A Strontium-90 Sequestrant for First-Aid Treatment of Radiation Emergency

Mamoru Haratake,*[,a] Eisuke Hatanaka,[a] Takeshi Fuchigami,[a] Makoto Akashi,[b] and Morio Nakayama*[,a]

[a] *Graduate School of Biomedical Sciences, Nagasaki University; 1–14 Bunkyo-machi, Nagasaki 852–8521, Japan: and* [b] *Research Center for Radiation Emergency Medicine, National Institute of Radiological Sciences; 4–9–1 Anagawa, Inage-ku, Chiba 263–8555, Japan.* Received May 10, 2012; accepted July 24, 2012

In this study, hydrophilic porous polymer beads with phosphonic acid groups (PGMA-EGDMA-TTA-MP) were synthesized, and assessed as a radioactive strontium-90 sequestrant for the treatment of the radiation emergency. Strontium ions were rapidly absorbed into the blood from the gastrointestinal (GI) tract after oral administration to rats, and distributed to the target organ, *i.e.*, bones. Over 40% of the administered strontium was absorbed into the blood, while the remainder was discharged in the feces within 48 h after the administration. When the PGMA-EGDMA-TTA-MP beads were administered to rats subsequent to the strontium solution, the strontium had accumulated less in the femur. Consequently, the oral administration of the PGMA-EGDMA-TTA-MP beads was effective in suppressing the absorption of strontium from the GI tract.

Key words strontium-90; radiation emergency; polymeric adsorbent

After the severe nuclear power plant accident at Fukushima in March, 2011, Japan has been suffering from the third most serious exposure to radiation following the Hiroshima and Nagasaki atomic bombs.[1,2] Since then, high levels (large amount) of the plant fuels and diverse fission products have been discharged from the plant facility into the environment. A similar accidental disaster has also occurred at the Chernobyl nuclear power plant in 1989. Strontium-90 (Sr-90) is one of artificial radioactive by-products of fission reactions within nuclear reactors that generate electricity. About 3% of the mass of the spent nuclear fuel consists of fission products including Sr-90.[3] Due to its high energy of 0.54 MeV and long physical half-life of approximately 30 years by β^- emission, Sr-90 is classified as a high-level waste. Its daughter radionuclide, yttrium-90, with a physical half-life of 64.1 h emits hard β^--particles with a maximum energy of 2.27 MeV. For a relatively fresh fission product mixture, another radioactive strontium, strontium-89 (Sr-89) with a maximum energy of 1.46 MeV and a half-life of 52.7 d by β^- emission, can also be present. Since strontium belongs to the alkaline earth metals, its chemical behavior very closely resembles that of calcium, leading to its enrichment in bone and teeth in various organisms. After the radioactive strontium species enter the organisms, it deposits in the bone and bone marrow, and exposure from contaminated food and water is linked to bone cancer and leukemia[4]; it takes hundreds of years to naturally decay to harmless levels (its biological half-life is over 50 years). Sr-90 is also considered the most important radioactive isotope in the environment and one of the most frequently occurring radionuclides in groundwater at nuclear facilities due to its long half-life. For instance, in Finland, the measured cumulative Sr-90 deposition in 1963–2005 averaged 1200 Bq/m^2, of which 150 Bq/m^2 originated from the Chernobyl accident a quarter century ago,[5] and other large fraction from the atmospheric nuclear weapons tests in the 1960s.[6,7]

From the viewpoint of radiation protection, it is quite important to suppress the absorption of Sr-90 from the environment and/or food by humans and other animals as low as possible. Especially, for the radiation emergency like the Chernobyl and Fukushima accidents, an effective first-aid treatment is urgently needed, since a large amount of the fission products including Sr-90 are released from the plant facility to us in a short duration. In addition, there are few reports of the absorption behavior of strontium in humans. A likely absorption pathway of Sr-90 is thought to be from the gastrointestinal (GI) tract. One of the effective procedures for the treatment of a radiation emergency is to discharge the radionuclides from the GI tract in feces using non-absorbable and non-toxic oral preparations with the adsorption capacity for the fission products. The use of water-insoluble cross-linked polymeric materials is appropriate for accomplishing such a purpose. In this study, the oral absorption behavior of strontium from the GI tract was initially examined, and polymer beads for sequestering the strontium in the GI tract were newly synthesized and their potential assessed in rats.

Experimental

Materials and Apparatus A radioactive tracer strontium-85 (Sr-85) chloride (257.73 Mbq/mL–0.5 M HCl) was purchased from PerkinElmer Japan Co., Ltd. (Tokyo, Japan). Glycidyl methacrylate (GMA) and 2,2′-azobis (isobutyronitrile) (AIBN) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ethylene glycol dimethacrylate (EGDMA) and methyl *iso*-butylketone (MIBK) were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). GMA and EGDMA were subjected to radical suspension polymerization without further purification. Triethylenetetramine (TTA) and a strontium standard solution (1000 g/L, atomic absorption spectrometry grade) were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). All other chemicals were of reagent or specified grade and used as received. A Z-5010 atomic absorption spectrometer (Hitachi High-Technologies Corp., Tokyo, Japan) was used for the determination of the strontium concentrations. An ARC-380 γ-ray counter (Hitachi Aloka Medical,

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: haratake@nagasaki-u.ac.jp; morio@nagasaki-u.ac.jp

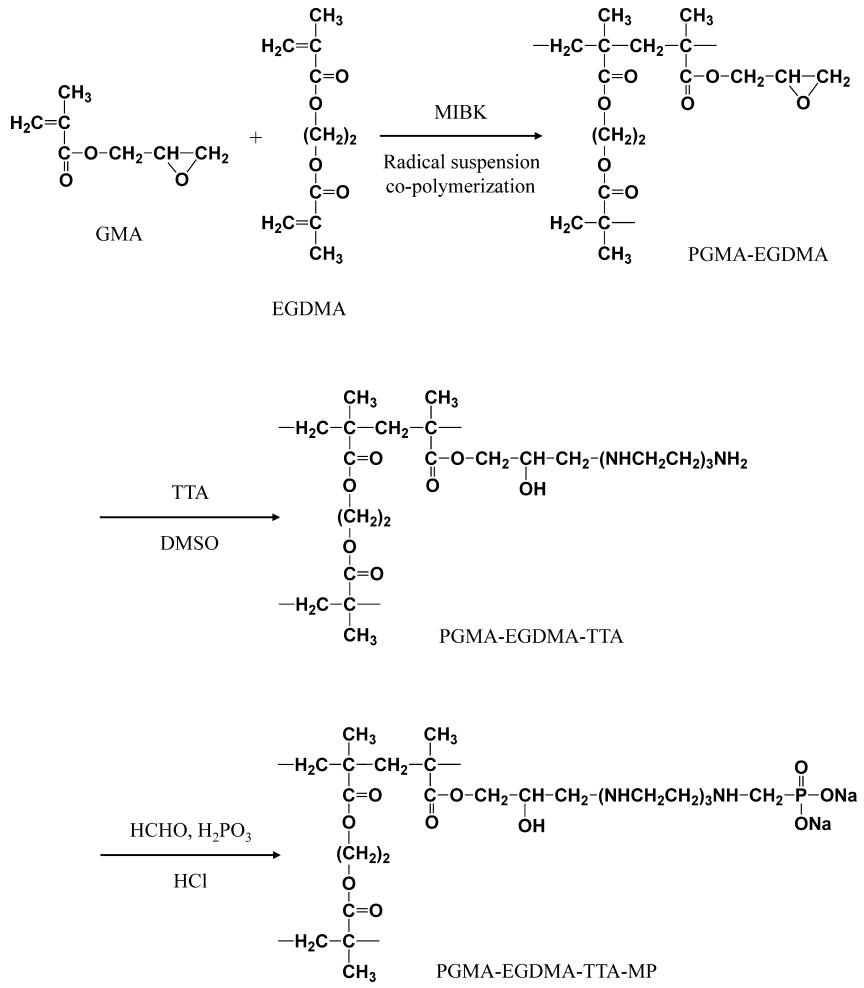

Chart 1. Synthetic Route of PGMA-EG-TTA-MP Beads

Ltd., Tokyo, Japan) was used for the determination of Sr-85.

Synthesis of PGMA-EGDMA-TTA-MP Beads Spherical PGMA-EGDMA-TTA-MP beads were synthesized according to the procedure shown in Chart 1.[8] An equal volume of GMA (25 mL) and EGDMA (25 mL) were mixed, and AIBN (1 w/v%) as the initiator was dissolved in the monomer mixture. After the addition of MIBK as a porogen, the resulting mixture (100 mL) was poured into a 0.04 w/v% gelatin solution containing 1.15 w/v% sodium sulfate (520 mL) with stirring at 500 min^{-1}. The radical suspension copolymerization was allowed to proceed at 50°C for 0.5 h, at 75°C for another half hour and finally at 95°C for 1.5 h to complete the reaction. The obtained milky white beads were thoroughly washed with deionized water and methanol to remove the gelatin and MIBK. After drying, the copolymer beads with a 75–150 μm diameter were fractionated by sieving and subjected to the subsequent chemical derivatizations. The PGMA-EGDMA beads (5 g) and dimethylsulfoxide (37.5 mL) were placed in a pressure-resistant thick glass tube, and the mixture was allowed to stand for several hours. After the addition of TTA (12.5 mL) to the glass tube, the reaction mixtures were further allowed to react in a water bath at 80°C for 4 h with occasional shaking by hand. After washing, the TTA moiety-attached PGMA-EGDMA beads (5 g) were combined with phosphonic acid (15 g), formalin (30%, 30 mL) and 3.6 M HCl (70 mL) in a pressure-resistant thick glass tube and heated in a water bath at 90°C for 3 h. The resulting polymer beads were washed with deionized water, and soaked in 0.1 M NaOH to form the Na salt, followed by a thorough washing with deionized water until the pH of the eluate became neutral. The obtained beads (PGMA-EGDMA-TTA-MP) were further washed with methanol to wash out any residual organic reagents in the beads. After drying at 50°C for 3 h, the beads were kept in a desiccator at ambient temperature until used.

After wet digestion of the phospholipid vesicle suspensions, using a 4 : 1 mixture of nitric acid and perchloric acid, the phosphorous content of the beads was determined based on vanadium (V) that is capable of forming a yellow complex with phosphate ion.[9] The color produced by the complexation was monitored at 440 nm. The contents of other elements were determined by the conventional combustion method. Elemental analysis [wt% (mmol/g)]: C, 51.71 (43.05); H, 7.43 (73.71); N, 4.44 (3.17); P, 2.00 (0.62).

***In Vitro* Adsorption Experiments of Strontium to PGMA-EGDMA-TTA-MP Beads** For the preparation of the adsorption isotherm, a series of strontium solutions was prepared by the appropriate dilution of the strontium standard solution (1000 g/L) with diluted HCl or sodium hydroxide in order to produce the solution pH of 5–6. PGMA-EGDMA (50 mg) and the strontium solution (50 mL, 2 to 5 g/L) were combined in an Erlenmeyer flask, and mechanically shaken at 80 min^{-1} and at room temperature for 24 h. An aliquot of the

strontium solution in the flasks was drawn at appropriate time intervals, and then diluted with 0.1 M nitric acid. Unless otherwise stated, the strontium concentrations were determined by radiometry for Sr-85. The strontium amount bound to the beads was calculated from the difference in its concentration between before and after shaking with the beads.

The adsorption rate study was carried out using a 200 mL three-necked round bottom flask equipped with a stirring apparatus at 25°C. One hundred and fifty milligrams of the strictly fractionated resins and a given volume of deionized water were placed in the flask, and allowed to stand for 1 h. The strontium stock solution was added to the flask to achieve the desired concentrations. One milliliter aliquots were withdrawn from the flask at appropriate time intervals. The samples were diluted, if necessary, and assayed by atomic absorption spectrometry.

***In Vivo* Absorption Experiments of Strontium in Rats** Four-week old male Wistar rats (weighing 100–120 g, specific pathogen free) were purchased from Kyudo Co., Ltd. (Tosu, Japan) and cared for in accordance with the guidelines of Nagasaki University on Animal Care. The rats were housed one per cage on a 12 h light/12 h dark schedule at 23±2°C and 60% relative humidity, and were given deionized water during all the experiments. Unless otherwise stated, all *in vivo* absorption experiments were carried out using 12 h-fasted rats before the administration of the strontium solutions. In the experiments of Fig. 4A, *ad libitum* deionized water and a regular diet CE-2 from Oriental Yeast Co., Ltd. (Tokyo, Japan) were supplied to rats after the administration of the strontium solutions.

The rats orally received a 0.5 mL Sr-85-spiked strontium nitrate solution (strontium nitrate concentration: 1 mM, the radioactivity of the solution: ≈50000 counts) using a stainless feeding needle (ϕ1.2×80 mm), and bred for 48 h under the conditions as already described. The feces were collected during the post-administration breeding. Under ether anesthesia, the GI tracts were removed and separated into the stomach, jejunum, ileum, cecum and colon. The radioactivity of the obtained GI tract samples was measured without removing the contents. To monitor the strontium concentrations in the blood, blood samples (0.1 mL each time) were sequentially withdrawn from the tail vein at appropriate time intervals after the oral administration of strontium.

PGMA-EGDMA-TTA-MP beads (1 g) were suspended in deionized water (10 mL). The bead suspension (0.1 mL) was orally administered using a stainless feeding needle (ϕ1.2×80 mm) just after the administration of the strontium solution as described in the previous section. The control group rats [bead (−) in Fig. 5] received deionized water of the same volume after the administration of the strontium solution. Blood and femur samples were collected 30 min after the administration of the beads and then subjected to measurement of the radioactivity.

Results and Discussion

Adsorption Behavior of Strontium by PGMA-EGDMA-TTA-MP Beads For the design of the Sr-90 sequestrant, its structural features are important factors affecting the adsorption behavior of strontium ions. PGMA-EGDMA-TTA-MP beads are hardly absorbable through the GI tract, because the beads consist of cross-linked polymers that are insoluble even in the presence of biosurfactants such as bile acids. To achieve the rapid adsorption of strontium in the GI tract, hydrophilic PGMA-EGDMA beads as the backbone polymer were prepared. The PGMA-EGDMA beads were rendered highly porous using MIBK as a porogen in the suspension polymerization of the PGMA-EGDMA beads (≈110 m^2/g-bead, determined by the nitrogen gas adsorption method).[8] The resulting large surface area could possibly provide an enhancement in the adsorption velocity of the strontium ions. The phosphonic acid groups were introduced to the PGMA-EGDMA beads as a functional group for the adsorption of the strontium ions. In addition, a spacer moiety, triethylenetetramine between the backbone polymer and the functional groups, which allows increasing freedom of the functional groups, was also introduced.

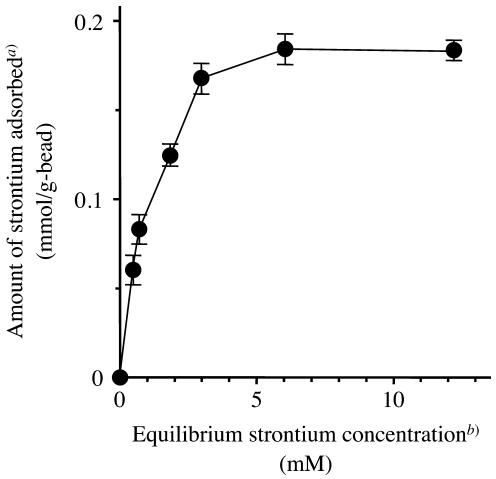

Fig. 1. Adsorption Isotherm of Strontium to PGMA-EGDMA-TTA-MP Beads

a) =(amount of strontium initially added)×[1−(radioactivity of sample)/(radioactivity of polymer-free control)]. *b*) Determined by atomic absorption spectrometry. Data express mean and standard error (n=3).

Shown in Fig. 1 is the adsorption isotherm of strontium by the PGMA-EGDMA-TTA-MP beads in deionized water at pH 5–6. The adsorbed amount of strontium increased with increases in its concentration and then reached a plateau value (≈0.2 mmol/g). The observed capacity of the beads for strontium was approximately one-third of its phosphorous content (0.62 mmol/g) under the conditions of this experiment. Cationic strontium ion can bind to the anionic phosphonic groups ($-PO_3^{2-}\cdot 2Na^+$) in the beads by electrostatic attractive forces. Thus, the phosphonic groups in the free form ($-PO_3H_2$) becomes less adsorptive for strontium ions, which is dependent on the degree of ionization that governs the pK_a values of the phosphonic acid (pK_{a1}=1.3 and pK_{a2}=6.7[10]) and pH of the medium. The physiological pH in the small intestine and the subsequent parts of the GI tract (5 to 7) can promote the ionization of the phosphonic acid groups, while the adsorbed amount of strontium is fairly low in the stomach where the phosphonic acid groups are mostly present in the free form.

The adsorption velocity of strontium ions to the sequestrants is an important factor for obtaining an effective sequestration from the GI tract. If the adsorption velocity is significantly lower than the absorption velocity from the GI tract, the strontium ions could be preferentially absorbed from the surface of the small intestine to the blood. The time–course of

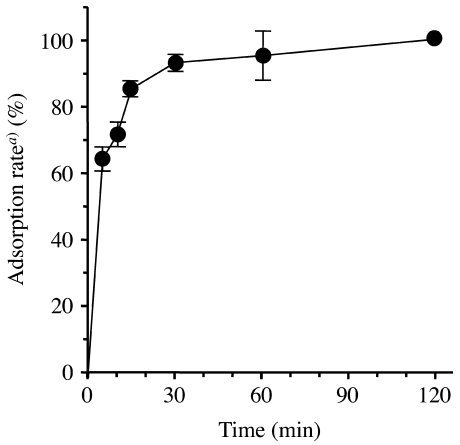

Fig. 2. Time–Course of Adsorption of Strontium to PGMA-EGDMA-TTA-MP Beads

a) =100×(adsorbed amount of strontium at time *t*)/(adsorbed amount of strontium at 120 min). Initial concentration of strontium nitrate: 0.1 mM. Data express mean and standard error (n=3).

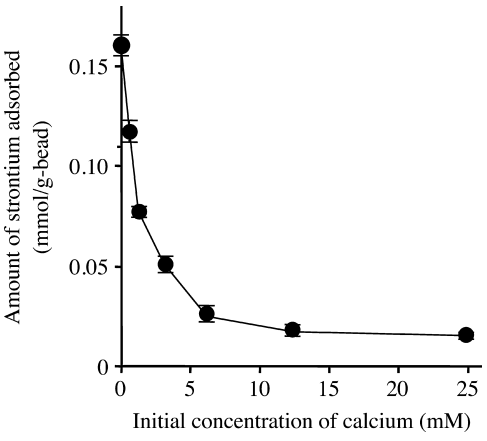

Fig. 3. Adsorption of Strontium to PGMA-EGDMA-TTA-MP Beads in the Presence of Increasing Calcium Ion Concentration

Strontium nitrate and calcium chloride concentrations: 6.2 and 0.0–24.8 mM. Data express mean and standard error (n=3).

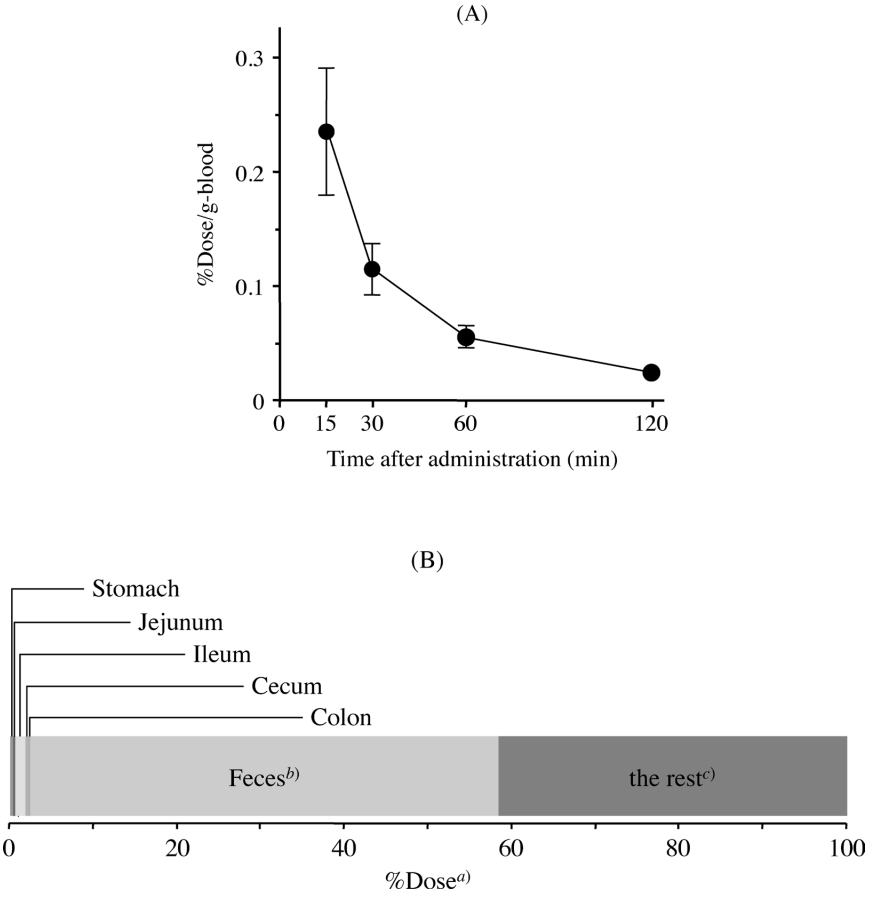

Fig. 4. Time–Course of Strontium Concentration in Blood after Oral Administration in Rats (A) and Absorption Fate of Orally Administered Strontium in the GI Tract of Rats (B)

a) The radioactivity of Sr-85 administered to rats was defined as 100%. *b*) Cumulative value for 48 h after the oral administration. *c*) =100−(the summation of the values for the other fractions). Data express mean and standard error (n=3).

the adsorption of strontium by the PGMA-EGDMA-TTA-MP beads is shown in Fig. 2. The adsorption rate promptly increased and then reached an equilibrium state at 30 min, which appears to be, in part, attributable to the porous structure of the PGMA-EGDMA-TTA-MP beads. Such a rapid adsorption could possibly be reflected in the adsorption of strontium in the GI tract.

Because the adsorption of strontium is based on the electrostatic attractive interaction with the phosphonic acid groups in the PGMA-EGDMA-TTA-MP beads, competition with other cations for the adsorption to the phosphonic acid groups can occur. The adsorption of strontium to the

PGMA-EGDMA-TTA-MP beads was examined in the presence of an increasing divalent calcium ion concentration (Fig. 3). The adsorbed amount of strontium decreased with the increasing calcium ion concentration; at the same concentration of calcium as strontium (6.2 mM), the adsorbed amount of strontium decreased to one-eighth of that in the absence of calcium ions. The PGMA-EGDMA-TTA-MP beads seem to preferentially bind the calcium ions. However, the adsorbed amount of strontium did not completely decrease to zero even at the higher concentration of calcium (24.8 mM), which indicates that the PGMA-EGDMA-TTA-MP beads possess the adsorption capacity for strontium even in the presence of a high calcium ion concentration. Strontium is an alkaline earth metal element that belongs to Group 2 of the periodic table and shares similar properties with calcium; *e.g.*, the ionic radius of strontium (valence state +2 and coordination number 6–12: 0.118–0.144 nm) is almost identical to that of calcium (valence state +2 and coordination number 6–12: 0.100–0.134 nm).[11] Strontium behaves chemically much like calcium, and therefore tends to concentrate in the bones and teeth. The observed higher adsorption capacity of calcium ions to the PGMA-EGDMA-TTA-MP beads may be due to its extremely high affinity for phosphonic acid (*cf.* the solubility product of the calcium ion and the phosphate ion, $[Ca]^3[PO_4]^2=2\times10^{-29}$).[12] Thus, the PGMA-EGDMA-TTA-MP beads do not differentiate between strontium and calcium, so they may become partly completed to the latter, since it is more abundant in the contents of the GI tract.

Absorption of Strontium from the GI Tract Currently, little is known about the absorption behavior of strontium from the GI tract. We initially examined the absorption behavior of strontium in rats by monitoring the Sr-85 radioactivity in the blood. The radioactivity in the blood was the highest at 15 min after the oral administration of the strontium solution, and then gradually decreased (Fig. 4A). Thus, orally administered Sr-85 ions seem to be promptly absorbed from the GI tract into the bloodstream. The strontium was then rapidly cleared from the blood. Separately, the radioactivity of the GI tract was determined at 48 h after the administration of the strontium solution. The cumulative radioactivity of the feces for 48 h after the administration was also measured (Fig. 4B). Nearly 60% of the administered strontium was found in the feces, while the radioactivity remaining in the GI tract was fairly low. The strontium ions did not show an organ-specific retention in the GI tract. As a result, a considerable amount of the strontium administered was thought to be absorbed from the GI tract into the blood. Consequently, strontium ions were rapidly absorbed from the GI tract at a significant absorption rate, which strongly suggests the necessity of treatment during a radiation emergency by Sr-90 sequestrants.

Inhibitory Effect of PGMA-EGDMA-TTA-MP on Absorption of Strontium from the GI Tract The inhibitory effect of the PGMA-EGDMA-TTA-MP beads on the absorption of strontium from the GI tract was assessed in rats. Because the absorption of strontium from the GI tract occurred within 30 min, the radioactivity of the blood and bone samples was determined at 30 min after the administration of the beads, and represents in %dose/g (Fig. 5). The radioactivity of the bone from the rats treated without the beads (control group) was approximately 5-fold higher than that of the blood sample (the radioactivity ratio of the bone to the blood: 5.47).

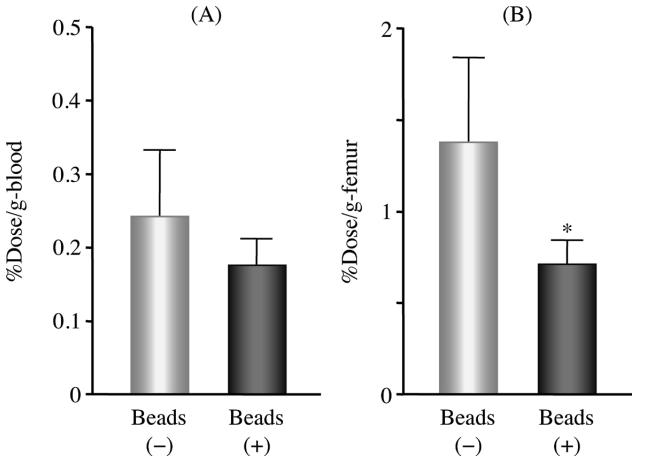

Fig. 5. Inhibitory Effect of PGMA-EGDMA-TTA-MP Beads on Absorption of Strontium in Rats at 30 min after Oral Administration

(A) Blood, (B) Femur. A half milliliter of Sr-85-spiked strontium nitrate dissolved in distilled water (50 mM). Data express mean and standard error (n=3). *Significantly different from the value for the control group [Beads (−)] with $p<0.05$ by the one-way ANOVA analysis.

Thus, the strontium that once appeared in the circulating blood seemed to rapidly distribute into the bone tissues. A remarkable difference in the radioactivity of the blood between the two groups was not observed at 30 min after the administration of the beads (Fig. 5A). The radioactivity of the bone from the rats treated with the PGMA-EGDMA-TTA-MP beads was significantly lower than that treated without the beads (Fig. 5B). The radioactivity ratio of the bone to the blood from the rats treated with the beads (3.90) was lower than that from the control rats. In addition, the Sr-85 radioactivity of the 48 h-feces increased nearly 25% in the rats treated with the PGMA-EGDMA-TTA-MP beads. These results demonstrate that the administration of the PGMA-EGDMA-TTA-MP beads was effective in suppressing the absorption of strontium from the GI tract.

Conclusion

In this study, hydrophilic porous polymer beads with phosphonic acid groups (PGMA-EGDMA-TTA-MP) were synthesized, and assessed as a radioactive Sr-90 sequestrant for the treatment of a radiation emergency. Strontium ions were rapidly absorbed into the blood from the GI tract after oral administration to rats, and distributed into the target organ bones. When the PGMA-EGDMA-TTA-MP beads were administered to rats subsequent to the strontium solution, the strontium had accumulated less in the femur. Consequently, these data suggested that the oral administration of the polymeric materials is effective in suppressing the absorption of strontium from the GI tract in the first-aid treatment of a radiation emergency. A problem to be overcome for the development of a better sequestrant was also observed in this study. We are now trying to improve the selectivity of the beads for strontium ions.

References

1) Brumfiel G., Cyranoski D., *Nature*, **471**, 273–275 (2011).
2) Brumfiel G., Fuyuno I., *Nature*, **483**, 138–140 (2012).
3) World Nuclear Association, "Radioactive Waste Management," London, United Kingdom (updated Apr. 2012): ‹http://tinyurl.

com/448n8k7› accessed 29 Apr. 2012.
4) U.S. Environmental Protection Agency, "Strontium," Washington, DC (updated Apr. 2012): ‹http://tinyurl.com/ye5y6g4› accessed 29 Apr. 2012.
5) Paatero J., Saxén R., Buyukay M., Outola I., *J. Environ. Radioact.*, **101**, 309–316 (2010).
6) Stamoulis K. C., Assimakopoulos P. A., Ioannides K. G., Johnson E., Soucacos P. N., *Sci. Total Environ.*, **229**, 165–182 (1999).
7) Froidevaux P., Haldimann M., *Environ. Health Perspect.*, **116**, 1731–1734 (2008).
8) Haratake M., Yasumoto K., Ono M., Akashi M., Nakayama M., *Anal. Chim. Acta*, **561**, 183–190 (2006).
9) Kitson R. E., Mellon M. G., *Ind. Eng. Chem. Anal. Ed.*, **16**, 379–383 (1944).
10) Haynes W. M., "CRC Handbook of Chemistry and Physics," 92nd ed., CRC Press, New York, 2011.
11) Jia Y. Q., *J. Solid State Chem.*, **95**, 184–187 (1991).
12) Greenwald I., *J. Biol. Chem.*, **143**, 703–710 (1942).