EXPERIMENTAL INFECTIVE PNEUMOCONIOSIS: EFFECT OF FIBROUS AND NON-FIBROUS SILICATES AND *CANDIDA ALBICANS* ON THE LUNGS OF GUINEA PIGS

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Abstract: The role of infection (*Candida albicans*) in modifying the fibrogenesis caused by a fibrous platy silicate (talc) and a particulate silicate (kaolin) was studied in the lungs of guinea pigs over a period of 180 days. Kaolin was found to be more fibrogenic in the presence of *Candida albicans* than the talc. The enhanced fibrogenicity has been attributed to the adjuvant activity of kaolin with polysaccharide glucan component of candida.

Key words: Silicates—Candida—Pulmonary fibrosis—Guinea pig

INTRODUCTION

Earlier studies by the authors using a low grade infective stimulus—*Candida albicans*—have shown that the infection enhanced the fibrotic potential of a fibrous silicate amosite much more than a particulate non-silicate manganese dioxide^{1,2}. The present studies have, therefore, been undertaken using a fibrous platy silicate (talc) and a non-fibrous silicate (kaolin) to investigate whether this property of *Candida albicans* is true only for fibrous dust or also holds good for particulate dust.

MATERIALS AND METHODS

Dust

The coarse sample of kaolin was obtained from Chakradharpur and the talc from Jaipur, India. The respirable size dust was prepared by gravitational method³. A weighed quantity of kaolin and talc was suspended separately in physiological saline at a concentration of 75 mg/1.5 ml and autoclaved at 6.8 kg pressure for 20 minutes.

Organisms

The culture of *Candida albicans* (Robin) Berkh was kindly supplied by the Antibiotics Division of Central Drug Research Institute, Lucknow, India. It was maintained on

Table.

Group	Number of animals	Material injected in 1.5 ml of sterile physiological saline per animal
I	25	Organism alone 500 μ g
II	25	Talc dust 75 mg
III	25	Talc dust 75 mg+organisms 500 μ g
IV	25	Kaolin dust 75 mg
V	25	Kaolin dust 75 mg+organisms 500 μ g

Sabourauds agar medium and a 48 hrs old culture was used for animal inoculation.

A series of preliminary experiments performed with varying doses of organisms either alone or mixed with 75 mg of the dust revealed that with 500 μ g of the organisms and dust there was no mortality of the animals during the 15 days period of observation. It was, therefore, decided to use this dose.

Animals and experiments

One hundred twenty five guinea pigs raised in ITRC animal house and weighing between 250~275 g were maintained on pellet diet (Hindustan Lever, Bombay), green leafy vegetables and water *ad libitum*. They were divided into five groups and inoculated intratracheally with dust or organisms as shown in the Table.

Two animals from I, III and V Groups were killed under ether anaesthesia for doing the viable count of the organisms. Two animals from each group were subsequently killed as above at 1, 7, 15, 30, 60, 90, 120 and 180 days after inoculation and the lungs collected for bacteriological and histopathological studies.

Pathological and bacteriological techniques

In order to obtain viable count of the organisms from the lungs, the cardiac lobe was aseptically removed, blotted and weighed. The lobe was then crushed in a Potter-Elvehjem type all glass homogenizer and diluted with normal saline so that each ml of the suspension contained 100 mg of the lung tissue. The suspension was serially ten-fold diluted, inoculated on Sabourauds agar plates and incubated at $37\pm 2^\circ\text{C}$. The colonies from each dilution obtained at 24 and 48 hours were counted and results expressed as number of organisms per gram of the lung.

The remaining tissue of the lung was gently inflated with 10% formal saline to their normal size. It was processed, embedded in paraffin and cut at 5 μ m thickness. Multiple sections were stained with hematoxylin and eosin, silver impregnated for reticulin⁽⁴⁾. Periodic acid Schiffs-Hematoxylin staining was used to demonstrate organisms in sections^(5,6).

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RESULTS

Viable count

In Group I, the viable count of the organisms increased at early periods and became negative by 15 days. In Groups III and V also, it increased but became negative after 30 days in talc and after 15 days in kaolin injected animals.

Group I (Candida albicans)

The animals killed at one day after inoculation revealed an intense polymorphonuclear leucocytic reaction to PAS positive organisms in the parenchyma which at places also involved bronchial and bronchiolar lumen. By 7 days, the acute reaction became replaced by macrophages confined to the interstitial spaces. The septal cells became hyperplastic and on PAS staining only an occasional intact organism could be seen. The reaction had further abated by 15 days and only a few areas of parabronchial accumulation of macrophages remained. Some vascular cuffing developed by 30 days, although rest of the parenchyma had regained normal architecture. At later periods, the histological picture did not change while at the termination of experiment (180 days) only a few isolated foci of lymphoid aggregations could be seen.

Group II (Talc dust)

The animals killed at one day revealed an acute inflammatory reaction and fibrinous exudations in the area of talc deposition. Subsequently by seven days, acute reaction subsided and many focal areas comprised of dust laden macrophages and syncytial giant cells appeared. Some lymphocytic infiltration also ensued adjacent to fixed structures. The interalveolar septa developed thin fibroblastic proliferation at 15 days and there was an attempt at encapsulation of dust. Some fibres also grew into the dust mass. By 30 days, the syncytial foreign body giant cells became prominent in the granulomatous area but the dust laden macrophages began to show degenerative changes. The gradual increase in the fibroblastic activity especially at the periphery of dust areas continued even at 60, 90 and 120 days.

At the termination of experiment (180 days), the dust areas developed mononuclear cell infiltration and thick reticulin fibroblastic proliferation in the interalveolar septa. It caused interstitial thickening and narrowing of alveolar ducts and alveoli.

Group III (Talc dust+Candida albicans)

Marked consolidation and fibrinous exudation over large areas were evident at one day post-inoculation. In addition, occasional PAS positive organisms could be detected in the dust areas. By 7 days, shrinkage of the lesions occurred forming focal areas of chronic granulomatous reaction, comprised of densely packed dust laden macrophages and multinucleated giant cells. The dust laden phagocytes involved the interstitial tissue resulting in increased thickening. By fifteen days, fibroblastic reaction ensued at the

Fig. 1. Guinea pig lung injected with talc and *Candida albicans* at 180 days. Loosely arranged reticulin fibres (reticulin stain $\times 160$).

periphery of dust deposits. The dust loaded macrophages exhibited, at places, varying degree of degenerative changes. Occasional small PAS positive elliptical structures were present in the dust lesions. In addition, areas of lymphoid hyperplasia appeared adjacent to fixed structures. At 30 days, the fibroblastic activity increased in the dust areas and at places intra-alveolar fibrosis ensued. The fibrotic reaction progressed further at 60, 90 and 120 days and, in addition, excessive hyperplasia of lymphoid tissue occurred especially around blood vessels and bronchioles.

By the end of the study (180 days), the dust areas developed increased fibroblastic activity in interstitial area occluding some alveoli. It formed a loosely arranged network of thick reticulin fibrosis (Fig. 1). The lymphoid hyperplasia increased around the blood vessels and adjacent to dust areas.

Group IV (kaolin dust)

The inoculation of kaolin at one day incited an acute inflammatory reaction comprised of polymorphonuclear leucocytes, cellular debris, marked oedematous thickening of inter-alveolar septa and occlusion of alveolar lumen. By seven days, marked phagocytosis of dust particles by septal cells and free macrophages ensued, causing degenerative changes as evinced by globular nuclei. The reaction resulted in proliferation of thin fibroblasts in the interalveolar septa and the dust mass. By 15 days, the reaction had retracted

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into small foci of foreign body syncytial giant cell granulomas. The parenchyma between the foci collapsed accompanied by mild septal fibroblastic thickening especially in those encircling the dust mass. By thirty days, the reaction constricted further, with more crowding of degenerating dust laden macrophages and multinucleate giant cells, and these areas revealed only thin reticulin fibres. At subsequent periods of 60, 90 and 120 days the fibrosis progressed slowly to thick reticulation in the dust foci and the interstitial areas.

At the termination of experiment (180 days), the dust foci became less cellular as most of dust cells developed degenerative changes and many ghost cells formed. Only a few dust laden giant cells persisted in some of the alveoli. Such areas revealed only a loose network of thick reticulin fibres in the dust foci and the interstitial area. The lymphoid hyperplasia in the vicinity of bronchioles and blood vessels persisted.

Group V (kaolin dust+Candida albicans)

The combined effect of kaolin and organisms at one day incited an acute inflammatory reaction similar to kaolin dust. By seven days, the reaction retracted to focal granulomas comprised of dust laden macrophages and syncytial giant cells. The dust foci adjacent to bronchi and bronchioles developed granulomatous changes more often than those in the parenchyma. The alveoli abutting on the alveolar ducts and respiratory bronchioles

Fig. 2. Guinea pig lung injected with kaolin and *C. albicans* at 180 days. Thick reticulin fibres with moderate collagenation (Ret. $\times 160$).

developed thick interstitial reticulin fibrosis obliterating the alveolar lumen with thinner and looser reticulin network. The focal granulomatous reaction involved larger areas of parenchyma by fifteen days. Some of the dust laden macrophages appeared swollen and developed foamy cytoplasm while others progressed to ghost forms or complete necrosis, releasing large amount of debris. Such areas developed only thin reticulin fibrosis. The bronchioles and blood vessels developed lymphoid hyperplasia in their vicinity. The focal granulomas developed interstitial thickening due to increased fibroblastic reaction by 30 days. The fibres formed a loose meshwork enclosing dust masses. At subsequent periods of 60, 90 and 120 days the dust granulomas showed reduced cellularity as more phagocytes developed degenerative changes. The dust foci adjacent to fixed structure like bronchioles and blood vessels developed compact reticulin meshwork with some collagenation.

By 180 days, the granulomas were comprised of a homogeneous dust mass interspersed with lightly staining nuclei of macrophages, giant cells and fibroblastic activity. Such areas developed compact meshwork of thick reticulin fibres and moderate collagenation (Fig. 2). The lymphoid hyperplasia adjacent to bronchioles and blood vessels persisted.

DISCUSSION

Association of an infective factor and dust has been known to modify the pneumoconiotic lesions⁷⁻¹¹, as SPF animals fail to produce characteristic nodular lesions of silicosis^{12,14}. In addition, physical and chemical characteristics of a dust also modify the lesions. In the present study, the biological activity of fibrous platy silicate dust like talc did not seem to depend solely on its structure as it produced only a thin reticulin fibrosis which was not enhanced in the presence of *Candida albicans*. Conversely, the combined action of *Candida albicans* and kaolin produced thick reticulin and collagenous fibrosis. Since kaolin has been known to possess adjuvant properties¹⁵, it would seem quite possible that it might have localised the degeneration products like glucan and other factors of *Candida*, which are known to produce granulomatous lesions^{16,17}. Possibly, this property of kaolin could have contributed to the enhanced biological activity of candida glucan and other somatic components. Further additional studies, with repeated doses of organisms, may provide evidence to support the possible role of glucan and other antigenic substances like mannan in aggravating the dust fibrosis.

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