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Changes in the Serum Antioxidant System and Lipid Peroxidation under the Influence of Asbestos

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UDC 616.152.21+616.153.915-39]-02:[613.633:622.367.6

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 8, pp. 145-147, August, 1994
Original article submitted December 26, 1993

The iron content, the state of the serum antioxidant system, and their relationship with the changes in lipid peroxidation in rat liver and lungs at the early stages of chrysotile-asbestos action, and the effect of the naturally occurring flavonoid rutin are studied. Intensification of lipid peroxidation in the liver and lungs and an increase in the oxyproline content, which correlates with the rise in serum antioxidant activity, are observed four weeks after a single intratracheal administration of 50 mg asbestos. The total serum iron content remains unchanged. Rutin has a pronounced anti-asbestos effect, inhibits the early stages of fibrosis, and facilitates normalization of the antioxidant system imbalance induced by asbestos.

Key Words: *asbestos; antioxidant activity; ceruloplasmin; transferrin; rutin*

Recently, the pathological effect of asbestos has been attributed to the potentiation of the free-radical processes (FRP) in the organism [4,10]. Intensification of lipid peroxidation (LPO) and changes in some parameters of the antioxidant system [7,12] during prolonged contact with asbestos have been revealed. Ceruloplasmin (Cp) and transferrin (Tf) are the main contributors to the serum antioxidant system (AOS) [3,8]. Their effect is based on oxidation of Fe^{2+} and binding of Fe^{3+} and on the interaction with oxygen radicals. The ratio of Cp and Tf electron paramagnetic resonance (EPR) signals, which reflects antioxidant activity (AOA) is recommended as a parameter displaying the best correlation with the severity of the pathological state [8]. The role of iron ions in FRP induction (these ions are present in asbestos fibers) has been extensively discussed. Of interest in this connection are the preparations with chelating and antioxidant activities, which may prevent the toxic effect of asbestos. For a better evaluation of the role of FRP in the pathogenesis of diseases provoked by asbestos the determination of both the LPO intensity and the assessment of total AOA are necessary. In the present study we investigated the levels of iron ions, the serum AOS, their relationship with changes in LPO in rat liver and lungs at the early stages of asbestos action, and the effect of the naturally occurring flavonoid rutin (the vitamin P group) on these parameters.

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0007-4888/94/0008-0834$12.50

MATERIALS AND METHODS

Experiments were performed on outbred male albino rats weighing g 150-180 g maintained under standard vivarium conditions. The animals were divided into 4 groups: group I, intratracheal administration of 1 ml 0.9% NaCl (controls); group II, a single intratracheal administration of asbestos (fiber length not more than 10 μ, Bazhenov deposits) in a dose of 50 mg/ml normal saline (37°C); group III, a single instillation of asbestos combined with intragastral administration of rutin (1 ml of 1 mM solution in normal saline once daily from the first day after asbestos administration); group IV, daily intragastral administration of rutin (1 ml of 1 mM solution). The rats were decapitated after a 4-week period, the livers and lungs were perfused with normal saline, and 6% and 3% homogenates, respectively, were prepared. The determination of TBA-reactive products was performed as described [9] with the following modification: 0.25 ml of the studied specimen was added to 3.0 ml 1.5% phosphoric and 1.0 ml 0.5% thiobarbituric acid, thoroughly mixed, incubated for 45 min at 100°C, and cooled to room temperature. n-Butanol (4 ml) was then added, the probes were shaken, and centrifugation was performed at 1800 g. The upper (butanol) fraction was aspirated, and its light absorbance at λ=532 nm was measured in an LKB Biochrom Ultrospec 4050 spectrometer. Serum was prepared by incubating blood in centrifuge vials for 30 min at 37°C (for better thrombus retraction) with subsequent centrifugation at 1800 g. The specimens were frozen in liquid nitrogen as tablets [2]. Electron paramagnetic resonance spectra were measured in a Varian E-4 radiospectrometer under the following conditions: UHF power 10 mW, time constant 1 sec, modulation amplitude 10 G, amplification 2.5×10, and field scanning rate 250 G/min. The oxyproline content in lung tissue was determined by Stegeman's method [13]. Antioxidant activity was measured spectrophotometrically with some modifications [14].

The total serum Fe content was determined after Johnson [11], and the total lipid content in the lung was measured as previously described [1].

RESULTS

The oxyproline content in the lungs increased significantly (Table 1) after 4 weeks of a single intratracheal administration of asbestos, indicating early stages of fibrosis. LPO in the liver and lungs increased 1.4- and 1.7-fold, respectively, compared with the control, while the lipid levels remained practically unchanged. Generally, when AOS is assessed, only the Cp level is measured, which increases in various acute inflammations and destructive processes. However, Cp is just one of the AOS components and cannot provide complete information regarding the state of AOS. Therefore, we determined not only Cp and Tf levels but also their ratios [8]. In the sera of experimental animals we measured the intensity of EPR signals with g=2.05 (Cp) and g=4.3 (Tf). Analysis of EPR spectra has shown that in group II rats the serum content of Cp is increased 1.4-fold and that of Tf is decreased 1.3-fold compared with the control. The Cp/Tf ratio was increased and corresponded to AOA measured spectrophotometrically. The activation of the Cp/Tf system is a result of LPO intensification due to the pathological effect of asbestos and reflects the activation of AOS under the given conditions. It is thought that a sharp increase in the intensity of the Cp EPR signal and in the Cp/Tf ratio coincides with the break of the compensatory-restorative reactions and with the

TABLE 1. Changes in Serum AOA and Tissue LPO under the Influence of Asbestos and Rutin

Parameter	Group of animals			
	I, control (n=8)	II, asbestos (n=8)	III, asbestos+ rutin (n=9)	IV, rutin (n=8)
Oxyproline, μg/100 g lung tissue	218±26	369±49	271±19	217±18
Lipids, % of lung wet weight	2.83±0.5	3.3±0.8	2.8±0.8	2.8±0.9
Lung LPO , nM/g tissue	196±45	325±78	320.7±58	209.7±34
Liver LPO, nM/g tissue	383±41	516±11.8	414±96	347±52
Serum iron, nM	22.4±3.2	22.72±3.42	9.9±3.5	17.71±0.61
Transferrin, rel. units	9.8±0.96	6.4±0.15	4.4±0.7	8.4±0.45
Ceruloplasmin, rel. units	3.2±0.19	4.3±0.3	2.3±0.14	3.24±0.5
Cp/Tf, rel. units	0.36±0.023	0.68±0.028	0.48±0.017	0.35±0.03
AOA, rel. units	0.31±0.13	0.71±0.15	0.49±0.09	0.3±0.08

severity of the organism's state [5]. Under the influence of asbestos (group II animals) the total serum Fe content was the same as in the control group. On the other hand, it is known that a balanced concentration of divalent Fe depends on the Cp and Tf contents as follows: $[Fe^{2+}]=[Tf]/[Cp]$ [8]. In the control group this ratio was equal to 2.8, whereas in group II rats it was equal to 1.47, which indicates a decrease in the serum Fe^{2+} content in asbestos-treated rats. This may be attributed to compensatory alterations of the Fe redox cycle in the organism under the influence of asbestos. Thus, at early stages of asbestos action LPO in tissues is intensified, which correlates with the increase in the blood AOA activity.

Potent antioxidant effects of flavonoids on various FRP, have recently been demonstrated. Being a naturally occurring nontoxic compound, rutin possesses an antioxidant activity due to its ability to interact with lipid radicals and active oxygen forms and to chelate Fe ions [6]. In the rats receiving rutin from the first day of asbestos instillation (group III) the oxyproline content in the lungs decreased significantly compared with group II animals; however, there were no changes in LPO in the lungs and liver. The total AOA and the Cp/Tf ratio in group III were decreased compared with group II and approximated the control values. However, the Cp and Tf levels in group III were lower than in groups I and II, i.e., the Cp level alone cannot be a significant parameter of AOS. Therefore, it is determination of the Cp/Tf ratio, rather than of each individual protein in isolation from the other, which is important. The total serum Fe content decreased almost 2-fold in group III rats compared with groups I and II. This is due to the chelating properties of rutin. In group IV animals. which received only rutin, all parameters were practically the same as in the control.

The naturally occurring flavonoid rutin elicits a potent anti-asbestos effect: it inhibits the early stages of fibrosis and helps prevent the asbestos-induced AOA imbalance. Undoubtedly, EPR spectroscopy, allowing the measurement of both Cp and Tf in one sample [3,8], is the most sensitive method of evaluating the AOS state. In our experiments the Cp/Tf ratio and the total serum AOA measured by the conventional spectroscopic method correlated in all the studied groups. A correlation was also established between the changes in the lung content of oxyproline and the serum AOS state, which allowed us to recommend assessment of the AOS state as a diagnostic test to evaluate the effect of asbestos on the organism and the effectiveness of therapy.

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