LESIONAL ASPECTS IN RAT EXPERIMENTAL ASBESTOSIS

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Abstract: Asbestos is a professional diseases related to various injuries including inflammation, fibrosis, even malignancy. The purpose of present paper was to reveal the gross and histological aspects occurring in rat experimental asbestosis. The experiment was carried out 240 days long, on Wistar rats, intraperitoneally injected once with aqueous asbestos suspension. In the end, animals were euthanasiated by prolonged narcosis. Necropsy was done and tissue samples were harvested from both affected and apparently healthy areas. A haematoxylin-eosin and Tricrom Masson staining was made from each sample. The slides were object to minutely examination. Gross evaluation revealed various size granulomas in various locations. Adherence among serous membranes and abdominal organs were also found. Histologically, granulomas, regardless of location, revealed in central area as bestos fibers, fagocytated or not, covered mainly by fibroblasts, capillaries and connective tissue. Those aspects reveal strictly fibroblastic and fibrotic proliferation. However, undifferentiated cell nests and abnormal mitotic figures were found; this cell nests might be developing points for malignant proliferation.

INTRODUCTION

Asbestos is a name for a couple of hydrated silicate fibers, sheared in serpentine and amphibole (Kamp and Wietssmann, 1999). Asbestos fibers generate a strong inflammatory response reflected not just in local injuries, but in various tissues and organs; their effects in patients are known as asbestosis. It occurs in various locations including peritoneum; asbestosis is the major cause of mesothelioma. Asbestos fibers are responsible for various inflammatory and fibrotic injuries but, in advanced stage, they are constantly related to malignancy (Baba, 2002). Spontaneous asbestos related injuries are well known, but just in advanced stages because of difficulties in early diagnosis. Therefore, experimental asbestosis provides a useful tool for understanding early preneoplastic stages, otherwise free for any clinical signs. Our purpose was to revealed early stages in peritoneal asbestosis, in order to understand initiation and promotion in asbestos mediated mesothelioma.

MATERIAL AND METHOD

Experiment was performed during 240 days long, on 18 white male Wistar rats, 150 g average body weight in the beginning and 250 g in the end of experiment, divided into two experimental groups: asbestos inoculated group 10 animals and 10 in placebo group.

Asbestos fibers (antofilit and tremolit) sterile suspension was intraperitoneally injected (1 ml) in experimental group, while the placebo group was injected with sterile saline solution 0.9%.

In the end animals were euthanatized by prolonged narcosis. Gross examination was made first, latter tissue samples from different tissue and organs were collected, fixed in 10% phosphate-buffered formalin and embedded in paraffin wax. Later samples were cut in 4µm
sections and mounted on slides. A haematoxylin-eosin and Tricrom Masson staining was made from each sample.

RESULTS AND DISCUSSIONS

**Gross evaluation** reveals various intensity lesions in all subjects in experimental group, located in serous membranes and abdominal organs. Autopsy revealed granulomas in various size, consistence or location. Sometime, they were the size of a needle top; others were 2-3 mm diameter. Granulomas were white, dens, well circumscribed, or conglomerated providing a proliferative structure spread to the margins of the serous membranes or capsules. They were found on the internal organs (stomach, gut, pancreas, liver or mesenteric linfonodes), in mesentery, peritoneum and abdominal wall.

Granuloma incidence occurs as follows:
- epiplooon was affected in five subjects.
- granulomas located on Glisson’s capsule were found in four subjects, whereas granulomas located under Glisson’s capsule within hepatic tissue were found in other 4 subjects.
- serous membrane of stomach was affected in four animals.
- serous membrane of gut was affected in three animals, whereas colon was affected in four.
- mesentery was affected in four cases.
- one rat showed granuloma located on surface of pancreas, other on abdominal wall.

The granulomas on serous membranes and capsule of internal abdominal organ induce various adherences. Adherences between liver and diafragm were found in four cases, among jejune, mesentery and colon in three, epiplooon and stomach in two cases and one case revealed adherence between stomach and intestine. Some cases showed even an adherential block involving all organs from abdominal cavity.

A particular aspect revealed, also, in previous rapports (Baba et. all, 2001), was hepatomegalia. In two cases, the liver showed distended capsule and rounded margins, lobules were evident. Splenomegalia was, also, seen.

The most commonly occurring lesion in gross evaluation is granuloma, in various size and locations. Often, granulomas induced adherences among epiplooon, mesentery and abdominal organs. Present lesions were similar to those described in earlier reports (Vasilieva et. all, 1998).

**Histology** revealed asbestos fibres in the granulomas core; they were thick and structured in clusters, or thin, spread within central area. Sometime, asbestos fibres were phagocytated by polinuclear cells, surrounded by fibroblasts, confined into poorly filamentous collagen. The fibroblasts were elongated showing tiny elongated nuclei, but fibroblasts with macrophagic appearance were also seen. Tiny asbestos fibres were found not just inside polinuclear cells but into histiocytes and gigantic cells as well. The granuloma periphery revealed high number capillaries confined into fibrillary, circular structures.

Some particularities according to granuloma size were found. In large granulomas, intense fibrocytic reaction was seen in central necrotic areas. Little granulomas revealed tiny asbestos fibres, mainly phagocytated, intense cellularity and lymphohistiocytic infiltration. On stomach and gut, the granulomas were bigger and confined into intense irrigated fatty tissue. Granulomas were attached to serous membrane, but, sometime, they were covered by those membranes; internal location revealed even more cellularity. Intestinal Payer patches
hypertrophy was responsible for villosities detaching and shrinking. Hepatic granulomas revealed thin and isolated asbestos fibres, heterogeneously dispersed within proliferative tissue; often, they were related to atrophy in correspondent parenchyma. Some granulomas were covered by poorly adherent, mesothelial like hypertrophic cells. In confluated granulomas, responsible for adherences among various serous membranes and capsules, fibrillary connective tissue, histiocytes, gigantic cells and many capillaries were found.

Intrahepatic granulomas were closely to portobiliary areas, or inside hepatic lobules. They were highly circumscribed, made by a little necrotic core, surrounded by lymphohistiocytes, epithelioid and gigantic cells. The external separating layer consisted in fibroblasts and fibrocites. The intrahepatic granulomas induced compression atrophy in the surrounding hepatocytes.

As inconstantly associated lesion, epithelial proliferation in kidney calice was found. These cells were infiltrated into adjacent mesenchima; lymphocyte infiltration in kidney mesenchima was also present. Many cases revealed insular granulo-vaculary hepatosis responsible for hepatomegalia described in gross examination.

In high number granulomas, regardless of location, were found undifferentiated cell nests and abnormal mitotic figures. These preneoplastic cells showed large nuclei, visible chromatin and poorly demarcated cytoplasm, sometime with sincitial appearance; they were more or less demarcated by fibroblasts and fibrocites. This cell nests might be developing points for malignant proliferation.

In conclusion, granulomas, regardless of location, revealed a similar structure. They are made by asbestos fibers core, covered mainly by fibroblasts capillaries and connective tissue. Those aspects reveal strictly fibroblastic and fibrotic proliferation. In this stage, expectedly, no malignant lesions were seen, because mesothelioma showed very long latency. However, some granulomas or even hepatocytes revealed discrete premalignant aspects. The significance of lymphohistiocytic infiltration within gut endothelium or in kidney medullar area is still unclear, but it might be related to systemic inflammation induced by asbestos fibers.

Pulmonary asbestosis was the choice for mostly previous experiments (Davis, 1994), but airway administrations for asbestos fibers is highly distressful for animals, and involves some risks for personal. Therefore, our choice was the intraperitoneally administration that seems to provide similar cellular and sistemic response. Moreover, peritoneum locations, even rare, are present in human (Zalay et all, 1992) and animal clinic (Smith et all, 1989). Previous rapports regarding experimental pulmonary asbestosis in rats revealed, after 24 mounts, granulomas containing epithelioid and fibroblastic cells, associated to preneoplastic lesions, otherwise similar to those described in present paper (Vasilieva et. all, 1998). Significantly, were no found other lesion described in spontaneous mesothelioma like pleurisy (Kishimoto et. all 1998) or ascites (Smith et. all, 1989). This might be easy explained by very early neoplastic lesions found in our experiment, as compared to advanced asbestos related mesothelioma described in clinic rapports.

CONCLUSIONS

Our findings proved that asbestos fibers are responsible for various lesions in relatively short period. Gross evaluation revealed granulomas in various size, consistence or location. The most affected locations were epiploon, liver (on capsule or within hepatic tissue), stomach, gut and mesentery. Confluated granulomas often induced adherence among internal
organs. Histologically, granulomas, regardless of the location, revealed in central area asbestos fibers, fagocytated or not, covered mainly by fibroblasts, capillaries and connective tissue. In some areas, undifferentiated cell nests with large nuclei, visible chromatin and poorly demarcated cytoplasm, sometime in sincitial appearance and abnormal mitotic figures were found; this cell nests might be developing points for malignant proliferation.

Those aspects reveal chronic inflammatory figures due to prolonged contact to asbestos fibers, histologically characterized by fibroblastic and fibrotic proliferation. In addition, early malignant lesions were found; possible starting points for latter mesotheliomas.

BIBLIOGRAPHY