Tissue Distribution of Intravenously Administered Insoluble Microparticles: Report II

Takuya Sugahara*1, Masaharu Kotani2, Keiji Kuramoto3, Kouichi Arai1, Tadashi Shiraishi1 and Tohru Shoji3

Department of Pharmacy, Yamagata City Hospital Saiseikan1,
Department of Molecular and Cellular Biology, School of Pharmaceutical Science, Ohu University2,
Laboratory of Clinical Pharmaceutics, School of Pharmaceutical Science, Ohu University2,
Division of Hospital Pharmacy, Yamagata University3

Received April 11, 2012
Accepted July 17, 2012

In this study, we investigated the in vivo distribution of insoluble fluorescent microparticles administered to rats through the central vein. We previously reported that the IMs increased by mixed operation of some injections. The number of 10-25 μm insoluble microparticles was 41 ± 1/mL. Therefore, the daily 10-μm fluorescent microparticle dose was set at 2000/day for the nutritional solution dose of 50 mL/day required to maintain the rats. The fluorescent microparticles were administered by bolus injection into the central vein on Days 1, 2, and 3. A fluorescence microscope was used to assess frozen thin sections of each organ that were prepared on Day 4 and urine samples. A large number of fluorescent microparticles were found in the lung tissue, with 1–5 particles in each of the 20-μm thick and 25-mm² area sections. In 20-μm thick and 50-mm² area kidney tissue sections, the number of observed fluorescent microparticles ranged from 0.1 to 0.2. No fluorescent microparticles were detected in tissue samples from the veins, cerebrum, cerebellum, heart, or liver. The total number of fluorescent microparticles excreted in the urine was 283.4 ± 113.0, which only accounted for approximately 4.7% of the dose administered over the 3-day period. These findings suggest that 10 μm insoluble microparticles administered intravenously, accumulate mainly in the lungs and kidneys and are excreted at low levels in the urine.

Key words —— insoluble microparticles, tissue distribution, admixture, accumulation, excretion in urine

Introduction

The Japanese Pharmacopoeia 16th Edition (JP) stipulates the permissible number of insoluble microparticles (IMs) per mL in injectables, as determined by light obscuration particle count test (Table 1).11 The pharmacopoeias of Japan, USA, and EU include the similar stipulations in this respect. We had previously reported that the IMs increased by mixed operation of some injections (50% Glucose preparation, amino acid preparation, 1 mol potassium chloride preparation, 10% sodium chloride preparation, maintenance fluid, vitamin preparation, and H2 blocker preparation).20 The number of 10 μm IMs exceeded the JP limits. Therefore, we reported that in-line filters are useful for avoiding the risks associated with the infusion treatment methods due to such microparticles and are essential in cases wherein a possibility of administration of 2 or more injectable drugs exists.21 In addition, after intravenous injection of mice with large doses of barium sulfate particles 1.5–10.0 μm in diameter, as surrogates for IMs, the particles were found to be distributed to all...
organs except the brain. 3)

The IMs of various types, forms, and a size are included in injection products. In this study, we examined the tissue distribution of them after injecting the different spherical fluorescent microparticles (FMs) from a rat central vein.

Materials and Methods

1. Experimental Animals

Seven-week-old male F344/DuCrlCrlj (Fischer 344) rats (Charles River Laboratories Japan, Inc.) were reared in the husbandry section of the Research Building for 6 days and used in the study at the age of 8 weeks. During the rearing period, the rats were allowed free access to solid feed for laboratory animals (CRF-1, Oriental Yeast Co., Ltd.) and water from the beginning of the quarantine and acclimation period. However, the rats were not provided with either food or water during the study period, and a nutritional solution comprising nutritional sources essential for maintaining life, such as sugars, amino acids, vitamins, and trace elements, was administered by continuous infusion for 3 days at 50 mL/day/rat through a central venous catheter placed in the external jugular vein. The central venous nutritional infusion solution, termed as total parenteral nutrition, provided nutrition to the rats and was prepared by mixing 1000 mL of PN Twin®-1 (Ajinomoto Pharmaceuticals Co., Ltd., Tokyo, Japan) on days 1–3 with 1 vial of Malatamin® vitamin complex (Daiichi Sankyo Co., Ltd., Tokyo, Japan) and 1 ampoule of Elementmic® essential trace element preparation for total parenteral nutrition (Ajinomoto Pharmaceuticals Co., Ltd.). 50 mL of the resultant mixture was administered to each rat.

2. Test Suspension

10 μm Fluorescent Polymer Microspheres (Catalog number G1000, Polystyrene, Fluorescent green (excitation 468 nm, emission 508 nm), Mean diameter 10.1 μm, 1.8 × 10^7/mL (Concentration 1% Solids), Particle density 1.05 g/cm³, Size (CV)³ uniformity <5%, Duke Scientific Corp.) were used as surrogates for IMs. The number of 10-25 μm IMs, formed during the admixture procedure simulating the clinical usage reported previously, 2) was 41 ± 1 /mL. The total number of particles in a 50 mL/day infusion was 2050 ± 50; therefore, the appropriate dose of the 10-μm FMs was set at 2000 /day, and the test suspension was prepared by suspending 2000 FMs in 1 mL of physiological saline solution. This volume was administered once per day per rat in the test group.

3. Study Method

The study schedule is shown in Fig. 1. At the beginning of the study, the rats were weighed and allocated without bias by the randomized block method in the order of their weight. On days 1–3,
the control and FM groups received either a 1-mL bolus of saline or test suspension per rat through a central venous line once daily. On each day, 24-h urine samples were collected at 10:00 hours before administration. In addition, because the study was completed at 16:00 hours on day 3, the 6-h (10:00–16:00 hours) urine samples were collected on that day.

4. Statistical Analysis

The statistical software used was StatView® for Windows version 5.0 (SAS Institute, Inc., Cary, NC, USA). All measured values are expressed as mean ± standard deviation. The weights of the rats in the control and FM groups at study initiation were compared using the Mann–Whitney U-test, and other comparisons between the groups were made using the unpaired two-group t-test. The significance level was set at \( P<0.05 \) in the two-sided tests.

Results

1. Body Weights

The body weights of the rats in the control group (176.6 ± 3.2 g, \( n = 8 \)) were not significantly different from those in the FM group (176.9 ± 3.7 g, \( n = 8 \)) (\( P = 0.9164 \)).

2. Amount of FMs Excreted in Urine

The volumes of the urine samples collected from the control and FM groups are shown in Table 2, and the number of FMs excreted is...
shown in Table 3. The urine samples collected at 10:00 hours on days 1, 2, and 3 were termed as day-1, day-2, and day-3-1 samples, respectively, and those collected at 16:00 hours on day 3 were termed the day-3-2 samples. The day-1, day-2, and day-3-1 urine samples were collected at 24 h, whereas, the day-3-2 urine samples were collected at 6 h.

The urine volumes in the control group were as follows: day-1, 32.4 ± 1.5 mL; day-2, 39.6 ± 2.2 mL; day-3-1, 35.3 ± 1.4 mL; day-3-2, 10.6 ± 1.1 mL; and total volume, 117.9 ± 4.8 mL. The urine volumes in the FM group were as follows: day-1, 30.8 ± 1.2 mL; day-2, 39.6 ± 2.2 mL; day-3-1, 34.4 ± 1.3 mL; day-3-2, 9.3 ± 1.8 mL; and total volume, 114.1 ± 3.8 mL. A difference in the urine volume was observed between the 2 groups only for the day-1 samples (P < 0.05); however, no differences were observed in the urine volumes between the day-2 and day-3-2 samples or in the total volumes between the day-1 and day-3-2 samples.

No FMs were observed in urine samples collected from the control group. The number of FMs in the urine samples collected from the FM group was as follows: day-1, 0 ± 0; day-2, 50.5 ± 37.1; day-3-1, 187.8 ± 74.9; day-3-2, 45.1 ± 27.6; and total number, 283.4 ± 113.0. The day-1 samples were collected immediately before the first administration of FM suspension or physiological saline solution; therefore, no FMs were excreted in either the control or FM group.

### Table 2 Volume of urine samples

<table>
<thead>
<tr>
<th>Day</th>
<th>Control groups (mL)</th>
<th>FM groups (mL)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>32.4 ± 1.5</td>
<td>30.8 ± 1.2</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Day 2</td>
<td>39.6 ± 2.2</td>
<td>39.6 ± 2.2</td>
<td>NS</td>
</tr>
<tr>
<td>Day 3-1</td>
<td>35.3 ± 1.4</td>
<td>34.4 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Day 3-2</td>
<td>10.6 ± 1.1</td>
<td>9.3 ± 1.8</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>117.9 ± 4.8</td>
<td>114.1 ± 3.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

The day-1, day-2, and day-3-1 samples were 24-h urine samples, whereas the day-3-2 samples were 6-h urine samples. Data are mean ± standard deviation. Unpaired two-group t-test. n = 8.
NS : not significant; FMs : fluorescent microparticles.

### Table 3 Number of fluorescent microparticles (FMs) excreted in the urine

<table>
<thead>
<tr>
<th>Day</th>
<th>Control groups (particles)</th>
<th>FM groups (particles)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>NS</td>
</tr>
<tr>
<td>Day 2</td>
<td>0 ± 0</td>
<td>50.5 ± 37.1</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Day 3-1</td>
<td>0 ± 0</td>
<td>187.8 ± 74.9</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Day 3-2</td>
<td>0 ± 0</td>
<td>45.1 ± 27.6</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Total</td>
<td>0 ± 0</td>
<td>283.4 ± 113.0</td>
<td>P &lt; 0.0001</td>
</tr>
</tbody>
</table>

The day-1, day-2, and day-3-1 samples were 24-h urine samples, whereas the day-3-2 samples were 6-h urine samples. Data are mean ± standard deviation. Unpaired two-group t-test. n = 8.
NS : not significant.

3. FM Distribution in the Tissues

FM distribution in the different rat organs is shown in Table 4, and FM images in lungs and kidneys are shown in Fig. 2. The number of FMs found in the lung sections, measuring 5 mm × 5 mm × 20 μm, ranged from 1 to 5, and the number found in the kidney sections, measuring 5 mm ×
10 mm × 20 μm, ranged from 0.1 to 0.2. However, no FMs were found in the veins, cerebrum, cerebellum, heart, or liver.

**Discussion**

In this study, the FMs were administered to rats through the central vein at doses equivalent to 41 ± 1 /mL for the 10-25 μm IMs. The FMs were used as a surrogate for the IMs formed during the admixture procedure simulating the clinical usage reported previously. We administered FMs to rats during the 3-days in order to confirm the accumulation of it to each organs. The mean number of FMs administered during the 3-day study period was 6000. As a result, we were able to measure all the FMs in the collected urine. However, the total number of FMs excreted in the urine was 283.4, which accounted only for approximately 4.7% of the total number of FMs administered. The glomerulus does not pass particles larger than 8 nm in diameter. Therefore, it is a possibility that the FMs of 10 μm diameter could not be excreted into the urine.

In addition, although the FMs were found to

---

**Table 4** Distribution of fluorescent microparticles (FMs)

<table>
<thead>
<tr>
<th></th>
<th>Control groups</th>
<th>FM groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veins</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lung</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>Heart</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Liver</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Kidney</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

++ : 1 to 5 FM/frozen section of 5 mm × 5 mm × 20 μm
+ : 0.1 to 0.2 FM/frozen section of 5 mm × 10 mm × 20 μm
– : not detectable
accumulate in the lungs and kidneys, no traces of FMs were found in the veins, cerebrum, cerebellum, heart, or liver. The number of FMs in the lung sections, measuring 5 mm × 5 mm × 20 μm, was 1–5 and those in the kidney sections, measuring 5 mm × 10 mm × 20 μm, was 0.1–0.2. In this study, since we aimed at a qualitative verification, we did not measure the weight of the internal organs of a rat. Therefore, we were not able to quantify cumulative dosage to each internal organs of a rat. However, as we collected the whole quantity for the urine of the rat, we were able to check the amount of particulate excretion into urine. It was guessed from the amount of urine excretion that the amount of accumulation in body was 95.3%. Probably, there were mostly distributed in the lungs.

In a previous study, large single doses of barium sulfate particles 1.5, 5.0, and 10.0 μm in diameter were administered to mice as surrogates for IMs. In addition, major organs were collected 5 min after administration and analyzed by radiography. We reported that the microparticles were distributed to all organs (lungs, heart, liver, and kidneys) examined, except for the brain. The blood–brain barrier may have prevented distribution of barium sulfate particles to the brain. The reason for the widespread distribution may have been because these organs were collected 5 min after the large-dose administration. Therefore, large quantities of barium sulfate particles remained in the circulating blood. In the present study, the organs were collected for analysis at least 22 h after the final FM administration. In addition, the FM dose in this study was lower than the barium sulfate particle dose in the previous study, and there is also a possibility of differences in the particle-size distribution. The 10-μm FMs administered through the central vein may have circulated in the blood immediately after administration; however, with time, most of the FMs in the systemic circulation may have been physically trapped in the pulmonary capillaries. The fraction of FMs that managed to pass through the pulmonary capillaries reached the kidneys, where the particles were trapped, and a portion of these particles was then excreted in the urine. The above results suggest that IMs formed during admixture procedures in clinical settings accumulate in the lungs and kidneys.

Kanke et al.5 reported that 3- and 5-μm spheres were localized and retained in the liver and spleen, whereas 7- and 12-μm spheres were mechanically filtered and retained for prolonged periods in the lungs following intravascular administration of 141Ce-labeled polystyrene divinylbenzene microspheres to beagle dogs. Kutscher et al.6 reported that after intravenous administration of fluorescent polystyrene microparticles (2, 3, 6, and 10 μm) to rats, 2- and 3-μm spheres readily passed through the lungs to the liver and spleen, 10-μm spheres were completely trapped in the lungs for the 1-week study duration, and approximately 84% of 6-μm spheres that were initially trapped in the lungs cleared over the next 2 days, with an additional 15% clearance over the remaining 5 days.

Our finding that most of the 10-μm FMs accumulated in the lungs and few accumulated in the liver is consistent with that reported by Kanke et al. and Kutscher et al. However, one difference between our findings and the previous two studies is that in the present study, the 10-μm microparticles also accumulated in the kidneys, and only approximately 4.7% of those administered were excreted in the urine. In addition, one surprising finding in the present study was seemed that the FMs in the lungs and kidneys were not observed within the blood vessels but rather in the extra-
vascular cells or intercellular substances. The reasons for this may be the increased vascular permeability and phagocytosis by reticuloendothelial cells that circulate in the bloodstream, migrate into tissues, and are then carried by lymph vessels. However, although Piskin et al.\(^7\) reported that granulocytes and macrophages phagocytosed microparticles up to 6 \(\mu\)m, it remains uncertain whether these leukocytes are able to internalize rigid 10-\(\mu\)m polystyrene microparticles and whether 10-\(\mu\)m microparticles can penetrate vessel walls. Itho et al.\(^8\) reported that the intravenous injection of precipitates-containing etoposide solution caused a marked increase in pulmonary vascular permeability and edema in rats. Furthermore, Sendo et al.\(^9\) reported that the silicon contamination aggravated pulmonary edema that occurred after the intravenous injection of contrast medium in rats. As a result, edema formation may be localized. From this experimental result, we presumed that a 10 \(\mu\)m particles increased vascular permeability and particles leaked out to internal fluid. The same result may happen also human.

Notwithstanding the above discussion, the results of the present study suggest that intravenously administered IMs do not always circulate within the blood vessels but instead accumulate in the body, thereby raising concerns about the risk of tissue damage.

Adverse events associated with the use of IMs or foreign microparticles include phlebitis, pulmonary embolism, pulmonary granuloma, local tissue infarctions, and capillary and arterial occlusions; fatal cases have been reported.\(^{10-13}\) In addition, Lehr et al.\(^{14}\) reported that the reduction in capillary perfusion due to the presence of microparticles was greater in muscle areas that suffered more severe damage and was dependent on the severity of muscle damage due to ischemia and/or reperfusion. This suggests that the administration of microparticle-containing drugs may cause damage to major organs because of factors, such as trauma, major surgery, and sepsis.

The different types of insoluble microparticles formed in clinical settings include rubber fragments from rubber stoppers, glass or plastic fragments from glass or plastic ampoules, silicone from plastic syringes, drug microparticles formed during changes on combination or dissolution of lyophilized agents, and matter formed from other infusion-related equipment or materials.\(^{15-19}\)

For preventing adverse events associated with the use of IMs, in-line filters can be used as they prevent IMs from entering the body. In-line filters are recommended in a number of guidelines,\(^{20-25}\) and we have always highly recommended their use.

In the present study, 0.1- and 1-\(\mu\)m particles were also administered using the same experimental system. However, because the 0.1-\(\mu\)m particles were fluorescent red and the 1-\(\mu\)m particles were fluorescent blue, their fluorescence intensities were low and thus could not be detected by our fluorescence microscopy system. In a previous study,\(^{21}\) a large number of microparticles 1–10 \(\mu\)m in diameter were detected. The distribution of IMs less than 10 \(\mu\)m in diameter may have been different from that observed in the present study. In addition, admixed drugs contain not only IMs more than 1 \(\mu\)m in diameter but also those that are 1–1000 nm in diameter, and concerns have been raised that these smaller microparticles may also have adverse effects.\(^{26,27}\) On the contrary, microparticle-type carrier agents with particle diameters in the \(\mu\)m range that made use of the diameter-dependent differences in distribution have also been reported.\(^{28}\) In the future, further consider-
ation should be given to materials used in such studies, and research should be extended to cover IM 10 μm in diameter or less, which are not covered by the JP stipulations.

Conclusions

Even if sufficient care is used during admixture of drugs, IM may form or mix with the agents. Because such microparticles are not visible to the naked eye, they can be administered unintentionally. Intravenously administered IM may accumulate in vital body tissues, which raises concerns about their potential negative effects on the human body. Therefore, we recommend the use of in-line filters for intravenous infusions. We believe that in particular, the use of in-line filters is essential in cases involving children, elderly patients, patients with microcirculatory damage to major organs, patients being treated in intensive care units who are at high risk for IM, and patients receiving multiple injectable drugs.

Acknowledgments

We are grateful to Kenta Kajiwara and Yukifulmi Kokuba (Ajinomoto Pharmaceuticals Co., Ltd., Parenteral Nutrition and Dialysis Research Laboratories) for their cooperation in this study.

References


15) Yorioka K, Oie S, Oomaki M, Imamura A, Kamiya A,


