Alginate Enhances Excretion and Reduces Absorption of Strontium and Cesium in Rats

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In March, 2011, Japan suffered an accident at a nuclear power plant following a severe earthquake, resulting in release of radioactive materials into the atmosphere. Human contamination from these materials, especially radioactive strontium ($^{90}\text{Sr}$, half-life=28.8 years) and cesium ($^{137}\text{Cs}$, half-life=30.17 years), which have long half-lives, is a critical public health issue.1,2) In particular, there is interest in medicines and foods that might help to reduce absorption and promote excretion of these radioactive materials. Since establishing the safety of new substances is time-consuming, attention has been focused on natural products or foods that are already known to be safe, and some promising results have been reported.3–5)

Alginate (ALA), which is an intercellular polysaccharide associated with brown algae, is used as a food additive, a health food and a medicine. Here, we first examined the adsorption of strontium (Sr) and cesium (Cs) by ALA in vitro, and then evaluated the effects of ALA on absorption and excretion of Sr and Cs in rats, in order to evaluate its potential usefulness for minimizing radiation damage from materials released after a nuclear accident. Both Sr and Cs were concentration-dependently adsorbed by sodium alginate (ALA-Na) in vitro. In rats given diet containing either ALA-Na or calcium alginate (ALA-Ca) for two weeks, the plasma concentration of Sr gradually decreased compared with the controls (normal diet); however, in the case of Cs, the plasma concentration was decreased only in the ALA-Ca group, but not the ALA-Na group. Moreover, we examined the effect of preadministration of diet containing either ALA-Na or ALA-Ca on absorption of Sr and Cs administered orally as the chloride salts to rats. Absorption of both Sr and Cs was reduced in the ALA-Ca group, while absorption of only Sr was reduced in the ALA-Na group. Safety assessments indicated that ALA-Ca is safer than ALA-Na. These results indicate that ALA-Ca reduces absorption and promotes excretion of both Sr and Cs, while ALA-Na does so only for Sr.

Materials and Methods

Chemicals and Animals Strontium chloride ($\text{SrCl}_2$) and cesium chloride ($\text{CsCl}$) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals and solvents were analytical-grade commercial products. The animal study was performed according to the Guidelines for the Care and Use of Laboratory Animals at the Takasaki University of Health and Welfare and approved by the Committee of Ethics of Animal Experimentation of the University. Specific-pathogen-free male Wistar rats at five or six weeks of age were purchased from SLC Japan (Hamamatsu, Japan), and housed 2 animals per cage in an animal room kept under controlled conditions (temperature of 23±3°C, humidity of 50±20%), with a 12-h light/dark cycle. ALA-Na and ALA-Ca were supplied by Kimica Corporation (Tokyo, Japan). Normal rat diet (CA-1) and diet containing 10% ALA-Na or ALA-Ca were supplied by Clea Japan Inc. (Tokyo, Japan).

In Vitro Experiments ALA-Na solutions at concentrations of 0 (control), 0.25, 1, 2.5 and 5 mg/mL were prepared, and $\text{SrCl}_2$ or $\text{CsCl}$ was added to give a final concentration of 0.2 mg/mL (namely 1.26 m$\text{SrCl}_2$ and 1.19 m$\text{CsCl}$). A mixture of $\text{CsCl}$ and $\text{SrCl}_2$ at the same final concentrations potentially cause hypertension, was examined in those studies. From this point of view, calcium alginate (ALA-Ca) may be more useful than ALA-Na, if it has an equivalent effect. ALA-Ca forms a gel in water, so it can be difficult to handle. However, gelled ALA-Ca might be advantageous in terms of uptake and/or adsorption of metals. In this study, we examined and compared the effects of ALA-Na and ALA-Ca on absorption and excretion of Sr and Cs in rats. Moreover, we examined the safety of ALA-Na and ALA-Ca.

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of 0.2 mg/mL was also used. These solutions were filtered by centrifugation (15000 × g, 20 min) through a membrane filter (Amicon® ultra 3K device, 3000NMWL, Merck Millipore, TN, U.S.A.). The amounts of Sr and Cs recovered in filtrates were measured by the graphite furnace method using an atomic absorption photometer (ContrAA® 700, Analytik Jena AG, Jena, Germany). The experiment was conducted twice and the concentrations of Sr and Cs are shown as a ratio to the control (ALA-Na 0 mg/mL). EC50 values were calculated. ALA-Ca was not used for in vitro experiments, because it forms a gel in water.

Promotion of Sr and Cs Excretion in Rats One week after purchase, rats were randomized based on weight (153–180 g) into control, ALA-Na, and ALA-Ca groups (n = 5 each). Blood samples were withdrawn from the jugular vein with a heparinized syringe under anesthesia induced with diethyl ether, and were centrifuged at 1700 × g for 10 min to obtain plasma. The initial concentration of native Sr or Cs in plasma was then measured. Normal diet, ALA-Na-containing diet or ALA-Ca-containing diet was individually given to the rats with free access for two weeks. The rats were provided with water ad libitum. After 7 and 14 d, blood samples were collected to measure the plasma concentrations of Sr and Cs.

Control of Absorption of Sr and Cs One week after purchase, rats weighing 153 to 174 g were randomized into six groups (n = 5 each), i.e., two normal diet groups (groups No. 1 and 4), two ALA-Na-containing diet groups (groups No. 2 and 5) and two ALA-Ca-containing diet groups (groups No. 3 and 6), and the appropriate diet was individually given to rats for two weeks. Then, SrCl2 (1 mg/5 mL/kg) for groups 1, 2, and 3 or CsCl (2 mg/5 mL/kg) for groups 4, 5, and 6 in distilled water was orally administered to the rats. Blood samples were collected at designated times for 3 h and the plasma concentration of Sr or Cs was determined. The maximum plasma concentration (Cmax); time to Cmax (tmax), and absorption rate constant (k) were determined from the observed data by subtracting the value before administration. The area under the plasma concentration–time curve from 0 to 3 h (AUC0–3h) was estimated by means of the linear trapezoidal method.

Safety Assessments One week after purchase, rats weighing 182 to 206 g were randomized into three groups (n = 5 each), i.e., a normal diet group, an ALA-Na-containing diet group and an ALA-Ca-containing diet group, and the appropriate diet was given individually to the rats for one week. Then, 2 mL of blood was collected from the jugular vein of each animal under ether anesthesia to obtain post-heparin plasma, which was used for measurement of biochemical parameters, namely, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (Cho), triglyceride (TG), phospholipid (PL), non-esterified fatty acid (NEFA), high density lipoprotein (HDL), low density lipoprotein (LDL), blood glucose (GLU), calcium (Ca), sodium (Na), potassium (K), and chloride (Cl). Moreover, at one week after purchase, rats with weights of 209 to 232 g were similarly randomized into three other groups (n = 5 each), and the three kinds of diet were individually given to the rats for two weeks. Body weight and diet intake were measured and the general condition of the animals was evaluated during this period. Then, blood was collected from each animal to obtain the plasma, which was used for the measurement of several biochemical parameters as described above. After blood sampling, the animals were euthanized by exsanguination (n = 3 each). Heart, liver, lung, kidneys, spleen, submandibular glands, parotid glands, mesenteric lymph nodes, stomach, intestines, testis, epididymis, thyroid glands, adrenal glands, white adipose tissue and brown adipose tissue were removed and immediately fixed in 10% neutral buffered formalin. The tissues were embedded in paraffin, cut at 4 μm in thickness, stained with hematoxylin and eosin, and examined microscopically.

Statistical Analysis Data are expressed as means ± S.D. Statistical comparisons were made using two-way repeated measurements of analysis of variance (ANOVA) with post hoc test. Values of p < 0.05 were considered significant.

RESULTS

In Vitro Experiments Sr alone was adsorbed by ALA-Na in a concentration-dependent manner and recovery of Sr in the filtrate was less than 11% of the control at ALA-Na concentrations of 1–5 mg/mL (Fig. 1). The EC50 value was 0.296 ± 0.001 mg/mL. Cs alone was also adsorbed by ALA-Na in a concentration-dependent manner, and Cs recovery in the filtrate was below the detection limit (1.0 × 10−4%) at ALA-Na concentrations of 2.5 and 5.0 mg/mL. The EC50 value of ALA-Na for Cs was 0.211 ± 0.002 mg/mL. On the other hand, when a mixture of Sr and Cs was used, adsorption of Cs by ALA-Na was less than in the case of Cs alone, whereas adsorption of Sr by ALA-Na was the same as in the case of Sr alone. The EC50 values of Sr and Cs were 0.308 ± 0.002 mg/mL and 1.073 ± 0.003 mg/mL, respectively.

Promotion of Sr and Cs Excretion The initial plasma concentration of native Sr was 301.8 ± 36.2 ng/mL and 63% (162.1 ± 33.4 ng/mL) at one week, and 77% (144.6 ± 32.4 ng/mL) and 66% (122.2 ± 16.5 ng/mL) at two weeks, respectively, compared with the control group (Fig. 2). On the other hand, the initial plasma concentration of native Cs was 118.8 ± 30.7 ng/mL in six-week-old rats, and this was significantly reduced (to 60% of the control: 122.0 ± 33.7 ng/mL) after seven and fourteen days of treatment with ALA-Na or ALA-Ca diets. The area under the plasma concentration–time curve from 0 to 3 h (AUC0–3h) was estimated by means of the linear trapezoidal method. The bars represent the mean recovery of Sr and Cs as percent of the amount added (n = 2). N.D.: Not detected (less than 1.0 × 10−4% of the amount added).
**Fig. 2.** Plasma Concentration of Sr in Rats during Two-Week Feeding with Normal Diet, ALA-Na-Containing Diet or ALA-Ca-Containing Diet

The data represent means±S.D. (n=5). The significance of differences between the control group and ALA-Na or ALA-Ca group was determined by ANOVA with post hoc test. *p<0.05.

**Fig. 3.** Plasma Concentration of Cs in Rats during Two-Week Feeding with Normal Diet, ALA-Na-Containing Diet or ALA-Ca-Containing Diet

The data represent means±S.D. (n=5). The significance of differences between the control group and ALA-Na or ALA-Ca group was determined by ANOVA with post hoc test. *p<0.05.

**Fig. 4.** Plasma Concentration of Sr after Oral Administration of SrCl₂ to Rats

The data represent means±S.D. (n=5).

**Fig. 5.** Plasma Concentration of Cs after Oral Administration of CsCl to Rats

The data represent means±S.D. (n=5).

**Table 1.** Pharmacokinetic of Sr Plasma Concentration after Oral Administration of SrCl₂ to Rats

| Group    | Parameter | Cmax (ng/mL) | Tmin (h) | kₘ (×10⁻³ h⁻¹) | AUC₀→₃h (ng·h/mL) |
|----------|-----------|--------------|----------|-----------------|-------------------|
| Control  |           | 108±43       | 0.6±0.2  | 4.7±0.8         | 80±37             |
| ALA-Na   |           | 58±34*       | 0.9±1.2  | 4.1±3.5         | 33±16*            |
| ALA-Ca   |           | 37±13*       | 1.6±0.9  | 1.9±3.5*        | 24±9*             |

The data represent means±S.D. (n=5). The significance of differences between the control group and ALA-Na or ALA-Ca group was determined by using ANOVA with post hoc test. *p<0.05.
Table 2. Pharmacokinetic of Plasma Cs Concentration after Oral Administration of CsCl to Rats

| Group     | Parameter | Parameter Value |
|-----------|-----------|-----------------|
|           | C<sub>max</sub> (ng/mL) | T<sub>max</sub> (h) | k<sub>a</sub> (×10<sup>−1</sup> h<sup>−1</sup>) | AUC<sub>0−3h</sub> (ng·h/mL) |
| Control   | 233±39    | 0.3±0.0         | 6.0±0.3      | 401±57            |
| ALA-Na    | 227±72    | 0.3±0.1         | 5.9±0.5      | 401±83            |
| ALA-Ca    | 142±13*   | 0.7±0.7         | 5.1±0.4      | 301±52*           |

The data represent means±S.D. (n=5). The significance of differences between the control group and ALA-Na or ALA-Ca group was determined by using ANOVA with post hoc test. *p<0.05.

Our in vivo experiments indicated that ALA-Ca is effective to promote excretion and decrease absorption of Sr and Cs. Because ALA is not absorbed in the gastrointestinal tract, a mechanism of the excretion promotion of Cs and Sr might be inhibition reabsorption of Cs and Sr which are excreted from the blood to the gastrointestinal tract. On the other hand, ALA-Na had similar effects on Sr, but appeared to be less effective in the case of Cs. This phenomena which was also supported by the results of in vitro study, is not simply due to ion exchange effects, because sodium (Na) and Cs are monovalent cations, whereas calcium (Ca) and Sr are bivalent cations. ALA is a macromolecular complex of two kind of sugars, namely β-d-mannuronic acid and α-l-guluronic acid.12,13) It is formed byionic bonds between the carboxyl groups of these sugars and metal ions such as Na and Ca. Further, the ionic radiiuses of Na, Ca, Sr, and Cs are 95, 99, 113, and 169 p.m., respectively.14) Therefore, it might not be as easy for the available sites in ALA to accommodate the large Cs ion in the presence of the smaller Sr ion. Besides, the easy gel formation of ALA-Ca might also have contributed to physical uptake and/or adsorption of Sr and Cs.

In addition, in in vitro experiments, ALA-Na decreased the adsorption of both Sr and Cs individually, but the effect on Cs was reduced in the presence of Sr, though the effect on Sr was not disturbed in the presence of Cs. It was reported that ALA could form a chelate with a divalent cation,15) but not with monovalent cations. As a result, it was considered that chelate of ALA with Sr was more stable than that with Cs.

The effects of ALA on excretion and absorption of Sr and Cs...
Cs raise the concern that it might have similar effects on trace elements that are indispensable in the body. Therefore, we investigated the safety of ALA. Mineral deposition, due to excessive ingestion of sodium, was seen in kidney epithelium of the ALA-Na group. No abnormality at all was detected in the ALA-Ca group. These results indicate that ALA-Ca is safer than ALA-Na, when taken daily for protection against radiation damage.

In conclusion, we found that ALA, especially ALA-Ca, is effective to promote excretion and to decrease absorption of Sr and Cs. Therefore it might be an effective protective agent after nuclear accidents involving release of radioactive material. In this study, we used 10% ALA-Na or ALA-Ca in the diet, whereas 4 g/body/day of ALA-Na is typically ingested by humans as a health food.\textsuperscript{16,17} This dosage of 4 g/body/day is also recommended by IAEA to remove radioactive Sr from the human body. Accordingly, clinical studies are needed to identify appropriate amounts of ALA-Ca for use as a protective agent.

Fig. 7. Changes of Biochemical Parameters in Rat Plasma during Two-Week Feeding Period

Plasma concentrations of Cho (a), TG (b), PL (c), NEFA (d), HDL (e), and LDL (f) were measured. The data represent means±S.D. (n=5). The significance of differences between the control group and ALA-Na or ALA-Ca group was determined by ANOVA with post hoc test. *p<0.05, **p<0.01.
### Table 3. Biochemical Parameters of Rats after a One or Two-Week Feeding Period

| Parameter      | Time (d) | Control       | ALA-Na       | ALA-Ca       |
|----------------|----------|---------------|--------------|--------------|
| Na (mmol/L)    | 0        | 148.1±4.9     | 148.1±4.9    | 148.1±4.9    |
|                | 7        | 144.7±4.7     | 146.1±4.8    | 139.0±11.2   |
|                | 14       | 132.7±16.5    | 133.2±11.9   | 140.9±4.5    |
| K (mmol/L)     | 0        | 4.5±0.7       | 4.5±0.7      | 4.5±0.7      |
|                | 7        | 4.8±1.2       | 4.8±1.2      | 3.7±0.5      |
|                | 14       | 3.7±0.5       | 4.2±0.5      | 4.6±0.5      |
| Ca (mmol/L)    | 0        | 12.3±1.7      | 12.3±1.7     | 12.3±1.7     |
|                | 7        | 10.9±0.2      | 10.4±0.7     | 9.7±1.1      |
|                | 14       | 9.3±1.4       | 9.2±1.2      | 9.9±0.7      |
| Cl (mmol/L)    | 0        | 104.6±4.1     | 104.6±4.1    | 104.6±4.1    |
|                | 7        | 104.8±2.9     | 106.4±3.0    | 98.1±8.6     |
|                | 14       | 95.7±13.3     | 94.1±6.8     | 100.5±3.7    |
| GLU (mg/dL)    | 0        | 331.6±133.9   | 331.6±133.9  | 331.6±133.9  |
|                | 7        | 240.4±21.1    | 274.0±68.7   | 213.6±59.4   |
|                | 14       | 258.0±21.9    | 209.4±26.0   | 213.8±29.3   |
| ALT (U/L)      | 0        | 35.2±11.0     | 35.2±11.0    | 35.2±11.0    |
|                | 7        | 33.4±4.4      | 27.2±5.8     | 30.2±3.7     |
|                | 14       | 33.2±6.7      | 26.8±5.4     | 34.0±4.3     |
| AST (U/L)      | 0        | 68.0±5.5      | 68.0±5.5     | 68.0±5.5     |
|                | 7        | 77.8±25.0     | 67.8±8.5     | 63.6±15.7    |
|                | 14       | 60.0±15.7     | 56.4±9.4     | 61.6±8.0     |

The data represent means±S.D. (n=5).

Fig. 8. Representative Histochemistry of Rat Kidneys after a Two-Week Feeding Period with Normal Diet (a-1–2) and ALA-Na-Containing Diet (b-1–3)

Figure 8a-2 is enlargements of Fig. 8a-1 black frames. Figures 8b-2 and 8b-3 are enlargements of Fig. 8b-1 red and blue frames, respectively. No abnormalities were seen in the epithelium of papilla (*) or pelvis (***) in kidneys of rats given normal diet. Mineral deposition was seen in pelvic (↓) and papillary epithelium (↓↓) in kidneys of rats given ALA-Na-containing diet. Hyperplasia or proliferation of transitional cells can be seen around the deposits.

### Table 4. Histopathological Findings in Rat Kidneys after Two-Week Feeding of Normal Diet, ALA-Na-Containing Diet and ALA-Ca-Containing Diet

| Findings                                      | Control | ALA-Na | ALA-Ca |
|-----------------------------------------------|---------|--------|--------|
|                                               | No. 1   | 2      | 3      | 1      | 2      | 3      | 1      | 2      | 3      |
| Mineral deposition in medulla                  | –       | –      | –      | ±      | –      | –      | –      | –      | –      |
| Mineral deposition in pelvic epithelum with inflammatory cell infiltration | –       | –      | –      | ++     | +      | –      | –      | –      | –      |
| Mineral deposition in papillary epithelum with inflammatory cell infiltration | –       | –      | –      | +      | –      | –      | –      | –      | –      |
| Proliferation of transitional cells in papilla | –       | –      | –      | +      | –      | –      | –      | –      | –      |
| Hyperplasia of pelvic epithelial cells         | –       | –      | –      | ++     | +      | ±      | –      | –      | –      |

Grade of changes: –, within normal limits; ±, slight; +, moderate; ++, severe.
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