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The removal of strontium from the mouse by diphenylphosphinyloxymethyl compounds

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Exposure to radionuclides implies an important health hazard from potential accidents in nuclear power plants. Strontium-90 (^{90}Sr) is one of the most potentially hazardous radionuclides, because of its long physical half-life. The administration of chelating agents has been indicated in cases of internal contamination following ingestion or inhalation of radiostrontium. Although a number of chelators have already been tested, most of these agents were found to be ineffective or yielded contradictory results in removing strontium from the body after absorption of this element (Catsch & Harmuth-Hoene, 1979). To increase the information on this question, the effects of several chelating agents on the distribution and excretion of strontium were recently evaluated in mice (Ortega et al 1989; Colomina et al 1991; Llobet et al 1991a,b, 1992). Nevertheless, most of the compounds tested were unable to remove significant amounts of strontium from the body.

Due to the interest in finding chelators, which were able to mobilize strontium from its target tissues, the present study was undertaken to evaluate the relative efficacy of a series of new chelating agents (diphenylphosphinyloxymethyl compounds) in increasing the strontium excretion and in decreasing tissue acumulation of strontium in mice. These compounds were also tested in an octanol/water system to assess whether this model could be used as a preliminary screening method in the study of the removal of strontium by chelating agents.

MATERIALS AND METHODS

Chemicals and animals: Four chelating agents: diphenylphosphinyloxymethyl ether (B.V.58),

11,2-Bis(diphenylphosphinyloxymethyl)ethan(D.21),1,5-Bis(diphenylphosphinyloxymethyl)-3-oxanentan (B.V.46) and 1,8-Bis(diphenylphosphinyloxymethyl)-3,6-dioxaoctane (B.V.47) were synthesized in the Institute of Physiologically Active Compounds (Moscow, Russia). These compounds were tested to evaluate their relative *in vivo* and *in vitro* efficacies to mobilize strontium from the body. Fifty Swiss male mice (Interfauna Ibérica, Barcelona, Spain) weighing 30-32 g were divided into five groups of ten animals. Food (Panlab diet, Barcelona) and tap water were allowed *ad libitum*.

Experimental: Strontium nitrate (E. Merck, Darmstadt, Germany) was administered s.c. at a single dose of 570 mg/kg (30% LD_{50}) (Colomina et al., 1991). Five minutes later the animals were treated with i.p. doses of B.V.58 (107 mg/kg), B.V.46 (158 mg/kg), B.V.47 (107 mg/kg) and D.21 (158 mg/kg). These doses were equivalent to 1/2 of the i.p. LD_{50} of the chelating agents. Control group received an equal volume of 0.9% saline. Animals were placed in metabolic cages and urine and feces were collected. Twenty four hours later, the mice were removed from the metabolic cages, weighed, and sacrified by ether inhalation. Samples of bone, muscle, brain, liver and kidney were removed. Tissue samples as well as urine and feces samples were processed to measure the strontium concentrations by inductively coupled plasma spectrometry (Jobin Yvon JY 38 VHR) as described previously (Llobet et al., 1991b, 1992).

An octanol/water system was developed according to Llobet et al. (1993). A physiological incubation medium served as the aqueous phase, whereas the organic phase consisted of n-octanol saturated with the aqueous phase for 24 hr. Each chelator was dissolved in the aqueous phase (to which 67.8×10^{-3} mmol $SrCO_3$ were added) at a concentration of 1×10^{-3}M. For each chelating agent, 4 ml of this solution were vigorously mixed with 4 ml of n-octanol saturated. The mixture was shaken at room temperature for 24 hr and then centrifugated at 700 g for 5 min to improve separation and to eliminate emulsions. One ml of octanol was removed and the rest was eliminated by aspiration. One ml of the aqueous phase was also removed for strontium determinations. Aqueous and octanol samples were evaporated in a thermoelectric plate and resuspended into 5 ml of 0.1 M nitric acid for strontium determinations by inductively coupled plasma spectrometry (Jobin Yvon JY 38VHR) (Llobet et al., 1993). Distribution coefficients ($D_{o/w}$) were determined as [concentration of strontium in octanol phase]/[concentration of strontium in aqueous phase] (Scherrer & Howard, 1977; Yokel & Kostenbauder, 1987; Llobet et al., 1993).

Statistics: Tests groups were compared to the control group at a level of significance of $p < 0.05$. Homogeneity of variances was analyzed by Bartlett's test. ANOVA/Kruskal-Wallis test was used to evaluate all groups simultaneously. Differences between groups were analyzed using Student's t test or Mann-Withney U test.

RESULTS AND DISCUSSION

The distribution and excretion of strontium by mice treated with the above chelating agents are summarized in Table 1. There were no significant differences between control and treated animals in bone, muscle, liver, or kidney, whereas the levels of strontium in brain of animals treated with D.21 were significantly increased. The excretion of strontium into urine and feces was not affected by any of the chelators tested (Table 1).

Table 1. Influence of the indicated chelating agents on tissue strontium concentration and strontium excretion.

CHELATING AGENTS	Tissue strontium concentrations[a] (μg/g)					Strontium excretion[a] (μg/g/24 hr)	
	LIVER	KIDNEYS	MUSCLE	BONE	BRAIN	URINE	FECES
Control	0.33±0.14	0.23±0.07	0.66±0.26	25.8±2.7	0.21±0.08	1219±527	6682±2482
B.V.58	0.32±0.18	0.24±0.06	0.46±0.32	26.7±7.05	0.26±0.07	1074±881	4471±510
B.V.46	0.40±0.26	0.29±0.14	0.72±0.54	29.9±6.30	0.34±0.14	573±426	4515±2469
B.V.47	0.23±0.11	0.17±0.03	0.51±0.43	22.7±6.50	0.25±0.08	688±273	6235±3344
D.21	0.44±0.33	0.25±0.03	0.69±0.51	22.9±5.50	0.39±0.15[b]	1478±1497	3950±2762

[a]Results are expressed as arithmetic means in each group ± SD.

[b]Significantly different from control group, $p < 0.05$.

The results obtained in the octanol/water system are shown in Table 2. None of the chelating agents significantly increased the strontium concentration in the aqueous system. In contrast, B.V.47 and B.V.58 increased the concentration of strontium in the organic phase. The distribution coefficients of B.V.58 and B.V.47 were also significantly higher than the $D_{o/w}$ of strontium in absence of the chelators.

Table 2. Solubilization of strontium by potential chelators in an octanol/water system[a]

CHELATING AGENTS	Strontium concentrations (μg/ml) in water	Strontium concentrations (μg/ml) in octanol	SAQ*	SOQ*	$D_{o/w}$
Control	2.30±0.84	0.03±0.03			0.02±0.02
B.V.58	3.15±1.07	0.37±0.20[b]	0.85±1.10	0.34±0.20	0.17±0.19[b]
B.V.46	2.58±0.85	0.06±0.02	0.28±0.86	0.03±0.01	0.03±0.01
B.V.47	2.51±0.80	0.31±0.26[b]	0.21±0.80	0.28±0.26	0.12±0.11[c]
D.21	2.91±0.49	0.04±0.11	0.61±0.50	0.01±0.01	0.01±0.00

*SAQ and SOQ (solubilization of Sr in aqueous and octanol solutions by chelators): Sr concentrations from samples containing chelators minus Sr concentrations from samples without chelators.

[a]Results are expressed as arithmetic means in each group ± SD.

[b,c]Significantly different from control group: $p < 0.05$, $p < 0.01$, respectively.

The results of this study indicate that although the chelators B.V.58 and B.V47 showed some abilities to remove strontium inside of the cells because of their slight lipophilicities, they would not be considered as good strontium mobilizing agents since they were not able to increase the solubilization of strontium in the aqueous phase. Thus, none of the chelating agents tested in the present investigation would be effective for strontium removal when this element has already been absorbed, transported, and taken up by tissue cells. Consequently, further investigations should be performed to search for new chelators which can reduce the uptake or hasten the elimination of strontium following internal contamination.

In contrast, the octanol/aqueous system would be a good model as a screening method to choose potentially mobilizing agents for strontium, which would be subsequently tested *in vivo*.

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