PLASMA BIOCHEMISTRY IN EXPERIMENTAL ASBESTOSIS

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Abstract: Asbestos are hydrated silicate fibres, responsible for wide range injuries like inflammation, fibrosis, even malignancy. The purpose of present paper was to reveal the plasma biochemical changes in rat experimental asbestosis. The experiment was carried out 240 days long, on Wistar rats, intraperitoneally injected once with aqueous asbestos suspension. Plasma samples were used in order to determine general metabolic status, inflammatory markers and oxidative stress. The values of uric acid, amylase, ALAT, ASAT, AP, triglycerides, cholesterol, urea, creatinin, glucose, bilirubin, total proteins, plasma ions (Ca\(^{2+}\), Mg\(^{2+}\), Fe\(^{3+}\), Na\(^+\), K\(^+\)) revealed no notable changes. Increased values were found in immunoglobulin levels (IgM and IgG), C reactive protein and complement 3 fraction. Oxidative stress markers as ceruloplasmin malondialdehyde and total hydrogen donor ability were also increased, but protein carbonyl revealed no significant differences. In conclusion, our findings revealed that asbestos fibers effects are far beyond the local proliferative lesions, but they induced a systemic inflammatory response. In blood, this response affects immune system compounds and induces a strong oxidative stress.

INTRODUCTION

Asbestosis is an occupational disease, related to inflammation, fibrosis and malignancy. It occurs in various localizations, including peritoneum, due to prolonged contact to asbestos fibers (Baba et al., 2001). Asbestos is a generic term including a couple of hydrated silicate fibers, sheared in serpentine and amphibole (Kamp and Wietsmann, 1999). Asbestos fibers generate a strong inflammatory response reflected not just in local injuries, but in various tissues and organs, including plasma biochemistry. In man, plasma inflammatory changes are even earlier than any clinical signs, so they might be a method for early detection. In this respect, experimental models are useful to evaluate the plasma biochemical abnormalities in various lezional stages. Unfortunately, no references regarding plasma inflammatory response in asbestos inoculated rats are available; therefore our issue is to evaluate minutely the plasma biochemical status, oxidative stress markers and inflammatory response in order to reveal whether rat experimental model is valuable model for spontaneous man asbestosis.

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%.

Blood was harvested in day 30 and day 240, from retroorbitar sinus under deep narcosis. Plasma samples were used in order to determine oxidative stress markers and other biochemical compounds. Subsequently, total leucocytes and leukocyte formula were done several times during experiment log.

**Biochemistry.** The values of uric acid, amylase, alanin aminotranferaze (ALAT), aspartat aminotransferaze (ASAT), alkaline phosfatase (AP), triglycerides, cholesterol, urea, creatinin, glucose, bilirubin and total proteins were measured by photometry. Plasma ions (Ca$^{2+}$, Mg$^{2+}$, Fe$^{3+}$, Na$^{+}$, K$^{+}$) were measured by using ISE (ion selected exchange) potentiometer. Plasma immunoglobulin (IgG and IgM), C reactive protein (CRP) and complement 3 fraction (C3) were measured by turbimetric method. All determinations were done by Konelab automatic analyzer.

**Oxidative stress markers.** Lipid peroxides were determined by measuring the production of tiobarbituric reactive substances according to the method of Burge and Aust, protein carbonyl were evaluated by using guanidine chloride. Ravin method (parafenildiamin dichloride) was the choice for measuring the plasma ceruloplasmin, while Hatano (diphenyl picrhydroxil) method was preferred for measuring hydrogen donor ability (Ciurdaru et all, 2001).

**Statistical analyses.** All data were expressed as the mean and standard deviation. T Student multiple range test from Excel Windows Software was used to assess the differences among groups. Differences p<0.05 were considered significant.

**Histology.** 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 staining was made from each sample.

**RESULTS AND DISCUSSIONS**

The values of uric acid, amylase, ALAT, ASAT, AP, triglycerides, cholesterol, urea, creatinin, glucose, bilirubin, total proteins, plasma ions (Ca$^{2+}$, Mg$^{2+}$, Fe$^{3+}$, Na$^{+}$, K$^{+}$) revealed no significant differences between asbestos inoculated group and placebo. Unexpectedly, this findings revealed that liver or other internal organs (pancreas, skeletal muscle, intestine etc.) do not suffer significant functional disturbances, however, severe asbestos related lesions were found (Sevastre et all, 2005). No data regarding metabolic profile in spontaneous or experimental asbestosis is available.

**Inflammatory proteins.** Immunoglobulin G (IgG), showed even in 30 days, slightly increased values (2233.4±327.5 mg/dl), but in the end, its levels were far over the normal values (3542.2±796.4 mg/dl) (p<0.05). Immunoglobulin M (IgM) had the higher value within 30 days (480.3±54.2 mg/dl), but finally the values were very close to control group (224.5±95.3 mg/dl) (p<0.05). C reactive protein (CRP) was highly increased in acute phase (30 days) 1.36±0.15 mg/dl (p<0.05) and in the end was close to control group as well (0.62±0.06 mg/dl) (p>0.05). Complement 3 (C3) fraction was insignificant increased in acute phase, but in chronic phase increasing in C3 level became relevant 345.5±85.3 mg/dl (p<0.05). Blood leucocytes confirm these biochemical data, early stages were related to high leukocytosis and neutrophilia,
typical for acute inflammation; whereas, in latter stage, certain signs for chronic inflammation (monocytosis and moderate leukocytosis) were found (Tab. 1).

Histology revealed, in limfonods, hypersecretory plasmocytic cells (Rieder cells) as certain sign for intense antibody synthesis. Moreover, signs of lymphocyte infiltration in glomerulus level were found, suggesting an early autoimmune type III glomerulonephritis.

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<th>Table 1. Blood inflammatory proteins values.</th>
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<td>IgG mg/dl</td>
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All this changes are not specific to asbestosis, but they are common in large number of inflammatory related diseases. However it is a certain connection between oxygen reactive species (ROS) and immunoglobulin; immunoglobulins are able to up regulate ROS synthesis in man macrophages (Klochars et all, 1989). One the other hand, “in vitro” studies showed that asbestos fibers are easier phagocytated by macrophages in contact to complement (Warheit et all, 1988). Various scientific rapports showed increase IgG, IgA levels (Nigam et all, 1993), or CRP (Kishimoto et all, 1998) in plasma of asbestos exposed workers, but no available data regarding experimental asbestosis were found. Therefore, our experimental data are in accord to those found in spontaneous man asbestosis at least in chronic stage. They are no available scientific reports regarding acute man or animal asbestosis.

**Oxidative stress markers.** Ceruloplasmin revealed high levels in asbestos group (26.29±3.61 mg/100ml) (p<0.001). Increased oxidative stress was proved by increased levels in malonildialdehyde (0.93 ±0.36 nmol/ml) (p<0.001) and total hydrogen donor ability (34.10 ±6.39%) (p<0.01), but protein carbonyl showed no relevant differences (1,42± 0,42 nmol/ml) (p>0.05) (Tab.2).

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<th>Table 2. Blood oxidative stress markers in 240 days.</th>
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<td>Ceruloplasmin (mg/100ml)</td>
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<td>Asbestos</td>
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Relation between asbestos fibers and oxidative stress, especially in unsaturated fatty acids level, is widely accepted; actual scientific reports involve asbestos fibers in lung lipid peroxidation, in intratracheal inoculated rats (Kaiglova et all, 1999). In asbestos exposed workers the malonildialdehyde (MDA) level rise proportionally to exposure period (Kamal et all, 1989). Our experimental data are in fully agreement to other reports; moreover we suggest that no only MDA, but ceruloplasmin and total hydrogen donor ability are available markers for asbestos related oxidative stress. Whether this systemic oxidative stress is directly liked to asbestos fibers or side effect in chronic inflammation is an exciting issue for further experiments.
CONCLUSIONS

Our findings revealed significant changes in immune system compounds, like immunoglobulin, c reactive protein and complement 3 fraction. In blood, harmful oxidative stress effects were indicated by increased ceruloplasmin, malondialdehyde, and total hydrogen donor ability levels. Unexpectedly, metabolic profile does not seem to be affected, at least during this time span.

Asbestos fibers effects are far beyond the local proliferative lesions, but they induce a systemic inflammatory response, acute in early stages and chronic in latter ones, reflected in plasma by changes in inflammatory proteins and oxidative stress markers.

BIBLIOGRAPHY