EFFECTS OF MINERAL DUSTS ON ULTRASTRUCTURE AND FUNCTION OF ALVEOLAR MACROPHAGES

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ABSTRACT

The effects of mineral dust on biomembrane and organelle in alveolar machrophages in vitro were studied. The results suggest:

1. When the mineral dusts were concentrated at the secondary lysosome in the alveolar macrophages in vitro it indicates that the structure of cytoplasma is always normal. Swelling and degenerative mitochondria, abnormal structure cytoplasma and separated karyolemma were observed when silicon dust spread in cytoplasma.
2. The investigation of the effects of dusts of quartz, asbestos, graphite, TiO_2, Be, Sb etc. on biomembrane of alveolar macrophages in vitro indicated that they differed greatly at the same concentration.
3. It is postulated that stress should be put on choice of drugs with protection effect on cytomembrane. Such as VitE, SOD, PVPNO and piperaquie in order to prevent cell damage.
4. The study on effects of the four kinds of mineral dusts on protein synthesis in alveolar macrophages was carried out by incorporation of ^{5}H-Leu.

INTRODUCTION

Alveolar macrophages play an important role in the onset and development of the diseases in the lung, especially during the process of pulmonary fibrosis in pneumoconiosis.[1-2]

Scientists in various country have paid special attention to the investigation of the relation between macrophages and the factors which cause diseases. In this investigation the electron microscopy had been applied to study the effects of different dusts on the morphorological changes of cell membrane and organellae and to observe the protective effects of several kinds of drugs to cell membrane. Radioactive isotope tracer technique has been used to determine the synthesis of protein to illustrate the toxicity of alternative way.

MATERIAL AND METHOD

Dusts

The content of free SiO_2 in quartz is 99%. All asbestos are UICC product produced in Germany. The Particles of Sb_2O_3 dust are less than 1 μm. No free SiO_2 was found in it. The purity of TiO_2 was more than 99%. All the dust particles were less than 5 μm. the particles of graphite and Be dust were less than 5 μm. There was no SiO_2 to be found. All of the dust was steriled by autoclaving and a 1 mg/ml solution was prepared with medium. Before using mixer. It was stirred thoroughly with a magnetic.

^{5}H-Leucine

The activity of the solution was 3.7×10^5. Bq/ml(100μ Ci/ml)

Drug

VitE was in capsules. SOD was obtained from the laboratory of Suzhou Medical College. Piperaquine and P_{204} were unprocessed powder.

Alveolar Macrophage (AM)

AM were obtained by pulmonary lavage of guinea pigs using RPMR-1640 at sterile condition. Lhe cell suspensions were cultured with quartz and TiO_2 dust at different concentrations. Control cultures were treated similarly except that the dusts were omitted. After 6 and 15 hr incubation respectively the AM were collected by centrifugation. The precipitates were fixed. The samples were examined in transmission EM. 0.5 ml cell suspension was transferred into culture bottle with a sterile cover glass slip. After 2 hr incubation the cover glass slips were taken out. Then the cover glass slips were placed in the bottles with 199 medium 100 μg of quartz, asbestos, Sb, Be, graphite and TiO_2 dust were added respectively with the exception of the control. After incubation, the samples were taken out at different intervals.

The experiment of protein synthesis in vitro was carried out

in Hank's solution.[3] One milliliter of AM suspension at a concentration of 1×10^6/ml was put in centrifuge tubes and the dust of quartz, asbestos, graphite or TiO_2 was added to make the final concentration at 100 mg/ml. All tubes and the control were incubated at 37°C for 2 hr. After incubation 7.4×10^4 BQ(2 μCi) labeled leucine was added to each aliquot and incubated for another 3 hr. The same amount of labeled leucine was added to the control after incubation. The protein was collected and the activity was counted by liquid scintillation counter.

RESULTS

Effect of SiO_2 on Damage of Organella

Under the TEM one can find that pseudopodia disappeared, lysome disrupted Si particles existed in cytoplasm freely, mitochondria expanded pyknosis, necrosis of some cells appeared losing normal cytostracture with vacuolar changes of matrix, presence of spare cytoplasm, nuclei were expanded to become round or oval, matrix vacuolar change in nuclei, heterochromatin condensed under the nuclear envelope, expending or nuclear envelope disrupting were observed. In some cases the content of nuclei became homogeneous and showed a medium electron density. It was completely impossible to distinguish euchromatin, heterochromatin and nucleolus. The boundary of cells became indistinct. Cells were disrupted finally.

Damage to cells caused by TiO_2 was less than that caused by SiO_2. In this case the majority of cells are in stress shown by expanding of cell volume, increasing of pseudopodia, phagosome and rougb surfaced endoplasmic reticulum. No necrosis cells were observed in the control.

The Effect of Quartz and Other Dusts on Cell Membrane

The investigation of the effects of quartz, asbestos, graphite, TiO_2, Sb, Be on the biological membranes has demonstrated that AM be different in response to different kinds of dusts. Among them the damage effect of quartz to membrane is the most obvious one. No abnormal changes of cell membrane were observed in the control in which cells had been cultured for different periods of time. The majority of these cultured adhesive AM cells in vitro coming from healthy rabbits were round, oval and astroid. There were evenly spreading ruffles at the cell membrane with irregular margin. Long or short filopodia, finger-like and pseudopodia were observed at cell membrane (Figure 1). The static pseudopodia were less and the active pseudopodia were more. When the pseudopodia accept a stimulating information it stretched itself to the foreign body and the cell at the opposite side then the cell moved to the foreign body (Figure 2).

The response of AM cultured in medium to Si dust was active. The change of membrane was characterized by the following features: (1) uneven ruffles were present first then disappear gradually and the cell membrane becomes homogeneous and smooth (Figure 3); (2) vacuoles were present at the surface of cell (Figure 4); (3) various size of holes were present at cell membrane (Figure 5); (4) pseudopodia and microvilli disappeared.

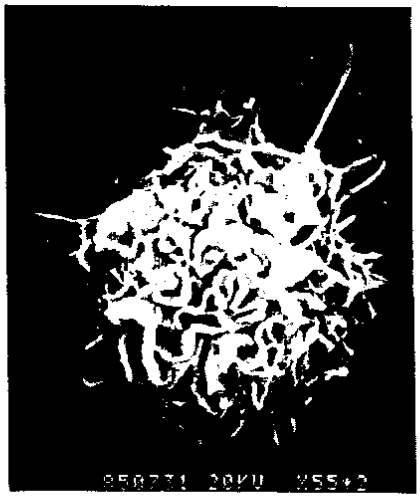


Figure 1. Control M ×5500.

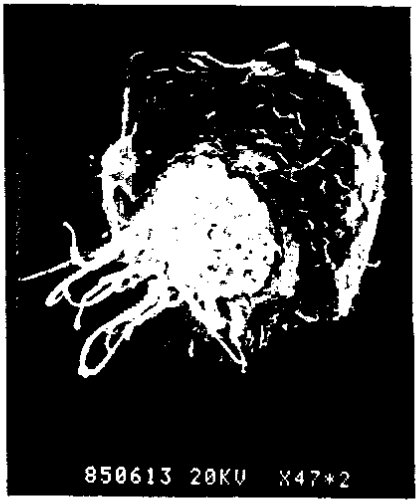
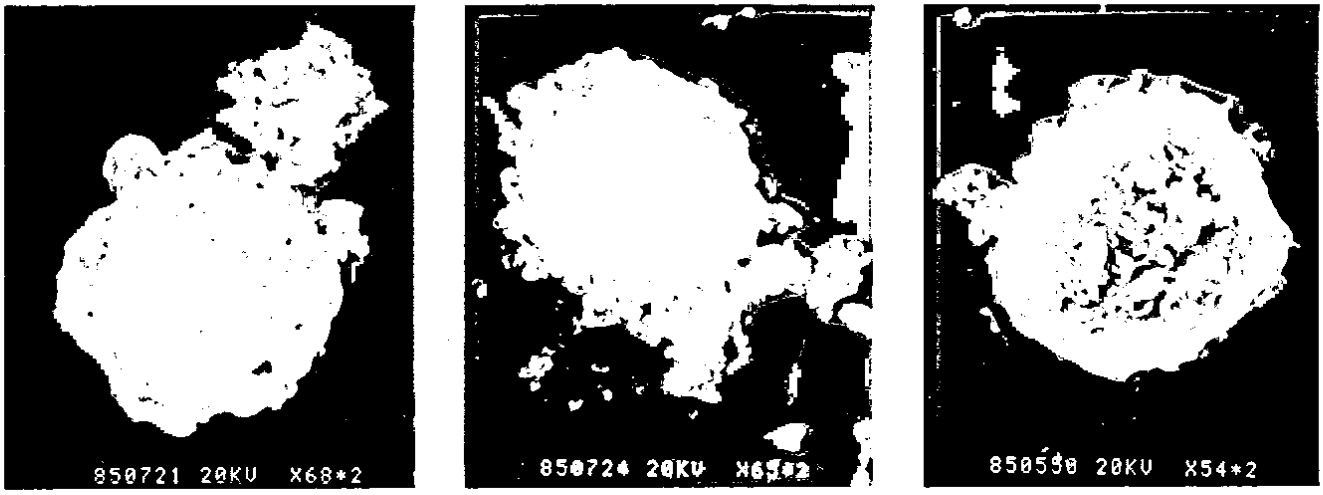


Figure 2. Pseudopodia and skirt margin ×4700.

In the AM cultured with asbestos fibers attachment was observed in the samples taken at the 5 min after the adding of the dust. The response of AM to different length of asbestos fibers was different. The short asbestos fibers were absorbed locally and were phagocytosed in situ. In this case the response was not obvious and the change of cell membrane was less. As the long asbestos fibers were concerned, the cells were phagocytosed in a sleeve-like fashion or phagocytosed the dust from the near end of the asbestos fibers by stretching numerous small pseudopodia at the surface of the cells. Some macrophages could pbagocytose a large amount of asbestos (Figure 6). Such kinds of cell were more often to be observed at the interval of 18 hr and 24 hr samples cultured and structure of cell membrane and its morphology were normal. The phaocytosis in the cells cultured with Si dust was different from that in the cells cultured with



Figures 3–5. After adding SiO_2 and incubated at 37°C for 30 min. Ruffles are uneven and pseudopodia disappear, ×5400. The vacuole structure is present at the surface of membrane. The membrane is even, ×6500. Various sizes of holes are present at the pyknosis membrame, ×6800.

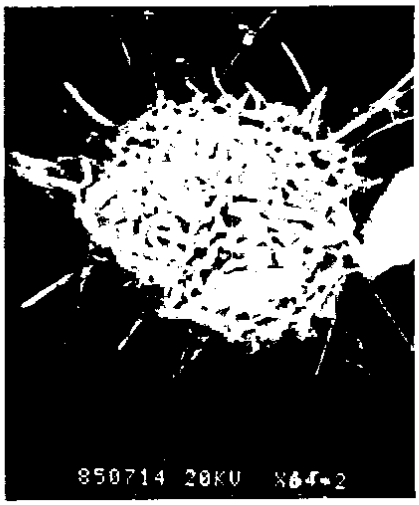


Figure 6. After adding asbestos fibers and incubating at 37°C for 10 hrs randomly fibers are present. ×6400

asbestos. In the latter case the cells aggregation should be observed on the cover glass slips and more cells phagocytosed a bundle of asbestos in common. As the culture time prolonged the dying cells increased gradually. The cell membrane interrupts before dying, the membrane ruffles disappeared and membrane dissolved locally. Comparing with that in Si group the dying of cell delays.

When Sb, Be, Graphite and TiO_2 dusts were added into the medium with AM and incubated for 5 hr the difference of cell membrane was very significant. In the group of Sb dust filopodia stretched themselves to various directions, and disorder of ruffles observed. The reaction of cells were very strong (Figure 7). In the group of Be dust straw-hat-like changes were observed in the majority of cells (Figure 8). In the group of graphite no obvious changes in the cell membrane were observed. In the group of TiO_2, large amount of absorbed TiO_2 particles which were phagocytosed in situ were observed. Some dust particles were taken by the flattened pseudopodia and there were partially morphorological changes of cell membrane. Ruffles at cell membrane could still be observed. It seems that TiO_2 has less effect on membrane structure.

Protection Effect of Drugs on AM Cell Membrane

SiO_2 was used as a cell damage agent. Cells were cultured in vitro and SiO_2, VitE, SOD, 4% P_{204}, and piperaquine were added to the cell suspension to make the final concentration to be 100 μg/ml, 40 μl/ml, 40 μl/ml, 100 μg/ml respectively. The samples for SEM were prepared at the 5 hr and 10 hr interval after the addition of drugs. All of the 4 drugs have protective effect on cell membrane. After the addition of these drugs AM were very active and the activity of pseudopodia was very frequent. Among them the effect of Vit E and SOD were more obvious.[4] Incorporation of H-Leucine in Cultured Cells

The effect of 4 kinds of dusts on protein synthesis in AM were shown that suppressive effects on protein synthesis the asbestos dust is the most significant one. The ratio to control counterparts for protein synthesis was 54.6%. The effect of graphite was the minimum.[4] (Table I)

DISCUSSION

AM is one of the most effective cells for phagocytosis. It plays an important role in removing foreign bodies in lung.

Figure 7. After adding Sb dust and incubating at 37°C for 5 hrs. filopodia are present, ×3300.

Figure 8. After adding Sb dust and incubating at 37°C for 5 hrs straw-hat like margin is present, ×4900.

Table I
Effects of 4 Kinds of Dusts on Protein Synthesis in AM

Sample	No	Viability %	Counts cpm/10^5 AM	Correcting to living cells	Ratio to the control
Control	7	96	26346	27443	100
Quartz	13	85	15838	18629	67.9
Asbestos	9	90	13478	14975	54.6
Graphite	8	89	22313	24792	91.2
TiO_2	10	87	17825	19375	74.7
*Blank			1221		

*H-Leu was addid at the end of incu bation in blank.

Recent investigations demonstrate that the function of AM have close relation with various pulmonary diseases e.g., infection, tumor and pulmonary fibrosis. Great attention has been paid to the investigation of morphorology, function and metabolism of AM and its interrelation to other kinds of pulmonary cells. Phagocytosis to 15 kinds of dust in AM in vitro has been investigated by successive photography by Diavert revert phase contrast microscope.[5] During the process of phagocytosis the change of cell membrane and response of cells were obvious. In order to know the mechanism of phagocytosis the effect of quartz dust etc. on organellae has been investigated. The results obtained demonstrate that the toxic effect of SiO_2 on lysosome is present most early. The toxicity of quartz is closely related to its concentration and the duration of action.

The response of cell membrane to different kinds of dust or different length of fiber dust particles of the same dust was different.[6] Brains[7] has reported that when the cells contact with foreign body, absorption occurs first and the change of the absorption depends on the chemical and physical properties of the dust particles. During phagocytosis, the increasing of the cell volume, energy metabolism and membrane receptors were observed. In this investigation the holes of cell membrane at the site of absorption and formation of phagocytosis vacuoles to quartz were observed in the process of AM.

The way of short asbestos fiber phagocytosis is the same to that of SiO_2. But the phagocytosis was slow and only a slight change of cell membrane was observed; while the long

asbestos fiber can be phagocytosed by one or more than one cell which caused aggregation of cells. The ability of phagocytosis of AM to dusts of quartz and asbestos may be related to C_3 receptor and Ig G receptor.[8-10] Sb dust caused the formation of a large amount of filopodia, intensive cell response and obvious morphological changes of cell membrane. Be dust caused the formation of more straw-hat-like cells which will be studied further.

In the aspect of morphological change of the cells the membrane change caused by SiO_2 was the fastest one and damage was obvious. Asbestos had less effect on AM. After phagocytosis of a large amount of fibers only less changes of cell membrane were observed until 24 hr after addition of dusts of asbestos. During the process of examination of AM cells in vitro by successive photography we found that cells can still move in peristalsis way and trasmigrate. There are some reports on interaction between mineral dusts and the membrane. The common viewpoint is that the change of membrane is concerned with the electronic structure at the surface of the mineral particle.

Both VitE and SOD are removing agents of free radicals. The picture of SEM demonstrates that they can protect the membrane from being damage. Schlipköter has demonstrated that P_{204} can form hydrogen bonds with SiO_2 in priority competitively to protect cell membrane piperaquie can make the membrane of lysosome stable. In the investigation of the effect of quartz and coal on phagocytosis, Comolli et al[11] found that labeled leucine tracer technique was the most sensitive one for the determination of protein synthesis. After addition of dusts, protein synthesis was observed 2 hr later. The effect of asbestos dust on protein synthesis was the most obvious one while the effect of quartz dust came the second. The mechanism of suppression of protein synthesis is waiting for further study.

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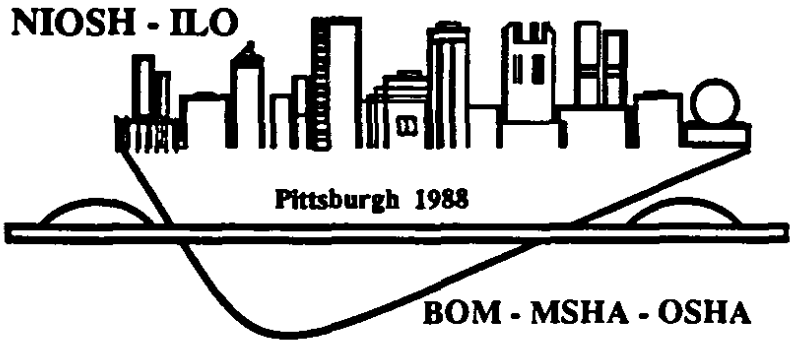

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