Increased Effective Half-Life of Intratracheal Administered

$^{51}\text{Cr}_2\text{O}_3$ by Respiratory Infection*

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D. A. Creasia, P. Kettesheim and Anna S. Hammons

Carcinogenesis Program, Biology Division, Oak Ridge National Laboratory

Oak Ridge, Tennessee 37830

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Consideration of the multiple factors that govern the degree of hazard from any exposure by inhalation is perhaps the most critical step in obtaining a realistic evaluation of the potential dose from airborne radionuclides. These factors not only include the physical, radiological and chemical properties, but also the degree of interaction of the radionuclide with the biological system (e.g., radiation or chemical damage). The interaction of the biological systems with the radionuclide, in turn, depends not only on physical properties but also on the "condition" of the biological system at the time of exposure. With airborne radionuclides the main route of entry into the biological system is through the respiratory tract. For insoluble (or poorly soluble) radionuclides that are deposited in the respiratory tract, the most important biological factor that will govern exposure, is respiratory clearance. It therefore becomes of paramount importance to ask the questions: what events, or under what conditions might respiratory clearance be altered? One possible condition that might cause alteration is respiratory infection. Despite the prevalence of respiratory infection, little attention has been given to the possible role that respiratory infections might have in altering pulmonary clearance. Because respiratory disease is so common and because pulmonary clearance is so important as a defense mechanism against inhaled substances, we felt it important to investigate their possible interaction.

MATERIALS AND METHODS

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(BC3F₁) (C57BL/6 Ba [SPF] X C3HF/Ba [SPF]) female mice 13-15 weeks old were used throughout the study. They were raised and maintained in a specific pathogen free mouse colony¹, housed 5-10 per cage and allowed free access to food and water. The mice were anesthetized with ethyl ether for viral inoculation and with 1.0 mg of sodium pentobarbital in saline given intraperitoneally for the intratracheal instillation of ⁵¹Cr₂O₃. The strain of influenza virus used was PR8-34-60 and was obtained from the University of Michigan (School of Public Health, Ann Arbor, Michigan).

The experimental design and techniques have been described in detail elsewhere². Briefly, the mice were intranasally inoculated with a 10% lung homogenate diluted 10^{-7.3} containing the influenza virus. This was usually sufficient to infect 95% of the mice, as indicated by hemagglutinin inhibition (HI) titers 2 weeks after virus inoculation. Control mice received an intranasal inoculum of appropriately diluted lung broth obtained from uninfected mice.

⁵¹Cr₂O₃ was used in this study because it had previously been shown to be insoluble and "physiologically inert"^{3,4}. The Cr₂O₃ was activated by neutron bombardment and had a geometric mean diameter of 0.12 μ. 10.0 μg of ⁵¹Cr₂O₃ (~250,000 cpm) contained in 25.0 μl of a suspension medium (sterile saline containing 100 IU/ml of penicillin and 50 μg/ml of streptomycin) were administered by intratracheal (i.tr.) injection using a 100 μl Hamilton syringe. Aggregation of particles was prevented by repeated ultrasonication.

Immediately after instillation of the $^{51}\text{Cr}_2\text{O}_3$, the amount of radioactivity delivered to each mouse was determined by whole body counting using an Armac whole body counter connected to a Packard scintillation counter. The data are expressed as percentages of the original lung burden.

RESULTS

Loosli⁵ has previously described in detail the pulmonary pathology developing from infection with PR8 influenza virus in mice. Our observations are very similar to his. Typical histopathological changes during the 2nd week of viral infection are shown in Fig. 1. Fifty uninfected mice (4 groups of 10 and 2 groups of 5 mice) were used as controls in the $^{51}\text{Cr}_2\text{O}_3$ elimination study. Originally each control group was age matched to an experimental group. Since the decrease in activity was very similar in all groups of pathogen free mice with little animal to animal variation, the data obtained from these animals were combined (see Fig. 2, shaded areas indicate range). It should be noted that the effective half life of $^{51}\text{Cr}_2\text{O}_3$ for these controls is approximately 7.5 days. At the termination of the experiment (90 days) the average amount of radioactivity remaining in control animals was 0.8% of the initial lung burden. Watson⁶ has recently shown that pulmonary clearance from the deep lung is independent of route of administration. Since our $^{51}\text{Cr}_2\text{O}_3$ particles are of an easily respirable size (0.12 μg GMD) we feel that the i.tr. application of the $^{51}\text{Cr}_2\text{O}_3$ has not created an extreme artifact.

When the animals were sacrificed at 90 days, the lungs were examined grossly and histologically. The distribution of the remaining $^{51}\text{Cr}_2\text{O}_3$ was determined with standard autoradiographic techniques. None of the control

mice showed any macro or microscopic lung lesions. The autoradiograms from these control animals show that the radioactive particles have remained well dispersed. The few remaining particles are mostly retained in alveolar wall cells (Fig. 3).

Eight experimental groups of infected animals received i.tr. administration of $^{51}\text{Cr}_2\text{O}_3$ at the following intervals before and after viral inoculation: -2 days, 0 time (i.e., within minutes following viral inoculation), 1, 3, 5, 7, 9, and 56 weeks. A total of 66 infected animals were used in this study and the data from each group represents the mean of at least five animals. Radioactivity was followed for >90 days after i.tr. injection of $^{51}\text{Cr}_2\text{O}_3$ except for the 56 week group which was terminated after 60 days. Immediately after the last whole body count, the mice were sacrificed and the extent of gross lung pathology determined. At this time the distribution of the remaining radioactivity in individual lobes was also determined.

In all eight groups tested, PR infection produced a marked inhibition of the pulmonary clearance mechanism. The inhibition of clearance was most notable when the $^{51}\text{Cr}_2\text{O}_3$ was administered 2 days before, simultaneously with, or 7 days after infection with the virus, i.e. prior to maximum development of pneumonia (see Fig. 4). The average effective half life ($T_{1/2}$) for these three intervals is 10, 13 and 16 days respectively as contrasted with the $T_{1/2}$ for the controls, which is only 7.5 days. Around day 90 the amount of radioactivity retained in the noninfected animals is approximately 6.08% while that retained in the infected mice is about 5.5% of the initially administered dose.

Autoradiograms from the lungs of mice in these three groups show that most of the radioactivity is found in completely consolidated lobes. Within the consolidated lobes the radioactive dust is usually found in large clumps often associated with lymphoid aggregates (Fig. 5). The tissue surrounding these "hotspots" probably receive a far greater radiation dose than would be indicated from the total lung burden.

The radioactivity found in the non-consolidated lobes, showing only microscopic evidence of pneumonia and scarring, tended to remain dispersed as in the non-infected animals. However, the retained activity was always ^{in these lobes} greater than in the lobes of non-infected animals.

In Fig. 6, the 3, 5, 7, 8 and 57 week intervals after viral inoculation are summarized. The one week interval (from Fig. 3) is included for comparison. At this time after viral inoculation the acute pneumonitis has subsided and fibrotic and metaplastic changes develop. The $T_{1/2}$ for $^{51}\text{Cr}_2\text{O}_3$ administered at these time points range from a high of 13.0 days (5 week interval) to a low of 9 days (7 and 9 week time points). When the animals were sacrificed 30 days after $^{51}\text{Cr}_2\text{O}_3$ deposition the residual activity remaining in the infected animals is 3 to 5 times greater than in the controls.

Evaluation of the autoradiograms from the lungs of mice included in these groups show that very few radioactive particles are retained in the consolidated lobes. Instead the majority of the retained activity is found in the non-consolidated lobes. These lobes show definite microscopic evidence of passed infection (i.e., focal fibrosis and thickened alveolar walls). The $^{51}\text{Cr}_2\text{O}_3$ particles are found in the thickened alveolar septa of these lung lobes (Fig. 7).

Figure 8 summarizes the data showing the effect of PR8 influenza infection on the Tl/2 for $^{51}\text{Cr}_2\text{O}_3$ and compares the effect on the Tl/2 of varying time interval between infection and radionuclide exposure. The Tl/2 of $^{51}\text{Cr}_2\text{O}_3$ in specific pathogen free mice is 7.5 days. In infected animals the Tl/2 is increased 1.3-2.1 times over that of the controls when the $^{51}\text{Cr}_2\text{O}_3$ is administered prior to the acute stages of the infection (time interval of -2 days, 0 time and 1 week). When the $^{51}\text{Cr}_2\text{O}_3$ is administered at a time in which the acute infectious episode is subsiding (>14 days after viral inoculation) the Tl/2 of $^{51}\text{Cr}_2\text{O}_3$ is slightly shorter; however, it is still 1.5 times that of the controls even when the $^{51}\text{Cr}_2\text{O}_3$ is administered more than one year after viral inoculation. Judging from the 57-week interval, the lung apparently never fully regains its normal ability to clear particles.

DISCUSSION

The data indicate that lower respiratory tract infections may produce a pronounced and long lasting effect on deep lung clearance. The impairment appears to be maximal when the dust exposure occurs prior to the acute stage of infection; more radioactivity is retained in lungs of infected than of uninfected mice 90 days after $^{51}\text{Cr}_2\text{O}_3$ application. Severely impaired particle clearance was still observed when the dust exposure occurred as late as 57 weeks after viral inoculation. In the case of PR8 influenza virus infection the involvement of lung parenchyma in the inflammatory process is widespread. An acute episode of pneumonitis (lasting 2-3 weeks) is followed by a chronic and even permanent phase of fibrosis and alveolar metaplasia. This may explain why the disturbance of the deep lung clearance from PR8 infection is so long lasting.

In this study we use ^{51}Cr as the radionuclide which has a relatively short half life of 27 days. In Fig. 4 it can be seen that, depending on when the $^{51}\text{Cr}_2\text{O}_3$ is deposited, the Tl/2 of $^{51}\text{Cr}_2\text{O}_3$ may be increased by the infection 2.1 times over control values. The information which cannot be gleaned from Fig. 4 is that physical decay of the ^{51}Cr radionuclide has become the controlling factor in determining the residual activity in the lung of infected animals. Fig. 9 shows that pulmonary clearance of $^{51}\text{Cr}_2\text{O}_3$ deposited before the acute stage of infection all but ceases. It therefore follows that the major portion of the radioactive decrease in the lung is through physical decay and the decrease in activity shown in Fig. 4 is mainly physical decay. If a radionuclide has a relatively long half life compared to the biological half life, physical decay will exert a minimum influence on the ^{amount of} residual radioactivity remaining in the lung. To stress the potential consequences of the interference of respiratory infection with clearance we recalculated the 0 time data from Fig. 4 using 1.0 year as an arbitrary physical half life for a radioactive particle otherwise similar to $^{51}\text{Cr}_2\text{O}_3$ (instead of the 27 day half life) (Fig. 10). With this type of calculation, the Tl/2 of noninfected mice is increased from 7.5 to 12 days. In the infected animals pulmonary clearance has almost ceased (see Fig. 9) and the curve indicating the residual activity remaining in these animals has been severely altered from the previous 0 time curve shown in Fig. 4. Ninety days after exposure to the hypothetical radionuclide there is still more than 50% of the initial radioactivity remaining in the lungs of the infected mice. In contrast, only 8% of the initial lung burden remains in the noninfected mice.

In conclusion, we have shown that an infection of the lower respiratory tract, such as influenza virus pneumonia can increase the duration of exposure to radiation from inhaled radioactive dusts. It must be pointed out, however, that infection per se is not responsible for the increase $T_{1/2}$. Rather it is the extent and persistence of inflammatory and scarring processes that determine the degree of radionuclide retention. The degree of tissue damage evolving from infections is not readily predictable nor can it be easily quantitated. This introduces a great uncertainty into calculating an effective half life for estimating a radiation dose sustained from inhaled radionuclides.

It is not unlikely that other types of respiratory infections and injuries induced by noninfectious agents (particularly those that cause long lasting fibrosis and metaplasia) may have similar effects.

LEGENDS

Fig. 1. Typical pathological findings in the lungs of mice infected with PR8 influenza virus. ~~Acute stage of pneumonia is shown.~~ Note (b) (2 months after virus inoculation (31x)) exudate and cellular infiltration in upper part of lung lobe, (a) hyperplastic response of bronchial epithelium and peribronchial infiltrate (10 days after virus inoculation (375x))

Fig. 2. Fraction of i.tr. administered $^{51}\text{Cr}_2\text{O}_3$ remaining in the respiratory tract of uninfected mice as a function of time. Solid line represents average of 50 mice; shaded area indicates high-low range of values encountered. Data are expressed as % of the initial lung burden.

Fig. 3. Autoradiogram from the lung of an uninfected mouse sacrificed 90 days after $^{51}\text{Cr}_2\text{O}_3$ deposition. Very few $^{51}\text{Cr}_2\text{O}_3$ particles remain ($\rightarrow$). Those remaining are mainly associated with cells of the alveolar septum.

H&E 375 X

Fig. 4. Fraction of ^{51}Cr remaining in the lungs of influenza infected mice as a function of time. $^{51}\text{Cr}_2\text{O}_3$ was administered by i.tr. injection at indicated times before and after viral inoculation. Data are expressed as a remaining percentage of the initially deposited activity. Averages of control animals are from 50 noninfected mice, means of PR8 infected groups are derived from groups ranging in size from 5-11 animals.

Fig. 5. Autoradiograms from the lung of an infected mouse which was i.tr. injected with 10 μCi $^{51}\text{Cr}_2\text{O}_3$ immediately after viral inoculation (day 0). The animal was sacrificed 90 days later. Aggregates of labeled material are found in the inflammatory and scar tissue of atelectatic lung. H&E 375 X

Fig. 6. Fraction of ^{51}Cr remaining in the lungs of infected mice in which the $^{51}\text{Cr}_2\text{O}_3$ was deposited after the acute stage of infection. The group receiving $^{51}\text{Cr}_2\text{O}_3$ 57 weeks after viral inoculation was sacrificed 60 days later and data are extrapolated to day 90 after i.tr. injection. Data are presented as percent of initial activity and are means from groups comprising at least 5 animals. One time interval (day 7) from a group receiving $^{51}\text{Cr}_2\text{O}_3$ before the acute infectious stage is shown for comparison.

Fig. 7. Autoradiogram from the lung of an infected mouse and sacrificed 90 days after $^{51}\text{Cr}_2\text{O}_3$ application. The mouse received 10.0 μg $^{51}\text{Cr}_2\text{O}_3$ 9 weeks after viral inoculation. Label is associated with cells of the alveolar septum ($\rightarrow$); preferentially in areas showing increased cellularity and thickening of the alveolar walls. H&E 375 X

Fig. 8. Summary of the effect of PR8 infection on the effective half life of i.tr. applied $^{51}\text{Cr}_2\text{O}_3$. Hatched area of the bars represent the mean $\text{TL}/2$ from 50 noninfected mice. *$T_{1/2}$ is the time needed for reduction of ^{51}Cr activity to 50% of initial lung burden.*

Fig. 9. Clearance of i.tr. injected $^{51}\text{Cr}_2\text{O}_3$ ~~particles~~ (corrected for physical decay) applied on the same day as viral inoculation. Closed circle (O) indicates mean of 50 noninfected mice. Shaded area represents high-low range. Open circles (O) represent values obtained from whole body counts of individual animals. Data are expressed as percent of the initial $^{51}\text{Cr}_2\text{O}_3$ dose.

Fig. 10. Effect of PR8 infection on the $\text{TL}/2$ of an hypothetical radionuclide with a physical half life of 1 year (and a biological half life equal to that of $^{51}\text{Cr}_2\text{O}_3$) administered on the same day as PR8 virus inoculation.

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