INFLUENCE OF PHYSICOCHEMICAL PROPERTIES OF BERYLLIUM PARTICLES ON CULTURED CELL TOXICITY

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Abstract — The toxicity of beryllium oxide (BeO), beryllium metal, and beryllium sulfate ($BeSO_4$) was studied in two cell lines, Chinese hamster ovary cells (CHO) and lung epithelial cells (LEC). Beryllium oxide particles were prepared at either 500 or 1000°C, and two different particle sizes of beryllium metal were used. Following a 20-h exposure to beryllium compounds, cells were grown in culture to quantitate cloning ability relative to controls as a measure of cell killing. The LEC cultures were more sensitive to beryllium cytotoxicity than the CHO cells. When expressed on the basis of the mass of material added to the cultures, the order of toxicity was $BeSO_4 \geq$ 500°C-BeO > 1000°C-BeO > Be metal (small) > Be metal (large). When cytotoxic effects were expressed on the basis of particulate surface rather than mass, the relative differences in toxicity between compounds was decreased. The order of toxicity was Be metal (small) ≈ Be metal (large) ≥ 500°C-BeO ≈ 1000°C-BeO. These data indicate that solubility influences beryllium toxicity to short-term cell cultures.

The use of beryllium in the aerospace, nuclear, and electronics industries is increasing.[1] However, the toxicity of beryllium has been recognized for many years. If inhaled in sufficient amounts, beryllium can cause a nonspecific chemical pneumonitis in the lungs, and at lower lung doses can cause a progressive granulomatous lung disease in susceptible individuals. Also, beryllium has been shown to cause cancer in experimental animals and is classified as a suspect human carcinogen.[2]

The mechanisms involved in the induction of these long-term responses are not well known. It is known that interactions between various types of lung cells and inhaled materials may, in part, determine the nature of potential chronic responses to inhaled toxicants. It has been shown that various beryllium compounds are cytotoxic when administered to alveolar macrophages,[3] and to epithelial cells and fibroblasts (1986-87 Annual Report, LMF-120, pp. 371-373).

This study was conducted to investigate the influence of different physical and chemical properties of beryllium compounds on their cytotoxicity for two different cell types *in vitro*. Specifically, we hypothesized that the two major factors influencing the cytotoxicity of beryllium particles are chemical form and specific surface area.

MATERIALS AND METHODS

We used two chemical forms of beryllium particles (beryllium oxide [BeO] and beryllium metal). For each chemical form, our hypothesis that specific surface area influenced toxicity was tested by converting the mass of beryllium compound added to the amount of surface of beryllium compound added.

Beryllium oxide particles were generated using methods described by Hoover *et al.*[4] Particles were prepared at either 500 or 1000°C by calcination for 16 h. Beryllium metal aerosols were obtained by dry powder aerosolization of Be metal powder (1986-87 Annual Report, LMF-120, pp. 45-49). The aerosol was passed into a five-stage aerosol cyclone, and particles obtained from

stages II and V of the cyclone were used. Specific surface area measurements of the particles were made in duplicate. Following outgassing of the samples for 16 h in flowing helium at 250°C, nitrogen adsorption isotherms were determined and data were reduced using a Brunauer-Emmett-Teller technique.[5]

Particle suspensions for administration to cell cultures were prepared on the day of exposure. Samples were weighed on a pan microbalance, culture medium was added, then samples were subjected to ultrasonic agitation for 30 min. Suspensions were stirred using a vortex mixer, diluted, then administered to cell cultures.

Cultured Chinese hamster ovary cells (CHO; a fibroblast cell type) and rat lung epithelial cells (LEC; Type II cells) were used. Cells were cultured in 6-well plastic tissue culture plates in a culture medium consisting of Ham's F12 medium supplemented with 10% newborn calf serum. Cell cultures were allowed to acclimate in a 37°C incubator in a humidified atmosphere of 5% CO_2/95% air for 24 h before exposure.

For beryllium particle exposure, the culture medium was withdrawn from the cell cultures, then particle suspensions in medium were added. Cultures were returned to the incubator for 20 h, removed, washed with medium three times to remove free particles, then trypsized to remove the cells. Either 200 (for CHO) or 500 (for LEC) cells were placed into triplicate culture dishes containing fresh medium. After 7 days, the cultures were fixed, stained, and the number of colonies counted. The relative cloning efficiency (RCE) was defined as the percentage of colonies formed compared to control cultures.

For each cell and particle type, the RCE was plotted as a function of the mass of beryllium added. The specific surface area of each particulate sample was used to convert the amount of mass added to the amount of surface added, and plots of RCE versus added surface were constructed. Replicate experiments were conducted for most of the assays, and when identical concentrations were repeated for a given sample, error bars representing + 1 standard deviation are shown on the figures. Values for the concentration required to reduce the RCE by 50% (LC_{50}) were determined using the probit procedures of the SAS software statistical package (SAS Institute Inc., Cary, NC).

RESULTS

The physicochemical characteristics of the beryllium compounds used in these studies are summarized in Table 1. All particles used were of respirable size. Beryllium oxide calcined at 500°C had the greatest specific surface area of any sample tested (189 m^2/g), and beryllium metal obtained from stage 2 of the cyclone had the least specific surface area (7.6 m^2/g).

Figure 1 shows the effect of BeO particles on CHO cell RCE. Two curves are shown for the 500°C-BeO because two different preparations were examined, each having slightly different amounts of surface area. It is readily apparent that, whereas the 500°C material is more toxic when expressed on the basis of mass, nearly identical cell killing is observed for the oxide produced at 1000°C when data are converted to surface area.

The effect of exposure to $BeSO_4$ or beryllium metal on RCE is shown in Figure 2 for CHO cells and in Figure 3 for LEC cells. Beryllium sulfate was more toxic to LEC than CHO cells, and was also more toxic than either beryllium metal particle sample. When normalized by specific surface area, similar cell killing curves were observed for both beryllium metal particles in either cell system.

Values for LC_{50} with associated ±95% confidence intervals are shown in Table 2. Because $BeSO_4$ is a soluble salt, reduction of RCE attributable to $BeSO_4$ could only be expressed on the basis of mass added to cell cultures. For mass-dependent cytotoxicity, significant differences can be seen

Table 1

Physical Properties of Beryllium Compounds Used in Toxicity Studies

Beryllium Sample[a]	Size[b] (μm)	GSD[c]	Specific Surface Area ($m^2/g \pm SD$)
$BeSO_4$	NA[d]	NA	NA
Be Metal - II	2.4	1.9	7.6 (1.2)
Be Metal - V	1.0	1.5	20.4 (4.0)
BeO - 500°C	1.8	1.9	146 (5) or 189 (3)[e]
BeO - 1000°C	1.9	1.8	35.8 (2.7)

[a]For beryllium metal, Roman numeral refers to the stage number of the cyclone separator from which the sample was obtained. For beryllium oxide, the temperature of calcination is given.

[b]For beryllium metal, the count median diameter (Feret's diameter) is given (1986-87 Annual Report, LMF-120, pp. 45-49). For beryllium oxide, the activity median aerodynamic diameter is given.[4]

[c]Geometric standard deviation (GSD) in size measurements.

[d]NA = Not applicable.

[e]Two separate preparations of 500°C-BeO were used.

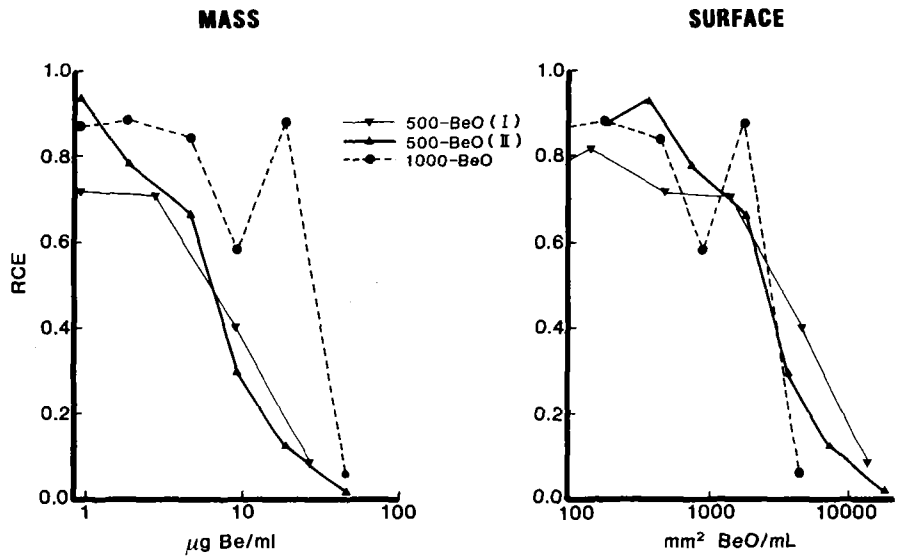

Figure 1. Reduction in CHO cell relative cloning efficiency (RCE) as a function of BeO added to cultures. At left, RCE is plotted against the mass of BeO added, and at right, against the surface of BeO added.

between either the two BeO samples or the two beryllium metal samples for each cell type. After conversion to surface area, however, the values for LC_{50} become nearly identical, and the 95% confidence intervals overlap in each case.

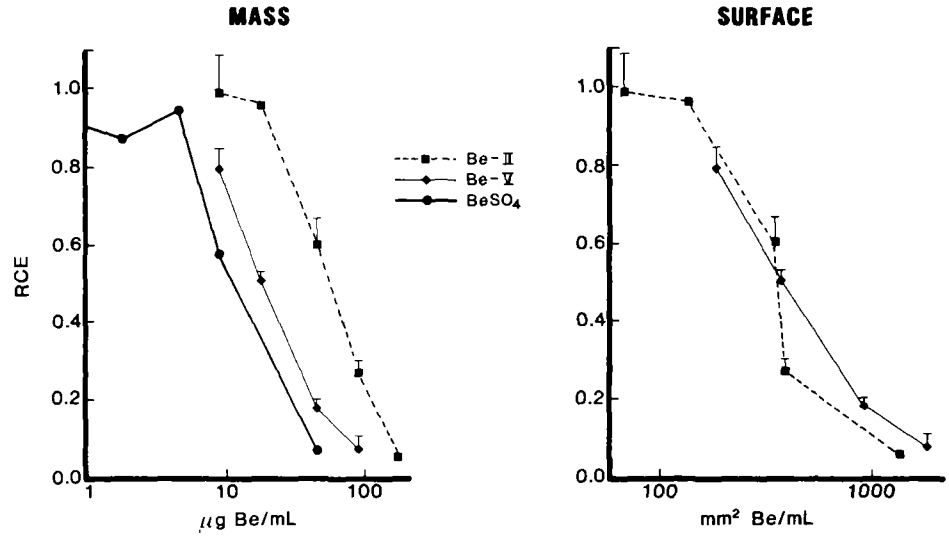

Figure 2. Reduction in CHO cell relative cloning efficiency (RCE) as a function of beryllium metal and $BeSO_4$. At left, RCE is plotted against the mass of beryllium metal or $BeSO_4$ added, and at right, against the amount of surface of beryllium metal added.

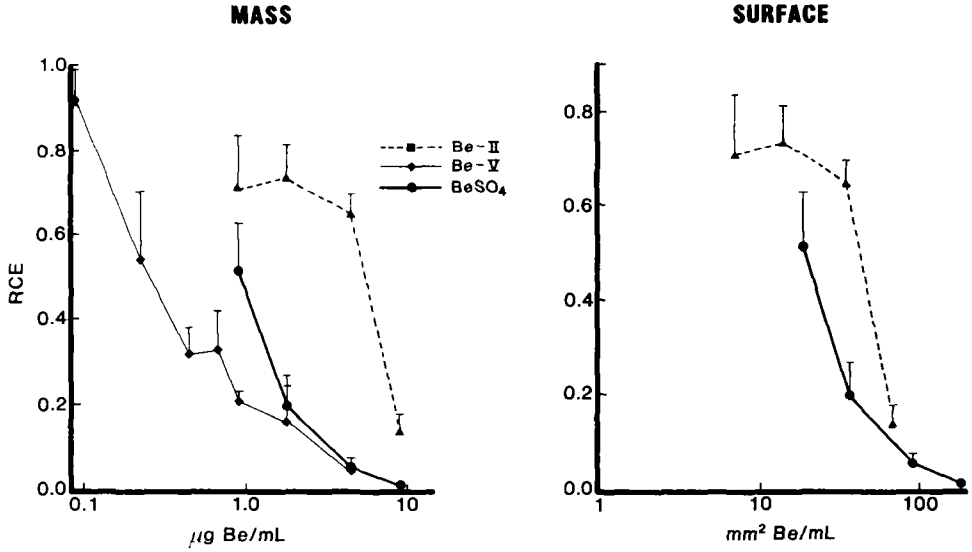

Figure 3. Reduction in LEC cell relative cloning efficiency (RCE) as a function of beryllium metal or $BeSO_4$ addition to cell cultures. At left, RCE is plotted against the mass of beryllium metal or $BeSO_4$ added, and at right, against the amount of surface of beryllium metal added.

DISCUSSION

We observed that CHO cell cultures were less sensitive to administered beryllium than LEC cultures for all beryllium compound types examined. This has also been observed following exposure of these cell types to nickel compounds (1986-87 *Annual Report*, LMF-120, *pp.* 371-373). Differences may be related to differing rates of particle phagocytosis or differing intrinsic susceptibility to the effects of metallic cations.

Depending on the particle source or preparation method, we observed considerable variability in the mass of beryllium metal or BeO particles required to depress the relative cloning efficiency of either CHO or LEC cell cultures. For both cell types, we found that (1) on an equal mass basis, the toxicity of BeO was greater than that of beryllium metal, (2) the toxicity of

Table 2

Cytotoxicity of Beryllium Particles to Cultured Cells

Cell Type[a]	Beryllium Compound[b]	LC_{50} µg/mL (±95% CI)[c]	LC_{50} mm^2/mL (±95% CI)[c]
CHO	$BeSO_4$	2.6 (1.6, 3.7)	NA[d]
CHO	BeO-500°C	3.4 (1.2, 12)	1800 (630, 2300)
	BeO-1000°C	19 (17, 22)	1900 (1700, 2200)
CHO	Be-II	56.2 (50.3, 62.7)	430 (380, 480)
	Be-V	19.3 (16.5, 22.2)	390 (340, 450)
LEC	$BeSO_4$	0.34 (0.21, 0.47)	NA
LEC	BeO-500°C	0.25 (0.09, 0.64)	130 (90, 340)
LEC	Be-II	3.7 (2.8, 4.6)	28 (21, 35)
	Be-V	0.88 (0.67, 1.07)	18 (14, 22)

[a]Chinese hamster ovary or lung epithelial cell cultures.

[b]Denotes particle type and preparation. For beryllium oxide, the temperature of calcination is given; for beryllium metal (Be), the cyclone stage from which particles were obtained is shown.

[c]The concentration of exposure compound required to reduce relative cloning efficiency by 50%. The mass of material required (µg/mL) was also corrected by specific surface area to give the amount of surface area required (mm^2/mL). The limits of the 95% confidence interval obtained from probit functions fitted to the data are given in parentheses.

[d]NA = Not applicable.

beryllium metal appeared greater than BeO when expressed on the basis of administered surface area of particles, and (3) the conversion of mass to surface area resulted in similar expressions of toxicity caused by different forms of the same beryllium compound.

These data agree with previous studies of the effects of beryllium compounds administered in vitro to cultured cells. We previously reported that the 500°C-BeO was more cytotoxic to cultured canine alveolar macrophages than the 1000°C-BeO;[3] however, normalization of these data by specific surface area gave similar values for LC_{50}.

Our observations support the hypothesis that beryllium metal is more toxic than BeO to cultured cells, when expressed on an equivalent surface-loading basis. In addition, we previously observed[6] that beryllium metal appears to be slightly more soluble than BeO in a nonbiological dissolution system when the effects of specific surface are removed.

Available evidence suggests that the form of administered beryllium is a crucial determinant of the expression of toxicity in cultured cell systems. Solubilized Be ion is poorly incorporated into cultured cells, whereas particulate or colloidal beryllium is more able to be internalized, probably because it can be phagocytized[7] (also see 1986-87 Annual Report, LMF-120, pp. 133-139). However, $BeSO_4$ was observed to be more toxic than BeO to cultured canine alveolar macrophages,[3] and here, we observed that the LC_{50} for $BeSO_4$ was either comparable to or slightly less than that for 500°C-BeO. Internalization of beryllium following exposure to $BeSO_4$ or other soluble beryllium compounds may occur through pinocytosis or phagocytosis of colloidal precipitates, and it is possible that although a relatively small fraction of administered beryllium may be internalized, the beryllium might be in a more bioavailable form, compared to phagocytized insoluble beryllium particles. Alternatively, it has been suggested that beryllium might have a direct toxic effect at the level of the cell membrane.[8]

In the case of relatively insoluble beryllium particles, we hypothesize that a plausible mechanism of action for the observed toxic effects involves phagocytosis followed by intracellular dissolution of beryllium ion within phagolysosomes. Because dissolution rate is a function of specific surface area (for relatively insoluble particles), it follows that once internalized, particles having equal amounts of surface area should dissolve (at least initially) at similar rates. Once dissolved, beryllium ion would be available to complex with cellular constituents and thus cause deleterious effects to the cell. This hypothesis can account for the observation that equal masses of particle samples that are chemically identical can result in the expression of differing toxicity, if the samples have different specific surface areas.

In conclusion, we have demonstrated that both beryllium metal and beryllium oxide, particle samples having the same chemical nature, yet different physical properties, induce toxic responses in either CHO or LEC cell cultures at differing concentrations. Over the exposure time examined (20 hours), specific surface area appears to most closely affect cell toxicity. Dissolution of beryllium ion from internalized particles appears to be important in the induction of toxic effects.

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