PET Tracing of Biodistribution for Orally Administered ⁶⁴Cu-Labeled Polystyrene in Mice

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Plastics are used commonly in the world because of their convenience and cost effectiveness. Microplastics, an environmental threat and human health risk, are widely detected in food and consequently ingested. However, degraded plastics are found everywhere, creating an environmental threat and human health risk. Therefore, real-time monitoring of orally administered microplastics to trace them in the body is tremendously important. Methods: In this study, to visualize their absorption path, we labeled polystyrene with ⁶⁴Cu-DOTA. We prepared radiolabeled polystyrene with ⁶⁴Cu. Afterward, ⁶⁴Cu-DOTA-polystyrene was orally administered to mice, and we evaluated its transit and absorption using PET imaging. The absorption path and distribution of ⁶⁴Cu-DOTA-polystyrene was measured using PET over 48 h. Ex vivo tissue radio-thin-layer chromatography (TLC) was used to demonstrate the existence of ⁶⁴Cu-DOTA-polystyrene in tissue. Results: PET images demonstrated that ⁶⁴Cu-DOTA-polystyrene began to transit to the intestine within 1 h. Accumulation of ⁶⁴Cu-DOTA-polystyrene in the liver was also observed. The biodistribution of ⁶⁴Cu-DOTA-polystyrene confirmed the distribution of ⁶⁴Cu-DOTA-polystyrene observed on the PET images. Ex vivo radio-TLC demonstrated that the detected γ-rays originated from ⁶⁴Cu-DOTA-polystyrene. Conclusion: This study provided PET evidence of the existence and accumulation of microplastics in tissue and cross-confirmed the PET findings by ex vivo radio-TLC. Information may be used as the basis for future studies on the toxicity of microplastics.

Key Words: microplastic; polystyrene; ⁶⁴Cu; ⁶⁴Cu-labeled polystyrene; PET

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Microplastics with diameters of less than 5 mm are recognized as a new environmental threat and human health risk (1). Microplastics have been observed to accumulate in many different marine animals, including fish (2–5), copepods (6,7), mussels (8–10), European flat oysters (11), and others (12–14). Fiber-type microplastics have been found in nitrates purchased at markets in Belgium (15). Considering that microplastics are widely detected in food, we can assume that microplastics are ingested along with the contaminated food. Therefore, it is highly likely that human consumption of microplastics is widespread. To understand the full significance of microplastic ingestion, the absorption path for microplastics ingested with foods needs to be visualized.

PET imaging is a powerful tool for observing absorption, distribution, metabolism, and excretion (16). PET can also be used to visualize the in vivo distribution of toxic substances labeled with radioactive isotopes, including diesel exhaust (17), and inhaled aerosols of toxic household disinfectants (18). Figure 1 shows a schematic of the study. We first identified the absorption path and distribution of microplastics using PET. Microplastic polystyrene was labeled with ⁶⁴Cu (⁶⁴Cu), to yield ⁶⁴Cu-DOTA-polystyrene and then was orally administered to mice. In a separate experiment, ⁶⁴Cu was orally administered as a control to assess the effects of the harsh stomach conditions on dechelated ⁶⁴Cu. PET was performed to monitor the absorption and distribution of ⁶⁴Cu-DOTA-polystyrene over 48 h. The biodistributions of ⁶⁴Cu-DOTA-polystyrene or ⁶⁴Cu were measured. Ex vivo tissue radio-thin-layer chromatography (TLC) was performed to identify whether γ-rays emitted from the tissue originated from ⁶⁴Cu-DOTA-polystyrene or from ⁶⁴Cu.

MATERIALS AND METHODS

Synthesis and Radiolabeling

To 300 μL of 0.1 M sodium carbonate buffer (pH 9.0), 2.5 mg of amino-polystyrene (0.2–0.3 μm; SpheroTech) were added. Then, 260 μg (471.70 nmol) of S-2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraacyclododecane tetraacetic acid (p-SCN-Bn-DOTA) in 50 μL of deionized water were added, and the mixture (pH 9.0) was shaken at 1,000 rpm and 25°C for 20 h. Unconjugated p-SCN-Bn-DOTA was removed using an Amicon centrifugal filter (30-kDa cutoff; Millipore). DOTA conjugation was confirmed using Fourier-transform infrared spectroscopy (Nicolet iS5; Thermo Fisher Scientific), and the resulting spectra were analyzed using Omnic software from Nicolet Instrument Corp. To determine the moles of DOTA per milligram of plastic, 50 μL of filtrate were analyzed by high-performance liquid chromatography (Waters). The quantity of DOTA in the filtrate was calculated from a standard curve (prepared from an analysis of known concentrations of DOTA). The conjugated moles of DOTA to polystyrene were then calculated by subtracting the moles of DOTA in the filtrate from the total moles of DOTA for the reaction. Physicochemical characterization of DOTA-polystyrene was performed using a field-emission scanning electron microscopy and dynamic light scattering. Concentrated DOTA-polystyrene was subsequently buffer-exchanged to isotonic buffered saline for use in the pet imaging study.
subsequent radiolabeling. The final concentration before radiolabeling was 2.5 mg/100 μL.

Cyclotron-produced [64Cu]CuCl2 was dried and redissolved in 0.01 N HCl (final concentration, 9.25 MBq/μL). In a 1.5-mL tube, 155.4 MBq of [64Cu]CuCl2 were added to 80 μL of 0.1 M NaOAc buffer (pH 5). DOTA-poly styrene (2 mg in 80 μL) was added, and the mixture was shaken in a Thermomixer C (Eppendorf AG) at 40°C and 1,000 rpm for 30 min. [64Cu]-labeled DOTA-poly styrene was purified using an Amicon centrifugal filter at 25°C, 3,000 rpm, for 30 min. By repeating this procedure, reaction buffer was exchanged to 1 × phosphate-buffered saline for further studies.

In Vitro Stability Study

[64Cu]Cu-DOTA-poly styrene in phosphate-buffered saline (1.85 MBq/30 μL) was diluted to 270 μL of phosphate-buffered saline, hydrochloric acid-potassium chloride buffer (pH 2), human serum, or mouse serum. Each sample was incubated at 25°C (buffer) or 37°C (serum) for 48 h. Percentage stability was analyzed using instant TLC (0.1 M citric acid in water as a mobile phase).

PET/CT

All animal experiments were performed under the institutional guidelines of the Korea Institute of Radiological and Medical Sciences. BALB/c nude mice (n = 5–7, 5 wk old; Shizuoka Laboratory Center) were used.

PET/CT images were acquired with an Inveon PET scanner (Siemens Medical Solutions). [64Cu]CuCl2 (4.81 MBq/100 μL) or [64Cu]Cu-DOTA-poly styrene (4.81 MBq/57.8 μg/100 μL) was orally administered to the mice. PET was performed at 1, 6, 12, 24, and 48 h afterward. The PET data were acquired for 15 min within 350–650 keV and were reconstructed using a maximum a priori with a Poisson distribution (SP-MAP) algorithm (target resolution 3). The voxel size was 0.776 × 0.776 × 0.796 mm. Regions of interest were drawn in the stomach, liver, and intestine using ASIpro (Siemens Medical Solutions) after coregistration of CT and PET images. SUV_{max} was then calculated.

Biodistribution Study

The accumulated radioactivity concentration (percentage injected dose [%ID]/g) in each organ was measured at corresponding times after administration of [64Cu]Cu-DOTA-poly styrene or 64Cu.

Ex Vivo Radio-TLC

Ex vivo radio-TLC assays were performed to determine whether the detected γ-rays emitted from the tissues were emitted from 64Cu or from [64Cu]Cu-DOTA-poly styrene. SUV_{max} is shown for the stomach, liver, and intestine. PET images demonstrate that [64Cu]Cu-DOTA-poly styrene remained in the stomach at later time points.

Statistical Analysis

The data are presented as the mean with SD. The Student t test was performed using Prism (version 5.0; GraphPad).

RESULTS

Synthesis and Radiolabeling

DOTA was conjugated by high-performance liquid chromatography and a Fourier-transform infrared spectrometer (Fig. 2A; Supplemental Fig. 1; supplemental materials are available at http://jnm.snmjournals.org). Pers milligram of polystyrene, 184.78 ± 0.26 nmol of DOTA were conjugated. The particle size of polystyrene and DOTA-poly styrene was 223–224 nm, and no aggregation was observed (in either set of results) after the DOTA-conjugation reaction (Figs. 2B and 2C). The radiochemical yield of [64Cu]Cu-DOTA-poly styrene was 92.07% ± 3.20%, and radiochemical purity was 96.39% ± 1.66% (Supplemental Fig. 2A).

In Vitro Stability Study

No significant dechelation was observed after 48 h in phosphate-buffered saline (96.34%), pH 2 (91.68%), human serum (93.23%), or mouse serum (96.83%). The in vitro stability study demonstrated that [64Cu]Cu-labeled polystyrene was stable for the period used in this study (Supplemental Fig. 2B).

PET/CT

Figure 3 and Supplemental Figure 3 show the representative PET data at 1, 6, 12, 24, and 48 h after oral administration of [64Cu]Cu-DOTA-poly styrene or 64Cu. The corresponding time–activity curve is shown for the stomach, liver, and intestine. PET images demonstrate that [64Cu]Cu-DOTA-poly styrene remained in the stomach for up to 24 h. The SUV_{max} of [64Cu]Cu-DOTA-poly styrene in the stomach was 35.42 ± 4.25 at 1 h, 36.22 ± 3.91 at 6 h, 37.32 ± 1.34 at 12 h, 22.68 ± 4.81 at 24 h, and 0.20 ± 0.03 at 48 h. Polystyrene began its transit to the intestine within 1 h. The SUV_{max} of [64Cu]Cu-DOTA-poly styrene in the intestine was 41.93 ± 22.59
at 1 h, 45.29 ± 19.79 at 6 h, 33.84 ± 7.10 at 12 h, 15.59 ± 3.22 at 24 h, and 0.72 ± 0.75 at 48 h.

The in vivo absorption and distribution pattern of ⁶⁴Cu on PET was statistically different at each PET measurement point (Fig. 3). The SUVmax in the stomach was 35.42 ± 4.25 for [⁶⁴Cu]Cu-DOTA-polystyrene and 8.39 ± 6.98 for ⁶⁴Cu at 1 h after administration. Compared with the SUVmax of ⁶⁴Cu, the SUVmax of [⁶⁴Cu]Cu-DOTA-polystyrene was 4.22-, 4.67-, 7.40-, and 7.83-fold greater in the stomach at 1, 6, 12, and 24 h, respectively. Moreover, the SUVmax in the liver was 0.04 fold lower than that of ⁶⁴Cu at the 1-, 6-, 12-, 24-, and 48-h time points, respectively. In the large intestine, the %ID/g of [⁶⁴Cu]Cu-DOTA-polystyrene was 0.36-, 3.95-, 2.28-, 3.11-, and 13.75-fold greater in the stomach that that of ⁶⁴Cu at the 1-, 6-, 12-, 24-, and 48-h time points, respectively. In the liver, the %ID/g of [⁶⁴Cu]Cu-DOTA-polystyrene was 0.10-, 0.22-, 0.18-, 0.49-, and 0.10-fold lower than that of ⁶⁴Cu at the 1-, 6-, 12-, 24-, and 48-h time points, respectively. Additionally, we observed transit of [⁶⁴Cu]Cu-DOTA-polystyrene to the liver, spleen, heart, blood, lung, kidney, bladder, and testis.

In contrast, most of the ⁶⁴Cu accumulated in the large intestine, stomach, and small intestine at 1 h after administration. ⁶⁴Cu then quickly transitioned to other organs, including the liver. The %ID/g was greater for ⁶⁴Cu than for [⁶⁴Cu]Cu-DOTA-polystyrene in all other organs, including the liver (9.59-fold), spleen (12.0-fold), heart (7.85-fold), blood (5.83-fold), lung (25.69-fold), kidney (26.92-fold), bladder (1.35-fold), brain (1.36-fold), and testis (6.35-fold).

**Ex Vivo Radio-TLC**

The ex vivo radio-TLC assay results for other tissues (liver, small, and large intestine) demonstrated that the radiation signal was from [⁶⁴Cu]Cu-DOTA-polystyrene, not from ⁶⁴Cu (Supplemental Fig. 4).

**DISCUSSION**

We first identified the in vivo distribution of microplastics in mice by labeling microplastic polystyrene with the radioisotope ⁶⁴Cu, orally administering [⁶⁴Cu]Cu-DOTA-polystyrene (radioisotope-labeled microplastic polystyrene) to mice, and using PET to trace its absorption and distribution. Next, ex vivo biodistribution studies confirmed [⁶⁴Cu]Cu-DOTA-polystyrene accumulation in specific organs. Ex vivo radio-TLC was used to confirm that the detected γ-rays originated from [⁶⁴Cu]Cu-DOTA-polystyrene. Exposure to microplastics in food and water through oral administration is a significant environmental and health problem (21–23). However, it is extremely likely that microplastics are widely distributed within the food we eat.

The advantage of PET is that it is possible to observe the in vivo absorption, distribution, metabolism, and excretion of substances labeled with radioactive isotopes without killing the animal (16). Although fluorescence is commonly used for in vivo exposure and biodistribution studies, fluorescence in animal bodies can be absorbed by bone and soft tissues, and prolonged exposure to ultraviolet light can result in bleaching and loss of fluorescence intensity (24). Therefore, quantification of fluorescent images is limited, compared with PET images. In addition, when microplastic-conjugated fluorescent dyes are used, animals must be killed to observe the absorption and accumulation of the microplastics over time.

**Biodistribution Study**

Figure 4 shows the biodistribution results for the organs of interest. Overall, the accumulation pattern was similar to that of SUV in the PET images. In the stomach, the %ID/g of [⁶⁴Cu]Cu-DOTA-polystyrene was 2.01-, 2.31-, 8.28-, 3.61-, and 13.27-fold greater than that of ⁶⁴Cu at the 1-, 6-, 12-, 24-, and 48-h time points, respectively. In the small intestine, the %ID/g of [⁶⁴Cu]Cu-DOTA-polystyrene was 6.89-, 0.92-, 3.44-, 2.50-, and 11.44-fold greater than that of ⁶⁴Cu at the 1-, 6-, 12-, 24-, and 48-h time points, respectively. In the large intestine, the %ID/g of [⁶⁴Cu]Cu-DOTA-polystyrene was 0.36-, 3.95-, 2.28-, 3.11-, and 13.75-fold greater in the stomach than that of ⁶⁴Cu at the 1-, 6-, 12-, 24-, and 48-h time points, respectively.
[64Cu]Cu-DOTA-polystyrene transit and absorption were observed within the same animal using PET, without killing the animal. In this study, we first observed the in vivo pathways (absorption, distribution, metabolism, and excretion) of microplastics labeled with a radioisotope using PET. To trace the polystyrene after oral administration, we selected 64Cu and p-SCN-Bn-DOTA for the radiolabeling of plastic particles. We subsequently confirmed that the detected radiation was emitted from the [64Cu]Cu-DOTA-polystyrene, not from 64Cu, using ex vivo radio-TLC. DOTA-N-hydroxysuccinimide ester and p-SCN-Bn-DOTA are frequently used chelators (19). DOTA conjugation was confirmed by Fourier-transform infrared spectroscopy, because the functional groups of DOTA show specific bands (Supplemental Fig. 1).

The biodistribution study also demonstrated that the distribution of [64Cu]Cu-DOTA-polystyrene was different from that of 64Cu. The biodistribution study provided quantification of [64Cu]Cu-DOTA-polystyrene accumulation in each organ, even at low levels of emitted γ-rays. Using the biodistribution, we observed the transit and accumulation of [64Cu]Cu-DOTA-polystyrene within the gastrointestinal tract (stomach, intestine, and liver), circulatory organs (heart, lung, and blood), renal system (kidney and bladder), and even brain, at 1 h after oral administration.

In contrast, orally administered 64Cu was rapidly removed from the stomach, small intestines, and large intestine, before transit to the other organs, including the liver (Fig. 4). We also observed a higher SUV in the liver on PET for the group that was orally administered 64Cu (Fig. 3). In a previous report, accumulation of 64Cu in the liver was observed on PET (20). For kidney and spleen, the levels of ID/g (1 < %ID/g < 10) at 1 h were 3.47 and 1.08, respectively. For bladder, testis, heart, lung, and blood, the levels of ID/g (%ID/g < 1) at 1 h were 0.70, 0.22, 0.55, 0.92, and 0.21, respectively. The rapid distribution of orally administered 64Cu to the other organs may have occurred because digestive fluid may facilitate solubilization of 64Cu in the stomach. 64Cu was partly cleared in feces after transit through the gastrointestinal tract, and the remaining 64Cu was distributed to other organs, including the liver.

In mice, the normal gastric pH is approximately 3.0 (25). During transit through the stomach, [64Cu]Cu-DOTA-polystyrene may encounter harsh conditions, possibly dechelating 64Cu. However, our ex vivo radio-TLC assay—through comparison data between 64Cu and [64Cu]Cu-DOTA-polystyrene—ensured that there was no dechelation of 64Cu in the stomach or liver at 1 h. According to the data, the detected signal from PET and the biodistribution at 1 h in all other organs, including the liver, was from...
Recently, several animal studies have been published on the effects of microplastics (26–29). Microplastic ingestion may induce behavioral disorders in mice (30). Therefore, it is important to observe how microplastics are distributed in the body after ingestion. Remarkably, biodistribution demonstrated that \( ^{64}\text{Cu}\text{-DOTA-polystyrene} \) was distributed to all tested organs, including the testis, even after a single dose. In contrast, most \( ^{64}\text{Cu} \) accumulated in large intestine, stomach, and small intestine at 1 h after administration. Subsequently, \( ^{64}\text{Cu} \) transited quickly to other organs, including liver, spleen, heart, blood, lung, kidney, bladder, brain, and testis, was greater for \( ^{64}\text{Cu} \) than for \( ^{64}\text{Cu}\text{-DOTA-polystyrene} \). n = 5. *P < 0.05, Student t test. **P < 0.005 Student t test. n.s. = not statistically significant.

\( ^{64}\text{Cu}\text{-DOTA-polystyrene} \), not from dechelated \( ^{64}\text{Cu} \). Although the acidity of the stomach did affect dechelation at 6 h after administration, the other organs were not influenced by dechelation of \( ^{64}\text{Cu} \) from radio-TLC (Supplemental Fig. 4). Therefore, each data point obtained from PET and biodistribution was confirmed with \( ^{64}\text{Cu}\text{-DOTA-polystyrene} \). Consequently, the dechelation of \( ^{64}\text{Cu} \) could be negligible (Fig. 4).

![Biodistribution results for \( ^{64}\text{Cu} \) and \( ^{64}\text{Cu}\text{-DOTA-polystyrene} \) in gastrointestinal tract (stomach, intestine, and liver), circulatory organs (heart, lung, and blood), renal system (kidney and bladder), and brain. Overall, accumulation pattern of biodistribution was similar to that of SUV in PET images. \( \%\text{ID/g} \) of \( ^{64}\text{Cu}\text{-DOTA-polystyrene} \) in stomach, small intestine, and large intestine was significantly higher than that of \( ^{64}\text{Cu} \). However, in liver, \( \%\text{ID/g} \) of \( ^{64}\text{Cu}\text{-DOTA-polystyrene} \) was lower than that of \( ^{64}\text{Cu} \). Additionally, \( ^{64}\text{Cu}\text{-DOTA-polystyrene} \) transited to gastrointestinal tract (liver and spleen), circulatory system (heart, blood, and lung), renal system (kidney and bladder), and even to brain and testis. In contrast, most \( ^{64}\text{Cu} \) accumulated in large intestine, stomach, and small intestine at 1 h after administration. Subsequently, \( ^{64}\text{Cu} \) transited quickly to other organs, including liver, spleen, heart, blood, lung, kidney, bladder, brain, and testis, was greater for \( ^{64}\text{Cu} \) than for \( ^{64}\text{Cu}\text{-DOTA-polystyrene} \). n = 5. *P < 0.05, Student t test. **P < 0.005 Student t test. n.s. = not statistically significant.](image-url)
individual organs such as digestive organs, circulatory organs, and excretory organs.

We used BALB/c nude mice because we aimed to assess the tumorigenesis after longitudinal polystyrene exposure for further study. When different strains of mice were used, possibly different degrees of absorption, distribution, metabolism, and elimination of polystyrene might be observed during PET.

The polystyrene used in these experiments was surface-coated with amines, and it seems likely that this process might affect their biodistribution. Polystyrene is a highly hydrophobic particle, and the addition of multiple primary amines (hydrophilic and positively charged at physiologic pH) and DOTA chelators (hydrophilic and negatively charged at physiologic pH) on the surface may influence biodistribution. Hydrophobic compounds and aggregates tend to show uptake and retention in the liver, and uptake in the liver may therefore be influenced by the surface modifications. Even if radiotracers were prepared from the same material, differences in size, shape, and surface charge can affect biodistribution and clearance. Generally, small nanoparticles penetrate capillary walls more easily than large nanoparticles, and positively charged nanoparticles are cleared more quickly by macrophages (32–34). Smaller and negatively charged silica nanoparticles have enhanced intestinal permeation by opening tight junctions (35). In this study, we selected a sphere-shaped and 0.2-μm-sized polystyrene and observed no significant differences in size or shape after DOTA conjugation. 64Cu-labeled DOTA-polystyrene contains uncoordinated carboxylic acids, which have negative charges, and free amines, which have positive charges at physiologic pH. These surface charges may affect the permeability of the gastrointestinal tract and distribution. Recent fluorescence-conjugated microplastic studies indicated that the biodistribution of microplastics was dependent on the size of the particles (26,36). According to the result of Deng et al. (26), the accumulation in the kidney and gut was greater for 5-μm microplastics than for 20-μm microplastics. Therefore, it is possible that a smaller amount of radioisotope-labeled microplastics might accumulate in mouse organs when larger microplastics are used.

CONCLUSION

Our results demonstrate the utility of PET for visualizing the absorption and distribution of polystyrene microplastics radiolabeled with 64Cu. PET provides information on the accumulation of microplastics in vivo and can provide information on how each organ might be affected after continuous microplastic exposure. The biologic effects of long-term exposure to microplastics in each organ affected in this study will be evaluated in future studies.

DISCLOSURE

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