Redox Status of Rats during Treatment by Ozonated Saline According to Different Protocols

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Repeated daily injections of ozonated saline to intact rats significantly modified their redox status. Intensification of free-radical processes paralleled by suppression of the antioxidant system creates prerequisites for failure of adaptation and compensation mechanisms of homeostasis.

Key Words: *ozonated saline; antioxidant system; lipid peroxidation*

Alternative therapeutic methods, including exposure to physicochemical factors, for example, ozone therapy, are more and more often used in modern medicine along with conservative drug therapy [1]. Numerous observations under natural conditions, experimental studies, and clinical data demonstrate a wide spectrum of therapeutic effects of ozone [2,3], favorable effects of minor doses of ozone causing activation of adaptation, defense, and compensatory mechanisms. Normalization of homeostasis prevents or compensates for pathological shifts in the system of metabolic regulation.

However, high concentrations of ozone and its solutions are toxic and cause pathological reactions. The leading mechanism of injury is intensification of free-radical processes paralleled by suppression of the antioxidant system.

The efficiency of ozonated solutions in the treatment of patients largely depends on correct choice of doses and protocols of ozone therapy as a component of combined treatment. This brings the best therapeutic corrective effect [4]. Parenteral methods of ozone treatment (injections of ozone dissolved in saline) are now most popular.

We evaluated the effect of ozonated saline (OS) on the components of antioxidant defense system (AOD) and lipid peroxidation (LPO) processes in intact rats injected with OS according to different protocols.

MATERIALS AND METHODS

Ozonated saline was prepared using an UOTA-60-01 ozonotherapeutic device with ozone destructor (Medozon).

Experiment was carried out on 35 male and female Wistar rats (200-300 g). Ozonated saline with 4 mg/liter ozone was injected intraperitoneally (0.006 ml/g). Two protocols were used: single injection (group 1; n=20) and 3 injections (once daily, 3 days running; group 2; n=15). Blood biochemistry (LPO products: conjugated dienes [3], lipofuchsin, Schiff's bases [2], and antioxidants: retinol and α-tocopherol [1]) was analyzed on days 1, 3, and 10 after OS injections.

RESULTS

The difference in shifts of the studied parameters in rats injected with OS according to different protocols increased during the first 3 days and remained unchanged throughout the whole study. After single injection of OS, the content of conjugated dienes increased by 8.57% in comparison with the initial level. On day 3 after the start of the experiment this parameter increased by 62.82% in comparison with the initial level in group 1, while

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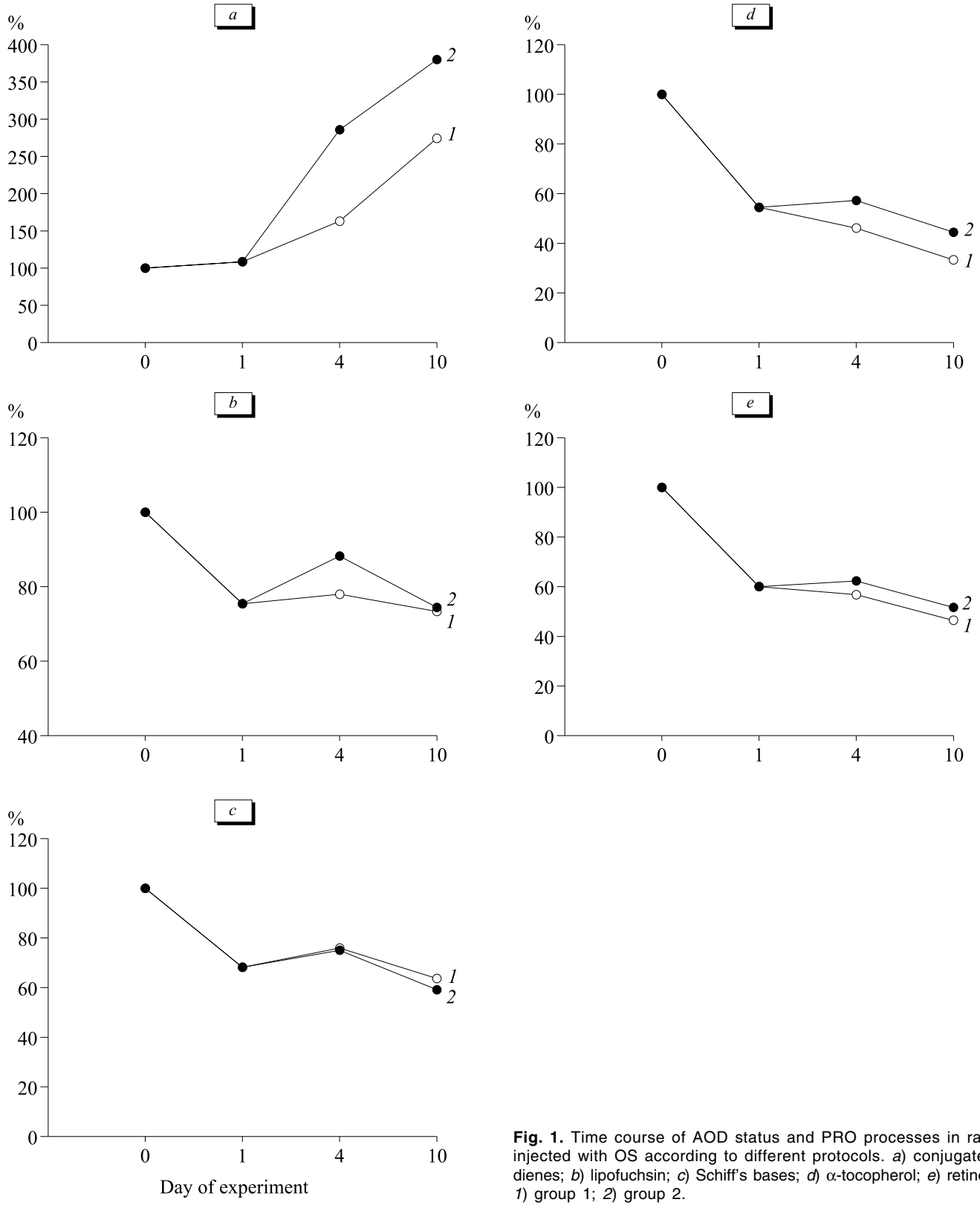

Fig. 1. Time course of AOD status and PRO processes in rats injected with OS according to different protocols. *a*) conjugated dienes; *b*) lipofuchsin; *c*) Schiff's bases; *d*) α-tocopherol; *e*) retinol. *1*) group 1; *2*) group 2.

in group 2 it increased 2.85 times in comparison with the initial level and 2.6 times in comparison with group 1. This indicates significant stimulation of the initial stages of free-radical oxidation and suppression of AOD first line, which is an unfavorable prognostic sign.

The concentration of lipofuchsin and Schiff's bases (products of deep lipid peroxidation) decreased after the first injection to 75.4 and 68.18% in groups 1 and 2, respectively. After 3 days, lipofuchsin formation in group 1 increased negligibly in comparison with the initial value. In group 2, this parameter increased 1.17 times in comparison with the initial value and 1.14 times in comparison with group 1. Lipofuchsin forms in the body as a result of reaction of protein amino groups with products of phospholipid transformation (MDA) and play a role in the formation of membrane cross-links. In addition, its presence indicates blockade of sulfhydryl groups in enzymes.

The content of Schiff's bases, most slowly forming during LPO as a result of nonenzymatic condensation of aldehydes with primary amines, changed almost identically in both groups during the first 3 days, which could be due to the duration of reaction between the initial components. These data suggest that deep LPO reactions were just slightly stimulated in our experiment.

Free-radical processes during ozone treatment were associated with reduction in the content of antioxidants utilized for neutralization of active O_2 metabolites. In group 1, the content of antioxidants almost linearly decreased throughout the 10-day experiment, which looked quite natural. More intense ozone treatment increased blood levels of antioxidants, indicating stress release of these substances into the blood as a result of more intensive modulation of hemodynamic and microcirculation processes. The difference in the content of antioxidants was permanent during the last 7 days of the experiment, their levels decreased in parallel in both groups.

These results indicate significant stress of the AOD system during repeated injections of ozone to intact (normal) rats. Presumably, injections of ozone to sick animals, when AOD system is stressed because of pathological processes, can lead to failure of the adaptation and compensatory potential.

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