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RELATIVE EFFECTIVENESS OF VARIOUS AGENTS FOR PREVENTING
THE INTERNAL DEPOSITION OF PLUTONIUM IN THE RAT

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ABSTRACT

The prompt administration of zirconium citrate was at least twice as effective as the prompt administration of the calcium disodium salt of ethylenediaminetetraacetic acid (CaEDTA) in preventing deposition of plutonium in the skeleton. On the other hand, CaEDTA was twice as effective as zirconium citrate in preventing the deposition of plutonium in soft tissues. The combined administration of zirconium citrate and CaEDTA was the most effective treatment for preventing the deposition of plutonium in the total rat. This combined treatment reduced by about two-thirds the plutonium retained after 30 days. Zirconium malate offered no advantage over zirconium citrate, and appeared to be somewhat more toxic. Three doses of the zirconium salts over a four-day period were no more effective than a single dose.

All forms of therapy (single injection) initiated 30 days after plutonium administration were ineffective in removing the plutonium fixed in the body of the rat.

Various skeletal segments were separately analyzed in both control and therapeutically treated rats and were shown to exhibit a comparable affinity for plutonium with the exception that bones of the skull were found to contain a lower concentration of plutonium by a factor of about two.

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INTRODUCTION

The search for agents effective in preventing the internal deposition of plutonium in the animal organism has turned up two substances possessing proven ability in this regard. Schubert and associates have shown that the administration of zirconium citrate, immediately prior to, or immediately following plutonium administration, results in a marked increase in the urinary excretion of plutonium^(1, 2). Foreman, Hamilton and Scott have found ethylenediaminetetraacetic acid (EDTA), its di, tri, tetra sodium, and calcium disodium salts, to be similarly effective in preventing the deposition of plutonium^(3, 4).

The mechanisms which have been proposed for action of these agents are quite different. Zirconium citrate, at the pH of body fluids, hydrolyzes to form colloidal aggregates which are ultimately excreted. In this process plutonium is apparently adsorbed or entrapped by the zirconium colloid and is excreted along with it⁽⁵⁾. This mechanism gains support from the fact that other metal salts which hydrolyze to form colloidal aggregates in the blood are similarly effective in plutonium removal⁽⁵⁾. The action of EDTA is quite different in that it effects the excretion of plutonium by forming a soluble chelate complex⁽³⁾. As one might anticipate from the difference in mechanism, these two agents have different effects upon the distribution of plutonium in the treated animal.

The objectives of the present study were to compare the therapeutic effectiveness of zirconium citrate, zirconium malate, and the calcium disodium salt of EDTA (CaEDTA) and more especially to determine whether combination of the two types of agents might result in additive effects. Zirconium malate was included in the experiments at the suggestion of Dr. Schubert⁽⁶⁾ on the premise that it might be expected to display less toxicity than the citrate.

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METHODS

Eleven different therapeutic treatments were employed. A description of these treatments and the number of animals so treated is given in Table I. All animals, including controls, were first injected intraperitoneally with one milliliter of a solution of plutonium(IV) citrate, pH 6, containing 8.7 μ c of plutonium. Animals administered prompt therapy were mature males of the Holtzman strain. Animals receiving delayed therapy were mature females of the same strain. Control animals listed in Table I include both males and females, and animals sacrificed 30 and 60 days after plutonium administration.

The zirconium citrate solutions were prepared according to Schubert⁽¹⁾ and contained 12.5 mg zirconium per milliliter. CaEDTA solutions¹ contained 200 mg of the salt per milliliter. Zirconium malate solutions contained 12 mg zirconium per milliliter and were prepared according to White and Schubert⁽⁵⁾. Each zirconium citrate or zirconium malate dose measured 4 milliliters (50 mg Zr) of solution except in the combined injections where the amounts administered were halved. Three milliliters of CaEDTA (600 mg) were administered as single doses, and 1.5 milliliters (300 mg) in the combined injections. All therapeutic agents were administered intraperitoneally.

Rats were housed in individual cages and maintained on a standard laboratory diet of Purina dog checkers and water ad libitum. After sacrifice by ether inhalation, the pelts and gastro-intestinal tracts were removed and discarded and the carcasses autoclaved for 30 minutes to facilitate the removal of the skeleton. Previous studies had shown that

¹ Sterile ampoules containing 1 gram of the salts per 5 ml were kindly furnished by the Riker Laboratories, Inc., Los Angeles, Calif.

TABLE I

RETENTION OF PLUTONIUM IN RATS FOLLOWING VARIOUS THERAPEUTIC TREATMENTS

* Delayed therapy initiated 30 days, prompt therapy, 30 minutes, following plutonium administration.

** Amounts administered: Zr citrate and Zr malate, 50 mg Zr; CaEDTA, 600 mg. Where two agents were administered, half these amounts were employed.

*** Doses administered 30 mins, 48 hrs, and 96 hrs after plutonium.

**** All animals sacrificed 30 days after initiation of therapy.

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such autoclaving resulted in no significant redistribution of plutonium between bone and soft tissue. Skeletons from rats in the prompt therapy studies were subdivided into the following segments: skull, vertebrae, ribs, limbs, and pelvic and pectoral girdles, in order to determine whether certain bones exhibited a special affinity for plutonium and whether this distribution was modified by the therapeutic treatments. The skeletons from rats in the delayed therapy studies were not subdivided but were analyzed in toto.

The soft tissues and skeletal fractions were digested in concentrated nitric acid, dried on a hot plate, and heated in a muffle furnace to a white ash, at 600 C. The ash was dissolved in known volumes of concentrated nitric acid and suitable aliquots were evaporated on stainless steel planchettes. The radioactivity present was measured on low background proportional counters (model PC-2, manufactured by the Nuclear Instrument Corp.) having a 52 per cent geometry and operating within the 95 per cent level of confidence.

RESULTS

Results of plutonium retention in the bone and soft tissue of the variously treated rats are summarized graphically in Table I. Thirteen of the treated animals died prematurely, due presumably to the large doses of therapeutic agents which were administered. The tissues of these animals were analyzed and the results included in the average, unless eliminated as statistically inconsistent. It should be noted that the animals were sacrificed at a constant interval (30 days) following therapy. Those animals given prompt therapy were therefore sacrificed 30 days after plutonium administration, while those given delayed therapy were sacrificed 60 days following plutonium administration. Control animals sacrificed 30 and 60 days following plutonium administration showed no significant difference in skeletal, or total plutonium content. Results from all controls

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are therefore combined in Table I, and it is felt that comparisons between treated groups are valid regardless of time of death or sacrifice.

Data pertaining to plutonium distribution in the various skeletal fractions are not presented in detail. When compared on a concentration basis, all skeletal fractions from the control animals exhibited a comparable affinity for plutonium except for the bones of the skull, which were significantly lower in plutonium concentration by a factor of about two. In the case of the therapeutically treated animals no correlation was found between plutonium distribution in the different skeletal segments and the types of therapy used.

DISCUSSION

All data were treated statistically, and the significance of differences noted in the ensuing discussion is at the 95 per cent confidence level.

None of the delayed therapeutic treatments were significantly effective in reducing the retention of plutonium in either bone or soft tissue. This finding does not exclude the possibility that repeated treatment might have resulted in eventual significant effect. However, the much greater effectiveness of prompt treatment is clearly indicated.

All of the prompt therapeutic treatments were effective in reducing the deposition of plutonium in the bone. Treatments involving zirconium salts were significantly more effective in this respect than was the administration of CaEDTA. On the other hand, CaEDTA was the only agent producing a significant decrease in soft tissue plutonium. The increased average retention of plutonium in soft tissues occasioned by the administration of zirconium salts was not statistically significant.

Combined treatment with zirconium citrate and CaEDTA resulted in an average total plutonium retention of 22 per cent which was significantly lower than that resulting from any other treatment. Simultaneous administration of these two agents combined the effects of low bone deposition

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attributable to the zirconium citrate, with the low soft tissue retention attributable to CaEDTA. The combination of zirconium malate with CaEDTA was somewhat less effective, although bone deposition in this case was lower than with CaEDTA alone, and soft tissue retention was lower than with zirconium malate alone.

The quantities of therapeutic agents administered in this study were maximal, as indicated by the 16 per cent mortality incurred. High dosages were employed in order to secure the largest possible effects from each agent. Over half of the deaths incurred were of animals treated with zirconium malate. While the experiment was not designed to evaluate relative toxicities, the suggested advantage of the malate over the citrate in this regard was certainly not borne out.

All data on bone deposition of plutonium reported in this paper are derived from analyses of the total skeleton. The uniformity noted in plutonium deposition in the various skeletal segments lends support to the more commonly employed practice of analyzing femurs and extrapolating such results to the total skeleton. It was also demonstrated that the effect of the therapeutic agents studied was not selective so far as the gross site of bone deposition is concerned.

While results of the present study should not be extended directly to the recommendation of procedures for the treatment of humans who may become contaminated with plutonium they do suggest the possibility of effectively utilizing a combination of chelating and colloiddally active agents, and re-emphasize the importance of prompt treatment for any such contamination.

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