Role of Alveolar Macrophages in Precipitation of Mineral Elements Inhaled As Soluble Aerosols

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Introduction

Previous investigations have shown that the phagolysosome of the lung macrophage has the capacity to dissolve solid particles of low aqueous solubility (1,2). We show in this paper that the inverse is also possible, namely, that the lysosome of the lung macrophage is also able to concentrate and precipitate several elements inhaled in water-soluble form. This particular lysosomal function may prevent the diffusion of many chemotoxic or radiotoxic elements to the bloodstream.

Materials and Methods

Animal Experimentation

The following water-soluble mineral salts were studied: chromium chloride (CrCl3), cerium chloride (CeCl3), uranyl nitrate (UO2(NO3)2•6H2O), and aluminum chloride (AlCl3). Submicron aerosols were generated from 1% solutions of these compounds and administered to rats in a facility that has been described previously (3). Six male Wistar rats, with a mean body weight of 250 g, were exposed for 5 hr per day for 5 days. The animals were killed on day 5, 3 hr after the termination of exposure. The particle number concentration measured in the chamber was $15 \times 10^3$/cm$^3$, resulting in a mass concentration of 10–30 $\mu$g/cm$^3$. The mean diameter of the particles was about 0.1 $\mu$m.

Microanalytical Methods

The ultrastructure of the cells in lung sections was examined using a Philips EM 300 microscope. The intracellular localization of the elements was determined using an electron probe Camebax Cameca microanalyzer equipped with thallium acid phthalate (TAP), pentaerythritol (PET), and lithium fluoride (LiF) crystals. An electron microscope specially adapted to this apparatus allowed us to examine ultra-thin sections, select zones of interest, and focus the electron beam on the intracellular organelle to be analyzed.

The conditions of analysis were as follows: electron acceleration voltage, 45 kV; probe diameter, 500 nm; probe current, 150 nA. Under these conditions, elements present under the microscope at a concentration greater than 100 ppm could be detected.

The $K\alpha$ line of phosphorus and aluminum were detected with the TAP crystal at $\sin\theta = 0.23969$ and $\sin\theta = 0.3463$, respectively. The $K\alpha$ line of chromium was detected with the PET crystal at $\sin\theta = 026072$. The $M\alpha$ line of uranium was detected with the PET crystal at $\sin\theta = 0.44692$ and the $L\alpha$ line of cerium at $\sin\theta = 0.29279$.

We made counts on the line of the element analyzed and measured background noise on either side. Mean background noise was then subtracted from the counts obtained on the line. This operation was repeated three times for each inclusion analyzed.

Results

Electron microscopy and microanalysis permit the determination of the precise intracellular localization of the deposit and its ultrastructural appearance for each element.

Lung Sections from Rats Exposed to Uranyl Nitrate Aerosol

Exposure of rats to UO2(NO3)2•6H2O caused abnormal deposits
in macrophages and cells, localized in the lysosomes that consisted of very dense and thick deposits of needles about 30 nm in length. The needles were sometimes isolated but most were clustered. The Kα line of phosphorus and the Lα line of uranium were detected in all these deposits by microanalysis (Fig. 1).

**Lung Sections from Rats Exposed to Aluminum Chloride Aerosol.** The deposits observed after exposure to AlCl₃ in the lysosomes of macrophages had two different ultrastructures. The first type consisted of dense spherules of different dimensions. Their contours are well defined, and they are composed entirely of aggregates of very fine granules. The second type consisted of either very dense aggregates (about 3 nm) or of undulated filaments within the lysosomes.

**Lung Sections from Rats Exposed to Chromium Chloride Aerosol.** In the alveolar macrophages, the deposits observed in phagolysosomes of rats exposed to CrCl₃ consisted of fine granules grouped in clusters. These were often concentrated in dense masses forming thick undulated filaments. In some lysosomes these filaments were abundant and formed parallel strata.

**Lung Sections from Rats Exposed to Cerium Chloride Aerosol.** In the macrophages of rats exposed to CeCl₃, we observed lysosomes containing dense deposits and other lysosomes containing myelinlike configurations. The deposits were localized in the dense lysosomes in the form of aggregates of either fine granules or of fine needles (30 nm in length). In the lysosomes containing myelinlike configurations, deposits were localized at the periphery in the form of granular aggregations. In the lysomes, the deposits were primarily in the form of fine needles. The Kα line of phosphorus and the Lα line of cerium were detected on all these deposits by microanalysis (Fig. 2).

**Discussion**

The role of lysosomes in the mechanism of concentration of certain mineral elements has been studied previously in renal cells (4-6). When the elements Al, U, Cr, and Ce are injected in soluble form, they are concentrated in the lysosomes of renal proximal tubule cells as insoluble phosphates. The intralysosomal concentration of these elements in phosphate form suggests a mechanism dependent on the enzymatic potential of the lysosome. It is known that this organelle contains numerous enzymes including acid phosphatases. These enzymes release free phosphate ions from different substrates. The phosphate ions thus released can precipitate elements such as Al, U, Cr, or Ce in an acid medium. This in vivo precipitation depends on a mechanism identical to that observed in vitro in the well-known cytochemical reaction of Gomori. The Gomori reaction is a chemical method that allows the microscopic visualization in a tissue section of organelles with high phosphatase activity. After incubation of a tissue section with a solution containing a soluble lead salt, the lead is precipitated as an insoluble lead phosphate salt in organelles containing acid phosphatase, namely, in lysosomes. Other elements such as Al or Ce can also be precipitated as insoluble phosphate salts in lysosomes by the same cytochemical method.
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