Removal of Strontium by the Chelating Agent Acethylamino Prophylidence Diphosponic Acid in Rats

S. Fukuda[1], H. Iida[1], Yueming Yan[2], Y. Xie[2] and W. Chen[2].
[1] National Institute of Radiological Sciences, Chiba 263-8555, Japan.
[2] Shanghai Institute of Materia Medica, Shanghai 200031, China.

INTRODUCTION

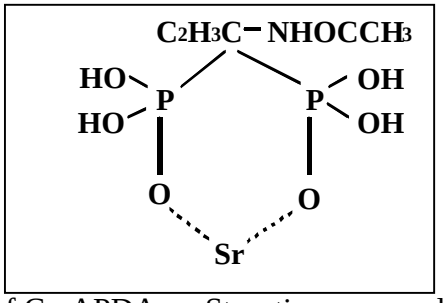
Radioactive strontium deposition in bone induces osteosarcoma, and is a difficult radioelement to remove (1-3). Any inhibition of strontium deposition in bone is desirable to reduce the risk of radiation-induced bone cancer. Of the compounds found in the literature, aluminium phosphate gel and alginate have been reported as effective in inhibiting the intestinal absorption of strontium (4-9).

We have synthesized a new drug, acetylaminopropylidinediphosphonic acid (APDA), for removal of strontium from the body. Our study focuses on the effects of the APDA chelating agent on the removal or inhibition of strontium absorption. Two modalities are considered: (1) parenteral administration of the chelating agent as a means to remove the strontium already deposited in the body (bone), and (2) oral administration as a mean to inhibit intestinal absorption of strontium. The effect of the chelating agent on the removal of strontium from the bone and the reduction of strontium deposition is tested in rats with the radioactive strontium injected or orally administered.

MATERIALS AND METHOD

APDA (Fig.1) was synthesized according to the scheme described by the Shanghai Institute of Materia Medica (10-12). For the preparation of the working solution of Ca-APDA, APDA was dissolved in a solution of CaCO3 (in equal molar concentrations to APDA) and was adjusted to pH 6.8 with NaHCO3 (molar).

Fig.1 Chemical structure of APDA



A. Effect of intraperitoneal administration of Ca-APDA on Strontium removal

To study the effect on the intraperitoneal administration of Ca-APDA, thirty-three male Wistar rats, 3 months of age, were pre-injected intravenously with 55,600 Bq of ^{85}SrCl solution, and the strontium radioactivity in the whole body was immediately measured by a Ge detector. Thereafter, for the chelating agent treatment, the animals were divided into four groups consisting of five to seven rats per group. In three groups of rats, 10 min after the pre-injection of radioactive strontium, the Ca-APDA solution was intraperitoneally administered, while the fourth group was kept as a control. The administered doses of the chelating agent were 150, 300 or 600 mg/kg, given in three equal doses over 3 days. Each rat was kept in an individual metabolic cage. The strontium radioactivity in the whole body of the rats, and in 24-h urine and feces samples, were measured at 1, 2, 3, 4 and 7 days. On day 7, the femur, liver, kidneys, spleen, and blood were removed and the strontium activity was measured.

In order to study the time dependent effect of Ca-APDA administration on strontium removal, twenty male rats, 3 months of age, were pre-injected with radioactive strontium as described above. Then, at variable times of 1, 10 or 30 min, post-injection of strontium, these three groups (n=5 per group), were injected intraperitoneally with 600 mg/kg Ca-APDA (one group was kept as a control). The whole body activity of strontium was measured after the parenteral injection of strontium and Ca-APDA. One day later, the strontium radioactivity was again measured in the whole body, and in the collected feces and urine. Rats were sacrificed, and the excised organs and blood samples were counted for strontium activity.

B. Effects of oral administration of Ca-APDA on inhibition of strontium intestinal absorption

Fifteen male rats, 3 months of age, were divided into three groups of five animals. Rats in the first group received the radioactive strontium via a probe (7400 Bq) simultaneously with the chelating agent, at a dose of 600 mg/kg Ca-APDA dissolved in
0.8 ml solution. Animals in the second group were given the same doses of both strontium and Ca-APDA as the first group; however, the Ca-APDA solution was given 10 min later than the radioactive strontium solution.

Rats in the third group received only the oral administration of radioactive strontium, and served as the control group.

The whole body activity was measured immediately after the strontium oral administration. All rats were individually kept in metabolic cages. After 1 day, the strontium radioactivity was once again measured in the whole body counter as well as in the collected urine and feces. The animals were sacrificed and the femur, liver, kidneys, spleen, blood, intestine, and feces were then removed and the strontium radioactivity measured. In the calculation of the whole body radioactivity, the intestine was excluded.

RESULTS

The ability of this agent to remove the parenterally administered radioactive strontium was screened at various doses between 150 to 600 mg/kg, as shown in Fig 2. The strontium retention in the whole body decreased after one day in all groups. The strontium retention in the 600 mg/kg Ca-APDA group decreased significantly from day 1 to 3, while those receiving the lower doses of 150 and 300 mg/kg showed a slower

Response, but still with a significant decrease after the day 2 to 4, compared with controls. The excreted radioactive strontium in the urine of animals receiving 600 mg/kg Ca-APDA increased significantly compared to that in the control group at 1 day after the injection (Table 1). In the group receiving the lower dose of 150 mg Ca-APDA, the radioactivity detected in the urine followed the trend observed in the whole body counting data (Table 1). However, in the group of animals receiving 300 mg/kg of Ca-APDA, whole body retention seemed to remain constant from days 2 to 3. Significant fecal excretion was observed at a dose of 600 mg/kg of Ca-APDA compared with the control group, at day 1, 3 and 4, but no significant differences were detected at days 2 and 7. Nevertheless, the main excretory pathway of the radioactive strontium undergoing the Ca-APDA treatment was the intestinal tract.

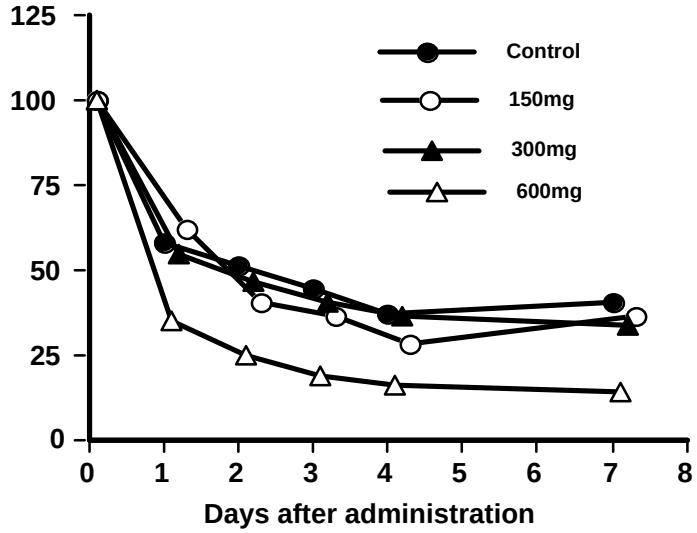


Fig. 2 Strontium activity (mean ± one SE) in the whole body of groups injected intraperitoneally with doses of 150, 300 or 600 mg/kg of Ca-APDA for 3 days, presented as percentages of activity immediately after strontium injection.

Strontium injection did not differ significantly from that of controls; large individual variability was observed, although the mean value was comparable to that of the group injected at 30 min. An increase of radioactive strontium in the urine and feces collected from all the Ca-APDA treated groups was observed; namely, the elevated radioactivity excretion in urine from animals injected at 1 and 30 min and in feces from those injected at 10 min were significantly different.

The strontium radioactivity in blood samples and in various organs excised from animals injected with different doses of the chelating agent is shown in Table 3. The administered dose ranged from 150 to 600 mg/kg Ca-APDA, 10 min after the radioactive strontium injection. The strontium activity in the blood of animals treated with 600 mg/kg Ca-APDA and in the bone of all of the Ca-APDA treated groups decreased significantly ($P < 0.05$ in the 150 and 300 mg/kg groups and $P < 0.001$ in the 600 mg/kg group) compared to the control.

For the oral administration, the whole body activity decreased significantly in both groups receiving the

Ca-APDA agent compared to controls. Strontium levels in urine also decreased significantly in both groups, but levels in feces remained unchanged as compared to controls (Table 4). The radioactivity measured in the excised organs from animals undergoing the oral administration treatment is also given in Table 4. In both groups receiving the Ca-APDA treatment, strontium activity decreased in the blood, followed by a significant increase of activity in the liver, kidneys and spleen. In those groups, the radioactivity levels in the femur also decreased significantly to 16.9% and 29.3 % of the levels observed in controls, respectively.

Ionic and total calcium levels were measured in the serum of animals treated with the Ca-APDA. The data indicated an increase of both ionic and total calcium, at 15 and 30 min after the administration of 5 mg/100g body weight of Ca-APDA injected intravenously (Table 5). In this experiment, rats injected with 600 mg/kg Ca-APDA intravenously died, showing a tetany-like reaction. Thereafter, following an observation of calcium depletion, the injected dose of Ca-APDA was gradually decreased and a dose of 5 mg/100g was selected. No untoward reactions were observed at this dose. Rats died when a dose of less than 5 mg/100g of APDA without Ca was injected.

Table 1 Excreted activity ($x10^3$Bq) of strontium in the urine and feces of groups injected intraperitoneally at doses of 150, 300 or 600 mg/kg of Ca-APDA for 3 days after the injection of the strontium.

Group	1 day	2 day	3 day	4 day	7 day
Urine					
Control group	18.1±2.5	2.4±0.2	1.3±0.2	1.4±0.3	0.9±0.1
150 mg/kg group	15.5±1.8	4.3±0.4[c]	3.1±0.3[c]	2.5±0.3[a]	1.0±0.1
300 mg/kg group	25.9±6.4	3.0±0.7	2.6±0.3[b]	1.3±0.1	1.1±0.1
600 mg/kg group	37.1±8.8[a]	2.5±0.2	1.8±0.1	0.8±0.2	1.3±0.7
Feces					
Control group	25.8±2.6	7.1±1.4	5.6±0.6	2.7±0.4	2.0±0.3
150 mg/kg group	36.8±7.5	6.4±1.1	6.3±0.8	3.2±0.4	2.7±0.1
300 mg/kg group	28.4±2.6	6.3±1.7	5.4±0.8	3.0±0.4	2.2±0.4
600 mg/kg group	45.3±4.9[b]	9.0±1.9	2.9±1.0[a]	1.7±0.2[a]	1.4±0.2

Values are mean ± one SE.

Significantly different from the control ([a]$P < 0.05$, [b]$P < 0.01$ and [c]$P < 0001$).

Table 2 Activity of strontium in the whole body, urine and feces of rats injected intraperitoneally with the Ca-APDA (600 mg/kg) 1, 10 or 30 min after injection of the strontium.

Group	Whole body	Urine	Feces
Control group	58.5±2.3%	18.1± 2.5	25.8±2.6
1 min after strontium	43.5±7.8	41.5±12.7[a]	35.7±7.3
10 min after strontium	36.5±2.4[c]	32.6±10.6	48.1±5.4[b]
30 min after strontium	45.9±5.0[a]	31.3± 5.3[a]	35.0±5.6

Values are mean ± one SE and are presented as percentages of the activity of whole body measured immediately after the injection of the strontium.

Table 3 Activity of strontium in the blood and organs of the groups injected intraperitoneally with doses of 150, 300 or 600 mg/kg of Ca-APDA for 3 days after injection of the strontium.

Group	Blood	Femur	Liver	Kidneys	Spleen
Control group	100.0 %	100.0	100.0	100.0	100.0
150 mg/kg group	111.0	67.3[a]	34.1	42.1	78.3
300 mg/kg group	96.9	64.8[a]	34.1	82.2	59.6
600 mg/kg group	49.2[a]	28.4[b]	26.9	39.3	29.8

Values are presented as percentages of the mean values of the control group.

*Significantly different from the control ([a]$P < 0.05$ and [b]$P < 0.001$)

Table 4 Strontium activity in the whole body, feces, urine, organs and blood (/ ml) 1 day after the oral administration of Ca- APDA(600 g/kg) administered after the strontium.

Group	Urine	Feces	Liver	Kidneys	Spleen	Blood
Control group	10.7±2.6 %	82.5±11.0	0.18±0.05	0.17±0.09	0.09±0.06	0.26±0.05
APDA together with Sr	1.2±0.2[b]	79.2±6.5	0.54±0.05[a]	0.21±0.05	0.18±0.25	0.11±0.03
APDA 10 min after Sr	1.9±0.2[b]	79.2±5.0	0.44±0.15	0.16±0.05	0.12±0.05	0.05±0.03

Values (mean ± one SE) are presented as percentages of that in the whole body measured immediately after the administration of strontium. Significantly different from the control ($^{a}P < 0.05$ and $^{b}P < 0.01$)

DISCUSSION AND CONCLUSION

Strontium-90 deposition in bones after accidental exposure is difficult to remove (1-3). The removal or the inhibition of uptake of radiostrontium from the skeletal system constitutes an important task due the great risk of radiation-induced bone cancer. Our interest focused in the applicability of a new chelating agent, a phosphoric acid derivative, the APDA, recently developed in the Shanghai Institute of Matreia Medica.

In the preliminary studies with this new agent, a tetany-like reaction was observed whenever administered to the experimental animals; this was assumed to be a symptom produced by an induced hypocalcemia. Therefore, the preparation of the corresponding calcium chelate was carried out, as described above and was used for the present chelation therapy studies. The administration of Ca-APDA induced no hypocalcemia, but instead, slightly increased calcium levels in serum.

In the present work, the parenteral administration of the chelating agent was tested as a mean to remove strontium already deposited in the body (bone). The data indicated the effectiveness of this administration route, with high doses of Ca-APDA (600 mg/kg) administered for 3 days, 10 min post strontium injection. The strongest effect was observed after the first Ca-APDA administration, inducing a reduction in activity to 35% of the control level (Fig.2), with a lesser effect after the second injection, as reflected in the radioactivity detected in urine and feces. These data may indicate the effectiveness of the agent Ca-APDA to form some strontium-chelate with the radiostrontium found free in extracellular fluids and the blood pool, but its ineffectiveness in removing any strontium already deposited in the tissues. The use of chelating agents has been shown to be highly efficient for removing radiometallic nuclides if treatment is initiated at an early stage post-intake; it has been suggested that the early rate of Sr deposition in bone from blood might be rapid, like that of Ca presented by a one parameter model (13).

The excretion route of the radiostrontium post Ca-APDA administration is through the kidneys and the hepatobiliary system, with the latter being more important (Table 1). The effectiveness of Ca-APDA on the removal of radiostrontium from various critical organs and the blood were clearly observed, with significant differences being detected in the femur of the 600 mg/kg Ca-APDA treated group (a reduction to 28.4% of the control value).

Oral administration of 600 mg/kg of Ca-APDA, either immediately after or 10 min post-radiostrontium contamination produced significant reduction of the administered radioactivity in the whole body. Most excreted strontium was eliminated via the gastrointestinal system, as no differences were detected in the tissue data compared with the control group, and excretion via the urinary system was low. Thus, the presence of Ca-APDA in the digestive tract might induce the formation of some less absorbable radiostrontium complexes. As a result, the radioactivity in the femur decreased to 16.9 % and 29.3% of that in the control group, if administered immediately or 10 min after the exposure, respectively. Some absorption of such a compound (perhaps a Ca-APDA - strontium complex) seems to induce a slight increase of strontium deposition in other tissues such as the liver, kidneys and spleen. However, these results indicated that the oral administration of Ca-APDA shortly after the radiostrontium intake inhibited the radioactive strontium absorption from the intestine.

In case of an accident related to accidental contamination with strontium, the most common route of the radioactive strontium intake is by inhalation (6,14-20). Thus, a slow intravenous drip of Ca-APDA solution might constitute an effective treatment for the radiostrontium inhalation. On the other hand, the oral administration of Ca-APDA as early as possible after the accidental contamination with radioactive strontium can be very effective in inhibiting the strontium absorption from the intestine. Aluminium phosphate gel and alginate have been reported to be effective drugs for the inhibition of strontium intestinal absorption. Aluminium phosphate taken orally with breakfast has reduced strontium levels by about 70-90 % in the plasma and increased the amount of excreted strontium in the feces in humans (18-21). Alginate administered orally reduced the strontium in the plasma and urine by 64 and 75 %, respectively, in rats. In the present experiment, we found that the oral administration of Ca-APDA inhibited the strontium intestinal absorption to a level of 16.9-29.3 % in bone, while the injection of Ca-APDA reduced the radiostrontium to a level of 35% in the whole body and to 28.4 % in bone. Therefore, Ca-APDA might constitute an effective decontamination agent in case of radiostrontium inhalation, if administered orally.

Throughout the present study, no untoward clinical or anatomical findings for any toxic effect of the

newly developed drug could be observed, from either the intraperitoneal injection or the oral administration of Ca-APDA. In a toxicological screening of this Ca-APDA, the LD30 measured for 30 days in mice injected intramuscularly was approximately 1,580 mg/kg. Perhaps the intravenous administration of the Ca-APDA, if necessary, should be done very slowly under careful control and electrocardiogram registration. Further toxicological studies are required before any clinical application, especially to study the long term effects after successive oral administrations.

In conclusion, our results indicate that the newly developed drug Ca-APDA, offers great potential for its applicability as a decontaminant agent at early times following the radiostrontium contamination. Ca-APDA was effective in removing radioactive strontium from the body when administered intraperitoneally, and in inhibiting the radiostrontium intestinal absorption when administered orally.

Although we demonstrated the effects of Ca-APDA on removal of strontium in the rat bone in the present study, further many examinations might be required to apply this drug for humans. Because it has been argued as to whether a rat is always an appropriate species to use as a model of humans, particularly due to its bone metabolism being very different from that of humans (22).

REFERENCES

1. Copp, D.H.; Axelrod, D. J.; Hamilton, J. G. The deposition of radioactive metals in bone as a potential health hazard. Am. J. Roentgenol. 58, 10-16(1952).
2. Barnes, D.W. H.; Carr, T. E. F.; Evans, E. P.; Loutit, J. F. ^{90}Sr-induced osteosarcoma in radiation chimeras. Int. J. Radiat. Biol. 18, 531-537(1970).
3. VanPutten, L. M.; DeVries, M. J. Strontium-90 toxicity in mice. J. Nat. Cancer Inst. 28, 587-603 (1962).
4. Carr, T.E.F.; Harrison, G.E.; Humphreys, E. R.; Sutton, A. Reduction in the absorption and retention of dietary strontium in man by alginate. Int J. Radiat. Biol. 14, 225-233 (1968).
5. Hesp, R.; Ramsbottom, B. Effect of sodium alginate in inhibiting uptake of radiostrontium by the human body. Nature. 208, 1341-1342 (1965).
6. Spencer-Laszlo, H.; Samachson, J.; Hardy E. P.; Rivera, J.; Strontium-90 metabolism during low strontium-90 intake in man. Radiat. Res. 22, 668-676 (1964).
7. Spencer, H.; Lewin, I.; Samachson, J.; Belcher, M. J. Effect of aluminum phosphate gel on radiostrontium absorption in man. Radiat. Res. 38, 307-320 (1969).
8. Sutton, A. Reduction of strontium absorption in man by the addition of alginate to the diet. Nature. 216, 1005-1007 (1967).
9. Sutton, A.; Harrison, G. E.; Carr, T. E. F.; Barltrop, D. Reduction in the absorption of dietary strontium in children by an alginate derivative. Int. J. Radiat. Biol. 19, 79-85 (1971).
10. Xie, Y.; Luo, M. Radiostrontium decorporation effect and toxicological studies on S-186 (APDA). Chinese Sci. Bull. 27, 350- 351 (1982).
11. Luo, Q.; Jiang, N.; Xie, Y. The dissoiation constants of 1-acetamido propylidene-1, 1-diphosphonic acid and 1-propionamido ethylidene-1, 1-diphosphonic acid. Chinese Sci. Bull. 28, 1570- 1571(1983).
12. .Luo, Q.; Shen, M.; Jiang, N.; Yan, Q.; Zhang, M; Xie, Y. The chelation of 1-acetamido-propylidene-1,1-diphosphonic acid and 1-propionamido ethylidene-1, 1-diphosphonic acid with alkaline earth metal ions. Acta Chimica Sinica. 41, 871- 875; (1983).
13. Goans, R. E.; Abrams, S. A.; Vieira, N. E.; Marini, J. C.; Perez M. D.; Yergey, A. L. A three-hour measurement to evaluate bone calcium turnover. Bone. 16, 33-38 (1995).
14. Cowan, F. P.; Farabee, L. B; Love, R. A. Health physics and medical aspects of a strontium 90 inhalation incident. Am. J. Roentogenol. 67, 805-809 (1952).
15. Rundo, J.; Williams, K.; A case of accidental inhalation of ^{90}SrCO3. Br. J Radiol. 34, 734-740 (1961).
16. Eve, I. S. A review of the physiology of the gastrointestinal tract in relation to radiation doses from radioactive materials. Health Phys. 12, 131-161 (1966).
17. Samachson, J; Spencer, H. Radioactive strontium: estimation of the amount accidentally ingested. Science. 148, 955-957 (1965).
18. Samachson, J. The gastrointestinal clearance of strontium-85 and calcium-45 in man. Radiat. Res. 27, 64-74 (1966).
19. Spencer, H.; Lewin, I.; Samachson, J. Inhibition of radiostrontium absorption in man. Int. J. Appl. Radiat. Isot. 18, 779-782 (1967).
20. Spencer, H.; Lewin, I.; Samachson, J. Radiostrontium absorption in man. The Lancet. 2, 156 (1967).
21. Cohn, S.H.; Spencer, H.: Samachson, J; Robertson, J. S. The turnover of strontium-85 in man as determined by whole-body counting. Radiat. Res. 17,173-185 (1962).
22. Frost, H. M.; Jee, W. S. S. On the rat model of human osteopenias and osteoporoses. Bone and miner. 18, 227-236 (1992).