Qualitative and Quantitative Study of Alveolar Macrophage Death after α-Irradiation in Primary Culture

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We have characterized alveolar macrophage cell death after α-irradiation in primary culture. The cells were obtained by pulmonary lavage of rats or two primates. Irradiation was performed 1 day after seeding cells using electrodeposited 241Am sources. The cell death process was characterized by vital staining with acridine orange and propidium iodine. Cell survival was measured taking into account the staining, which visualizes apoptotic and necrotic processes, and cell density. Kinetics of cell death was estimated up to 3 days after irradiation. Macrophages appear as radioresistant cells (rat $D_0 \approx 60$ Gy, primate $D_0 \approx 140$ and 100 Gy). The death process was usually slow and could involve both apoptosis and necrosis. Assuming an in vivo macrophage half-life of 10 days, an obvious toxicity of aerosols having an activity median aerodynamic diameter of 5 µm is expected for actinide oxides with a specific α activity similar to or above that of $^{239}$PuO$_2$.

Keywords: alveolar macrophage; α-irradiation; apoptosis; necrosis; actinide oxides

INTRODUCTION

After γ-irradiation alveolar macrophages become radioresistant (Kubota et al., 1994) but could be one of the main target cells after inhalation of aerosols containing insoluble α-emitters which have a short range, such as actinide oxides. A few studies have concerned macrophage death induced after α-irradiation (Taya and Mewhinney, 1992) but no actual dose–effect relationship is available. We have previously reported a dose–effect relationship for rat alveolar survival after α-irradiation in primary culture based on cell density measurements 1 h after the end of the irradiation. The doses were expressed as fluences (Lizon et al., 1997). No significant death was observed for a fluence $< 3 \alpha$ hits/µm, which corresponds to irradiation performed for $< 9$ h. Recently, we characterized the chemical toxicity of some lanthanides and actinides towards rat alveolar macrophages (Lizon and Fritsch, 1999). The process of cell death was apoptosis and its kinetics were slow; it would need several days before the process was achieved. Thus, the dose–effect relationship we previously reported for macrophage survival after α-irradiation might be overestimated.

The aim of this study was to characterize the kinetics of the alveolar macrophage death process and the dose–effect relationship after in vitro α-irradiation in terms of fluence, dose and number of α hits per cell. Results obtained in both rats and primates are used to estimate the in vivo cell response after inhalation of actinide oxides.

MATERIALS AND METHODS

Cell culture and α-irradiation

Alveolar macrophages were harvested by pulmonary lavage after repeated instillations of physiological buffer (0.9% NaCl). This was performed in anaesthetized animals, either 2-month-old male Sprague–Dawley rats or two baboons. After centrifugation (200 g for 10 min) cells were seeded at a density of $\sim 80000$ per cm$^2$ in polyethylene flasks, the bottom of which was covered with 3.5 µm thick mylar foil. The culture medium, RPMI 1640, was complemented with 10% serum substitute (BMS), which allows control cells to be maintained at a constant population size for at least 4 days in culture (Lizon and Fritsch, 1999).

The day after lavage the flasks were directly put on an electro-deposited 241Am source (flux between...
2.3 \times 10^{-4} \text{ and } 3.2 \times 10^{-4} \mu \text{m}^2) \text{ sealed with } 3.5 \mu \text{m thick mylar foil. The contact duration was calculated to obtain an exposure of } \sim 7 \text{ and } 14 \alpha \text{ particles/} \mu \text{m}^2. \text{ Unirradiated cells were used as controls.}

**Cell death measurement**

Vital staining was performed at the end of irradiation and then up to 3 days later. Acridine orange (10 \mu g/ml) and propidium iodide (10 \mu g/ml) were added to the culture medium. Cells were readily stained and observation had to be performed within 5 min to avoid stain toxicity. Images were recorded using a tri-CCD refrigerated camera attached to an inverted fluorescent microscope. For each sample eight images (422 \times 566 \mu \text{m}) were recorded using a 20\times objective. Survival measurement was performed taking into account cell density of control and irradiated cells (mean number of cells per image) and mean cell composition in terms of normal, necrotic, apoptotic, post-apoptotic necrotic and unstained cells as previously defined (Lizon and Fritsch, 1999).

**Dosimetry**

The entire cell was considered as the irradiation target. After vital staining morphometric parameters were measured in control cells on day 1 after the beginning of culture. Cell images were recorded as previously described. Mean cell diameters were then measured assuming a spherical shape (Lizon et al., 1997). Simulations were performed with custom software developed in the authors’ laboratory (LeFoll et al., 1998), taking into account geometric and morphometric parameters to provide doses and \alpha \text{ hit probability.}

**RESULTS**

Slow kinetics of \alpha\text{-irradiation-induced cell death were observed in rats and in one of the two primates. Cell death needed at least 1 day to be achieved. For the other primate most cell death was achieved before 1 day post-irradiation. As shown in Fig. 1, in rats cell death involved both apoptosis and necrosis and the necrotic–apoptotic ratio appeared to increase with fluence. In contrast, in primates no significant necrosis was observed (Fig. 2). The morphometric study has shown different mean cell diameters after 1 day in culture. It was 10.6 and 18.6 \mu m for rat and primate, respectively. These values were used for simulation calculations of dose and number of \alpha \text{ hits per cell.}

Figure 3 shows the dose–effect relationship for rat alveolar macrophage survival as a function of \alpha \text{ fluence at different times after the end of irradiation. Assuming that cell death was achieved after 3 days, the dose–effect relationship fits a single negative exponential function (r^2 = 0.977); a fluence of 5.2 \alpha \text{ particles/} \mu \text{m}^2 \text{ induces } 63\% \text{ cell lethality. Similar dose–response curves were observed in primates with a fluence of either 11.9 (r^2 = 0.981) or 8.6 \alpha \text{ particles/} \mu \text{m}^2 (r^2 = 0.593), which induced the same death. After simulation calculations these values corresponded to } D_0 \text{ values of 60, 140 and 100 Gy or 290, 1400 and 1000 \alpha \text{ hits/cell for rats, primate 1 and primate 2, respectively.}

![Fig. 1. Dose–effect relationship for \alpha\text{-irradiation-induced lethality in rat expressed as a fraction of the different kinds of cells encountered 3 days after the end of irradiation. Mean values for three animals } \pm \text{ SD.}](https://academic.oup.com/1281/67.17.595/fig/1)
Alveolar macrophage death after α-irradiation

DISCUSSION

This study shows that cell death induced after α-irradiation might involve different processes depending on the mammalian species studied. In fact, preliminary results obtained after α-irradiation of rat macrophages kept for several days in culture have shown that cell death was due only to apoptosis. The necrosis observed here might involve reversible rat cell alterations due to the lavage procedure. Thus, we can conclude that in vitro, as reported after γ-irradiation (Kubota et al., 1994), cell death induced by α-irradiation is due to apoptosis. Concomitantly, we have performed some pilot experiments after vital staining of rat macrophages obtained in animals previously exposed to 237NpO2 or industrial PuO2 aerosols (lung deposit of several kBq). No apoptosis could be observed in the lavaged cells but significant death occurred in vitro after 6–8 h in culture, which was not observed in the controls. From these results it appears difficult to extrapolate from in vitro to in vivo conditions as concerns radiation-induced cell death. However, one could expect that the half-life of apoptotic cells is very short in vivo, so that the probability of observing such cells is very low. This agrees with the fast disappearance of millions of endoalveolar polymorphonuclear cells in vivo, which occurs within a few hours (Fritsch and Masse, 1992).

CONCLUSION

Because of the slow death induced in vitro after α-irradiation of alveolar macrophages, the dose-
effect relationship we have previously reported for cell survival was overestimated by about a factor of three. Nevertheless, both rat and primate alveolar macrophages appear as relatively radioresistant cells (single negative exponential function of dose with a \(D_0\) of \(\sim100\ \text{Gy}\)). A similar radiosensitivity is expected in humans.

Simulations were performed to estimate the toxicity of different inhaled actinide oxides assuming that 1000 \(\alpha\) hits/cell are needed to kill 63% of the alveolar macrophage population (each macrophage is supposed to contain one particle). We have considered the default granulometry in the workplace (activity median aerodynamic diameter of 5 \(\mu\)m, \(\sigma_g = 2.5\)) (ICRP, 1994), which corresponds to a median aerodynamic diameter of \(\sim0.4\ \mu\text{m}\). Assuming a density of 9 and a shape factor of 1.5, the geometric diameter was 0.16 \(\mu\text{m}\). For a \(^{239}\text{PuO}_2\) particle we calculated that 1000 \(\alpha\) particles were emitted in about 2.5 days. This value is far lower than 7–10 days, the half-life of alveolar macrophages measured \textit{in vivo} (Fritsch and Masse, 1992). Thus, as previously reported (Lizon et al., 1997), significant alveolar macrophage death could be induced after inhalation exposure to actinide oxide aerosols which have a similar or larger \(\alpha\) specific activity than \(^{239}\text{Pu}\).

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\textbf{REFERENCES}


