Bull. Environ. Contam. Toxicol. (1998) 61:289-296

Bulletin of Environmental Contamination and Toxicology

Repeated Intraperitoneal Administration of Chelating Agents in Removal of Cesium from Mice

J. M. Llobet,[1] A. de la Torre,[2] J. Corbella,[1] J. L. Domingo[2]

[1] Toxicology Unit, University of Barcelona, Joan XXIII s/n, 08028 Barcelona, Spain
[2] Laboratory of Toxicology and Environmental Health, School of Medicine, "Rovira i Virgili" University, San Lorenzo 21, 43201 Reus, Spain

Received: 10 June 1998/Accepted: 21 July 1998

Although radiological incidents due to radiocesium are relatively rare events, in recent years some accidents involving ^{137}Cs have occurred. Among them, the Chernobyl nuclear accident in April 1986 is well known. That disaster demonstrated that large areas of land might be contaminated in an accidental release of radionuclides. Fallout from the Chernobyl accident resulted in a significant increase in the inventory of radiocesium in many areas over the world (Higgitt et al. 1993; Irlweck et al. 1993; Dahlgaard 1994; Kirchner 1994; Bunzl et al. 1995; Igarashi et al. 1996). In addition to ^{137}Cs, the shorter-lived radioisotope ^{134}Cs was also deposited (Higgitt et al. 1993).

Despite the fact that in the last decades of this century the Chernobyl accident has been the most serious nuclear incident, other incidents involving Cs have been also reported. In the Goiania accident (Brazil, 1987), about 250 individuals suffered external and internal contamination after ^{137}Cs exposition, mainly via ingestion and penetration through wounds, from an abandoned radiotherapy unit (Oliveira et al. 1991; Rosenthal et al. 1991). More recently, radiocesium contamination also occurred at a steel plant in Ireland. Fortunately, the radiological impact of that incident on both the personnel and the environment was negligible (O'Grady et al. 1996).

After deposition on the soil surface, Cs can be transferred to man via terrestrial pathways (plant uptake, resuspension into the air, contamination of the groundwater) (Bunzl et al. 1995). In turn, widespread Cs contamination of the food chain can also result by transfer from grass to meat and milk (Assimakopoulos et al. 1993; Belli et al. 1993; Karlen et al. 1995).

In humans, the main adverse effects due to environmental radiocesium are based on the radiation damage caused by internal contamination. In the body, due to similar chemical properties, Cs has a pattern of distribution similar to potassium. Although Prussian blue salts can be given to inhibit the intestinal Cs absorption from contaminated foodstuffs (Nielsen et al. 1991), the most serious problem appears when radiocesium has already been absorbed. In those cases, treatment with chelators should be initiated as soon as possible in order to eliminate radiocesium

Correspondence to: J. L. Domingo

from the body.

In a recent investigation the ability of 21 chelating agents to remove Cs from Cs-exposed mice was assessed (De la Torre et al. 1996). Although the study included the main chelator groups (aminocarboxylic acids, salicylic derivatives, crown ethers, cryptates and pyridones), the results were rather disappointing for most compounds. However, 5-aminosalicylic acid (5AS), sodium p-aminosalicylate (PAS), 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]-hexacosane (Kryptofix® 222), and 1,4,7,10,13-pentaoxacyclopentadecane (15-crown-5) significantly enhanced Cs excretion into urine.

Since in that study chelation treatment consisted of a single dose of each agent (De la Torre et al. 1996), to extend the knowledge on the potential use of chelators in the removal of Cs from the body, the purpose of the current study was to test in mice the relative efficacy of repeated intraperitoneal administration of a number of chelators in increasing Cs excretion and decreasing Cs tissue accumulation after an acute oral exposure.

The polyaminocarboxylic acids disodium calcium ethylenediaminetetraacetate ($Na_2CaEDTA$, EDTA) and ethylene-glycol-bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) were also included. It is well established that the first l-3 hours following an accident with internal contamination usually provides the best and perhaps the only opportunity to prevent uptake of radionuclides. Therefore, chelation therapy was initiated 10 minutes after Cs exposure.

MATERIALS AND METHODS

Cesium chloride and disodium calcium ethylenediaminetetracetate ($Na_2CaEDTA$) were purchased from E. Merck (Darmstadt, Germany). Ethylene-glycol-bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), sodium p-aminosalicylate (PAS) and 5-aminosalicylic acid (5AS) were obtained from Sigma Chemical Co (St. Louis, MO, USA). 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]-hexacosane (Kryptofix® 222) and 1,4,7,10,13-pentaoxacyclopentadecane (15-crown-5) were purchased from Aldrich (Steinheim, Germany). The disodium calcium salt of EGTA was prepared by adding 2:1:1 mole ratio amounts of NaOH, EGTA, and $Ca(OH)_2$, in that order, to 0.9% saline. The sodium salt of 5AS was prepared by adding 1:1 mole ratio amounts of NaCl and 5AS respectively to 0.9% saline.

Male Swiss mice (Interfauna Iberica, Barcelona, Spain) weighing 32 ± 2 g were used. Animals were housed in a fully air-conditioned facility with a constant day-night cycle (dark period from 8:00 pm to 8:00 am) at a temperature of 22 ± 2°C and a relative humidity of 50 ± 10%. Food (Panlab rodent chow, Barcelona, Spain) and drinking water were allowed *ad libitum.*

After a quarantine period of 10 days, mice were randomly distributed into six groups

each consisting of 20 animals. Each mouse was given by gavage a single oral dose of aqueous CsCl(1.016 mmol/kg). In a preliminary study, the oral LD_{50} of CsCl was estimated to be 10.16 mmol/kg. Ten minutes after Cs administration, chelation treatment was initiated. Chelators were injected ip for five consecutive days in two equally divided administrations at a total daily dose equal to 1/4 of their respective LD_{50} (LD_{50} values: $Na_2CaEDTA$, 17.5 mmol/kg; $Na_2CaEGTA$, 23.7 mmol/kg; PAS, 38.9 mmol/kg; 5AS, 13.5 mmol/kg; Kryptofix® 222, 1.3 mmol/kg; 15-crown-5,2.1 mmol/kg). An additional group of mice (control group) received 0.9% saline instead of chelators. Immediately after the first injection, mice were placed in metabolic cages (five animals per cage) and urine and feces were collected daily from each group for seven consecutive days. The animals were then anesthetized with diethyl ether and euthanized by cervical dislocation. Blood samples were obtained by inferior cava puncture. Liver, kidneys, muscle (gastrocnemius), testes, spleen, bone (femur), and brain were removed. The concentration of Cs in urine, feces and tissues were determined by atomic emission spectrophotometry (Philips PU 9200X) after sample digestion (Llobet et al. 1991 ,1992; De la Torre et al. 1996).

Data for excretion and distribution studies were compared by a one-way analysis of variance (ANOVA). When the analysis indicated that a significant difference existed, the chelator-treated groups were compared with the control group by Duncan's new multiple range test. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

As for other radionuclides, internal Cs contamination of human population requires to maximize protection. If given early, hexacyanoferrates suppress the absorption of approximately 99% of the oral doses of Cs (Catsch and Harmuth-Hoene 1979; Dresow et al. 1993a). However, when Cs has been absorbed administration of an effective antidotal treatment is essential. Although Prussian blue salts are used in clinical practice as antidotes for the treatment of humans contaminated with radioactive Cs (Lipsztein et al. 1991; Dresow et al. 1993b; Verzijl et al. 1993), patients with radiocesium contamination need to be treated with those salts for long time periods. Moreover, long treatments can be associated with chronic exposure to low doses of cyanide (Verzijl et al. 1993).

Figure 1 shows the effects of various chelating agents on Cs excretion into urine and feces. Amounts depicted correspond to the percentage of Cs excreted during the period of treatment (5 days) as well as the total period of observation (7 days). In all groups, most Cs was excreted during the first day decreasing over subsequent days (data not shown). However, seven days after Cs exposure (two days after the end of the chelation therapy) remarkable amounts of Cs were still excreted into urine. During chelation treatment, urinary elimination of Cs was significantly enhanced by 5AS (15%), EGTA (7%), 15-crown-5 (22%), Kryptofix® 222 (21%) and PAS (19%). In contrast, none of the chelators significantly increased the percentage of Cs excreted into feces, a minor Cs excretion route (De la Torre et al. 1996).

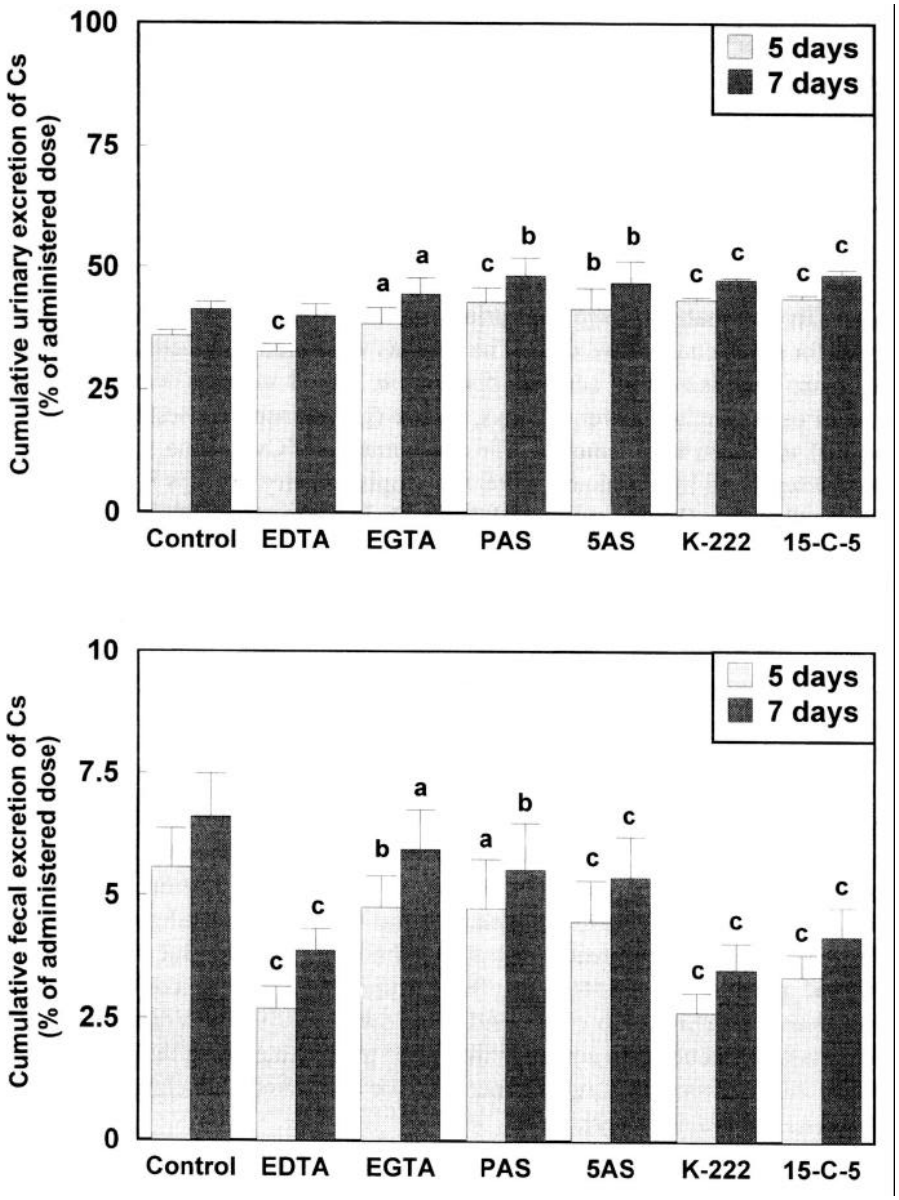

Figure 1. The effect of parenteral administration (five days) of the indicated chelators on the cumulative urinary (top) and fecal (bottom) excretion of Cs five and seven days after an acute oral exposure to Cs.

[a,b,c]Significantly different from the control group: [a]$p < 0.05$, [b]$p < 0.01$,[c]$p < 0.001$, respectively.

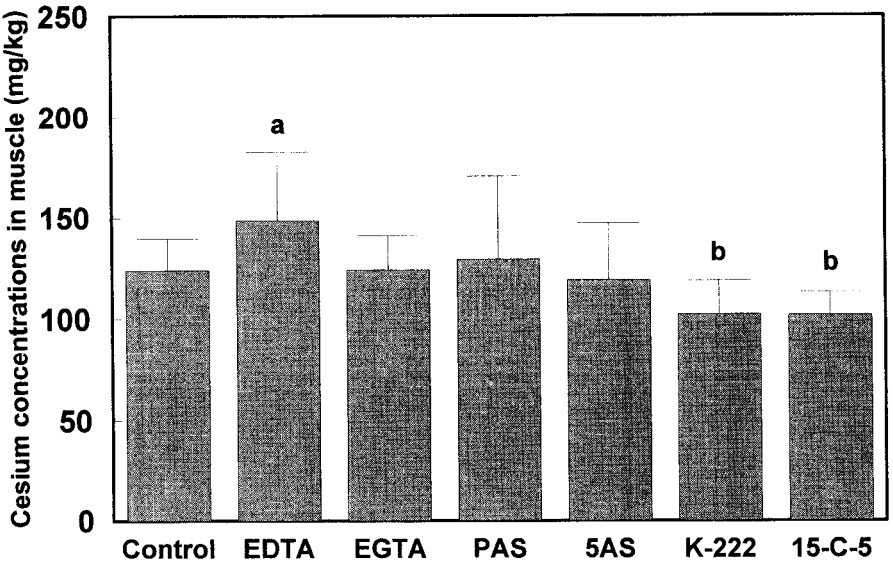

Figure 2. The effect of parenteral administration (five days) of the indicated chelators on Cs concentrations in muscle of mice sacrificed seven days after an acute oral exposure to Cs.
[a,b]Significantly different from the control group: [a]$p < 0.05$, [b]$p < 0.001$, respectively.

The effects on tissue Cs distribution by repeated parenteral administration of the six chelators tested are shown in Figure 2 (muscle) and Table 1 (blood, testes, brain, kidneys, bone, liver and spleen). The muscle is the primary storage site of Cs under normal conditions (De la Torre et al. 1996). Only Kryptofix® 222 (18%) and 15-crown-5 (17%) significantly diminished ($p < 0.001$) the levels of Cs in muscle. In contrast, EDTA (20%) increased the Cs concentrations in that tissue. Kryptofix® 222 reduced the levels of Cs in the remaining tissues analyzed, while Cs concentrations in blood, testes, brain and kidneys were also decreased by treatment with 15-crown-5. In turn, bone and kidney concentrations were significantly reduced by PAS and 5AS administration. Notwithstanding, treatment with EGTA diminished the levels of Cs in kidneys, bone and spleen. No beneficial mobilizing effects of EDTA could be observed (Table 1).

The above results suggest that both Kryptofix® 222 and 15-crown-5 could be effective chelators to reduce the body burden of Cs following an acute oral exposure to this element. However, before considering those chelating agents as potential alternatives to the use of Prussian blue salts in Cs mobilization, some questions still require further investigations. Those studies should include the toxicological evaluation of the chelators, as well as the assessment of the therapy effectiveness in relation to the time interval between Cs exposure and the beginning of the treatment.

Table 1. Effects of chelating agents on cesium concentrations (mg/ kg) in mouse tissues exposed to 1.016 mmol/kg cesium chloride

	BLOOD	KIDNEY	LIVER	TESTES	SPLEEN	BONE	BRAIN
Control	8.2±1.4	59.4± 8.5	32.0± 5.7	68.0±10.1	34.4± 6.0	37.8± 6.5	29.6± 3.3
EDTA	9.9±3.7	69.6±22.0	34.5± 7.8	86.9±21.6 [b]	42.1±13.0	35.1± 9.4	37.6± 8.3 [b]
EGTA	8.1±1.6	43.2± 7.4 [c]	29.6± 6.3	68.2±10.1	27.5± 6.5 [b]	32.1± 5.7 [b]	31.7± 4.9
PAS	8.4±2.8	46.1±13.2 [b]	29.7± 8.1	65.7±15.0	31.0±12.8	32.3±13.5 [a]	32.7± 9.5
5AS	8.7±2.4	45.7±14.9 [b]	28.0±10.8	70.0±21.0	24.5±10.0 [c]	26.6± 7.9 [c]	31.0± 7.6
Kryptofix® 222	6.4±1.3 [c]	41.1± 8.3 [c]	24.5± 5.6 [c]	47.9±10.6 [c]	24.9± 5.1 [c]	22.4± 7.3 [c]	25.9± 3.8 [b]
15-Crown-5	6.6±1.6 [b]	39.5± 9.0 [c]	32.6± 7.2	55.7± 8.7 [c]	37.2± 6.7	34.4±13.9	25.3± 3.2 [c]

Results are expressed as means ± SD. [a]Significantly different from control group ($p<0.05$). [b]Significantly different from control group ($p<0.01$). [c]Significantly different from control group ($p<0.001$).

Acknowledgments. This study has been funded by the Interministerial Commision of Science and Technology (CICYT), Spain (grant SAF93-0373). The authors thank Amparo Aguilar, Josep Mª Simón and the Servei d'Espectroscópia, University of Barcelona, for valuable technical assistance.

REFERENCES

Assimakopoulos PA, Ioamrides KG, Pakou AA, Mantzios AS, Pappas CP (1993) Transport of radiocesium from a sheep's diet to its tissues. Sci Total Environ 136: 1-11

Belli M, Sansone U, Piasentier E, Capra E, Drigo A, Menegon S (1993) ^{137}Cs transfer coefficients from fodder to cow milk. J Environ Radioact 21: 1-8

Bunzl K, Schimmack W, Krouglov SV, Alexakhin RM (1995) Changes with time in the migration of radiocesium in the soil, as observed near Chernobyl and in Germany, 1986-1994. Sci Total Environ 175: 49-56

Catsch A, Harmuth-Hoene AE (1979) Pharmacology and therapeutic applications of agents used in heavy metal poisoning. In: Levine WG (ed), The Chelation of Heavy Metals, Pergamon Press, Oxford, pp 190-193

Dahlgaard H (1994) Sources of ^{137}Cs, ^{90}Sr and ^{99}Tc in the East Greenland current. J Environ Radioact 25: 37-55

De la Torre A, Llobet JM, Domingo JL, Corbella J (1996) Evaluation of the effects of various chelating agents on the removal of cesium from the mouse. Res Commun Pharmacol Toxicol 1: 187-198

Dresow B, Asmus J, Fischer R, Nielsen P, Heinrich HC (1993a) Inhibition of intestinal radiocesium absorption from Chernobyl contaminated whey by hexacyanoferrates(II) in pigs. J Environ Radioact 21: 47-54

Dresow B, Nielsen P, Fischer R, Pfau AA, Heinrich HC (1993b) In vivo binding of radiocesium by two forms of Prussian blue by ammonium iron hexacyanoferrate (II). Clin Toxicol 31: 563-569

Higgitt DL, Rowan JS, Walling DE (1993) Catchment-scale deposition and redistribution of Chernobyl radiocesium in upland Britain. Environ Int 19: 155- 166

Igarashi Y, Otsuji-Hatori M, Hirose K (1996) Recent deposition of ^{90}Sr and ^{137}Cs observed in Tsukuba. J Environ Radioact 31: 157-169

Irlweck K, Khademi B, Henrich E, Kronraff R (1993) $^{239(240),238}$Pu, ^{90}Sr, ^{103}Ru and ^{137}Cs concentrations in surface air in Austria due to dispersion of Chernobyl releases over Europe. J Environ Radioact 20: 133-148

Karlen G, Johansson KI, Bertilson J (1995) Transfer of ^{137}Cs to cow's milk: investigations on dairy farms in Sweden. J Environ Radioact 28: 1-5

Kirchner G (1994) Transport of iodine and cesium via the grass-cow-milk pathway after the Chernobyl accident. Health Phys 66: 653-665

Lipsztein JL, Bertelli L, Oliveira CA, Dantas BM (1991) Studies of Cs retention in the human body related to body parameters and Prussian blue administration. Health Phys 60: 57-61

Llobet JM, Colomina MT, Domingo JL, Marti JB, Corbella J (1991) Evaluation of the effects of chelation therapy with time following strontium exposure to mice. Arch Environ Contam Toxicol 21: 612-620

Llobet JM, Colomina MT, Domingo JL, Corbella J (1992) Lack of effectiveness of several chelators in removing internally deposited strontium from mice following repeated parenteral administration. Vet Hum Toxicol 34: 7-9

Nielsen P, Dresow B, Fischer R, Heinrich HC (1991) Inhibition of intestinal absorption and decorporation of radiocesium in humans by hexacyanoferrates(II). Nucl Med Biol 18: 821-826

O'Grady J, Hone C, Turvey FJ (1996) Radiocesium contamination at a steel plant in Ireland. Health Phys 70: 568-572

Oliveira AR, Hunt JG, Valverde NJL, Brandao-Mello CE, Farina R (1991) Medical and related aspects of the Goiania accident: an overview. Health Phys 60: 17-24

Rosenthal JJ, de Almeida CE, Mendonça AH (1991) The radiological accident in Goiania: the initial remedial actions. Health Phys 60: 7-15

Verzijl JM, Joore HCA, van Dijk A, Wierckx FCJ, Savelkoul TJF, Glerum JH (1993) *In vitro* cyanide release of four Prussian blue salts used for the treatment of cesium contaminated persons. Clin Toxicol 31: 553-562