PHARMACOLOGY AND TOXICOLOGY

Damage to Erythrocyte Membranes as the Mechanism for Acrylate Toxicity

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Acute poisoning with acrylates (acrylamide and acrylonitrile) was associated with damage to erythrocyte membranes: early stages of acrylamide poisoning were characterized by impairment of the acid resistance of erythrocytes, while later terms of acrylate intoxication were accompanied by condensation of erythrocyte membranes. Activation of lipid peroxidation in blood plasma and erythrocytes was observed during the early stage of intoxication, while at later terms LPO intensity in erythrocytes decreased.

Key Words: *acrylamide; acrylonitrile; lipid peroxidation; erythrocytes*

Industrial monomers acrylamide (AA) and acrylonitrile (AN) are used in various branches of industry [1,2]. The pathochemical mechanisms for the action of these industrial poisons on human organs and systems were extensively studied over the last decades.

Here we studied the effect of acute intoxication with acrylates on the erythrocyte membrane. The state of erythrocyte membranes is a nonspecific integral criterion for the effect of membrane toxins. This criterion reflects the state of biomembranes in various organs and tissues [4,7].

MATERIALS AND METHODS

Experiments were performed on male outbred rats weighing 150-200 g. AA and AN were injected intraperitoneally in single doses of 1.4 and 0.76 mmol/kg, respectively. A hepatotropic poison CCl_4 (model compound) was injected intramuscularly in a dose of 0.16 ml per 100 g body weight. The state of erythrocyte membranes was studied by the method of acid erythrograms [3] using a computer system. HCl (0.002 M) served as a hemolytic. The study was performed at 24°C. The maximum rate of hemolysis was estimated by the most significant difference between optical densities of the solution (2 successive time points after addition of the hemolytic administration).

The study of erythrocyte hemolysis in hyposmotic medium was performed after acute poisoning of rats with AA, AN, and CCl_4 as described previously [9]. The percentage of erythrocyte hemolysis was calculated. Optical density of complete hemolysis was taken as 100%. The intensity of lipid peroxidation (LPO) in biomembranes was estimated by accumulation of malonic dialdehyde (MDA) in the plasma and erythrocytes [8]. The concentration of erythrocyte hemoglobin was measured by the hemiglobincyanide method [6]. The results were analyzed statistically. The data were expressed as $M \pm m$. The significance of differences was evaluated by Student's t test (Microsoft Excel software). The differences were significant at $p<0.05$.

RESULTS

Hemolytic resistance of erythrocyte decreased in rats with AA poisoning, which is seen from shorter

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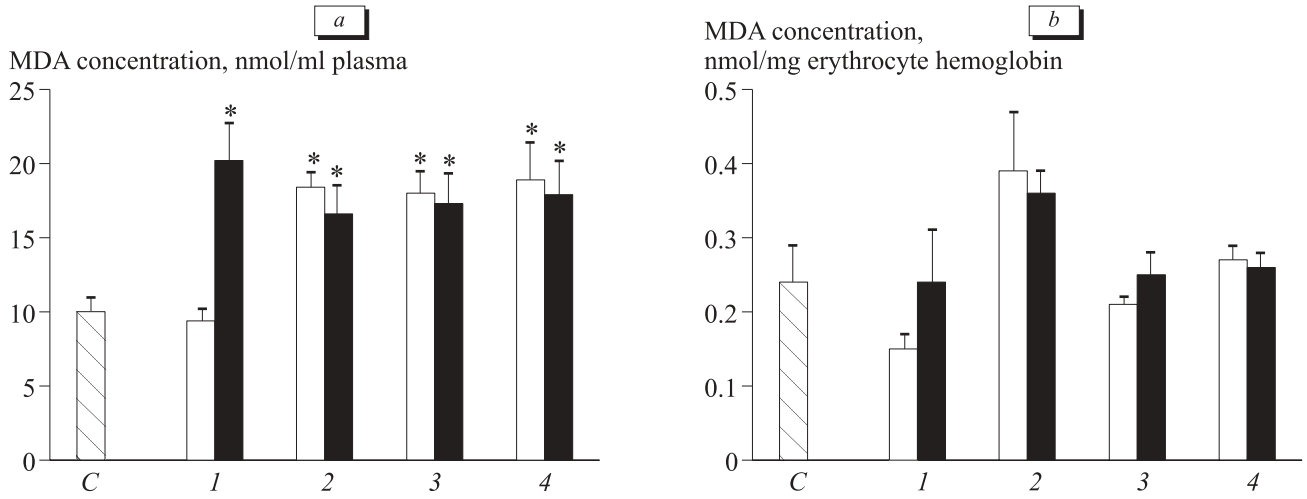

Fig. 1. Effect of acute poisoning with AN (light bars) and CCl_4 (dark bars) on MDA concentration in rat plasma (*a*) and erythrocytes (*b*). *C*, control. **p*<0.001 compared to the control.

time of attaining the maximum hemolysis rate (3 h after poisoning). This parameter in control and AA-poisoned rats was 4.4±0.3 and 3.4±0.2 min, respectively ($p<0.01$). This period corresponds to injury of biological membranes in hepatocytes after AA poisoning [5].

The time-effect relationship was evaluated to study the pathogenesis of AA-induced damage. The concentration of MDA (end product of LPO) was measured in various periods after monomer administration.

MDA concentration in the plasma and erythrocytes increased 1 and 3 h after AA injection, respectively (Table 1).

The state of erythrocyte membranes in hyposmotic medium after intoxication with AA and AN was compared to that observed in experiments with hepatotropic necrosis-inducing poison CCl_4. The study was performed 24 h after CCl_4 administration, which corresponded to the decrease in blood MDA concentration during intoxication with AA (Table 1) and AN and inactivation of liver-specific fructose monophosphate aldolase under conditions of acute acrylate poisoning [5].

TABLE 1. Effect of Acute Poisoning with AA on MDA Concentration in Rat Blood (*M*±*m*)

Time after AA injection, h	MDA concentration, nmol/ml plasma	MDA concentration, nmol/mg erythrocyte hemoglobin
Control	10.0±1.0	0.24±0.05
1	15.7±0.6*	0.34±0.03
3	16.3±2.5*	0.38±0.03**
12	9.3±1.2	0.20±0.01
24	10.9±1.0	0.40±0.11

Note. **p*<0.05 and ***p*<0.01 compared to the control.

Erythrocyte resistance in hyposmotic medium increased under all experimental conditions. The degree of hemolysis in hyposmotic solution was the following: control, 44.3±2.5%; AA, 37.7±2.0% ($p<0.05$); AN, 34.2±3.0% ($p<0.01$); and CCl_4, 26.1±3.0% ($p<0.05$). The rats receiving acrylate and CCl_4 significantly differed from control animals.

The time-effect relationship was estimated from the intensity of LPO in the blood after intoxication with AN and CCl_4. MDA accumulation in blood plasma of rats increased 3 h after CCl_4 injection (Fig. 1). Similar changes were observed 6 h after AN administration and persisted until the end of the study (Fig. 1). MDA concentration in erythrocytes of treated rats did not increase compared to the control (Fig. 1).

Our results indicate that changes in physicochemical characteristics of biological membranes, decrease in erythrocyte acid resistance, and activation of LPO are observed at the early stage after administration of AA and structurally similar AN. These changes are accompanied by a decrease in erythrocyte count and accumulation of LPO products in erythrocyte membranes. Our previous studies showed that LPO plays a role in the pathogenesis of acrylate intoxication [5]. However, erythrocyte resistance in hyposmotic NaCl solution increases during the late stage of acrylate poisoning (24 h after treatment). These changes are typical of CCl_4 poisoning. Similar changes in erythrocyte resistance were revealed during viral hepatitis, intrahepatic cholestasis, and intoxication with structurally different hepatotropic poisons [7]. It may be suggested that intoxication with AA and AN is

followed by liver disorders and condensation of erythrocyte membranes. These changes probably result from cholesterol accumulation due to liver dysfunction [7].

REFERENCES

1. *Acrylamide. Hygienic Criteria for Environmental Health* [in Russian], Geneva (1987).
2. *Acrylamide. Hygienic Criteria for Environmental Health* [in Russian], Geneva (1989).
3. I. I. Gitel'zon and I. A. Terskov, *Erythrograms as the Method for Clinical Study of Blood* [in Russian], Krasnoyarsk (1959).
4. S. N. Golikov, I. V. Sanotskii, and L. A. Tiunov, *General Mechanisms of Toxicity* [in Russia], Moscow (1986).
5. V. V. Ivanov, Yu. V. Kotlovskii, L. G. Klimatskaya, *et al*., *Molecular and Cellular Mechanisms for Toxicity of Xenobiotics and Occupational and Ecological Diseases* [in Russian], Novosibirsk (2004).
6. V. V. Men'shikov, *Laboratory Methods of Studies in Clinical Practice* [in Russian], Moscow (1987).
7. T. N. Sniedze and V. A. Vasarinya, *Experimental Study of Pathological Processes* [in Russian], Riga (1988), pp. 67-72.
8. *Modern Biochemical Methods* [in Russian], Moscow (1977).
9. F. C. Jager, *Nutr. Dieta. Eur. Rev. Nutr. Diet*., **10**, No. 3, 215-223 (1968).