Effect of Desferrioxamine B-Methane Sulfonate (DFOM) on Removal of Plutonium *in vitro* and *in vivo*.* (29686)

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The practical value of a simple test *in vitro* of potentially useful complexing agents for removal of radioelements deposited in animal tissue is obvious. A technique has been described(1,2) in which the ultrafilterability of a hydrolyzable radioelement through cellophane tubing serves as an easily and rapidly obtained measure of the ability of a test substance to form a diffusible complex with the radioelement. The present paper describes the application of this ultrafiltration technique for screening new agents against plutonium in the presence of blood plasma proteins. The effectiveness of desferrioxamine B-methane sulfonate† (DFOM) in removing plutonium from plasma was compared with that of diethylenetriaminepentaacetic acid (DTPA), a chelating agent of established therapeutic effectiveness. The effect of DFOM in removing plutonium from the mouse was also studied. DFOM, one of a group of chelating agents naturally occurring in Actinomycetes, was selected for testing because it has proved effective in removing iron from patients with chronic iron overload(3,4) and also prevents death of guinea pigs acutely poisoned with ferrous sulfate (5). The DFOM complex formed in the body is water soluble and excreted in the urine. Further, the high affinity of DFOM for trivalent iron(5,6) suggested that it would be even more effective in binding tetravalent plutonium.

Methods. Screening procedure in vitro. A monomeric solution of plutonium (about 90% ultrafilterable), consisting of $Pu(NO_3)_4$ in 1% sodium citrate, was magnetically stirred for 1 hour with 6 volumes of fresh dog plasma. Aliquots of 2.0 ml of plutonium-plasma were then mixed, by mechanical swirling for 1 hour, with 0.1 ml of either physiological saline or of DFOM or DTPA in saline to yield concentrations of either agent of 10^{-6} M, 10^{-4} M or 10^{-2} M. Duplicate solutions at each concentration were immediately ultrafiltered by centrifugation at $1000 \times g$. Duplicate aliquots of each ultrafiltrate, as well as of each solution before filtration, were analyzed for alpha activity in an automatic argon-methane proportional flow counter. The procedures followed in Exp. I, above, were repeated with different solutions of plutonium, plasma, and DFOM (Exp. II).

In vivo procedures. Each of 18 CF #1 female mice, 5½ months old and weighing 27.5 to 30.0 g, received an intravenous injection of 0.18 μc of Pu^{239}. This plutonium, which was about 90% ultrafilterable, was prepared by diluting the solution used in Exp. I with 1% sodium citrate. Three days later, at near maximal deposition of plutonium in bone and liver, 11 once-daily intraperitoneal injections of 0.20 ml of either DFOM in saline (about 300 mg or 5×10^{-4} moles per kg) or of saline solution were initiated. Six mice were killed 3 days after plutonium administration, the remainder at 14 days. Livers, both femurs, and periodic urine samples were ashed with concentrated nitric acid and the plutonium content determined as above.

Results and discussion. In vitro. A minimum concentration of 10^{-4} M DFOM, approximating a molar ratio of DFOM to Pu of one, was required to increase significantly the fraction of ultrafilterable plutonium (Table I). At this molar ratio DFOM was more effective than DTPA. But in comparison with DTPA, the effectiveness of which increased as the molar ratio was raised to ten,

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† 1-amino-6, 17-dihydroxy-7, 10, 18, 21-tetraoxo-27-(N-acetyl-hydroxylamino)-6, 11, 17, 22-tetraazaheptaeikosan-methanesulfonate ("Desferal"), kindly supplied by CIBA Ltd., Basle, Switzerland.

TABLE I. Effect of DFOM and DTPA on Ultrafiltration of Monomeric Plutonium from Blood Plasma.

Agent	Molar concentration in plasma	% Ultrafiltered* Exp I	Exp II
Saline controls	—	44.5 ± .8	53.2 ± 1.0
DFOM	10^{-6}	46.3 ± .9	53.0 ± 1.0
"	10^{-5}		57.3 ± .2
"	10^{-4}	80.0 ±1.6	84.4 ± .7
"	10^{-3}		77.1 ± .4
"	10^{-2}	79.9 ±1.9	80.7 ± 1.5
DTPA	10^{-6}	45.8 ± .1	
"	10^{-4}	50.2 ± .6	
"	10^{-2}	97.9 ± .3	

* Results of the 2 separate determinations ± average deviation.

TABLE II. Effect of DFOM on Tissue Distribution and Urinary Excretion of Plutonium.

	% Injected dose* Pu only † 3 days	Pu + saline 14 days	Pu + DFOM 14 days
Femurs	2.20	3.05 ± .26	2.15 ± .15
Liver	40.7	22.3 ± 1.3	19.0 ± 2.0
Cumulative urine/mouse	8.61	9.35	13.51

* Each value given is mean of determinations from 3 pairs of mice except those in the 3-day sacrifice group which are means of 2 determinations, each on 3 mice. Urine values are means from 2 cages, each containing 3 mice. Deviations shown are standard error of mean.
† Sacrifice time.

a 10-fold increase in the molar ratio of DFOM produced no further effect.

After ultrafiltration the systems prepared for Exp. I were stored for 6 days at 4°C and again ultrafiltered to determine the effect of time of contact between the agent and plutonium in the plasma. With the exception of a rise of about 10% in the fraction of plutonium ultrafiltered in each case, the results were the same as previously determined.

In vivo. Therapy with DFOM significantly increased the urinary excretion of plutonium (Table II). The daily increments, though gradually decreasing with time, added up to a 45% increase in urinary plutonium by the end of therapy. The total additional amount of plutonium removed by DFOM, however, was only 4% of the injected dose. The plutonium level in the femurs of treated mice was the same as that in the 3-day controls. No plutonium was removed from the liver.

The ultrafiltration screening technique is valuable in that negative results in the test tube, if achieved with concentrations of test substances obtainable *in vivo,* probably eliminate the need for animal testing. On the other hand, favorable results *in vitro* suggest, but do not necessarily ensure, a significant effect in the animal. In the present case an agent was tested that increased the ultrafilterability of plutonium from blood plasma but did not remove plutonium deposited in mouse tissues. If therapy had been given earlier when less plutonium was deposited in bone the effect of DFOM would undoubtedly have been greater. This is supported by the present work in which initiation of therapy at 3 days prevented further skeletal deposition of plutonium and caused a concomitant increase in urinary excretion.

Summary. An ultrafiltration screening technique was used to determine the effectiveness of desferrioxamine B-methane sulfonate (DFOM), a therapeutic agent that forms complexes with ferrous and ferric iron, in binding plutonium under physiological conditions. Although DFOM increased the fraction of plutonium ultrafilterable from blood plasma, the only effects when given intraperitoneally to plutonium-poisoned mice were the prevention of further uptake of plutonium in the bone after initiation of therapy and a small rise in urinary plutonium.

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