Conf-940437--5

PNL-SA-23309

EFFECTS OF INHALED PLUTONIUM NITRATE ON BONE AND LIVER IN DOGS

G. E. Dagle
C. R. Watson
R. E. Weller
R. L. Buschbom

April 1994

Presented at the
Health Effects of Internally
Deposited Radionuclides:
Emphasis on Radium & Thorum Conference
April 18-22, 1994
Heidelberg, Germany

Prepared for
the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
Richland, Washington 99352

EFFECTS OF INHALED PLUTONIUM NITRATE ON BONE AND LIVER IN DOGS

G.E. DAGLE, R.E. WELLER, C.R. WATSON, and R.L. BUSCHBOM
Pacific Northwest Laboratory, Biology and Chemistry Department
Richland, WA 99352

ABSTRACT

The life-span biological effects of inhaled soluble, alpha-emitting radionuclides deposited in the skeleton and liver were studied in 5 groups of 20 beagles exposed to initial lung depositions ranging from 0.48 to 518 Bq/g of lung. Average plutonium amounts in the lungs decreased to approximately 1% of the final body deposition in dogs surviving 5 years or more; more than 90% of the final depositions accumulated in the liver and skeleton. The liver-to-skeletal ratio of deposited plutonium was 0.83. The incidence of bone tumors, primarily osteogenic sarcomas causing early mortality, at final group average skeletal depositions of 15.8, 2.1, and 0.5 Bq/g was, respectively, 85%, 50%, and 5%; there were no bone tumors in exposure groups with mean average depositions lower than 0.5 Bq/g. Elevated serum liver enzyme levels were observed in exposure groups down to 1.3 Bq/g. The incidence of liver tumors at final group average liver depositions of 6.9, 1.3, 0.2, and 0.1 Bq/g, was, respectively, 25%, 15%, 15%, and 15%; one hepatoma occurred among 40 control dogs. The risk of the liver cancer produced by inhaled plutonium nitrate was difficult to assess due to the competing risks of life shortening from lung and bone tumors.

1. Introduction

The life-span biological effects of inhaled plutonium nitrate were studied in dogs to help predict risks of accidental exposure in man. This study was part of a multilaboratory program to evaluate different radionuclides and routes of exposure in dogs.[1] Lung tumors have been the principal lesions resulting from plutonium inhalation observed in our laboratory, although bone and liver lesions were observed following translocation to those tissues.[2,3] Preliminary results on the effects of inhaled plutonium nitrate on the liver and skeleton will be presented in this paper.

2. Methods

Groups of 20 young adult beagles, equally divided by sex, were given single 5- to 30-minute, nose-only exposures to aerosols of $^{239}Pu(NO_3)_4$. Initial lung depositions were estimated from external thoracic counts at 2 weeks after exposure. The dogs were held for life-span observations that included clinical examinations, periodic hematologic and clinical chemical evaluations, and thoracic radiographs. Group definitions, average lung depositions, and survival are shown in Table 1.

Radioanalysis and histopathologic examination of all tissues were made after death or euthanasia of each dog. Plutonium deposition and translocation were determined by radioanalysis of specimens using co-precipitation techniques, with liquid scintillation at lower levels and alpha spectroscopy techniques at higher levels. Histopathologic

occasional other systemic sites. Peritrabecular fibrosis was observed as a deterministic finding in all groups that produced bone tumors.

Liver damage was evaluated by observing serum levels of liver enzymes. Elevated serum chemistry values first occurred at 4.1 years after exposure in group-5 dogs when the group average accumulative dose was 2.8 Gy. At this time, the dose rate was 60 to 70 cGy per year. There were biphasic elevations of serum alkaline phosphatase (ALP) and serum glutamic-pyruvic transaminase (GPT) in individual dogs. An early increase was followed by a return to control values, and a later effect was characterized by persistent, increased elevations of both ALP and GPT. GPT and ALP values in dose-level groups 3 and 4 also became significantly higher ($P \leq 0.05$) than those for controls.

Ten intrahepatic bile-duct tumors occurred in 5 dogs from group 4; 4 cholangiocarcinomas (one fatal), 4 cholangioadenomas, 1 hepatoma, and 1 leiomyosarcoma. Intrahepatic bile-duct tumors also were present in 3 dogs from group 3, 1 dog from group 2, and 2 dogs from group 1; these consisted of 4 cholangiocarcinomas and 2 cholangioadenomas. Fatal hepatocellular carcinomas occurred in 2 dogs from group 2, and nonfatal hepatocellular carcinomas were present in 1 dog from group 1 and 1 control dog.

The mean of ratios of plutonium deposition in liver to that in skeleton at death for each individual dog was 0.83 ± 0.04 (range 0.15 to 2.1). The mean of ratios of concentrations (Bq/g) was 3.56 ± 0.18 (range 0.85 to 7.7). The ratios were not correlated with time after exposure, exposure level, or final body deposition.

Plutonium-induced lung tumors directly contributed to early mortality in 10 dogs (1 from group 5, 5 from group 4, and 4 from group 3); 32 dogs had lung tumors. Another plutonium-induced biological effect, with no direct relationships to bone or liver damage, was leukocytopenia (decreased neutrophils, lymphocytes, and eosinophils) in the highest exposure group. Malignant lymphoma, myeloma, or leukemia did not occur, despite the dose to lymph nodes and bone marrow.

4. Discussion

Because inhalation is a natural route of exposure, the comparison of bone-tumor risk estimates obtained from this study to previous studies with intravenously injected plutonium citrate in dogs will be of interest.[4] The bone-tumor risk with inhaled $^{239}Pu(NO_3)_4$, not adjusted for competing risks, was similar to the 76% incidence per Gy skeletal dose calculated for dogs intravenously injected with ^{239}Pu-citrate. The distribution of bone tumors in the dogs exposed to plutonium, whether by inhalation or intravenous injection, tended to be axial, in contrast to the appendicular distribution of spontaneously occurring bone tumors.

Preliminary comparisons, using neutron-induced fission tracks, of the microdistribution in bone of plutonium administered by the two routes (one dog each for injection and inhalation) showed that the plutonium concentration on the trabecular and periosteal surfaces was a factor of about 2 higher for the injection route.[5] This observation does not support the supposition that endosteal exposure is important for

Table 1. Skeleton and Liver Effects in Dogs Exposed to $^{239}Pu(NO_3)_4$

Study Summary

Group	5	4	3	2	1	V[1]	C[2]
Number of Dogs	20	20	20	20	20	20	20
Initial Lung Deposition[3] (kBq)	63±30	11±5.3	2.2±.14	.32±.03	.06 ±.02	—	—
Initial Lung Concentration[3] (Bq/g)	518±51	91±7	19±2	2.6±.3	.48±.12	—	—
Median Survival (m)	62	114	135	157	150	154	139

Skeleton

Deposition[3] (kBq)	23.4±3	3.4±.3	.72±.05	.10±.01	.05±.01	—	—
Concentration[3] (Bq/g)	15.8±2.1	2.1±.2	.49±.06	.07±.01	.03±.01	—	—
Dose[3,4] (cGy)	174±14	53±3	13±2	1.8±.2	.7±.1	—	—
Tumor Incidence (%)	85	50	5	0	0	0	0

Liver

Deposition[3] (kBq)	16.1±2	2.9±.3	.49±.04	.08±.01	.04±.01	—	—
Concentration[3] (Bq/g)	61.0±8.5	6.9±.9	1.3±.12	.24±.04	.12±.04	—	—
Dose[3,4] (cGy)	310±23	118±8	33±5	4.5±.5	1.8±.4	—	—
Tumor Incidence (%)	0	25	15	15	15	0	5

*[1]V=Exposed to nitric acid as **Vehicle** controls. [2]C=Untreated **Controls**.*
[3]Values shown are mean ± standard error. [4]Dose accumulated to one year prior to death.

examinations were made on hematoxylin- and eosin-stained paraffin sections; special stains and transmission electron microscopic examination of selected tissues.

3. Results

Bone tumors were the main cause of death or euthanasia in the 2 highest exposure groups (Table 1); 22 came from the axial skeleton (lumbar vertebra, 6; cervical vertebra, 4; thoracic vertebra, 3; sacrum, 3; ribs, 3; facial bones, 2; nasal turbinate, 1) and 14 from appendicular skeleton (humerus, 6; pelvis, 4; femur, 2; radius, 1; scapula, 1). Some dogs had multiple sites. The tumors were osteogenic sarcomas arising from endosteal surfaces, except for 2 hemangiosarcomas in vertebra of 2 dogs from group 2 and an anaplastic osteosarcoma in the radius of 1 dog from group 3. Metastases occurred to the lungs of 10 dogs, kidneys of 4 dogs, regional lymph nodes of 2 dogs, and

(described above) in dogs exposed by inhalation (compared to intravenously injected).

The importance of the liver damage produced by inhaled plutonium nitrate was difficult to assess given the competing risks from lung and bone tumors. The liver tumors related to plutonium were bile-duct tumors, similar to those seen with intravenously injected plutonium[6] but in contrast to the fibrosarcomas observed in dogs exposed to inhaled $^{238}PuO_2$.[7] Autoradiographic studies of liver in dogs exposed to inhaled plutonium in our laboratory show, in general, single tracks arising from hepatocytes in dogs exposed to $^{239}Pu(NO_3)_4$, alpha stars in dogs exposed to $^{239}PuO_2$, and both single tracks and alpha stars in dogs exposed to $^{238}PuO_2$.

The liver-to-skeletal ratio of plutonium deposition is similar to the ratio in humans, although in humans the ratio declines from about 0.7 at 10 years to 0.5 at 40 years after intravenous injection.[8] These similarities further validate the utility of the dog model in helping to assess the risks of accidental plutonium deposition in humans.

5. Acknowledgements

This work is supported by the U.S. Department of Energy (DOE) under Contract DE-AC06-76RLO 1830. Pacific Northwest Laboratory is operated by Battelle Memorial Institute for the DOE.

6. References

1. R.C. Thompson, *Life-Span Effects of Ionizing Radiation in the Beagle Dog* (PNL-6822, UC-408, U.S. Department of Energy, Office of Health and Environmental Research, Richland, Washington, 1989).
2. W.J. Bair, J.F. Park, G.E. Dagle, and A.C. James, *Radiat. Prot.* **26** (1989) 125.
3. G.E. Dagle, J.F. Park, E.S. Gilbert, and R.E. Weller, *Radiat. Prot.* **26** (1989) 173.
4. C.W. Mays, R.D. Lloyd, G.N. Taylor, and M.E. Wrenn, *Health Phys.* **52** (1987) 617.
5. J.M. Smith, W.S.S. Jee, G.E. Dagle, D.R. Atherton, and G.N. Taylor, in *Research in Radiobiology COO-119-257,* ed. S.C. Miller (University of Utah, Salt Lake City, 1982) pp. 52-62.
6. G.N. Taylor, C.W. Mays, M.E. Wrenn, L. Shabestari, and R.D. Lloyd, in *Life-Span Radiation Effects Studies in Animals: What Can They Tell Us?* eds. R.C. Thompson and J.A. Mahaffey (U.S. Department of Energy, CONF-830951, 1986) pp. 268-285.
7. N.A. Gillett, B.A. Muggenburg, J.A. Mewhinney, F.F. Hahn, F.S. Seiler, B.B. Boecker, and R.O. McClellan. *Am. J. Pathol.* **133** (1988) 265.
8. International Commission on Radiation Protection, Report No. 56 (Pergamon Press, Oxford, England, 1990).