TOXICOKINETICS OF BERYLLIUM FOLLOWING INHALATION OF BERYLLIUM OXIDE BY BEAGLE DOGS. III.

PRINCIPAL INVESTIGATORS

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Abstract — Young adult Beagle dogs inhaled radiolabeled beryllium oxide aerosols (^{7}BeO) prepared at either 500° or 1000°C to achieve one of two initial lung burdens (ILBs) of BeO. After exposure, animals were monitored by whole-body counting for ^{7}Be, and excreta, clinical, and radiographic data were collected. One group of dogs was assigned for serial sacrifice for quantitation of beryllium clearance from lung, translocation to other organs, and histopathologic analysis of lung and lymph nodes. A second group of dogs was subjected to periodic bronchopulmonary lavage for analysis of lymphocyte responsiveness to beryllium. These latter dogs were subsequently re-exposed to the high ILB level of BeO prepared at 500°C. ILBs following the second exposure were higher than that after the first exposure (74 vs. 42 µg BeO/kg, respectively). Except for one dog that exhibited enhanced beryllium retention after the second exposure, patterns of whole-body clearance were similar to those observed after the initial exposures to the 500°C-BeO. Analysis of lymphocyte responsiveness to beryllium in the second group of dogs is continuing.

Both acute and chronic effects can result from the deposition of beryllium compounds in the respiratory tract. Of particular concern is chronic berylliosis, an immunologically-mediated disease resulting from the deposition of relatively insoluble beryllium compounds in the lung, and characterized by the formation of pulmonary granulomas.[1] The effects of inhalation of beryllium particles such as beryllium oxide (BeO) and beryllium metal on human health have been recognized for many years; however, a suitable animal model of the chronic disease in humans has not been available. In the case of BeO, a relatively insoluble particle, decreased temperature of formation is associated with increased solubility and increased severity of biological effects.[2] However, little quantitative information is available that correlates BeO physicochemical factors with lung retention, translocation to other tissues and organs, excretion, the expression of lung lesions, and immunological events.

This report describes the current status of a study in which Beagle dogs inhaled BeO aerosols. Animals were acutely exposed to one of two initial lung burdens (ILBs) of BeO prepared at 500° or 1000°C. Previous reports described patterns of beryllium whole-body retention, lung clearance, translocation to other tissues and organs, and excretion of beryllium based on results from analysis of tissues from dogs thus exposed and sacrificed at times ranging from 8 to 365 days (1985-86 Annual Report, LMF-115, pp. 114-120; 1986-87 Annual Report, LMF-120, pp. 146-153). The BeO prepared at 1000°C was retained more tenaciously in the lungs than the 500°C-treated BeO. Beryllium clearing from the lungs and not excreted, translocated mostly to the tracheobronchial lymph nodes, liver, blood, and skeleton.

Additional animals were held for immunologic studies. Both blood and pulmonary lymphocytes were periodically obtained from these dogs for the assessment of lymphocyte reactivity to beryllium (1985-86 Annual Report, LMF-115, pp. 291-295; 1986-87 Annual Report, LMF-120, pp. 378-383; this report, pp. 311-315). The greatest immunologic response was observed in animals that received the high ILB level of BeO prepared at 500°C. Immunoreactivity peaked at approximately 6 to 8 mo after exposure, then declined for 2 yr thereafter.

We report here the re-exposure of these dogs to achieve a high ILB of 500°C-BeO. The decision to re-expose the animals, rather than sacrifice them as originally planned, was based on several factors. We observed a beryllium-specific pulmonary immune response in these dogs; however, it did not persist as is seen in humans with berylliosis. In humans, either chronic or multiple episodic exposures may be required to induce the chronic disease. In our dog model, the immunoresponsiveness may not have progressed to a persistent chronic response because insufficient beryllium remained in the lungs of the dogs. We, therefore, decided to re-expose the dogs in order to (1) investigate the temporal kinetics of the immune response after re-exposure compared with the first exposure, (2) provide a repeated exposure which is more typical of exposure modes in humans with berylliosis, and (3) provide an opportunity to sacrifice dogs at the time of immunologic reactivity, thus enabling correlations with tissue pathological responses. Following re-exposure, the patterns of whole-body clearance of beryllium were similar to those noted after the first exposure. Assessment of the immunoresponsiveness of these dogs is continuing.

MATERIALS AND METHODS

We used Beagle dogs born and reared in the Institute's colony. For the first exposure of the dogs, BeO particles radiolabeled with ^{7}Be were produced from a suspension of $Be(OH)_2$ (1984-85 Annual Report, LMF-114, pp. 20-24), calcined at either 500° or 1000°C for 16 h, resuspended in distilled water, then nebulized for nose-only exposures (5-42 min) of tranquilized dogs (1985-86 Annual Report, LMF-115, pp. 114-120). Dogs were exposed to achieve one of two ILBs. Control dogs were sham-exposed to nebulized distilled water for 30 min. For the second exposure, BeO produced, as described above, was calcined at 500°C, and dogs were exposed (30-97 min) to achieve an ILB similar to the highest level used in the first exposure (see Table 1 for the experimental design and Figure 1 for a diagram of the study timecourse). Group 1 dogs were designated for sacrifice at various times after exposure. The second group was subjected to periodic subsegmental pulmonary lavage at approximately 3 mo intervals, then re-exposed as described below.

After both exposures, dogs were held in metabolism cages for collection of urine and feces. Collections were made for the first 21 days, then for 3-day periods at 6-wk intervals after exposure. Excreta levels of ^{7}Be were determined by external gamma-ray counting. Whole-body counts for ^{7}Be activity were made on each dog for the first 7 days after exposure, twice weekly for 2 to 3 mo, then twice monthly thereafter. The whole-body count at 4 days after exposure was defined as the ILB. Dogs in Group 1 were sacrificed for determination of beryllium levels in various tissues; data from this portion of the study were reported previously (1986-87 Annual Report, LMF-120, pp. 146-153).

Dogs that were re-exposed to BeO prepared at 500°C were lavaged at selected times after exposures. Results from immunological evaluations obtained for a period of 2 1/2 yr after the initial BeO exposures are presented elsewhere in this report (this report, pp. 311-315). After the second exposure, dogs were lavaged at 2 and 4 wk after exposure, then at monthly intervals thereafter. This portion of the study is currently in progress, and the results to date are reported here.

Table 1

Experimental Design for BeO Inhalation Study

Group[a]	Sacrifice Time[b]	Low ILB[c] BeO Calcined at 500°C	Low ILB[c] BeO Calcined at 1000°C	High ILB BeO Calcined at 500°C	High ILB BeO Calcined at 1000°C	Unexposed Controls
Group 1	8	2[d]	1			
	32	2	2			
	64	2	2			
	180	2	2	2	2	
	365	2	2	2	2	
Group 2[e]						
First exposure	NA	4	4	4	4	4
Second exposure	NA			16		4

[a]Animals in Group 1 were exposed to BeO one time then sacrificed at various times after exposure to study the disposition of beryllium. Group 2 dogs were exposed twice and monitored for immunologic responses to beryllium.

[b]Number of days after exposure. NA = Not applicable.

[c]ILB = Initial lung burden; defined as the whole-body count 4 days after exposure. See table 2 for initial lung burdens achieved in Group 2 dogs.

[d]Number of dogs per group. Equal number of males and females were assigned to each exposure group.

[e]Number of Group 2 dogs shown for BeO exposure material during the first and second exposures.

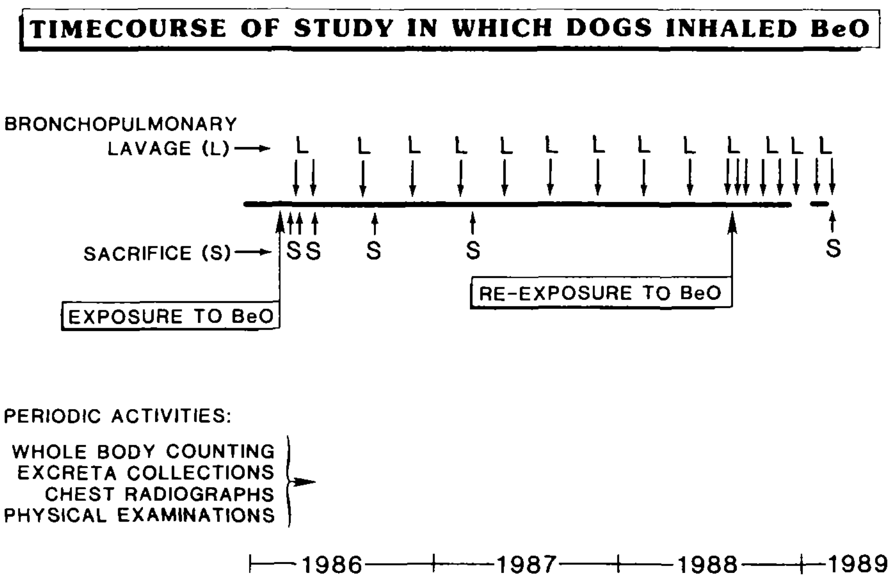

Figure 1. Schematic timeline of study of the toxicokinetics of beryllium in dogs. BeO exposures, lavages, (L), and sacrifices (S) are indicated as a function of time.

RESULTS AND DISCUSSION

Dogs were successfully re-exposed to ^{7}BeO calcined at 500°C approximately 2 1/2 yr after their first exposure. Lung burdens of BeO received by the dogs for both exposures are shown in Table 2. Characteristics of the aerosol were similar for the two exposures. The BeO aerosol mass concentration was 28 and 22 mg/m^3 for the first and second exposures, respectively. Whereas the first exposure used BeO calcined at 500° or 1000°C and was administered at one of two ILB levels, the second exposure was only to a high ILB level of BeO prepared at 500°C. The mean value of the ILB of BeO during the second exposure was 74 µg BeO/kg body weight (75% higher than the 42 µg BeO/kg level achieved during the first exposure). Pneumonitis was observed in several of the dogs; these animals had elevated respiratory rates that declined to normal within approximately 1 mo after exposure.

Table 2

Exposure Data for Dogs Assigned to Lung Lavage Group[a]

	First Exposure				Second Exposure			
		Aerosol[c]		ILB[d]		Aerosol		ILB
Exposure Group[b]	N	AMAD	σ_g	(µg/kg)	N	AMAD	σ_g	(µg/kg)
Control	4	NA[e]	NA	NA	4	NA	NA	NA
High-500°C	4	1.9	2.0	42 (11)	16	1.7	1.8	74 (19)
Low-500°C	4	1.7	1.9	18 (3)				
High-1000°C	3	2.0	2.0	41 (20)				
Low-1000°C	5	1.8	1.7	18 (2)				

[a]Group 2 dogs as defined in the experimental design (Table 1). Dogs were exposed for the first time in March 1986 and for the second time in August 1988.

[b]Level of ILB and temperature preparation shown for the first exposure. Numbers of dogs are not identical to those given in the experimental design (Table 1) because of re-grouping on the basis of dose actually received. For the second exposure, all dogs received BeO prepared at 500°C.

[c]Activity median aerodynamic diameter (AMAD) and geometric standard deviation (σ_g) of the ^{7}BeO aerosol shown.

[d]ILB = Initial lung burden. Standard deviation shown in parentheses.

[e]NA = Not applicable.

After the second BeO exposure, the whole-body retention of beryllium was again monitored by radioactivity counting of ^{7}Be. Clearance of beryllium through 70 days after the second exposure was best described by a single-component negative-exponential function (Fig. 2). Interestingly, one dog had a clearance pattern more typical of that of high-fired BeO; the clearance half-time for this dog was 260 days. This dog was exposed to the high ILB level of 1000°C during his first exposure. The F-statistic[3] showed that the whole-body clearance of beryllium for this dog was significantly different ($p \leq 0.05$) from that of the other 15 dogs. The cause for this enhanced retention of BeO in this dog is not known. The clearance half-time for the remaining 15 dogs was 98 days (standard error range 96-100 days; fitted function shown in Fig. 2), which was similar to the value of 54 days (standard error range 32-170 days) observed for the first component of

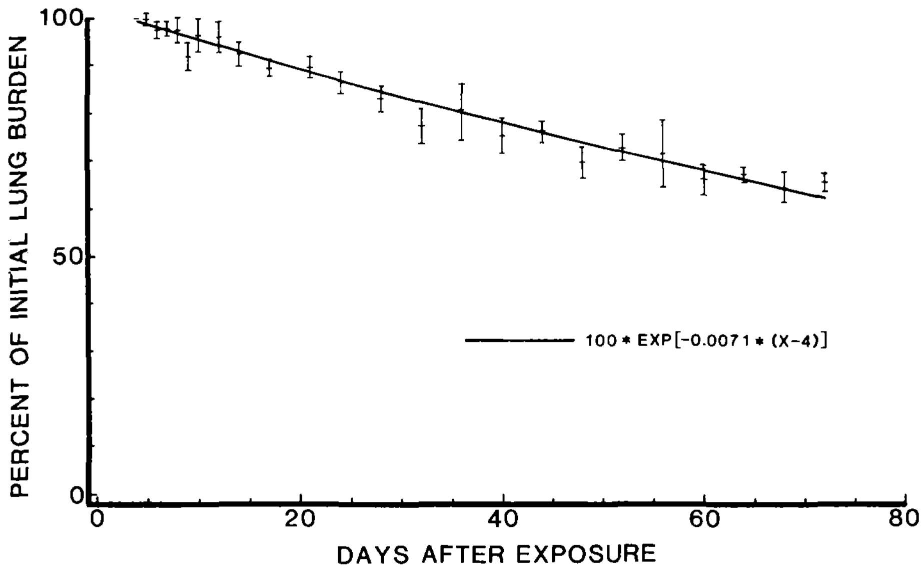

Figure 2. Whole-body clearance of beryllium as a function of time after the second exposure to BeO calcined at 500°C. Data from all 16 exposed dogs are shown. A single component negative exponential function fitted to 15 of the dogs is also shown; one dog was eliminated from this analysis because its pattern of clearance was different ($p \leq 0.05$) from the other 15 animals.

clearance after the first exposure (1986-87 Annual Report, LMF-120, pp. 146-153). Sufficient whole-body counting data have not yet been collected to permit a determination of whether a second component of clearance will become evident, as was the case after the first exposure to 500°C-BeO. With the exception of the dog noted above, there were no differences in clearance of the 500°C-BeO depending on the dose or BeO aerosols the dogs received during the first exposure.

Excreta collections, bronchopulmonary lavages, whole-body counting for patterns of beryllium clearance, and clinical examinations (including chest radiographs) of these dogs are continuing. The dogs will be sacrificed immediately after peak immunologic responses begin to decline, to provide correlations between immunoreactivity to beryllium and the nature of lesions present. It is anticipated that this study will yield significant new information regarding the suitability of the Beagle dog model for chronic human beryllium lung disease.

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