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REMOVAL OF INTERNALLY DEPOSITED TRANSURANIUM ELEMENTS BY Zn-DTPA

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(*Presented by* A. SEIDEL)

Abstract—The comparative effectiveness of Ca-DTPA and Zn-DTPA in removing internally deposited ^{239}Pu, ^{241}Am and ^{242}Cm was tested in the rat. The radionuclides were administered in a citrate solution and treatment with three chelate injections (1 mmol · kg^{-1} · d^{-1}) was begun 6 days later. No essential differences were observed between the two chelates tested. The data indicate that the removal of the isotopes from the liver cannot be a suitable criterion for the effectiveness of treatment in other soft tissue organs. The liver isotope content was reduced to 10% of the controls, whereas the content of other organs, including bone, was never reduced to less than 40% of the control. In liver and lung, the response to treatment was different for ^{241}Am and ^{242}Cm as compared to ^{239}Pu. There was no such difference as far as other organs are concerned.

INTRODUCTION

THE CALCIUM chelate of diethylenetriaminepentaacetate (Ca-DTPA) is commonly used for treatment of internal contamination with transuranium elements. However, there is still some reluctance to use Ca-DTPA, because of the toxicity of chelates observed in animals[1–5] and following administration of ethylenediaminetetraacetate in man.[6] It has been assumed that the toxic side effects might be due to the interaction of chelating agents with trace metals, since their chelates possess considerably higher stability constants than the corresponding calcium chelates[6] that are used for therapy. This assumption is supported by an enhanced excretion of these metals, especially of Mn and Zn,[7–9] after chelate administration. Therefore, by injection of the Zn-chelate instead of the Ca-chelate the toxicity of DTPA should be reduced. This was indeed proved for the lethal and toxic effects of Zn-diethylenetriaminepentaacetate (Zn-DTPA).[3,4,10–14] Thus, similar effectiveness of Ca- and Zn-DTPA in removing incorporated radionuclides would mean a higher therapeutic index of Zn-DTPA.

First results with ^{239}Pu,[15] ^{91}Y and ^{144}Ce[16] indicated a higher effectiveness of Ca-DTPA in removing these elements from the rat, as compared to Zn-DTPA. However, in recent studies with ^{147}Pm,[17] ^{238}Pu[18] and ^{239}Pu[19] an equal efficacy of Ca- and Zn-DTPA was found, at least for higher dosages. In the present study the comparative effectiveness of Ca-DTPA and Zn-DTPA on the removal of ^{241}Am and ^{242}Cm is evaluated; in addition, further data on ^{239}Pu are given.

METHODS

Female albino rats of the Heiligenberg strain, weighing 180–200 g, were injected intravenously with 0.1–0.3 μCi of ^{239}Pu, ^{241}Am, or ^{242}Cm, dissolved in 0.25 ml of a 1% sodium citrate solution. The animals had free access to food (Standard Pellets) and water. In the first experiment, groups of 20 rats were sacrificed at various time intervals up to the 10th week after administration of the isotope; they received no therapy at all. In further experiments, Ca-DTPA or Zn-DTPA was administered intraperitoneally on the 6th, 8th and 11th day after isotope injection at a dosage of 1 mmole/kg of body weight per day. Control animals received 0.9% NaCl. The animals were killed by desanguination on the 13th day after the isotopes had been administered. Using the same treatment schedule, in one experiment with ^{239}Pu, sacrifice was postponed until the 19th day. An additional group of untreated animals was always sacrificed on the first day of treatment (6th day).

The isotopes were maintained in stock

solutions of 3 N HNO_3, with a concentration of 50, 76 and 200 μCi/ml for ^{239}Pu, ^{241}Am and ^{242}Cm, respectively. The radiochemical purity amounted to 95 % for ^{239}Pu and 98 % for ^{241}Am and ^{242}Cm. The citrate injection solutions were prepared according to TAYLOR.[20] The pH, adjusted between 6.9 and 7.1, regularly rises to values of about 8 when the injection solution is allowed to stand overnight. We performed an auxiliary experiment on rats using the above mentioned procedure and varying the pH of the injection solution between 2.5 and 9.0. This did not influence the distribution pattern of ^{239}Pu, ^{241}Am or ^{242}Cm to a statistically significant degree.[21] Since aliquots of the injection solution were always counted together with the tissue samples, no correction for radioactive decay was necessary in the case of ^{242}Cm.

The chelates tested were Na_3[Ca-DTPA] and Na_3[Zn-DTPA], which were prepared from H_5DTPA, NaOH and $CaCl_2$ or ZnO. The concentration of the chelate in solution was 200 μmole/ml, the pH-value was adjusted to 7.4.

Radioactivity of the organs was assayed by alpha liquid scintillation counting using a method described elsewhere.[22] Small organs were analyzed as a whole. The skeletal content was calculated by multiplying by 10 the activity of two femurs. The variation of the activity concentration within the liver was low; therefore, three 400-mg pieces were analyzed from the liver and the total content was calculated by appropriate multiplication by the weight factor.

RESULTS

The retention up to 2 months of ^{239}Pu, ^{241}Am and ^{242}Cm in bone and liver of the untreated animals is shown in Fig. 1, additional control values are listed in Tables 1 and 3.

With all three radionuclides, the skeletal burden remains nearly constant, while there is a rapid decline of the liver burden and an increasing accumulation in the spleen. The initial ^{239}Pu retention by skeleton, spleen and adrenals exceeds by a factor of 2–3 that of ^{241}Am and ^{242}Cm, while the initial ^{239}Pu burden of the liver amounts only to about one-third of the corresponding values for ^{241}Am and ^{242}Cm. Since, however, ^{241}Am and ^{242}Cm are more

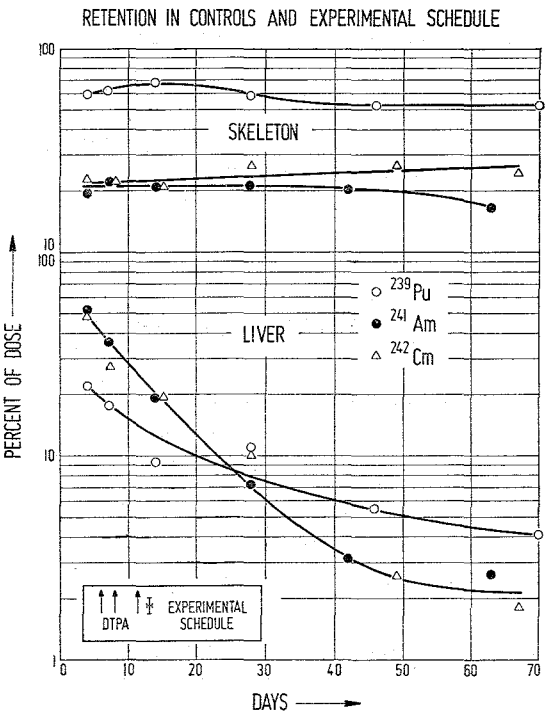

FIG. 1. Radionuclide retention in the skeleton and liver of untreated rats.

rapidly removed from the liver, the ^{239}Pu burden of this organ becomes relatively higher in later observation periods. The deposition in kidneys, lung and ovaries is virtually identical for all nuclides. The ^{241}Am- and ^{242}Cm-content of the thyroid is slightly higher than that of ^{239}Pu.

The treatment schedule is indicated in Fig. 1, the effect of the two chelates tested on the removal of ^{239}Pu, ^{241}Am and ^{242}Cm is presented in Table 1. In Table 2, these values are expressed as a percentage of the corresponding controls on the 13th day. The results of the experiment, in which the sacrifice was delayed to the 19th day, are given in Table 3.

The chelate administration results in a statistically significant diminution of the organ content of isotopes as compared to the control animals, with the few exceptions indicated in Table 1. There are no statistically significant differences between the effectiveness of Ca-DTPA and Zn-DTPA, except for the two cases indicated in Table 1. With the exception of the liver, the mobilized fraction depends neither

Table 1. Influence of Ca-DTPA *and* Zn-DTPA *on the removal of* ^{239}Pu, ^{241}Am *and* ^{242}Cm *from the rat*

Treatment	None	Saline	Ca-DTPA	Zn-DTPA
		(% of injected dose)		
Time of sacrifice	6th day	13th day	13th day	13th day
Isotope		^{239}Pu*		
Skeleton	62.0 ± 1.09	56.5 ± 0.54	41.5 ± 2.20	40.9 ± 0.79
Liver	14.6 ± 0.88	7.15 ± 0.71	1.44 ± 0.07	1.64 ± 0.09
Spleen	0.24 ± 0.02	0.32 ± 0.02	0.16 ± 0.01	0.18 ± 0.01
Kidneys	1.04 ± 0.04	0.74 ± 0.05	0.45 ± 0.04	0.36 ± 0.02‡
Lung	0.14 ± 0.01	0.12 ± 0.01	0.047 ± 0.003	0.054 ± 0.005
Thyroid	0.022 ± 0.003	0.016 ± 0.001	0.009 ± 0.001	0.008 ± 0.001
Adrenals	0.012 ± 0.001	0.013 ± 0.001	0.006 ± 0.001†	0.006 ± 0.0003†
Ovaries	0.017 ± 0.001	0.016 ± 0.002	0.010 ± 0.001	0.009 ± 0.001
Isotope		^{241}Am		
Skeleton	18.66 ± 1.46	18.71 ± 1.57	13.56 ± 0.16	14.39 ± 0.50
Liver	43.33 ± 1.16	24.07 ± 3.05	1.92 ± 0.22	2.23 ± 0.21
Spleen	0.062 ± 0.004	0.070 ± 0.005	0.047 ± 0.002	0.042 ± 0.005
Kidneys	0.823 ± 0.043	0.677 ± 0.034	0.449 ± 0.035	0.461 ± 0.049
Lung	0.107 ± 0.006	0.088 ± 0.009	0.069 ± 0.007§	0.078 ± 0.006§
Thyroid	0.021 ± 0.001	0.028 ± 0.003	0.014 ± 0.002	0.020 ± 0.003§
Adrenals	0.005 ± 0.001	0.005 ± 0.001	0.003 ± 0.0004†	0.003 ± 0.001†
Ovaries	0.009 ± 0.001	0.008 ± 0.001	0.005 ± 0.001§	0.006 ± 0.001§
Isotope		^{242}Cm		
Skeleton	22.25 ± 1.06	25.74 ± 0.66	16.02 ± 0.44	17.21 ± 0.41
Liver	42.71 ± 2.89	22.74 ± 2.91	1.73 ± 0.31	1.98 ± 0.06
Spleen	0.070 ± 0.007	0.082 ± 0.004	0.043 ± 0.003	0.043 ± 0.003
Kidneys	0.803 ± 0.092	0.620 ± 0.050	0.425 ± 0.036	0.409 ± 0.033
Lung	0.122 ± 0.009	0.092 ± 0.010	0.062 ± 0.004	0.068 ± 0.006§
Thyroid	0.024 ± 0.002	0.022 ± 0.001	0.015 ± 0.001	0.011 ± 0.001‡
Adrenals	0.004 ± 0.0004	0.004 ± 0.0004	0.002 ± 0.0004†	0.002 ± 0.0002†
Ovaries	0.013 ± 0.003	0.011 ± 0.001	0.006 ± 0.001	0.006 ± 0.001

Values indicate arithmetic means ±S.E., 4–5 rats per group.
* Values cited from Ref. 19.
† Counting error up to 15%.
‡ Statistically significant differences between Ca-DTPA and Zn-DTPA.
§ No statistically significant differences between treated and control animals (13th day).

Table 2. Influence of Ca-DTPA *and* Zn-DTPA *on the removal of* ^{239}Pu, ^{241}Am *and* ^{242}Cm *from the rat*

Isotope	^{239}Pu		^{241}Am		^{242}Cm	
			(% of control)			
Treatment	Ca-DTPA	Zn-DTPA	Ca-DTPA	Zn-DTPA	Ca-DTPA	Zn-DTPA
Skeleton	73 ± 4	72 ± 2	72 ± 6	77 ± 7	62 ± 2	67 ± 2
Liver	20 ± 2	23 ± 3	8 ± 1	9 ± 1	8 ± 2	9 ± 1
Spleen	50 ± 5	56 ± 5	67 ± 6	60 ± 7	52 ± 4	52 ± 4
Kidneys	61 ± 6	49 ± 4	66 ± 6	68 ± 8	69 ± 8	66 ± 8
Lung	39 ± 3	45 ± 5	78 ± 11	89 ± 11	67 ± 9	74 ± 10
Thyroid	56 ± 4	50 ± 4	50 ± 9	71 ± 13	68 ± 5	50 ± 5
Adrenals	46 ± 5	48 ± 4	60 ± 14	60 ± 23	50 ± 11	50 ± 7
Ovaries	63 ± 9	56 ± 9	64 ± 15	75 ± 16	55 ± 10	55 ± 10

Values calculated from Table 1, representing percent of the respective control values of the 13th day. S.E. calculated according to PARRAT.[26]

Table 3. Removal of ^{239}Pu *by* Ca-DTPA *and* Zn-DTPA *as observed after delayed sacrifice*

Treatment	None	Saline	Ca-DTPA		Zn-DTPA	
Time of sacrifice	6th day	19th day	19th day		19th day	
	(% dose)	(% dose)	(% dose)	(% of control)*	(% dose)	(% of control)*
Skeleton	62.0 ± 1.1	56.5 ± 1.1	35.0 ± 0.6	62 ± 2	41.4 ± 1.4	73 ± 3
Liver	14.6 ± 0.9	4.5 ± 0.2	1.2 ± 0.1	27 ± 3	1.6 ± 0.2	35 ± 4
Spleen	0.24 ± 0.02	0.28 ± 0.02	0.14 ± 0.02	50 ± 8	0.15 ± 0.01	54 ± 5
n	6	4	3		5	

Arithmetic means ±S.E.

* S.E. calculated according to PARRAT.[26]

on the organ nor on the element. Markedly more activity, however, can be removed from the liver, its response to treatment being higher for ^{241}Am and ^{242}Cm than for ^{239}Pu. The lung is the only organ from which ^{239}Pu is removed to a higher degree than ^{241}Am and ^{242}Cm.

A comparison of the values in Table 1 with those in Fig. 1 shows that following DTPA-treatment the ^{241}Am- and ^{242}Cm-content of the liver on the 13th day is virtually identical with values reached in control animals on the 60th day. The same holds for ^{241}Am in the skeleton, whereas the ^{242}Cm-content of the skeleton as well as the ^{239}Pu-content of both organs are lower in treated animals.

The results after delayed sectioning (Table 3) indicate that Ca-DTPA exerts an action on the skeletal ^{239}Pu sustained over several days, whereas both chelates have no such influence on the other organs. It should be noted that the variation between the animals treated with Ca-DTPA is rather low, although there are only 3 animals in this group.

DISCUSSION

The aim of the present study was to make a preliminary evaluation of the effectiveness of Zn-DTPA, as compared to Ca-DTPA, in removing internally deposited transuranium elements. In this respect there was clear evidence that Zn-DTPA equalled Ca-DTPA under the conditions of our experiments. The retention values given in Fig. 1 indicate that the isotopes were injected in "essentially monomeric" form.[20] This is of considerable importance since the effectiveness of the chelating agents depends on the chemical form of the injected isotope.[6]

At the time of the first chelate injection the skeletal deposition of all three isotopes was almost completed. On the other hand, there was still a rapid natural decline of the liver activity during the administration of the chelates. Both chelates possibly eliminated that isotope fraction which, even under normal conditions, would have been removed from the liver with a relatively short half time. Though, in our experiments, the final results of chelation therapy in the liver did not seem to be remarkably better than those of natural excretion up to two months, they suggest a considerable reduction of the radiation dose delivered to the organ during the early phase of deposition.

As far as the skeleton is concerned, the different behavior of ^{241}Am and ^{242}Cm should be noted: There was a steady increase in the skeletal ^{242}Cm, whereas the ^{241}Am in bone tended to decreasing values after the 30th day. Thus, chelation therapy gave better results for ^{242}Cm than for ^{241}Am when compared to the control values reached in the skeleton at later observation times. Considering the absolute amount of activity remaining in one organ after treatment, ^{239}Pu in bone proved to be the most difficult problem for chelation therapy.

Evidently, our chelate doses exceed by a factor of about 15 those which would be used for human therapy; extrapolation to lower doses should be made very carefully. Furthermore, some differences between Ca-DTPA

and Zn-DTPA have been reported[18] in the prompt treatment of ^{238}Pu incorporation and we have also observed that at very early stages of internal ^{241}Am contamination high doses of Zn-DTPA are less effective than equal doses of Ca-DTPA.[21] No differences, however, were found in the delayed treatment of ^{238}Pu incorporation by multiple chelate injections.[18] In rats, even the application of 3 doses of 2 mmol Ca-DTPA $\cdot$ $kg^{-1} \cdot d^{-1}$ caused only slight toxic reactions of the blood picture and of the renal functions.[4] It seems, therefore, improbable that our results were affected by toxic side effects of the chelates.

A sustained action of Ca-DTPA has been shown for ^{239}Pu[23] as well as for ^{241}Am.[21,24] The results which are reported in Table 3 for ^{239}Pu indicate, however, that our values are not markedly changed if sacrifice is delayed to the 19th day. The sustained action of Ca-DTPA in the skeleton, as compared to Zn-DTPA, deserves further studies as it could reveal possible differences in the effectiveness of both chelates.

Our data indicate that the effectiveness of the chelates in the liver cannot be a suitable criterion for their effectiveness in the other soft tissue organs. Following treatment, the decrease of the isotopes tested in spleen, kidneys and endocrine glands is rather similar to that in the skeleton. This is of some interest because in the beagle a higher concentration of ^{241}Am has been reported in the thyroid than in the skeleton.[25]

Bearing in mind the above mentioned lower toxicity of Zn-DTPA, our results encourage further studies relating to the question of whether Zn-DTPA could replace Ca-DTPA in the treatment of internal contamination with transuranium elements. These studies should be extended, preferably, to chronic treatment, because the possible gain in the therapeutic index of Zn-DTPA, as compared to Ca DTPA, is expected to be especially high in this respect.[10]

REFERENCES

1. H. Foreman, C. Finnegan and C. C. Lushbaugh, *J. Am. Med. Ass.* **160,** 1042 (1956).
2. K. M. Weber, *Virchows Arch. Abt. B Zellpath.* **5,** 39 (1970).
3. K. M. Weber, *Z. ges. exp. Med.* **150,** 354 (1969).
4. A. Seidel, *Strahlentherapie* **139,** 603 (1970).
5. F. A. Ahrens and A. L. Aronson, *Fedn. Proc.* **27,** 1401 (1968).
6. A. Catsch, *Dekorporierung radioaktiver und stabiler Metallionen,* K. Thiemig, München, 1968.
7. P. Dvořák, *Strahlentherapie* **139,** 611 (1970).
8. W. Nadolny, *Strahlentherapie* **141,** 100 (1971).
9. F. Havlíček, *Strahlentherapie* **134,** 296 (1967).
10. A. Catsch, E. Wedelstaedt, *Experientia* **21,** 210 (1965).
11. A. Catsch, *Naunyn-Schmiedeberg's Archs. exp. Path. u. Pharmak.* **246,** 316 (1964).
12. V. Nigrović, *Naunyn-Schmiedeberg's Arch. exp. Path. u. Pharmak.* **249,** 206 (1964).
13. F. Bohne, *Strahlentherapie* (1972). In Press.
14. H. Bömer, *Strahlentherapie* **142,** 349 (1971).
15. V. H. Smith, USAEC Document BNWL-280, 81 (1966).
16. A. Catsch, D.Kh. Lê and D. Chambault, *Int. J. Radiat. Biol.* **8,** 35 (1964).
17. V. H. Smith, USAEC Document BNWL-1050, part 1, 5.3. (1970).
18. V. H. Smith, Personal communication.
19. A. Seidel, V. Volf and A. Catsch, *Int. J. Radiat. Biol.* **19,** 399 (1971).
20. D. S. Popplewell, G. Boocock, D. M. Taylor and C. J. Danpure, *Radiation Protection Problems Relating to Transuranium Elements,* S. 205, EUR 4612 d-f-e, Luxembourg, 1971.
21. A. Seidel and V. Volf, unpublished observation.
22. A. Seidel and V. Volf, *Int. J. Appl. Radiat. Isotopes* **23,** 1 (1972).
23. J. F. Fried, E. H. Graul, J. Schubert and W. M. Westfall, *Atompraxis* **5,** 1 (1959).
24. J. C. Nénot, M. Morin and J. Lafuma, *Health Phys.* **20,** 383 (1971).
25. R. D. Lloyd, C. W. Mays, G. N. Taylor and D. R. Atherton, *Health Phys.* **18,** 149 (1970).
26. W. G. Parrat, *Probability and Experimental Errors in Science,* Wiley, New York, 1961.