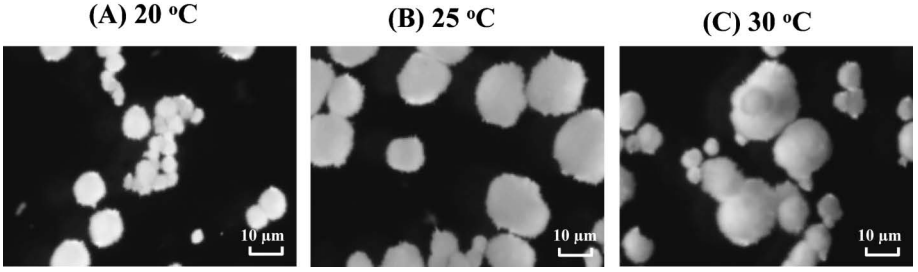

Fig. 4. Stereomicroscopic Photographs for Insoluble Microparticles in the Solution

Temperature at (20°C (A), 25°C (B) or 30°C (C)) at 6 h after sample preparation.

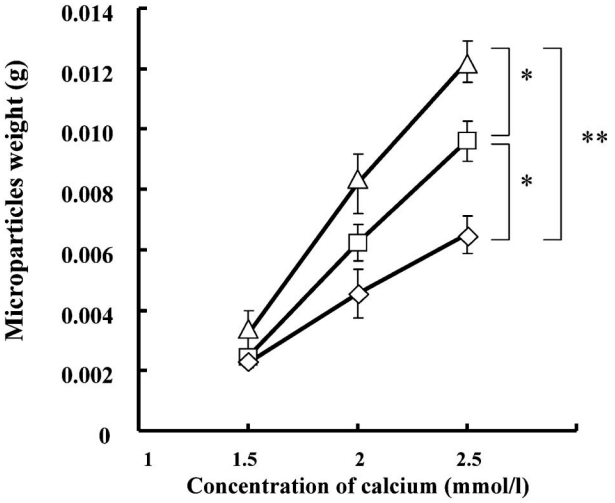

Fig. 5. Weight of Precipitate ($n=3\pm$S.D.) for Each Sample Stored

Temperature at (20℃ (◇), 25℃ (□) or 30℃ (△)) at 6 h after sample preparation. Tukey's HSD test; *$p<0.05$; ** $p<0.01$.

30, 300, 1000 μg/ml ceftriaxone with 1.25 mmol/l calcium chloride solution was measured, the numbers of insoluble microparticles with a diameter less than 10 μm 6 h after sample preparation were determined to be 37.3 ± 3.1, 211.5 ± 111.0, and 731.0 ± 240.4, respectively, while the numbers of insoluble microparticles with a diameter 10 μm or greater were 3.3 ± 1.5, 3.7 ± 1.5, and 23.5 ± 0.7, respectively. In all cases, the numbers of insoluble microparticles were within the permissible range and no precipitation was visible.

DISCUSSION

In this study, precipitation occurred in mixtures of ceftriaxone sodium with calcium-containing products as a function of ceftriaxone or calcium ion concentration, storage temperature, and shaking. Even at low concentrations of ceftriaxone, the number of insolu-

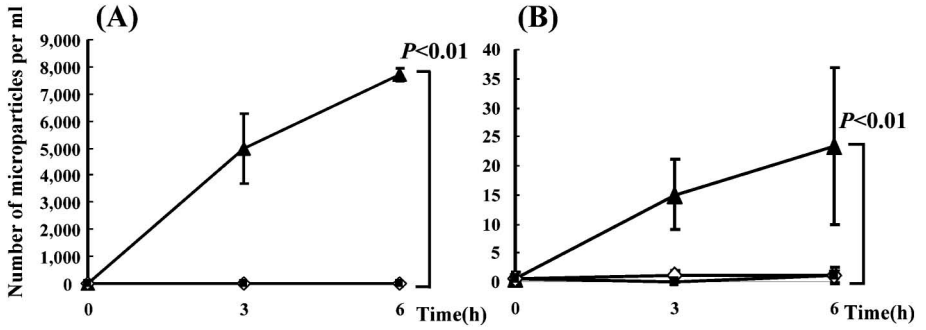

Fig. 6. Influence of Shaking on the Number of Insoluble Microparticles with a Diameter 10 μm or Greater (A) and 25 μm or Greater (B) Using a Light Obscuration Particle Counter

The influence of shaking (120 cycles/min) on the number of insoluble microparticles with a diameter 10 μm or greater (A) and 25 μm or greater (B) ($n=3\pm$S.D.). As analysed by two-way ANOVA with repeated measures; (▲) Ca: 1.25 mmol/l, 120 cycles/min, (◇) Ca: 1.25 mmol/l, without shaking, (■) Ca: 0 mmol/l, 120 cycles/min.

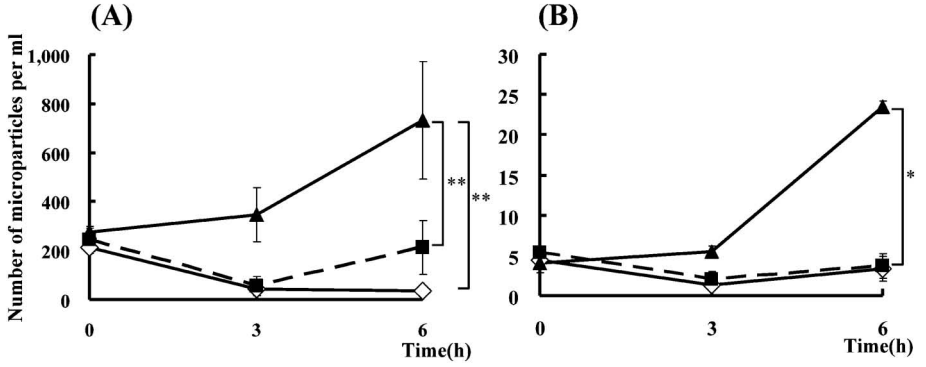

Fig. 7. Number of Insoluble Microparticles with a Diameter Less than 10 μm (A) and 10 μm or Greater (B) in Low Concentrations of Ceftriaxone in Isotonic Sodium Chloride Solution

The number of insoluble microparticles with a diameter less than 10 μm (A) and 10 μm or greater (B) in low concentrations of ceftriaxone in isotonic sodium chloride solution ($n=3\pm$S.D.). 30 μg/ml (◇), 300 μg/ml (■), and 1000 μg/ml (▲) mixed and shaken at 120 cycles/min at 30℃. Tukey's HSD test; *$p<0.05$; ** $p<0.01$.

ble microparticles arising due to precipitation with calcium was increased. The higher the storage temperature, the fewer insoluble microparticles precipitated, but the greater the weight of the precipitate. Thus, in the initial stage of the interaction between ceftriaxone and calcium ion, insoluble microparticles with a small particle diameter precipitate; this is followed by aggregation to form insoluble microparticles with larger diameter. In other words, the total number of insoluble microparticles detected by the light obscuration particle counter decreased as the proportion of insoluble microparticles with a large particle diameter increased. The increase of particle diameter at higher temperatures seems to be due to aggregation of precipitated insoluble microparticles. The observations made using the stereomicroscope supported the findings made using the light obscuration particle counter. The aggregation in perikinetic (diffusive) systems, in general, is accelerated by increasing particle and temperature.[15] And, diffusivities are proportional to the temperature based on Einstein relation.[16] It was thought that the particle diameter grew larger due to the aggregation at higher temperature, since diffusivity increased the particle collision based on the Brownian motion. The lower number of insoluble microparticles at higher temperatures seems to be differences in the sedimentation rate for individual particles. In general, insoluble particles sediment according to Stokes' Law.[17] Since aggregated particles with larger diameter sediment with a comparatively greater speed, there may be a risk of underestimating the total number of microparticles suspended in the sample solution when this includes particles with a comparatively large particle size. Therefore, it would be an advantage to develop a method for measuring the number of insoluble microparticles by gradually stirring without generating bubbles.

In our study, shaking increased the observed number of insoluble microparticles significantly, suggesting that physical stimulation affects the precipitation of ceftriaxone with calcium. In the previous study for the precipitation of calcium oxalate monohydrate from supersaturated solutions,[16] in any precipitation system there are two mechanisms for size enlargement: growth, by which is meant the deposition of ionic or molecular species on crystal surfaces, and aggregation, the process by which crystals collide, adhere to each other, and form new, stable particles. In orthokinetic (stirred) system, particle collision frequencies can be increased under the influence of stirring.[15] These studies suggest physical stimulation affects the collision of insoluble microparticles caused by incompatibility, and new particles are formed in the precipitation of ceftriaxone-calcium salt from supersaturated solutions. In a drop infusion using a peristaltic infusion pump, local stirring and stimulation may therefore encourage ceftriaxone-calcium precipitation because the fingerboard of the pump frequently pushes the line of the infusion device.[8,18] The influence of the bubbles by the shaking is neglected in this examination which evaluated for the number of insoluble microparticles with a diameter 10 μm or greater. Because the number of insoluble microparticles in the control solution which has not added calcium have not changed, and the gas bubbles 10 μm or greater was eliminated by standing for 2 min.[19,20]

The number of insoluble microparticles with a diameter less than 10 μm in 30 μg/ml ceftriaxone with 1.25 mmol/l calcium chloride solution was decreased. The reason seems to be due to gas bubbles, which was not removed before measurement. The incompatibility of ceftriaxone with calcium may occur immediately after the mixing, and is influenced by the vibration of the supersonic wave. Gas bubbles 10 μm or greater in the sample solution were eliminated by standing for 2 min in this experiment. However, standing for 30 min to 1 h is necessary to eliminate 2 μm gas bubbles.[19,20] There were no significant differences of the number of insoluble microparticles with a diameter less than 10 μm between 3 h and 6 h after sample preparation. And there were no significant differences of the number of insoluble microparticles with a diameter 10 μm or greater among immediately after mixing and 3 and 6 h later. Therefore, the number of insoluble microparticles with a diameter less than 10 μm in 30 μg/ml ceftriaxone with 1.25 mmol/l calcium chloride solution at 3 h after sample preparation might be decreased by eliminating gas bubbles which were not eliminated immediately after making of the mixture. Symptomatic biliary sludge made of ceftriaxone has frequently been observed in children.[21–23] There is also a report in an adult without gall bladder inflammation.[24] In these experiments on the incompatibility of various concentrations of ceftriaxone isotonic sodium chloride solution with calcium-containing products, the number of insoluble microparticles increased after mixing 1000 μg/ml of ceftriaxone, simulating the ceftriaxone concentration in biliary

sludge, with 1.25 mmol/l calcium isotonic sodium chloride solution, simulating the postulated plasma calcium ion concentration. Therefore, the possibility of the precipitation of ceftriaxone in mixtures with calcium-containing products *in vitro* and/or *in vivo* cannot be excluded, even in adults.

According to the FDA Alert,[4] deaths due to insoluble ceftriaxone–calcium precipitation in the lungs and the kidneys have occurred in newborn and premature babies following administration of higher dosages of ceftriaxone and higher concentrations of calcium-containing medium. The temperatures at which this occurred were probably higher than normal room temperature. It is also conceivable that the precipitation of ceftriaxone with calcium may occur in adults receiving total parenteral nutrition from an infusion pump.

CONCLUSIONS

Precipitation of insoluble microparticles occurred in mixed solutions of ceftriaxone and calcium-containing products. The amount of precipitation depended on the calcium ion concentration, temperature, storage time, and shaking. The precipitate could not be redissolved in water, even after agitating strongly.

Overall, not only calcium ion concentration, but storage temperature and shaking affected the extent of precipitation of ceftriaxone with calcium. Ceftriaxone has a prolonged biological half-life and may remain in circulation for a comparatively long time, with a concomitant risk of incompatibility even at low concentrations of calcium ion.

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