Effect of Antioxidants, Antihypoxants, and Actoprotectors on the Binding Ability of Serum Albumin During Intoxication With Tetrachloromethane

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A decline in serum albumin content and a tendency toward a decrease in the binding constant of the negatively charged fluorescent probe 1-anilinonaphthalene-8-sulfonate are observed in acute intoxication with tetrachloromethane. The mean number of binding sites per albumin molecule increases, therefore, the total concentration of the binding sites for serum albumins remains practically unchanged. Pretreatment with antioxidant, antihypoxant, or actoprotector increases serum albumin content (tomerzole) and partially normalizes its conjugational parameters: binding constant (sodium γ-oxybutyrate) and mean number of binding sites per molecule (dibunol, tomerzole).

Key Words: *serum albumin; conjugation; tetrachloromethane; antioxidants; antihypoxants; actoprotectors*

The course and outcome of pathologies caused by chemical factors depend on the state of serum albumins (SA): concentration, binding ability, and variations in the population of SA molecules [2,6]. The significance of conjugative and transport functions of SA increases during acute intoxication. The development of intoxication is determined by the efficiency of toxin binding by SA and its transport to the liver and kidneys. This is confirmed by the fact that the binding ability of SA increases after efferent therapy [11]. Changes in the binding ability of SA may be caused by mutation of the corresponding gene, impaired post-translational processing of albumin molecule under the action of lipid peroxidation products, glucose, etc., and inhibitory effects of structural analogs (bilirubin, hippurate, indican, etc.) [13,14]. Restoration of the binding ability of genetically defective SA is problematic, while in two other cases it is possible by controlling the formation of endogenous modifiers and inhibitors of binding (incompletely oxidized products and metabolites of free-radical oxidation of fats). Based on this hypothesis, we studied the binding ability of SA and the state of binding sites on SA molecule during acute intoxication with tetrachloromethane after administration of an antioxidant, antihypoxant, or actoprotector which may be helpful in the treatment of this condition [4,8,12].

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MATERIALS AND METHODS

Male outbred albino rats (n=58) weighing 180-210 g were used. Tetrachloromethane (TCM, 3.2 g/kg body weight, 50% oil solution) was subcutaneously injected into 30 rats; other rats served as the control. In series I, both experimental and intact rats were decapitated 24 after intoxication. In series II, III, and IV, the

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TABLE 1. Changes in the Binding Parameters of Serum Albumin in TCM Intoxication Treated with Antioxidant, Antihypoxant, or Actoprotector (*M±m*)

Parameter	Intact rats	TCM	TCM+ dibunol	TCM+ sodium γ-oxybutyrate	TCM+ tomerzole
Serum albumin concentration, μM	391.1±27.8	325.5±7.4	289.2±15.4	297.8±9.1	512.2±4.3+
Mean number of binding site per albumin molecule	1.39±0.13	1.51±0.13	2.14±0.13	2.12±0.08	0.83±0.07+
Serum concentration of binding sites[1], μM	524.7±37.2	487.2±40.2	616.4±31.7	631.6±35.1	384.2±55.6
Binding constant, μM^{-1}	4.21±0.78	3.50±0.60	2.26±0.42	4.17±0.60	2.87±1.08
Mean binding ability of albumin molecule[2], μM^{-1}	5.80±0.92	4.71±0.70	4.84±0.98	7.69±1.71	3.19±0.77
Reserve binding of albumin	3.77±0.57	4.67±0.48	7.52±0.69+	7.12±0.28+	1.90±0.38+
Mean binding ability of serum	2152.9±283	1768.0±386	1382.3±257	2311.0±519	2095.1±560
Quantum efficiency of ANS molecule	0.015±0.001	0.051±0.01*	0.013±0.00+	0.015±0.00+	0.051±0.009

Note. Calculated as product of: [1]total serum albumin concentration by mean number of binding sites per albumin molecule; [2]K_b by the mean number of binding sites per albumin molecule. $p<0.05$: *compared with intact rats, +compared with TCM-treated rats.

animals received dibunol (2,6-di-tret-butyl-4-methylphenol, 25 mg/kg in 3% Tween-80, intraperitoneally), sodium γ-oxybutyrate (100 mg/kg, intramuscularly), and tomerzole (5-ethoxy-2-ethylthiobenzimidazole, 35 mg/kg intramuscularly), respectively, 12 h before and 12 h after intoxication. The animals were sacrificed 24 h after intoxication. Intact rats injected with the same doses of the preparations served as controls. All rats were deprived of food for 16-18 h before decapitation. Serum was prepared by centrifugation of whole blood at 700*g*. The total albumin content was determined spectrophotometrically with the use of bromcresol green [5]. The binding ability and the state of binding sites of SA were assessed by the method of fluorimetric titration [10] using 1-anilinonaphthalene-8-sulfonate (ANS). Fluorescence was measured at a right angle to exciting light in cylindric cuvettes (inner diameter 14 mm) with a photomultiplier cell placed at the cuvette wall. The measurements were carried out in an apparatus based on an SF-46 spectrofotometer. The excitation wavelength was 380 nm, and the emission wavelength was 480 nm (filter with a 50% light transmission). The binding constant (K_b) and the number of binding sites per albumin molecule were calculated by linear approximation of the fluorimetric titration curve. A solution of ANS in butanol (10 μM, quantum efficiency 0.66 [1]) was used as a fluorescence standard. The quantum efficiency of SA-bound probe (QE_{SA}) was calculated from the following formula: $QE_{SA} = 0.66 \times FI_{SA}/FI_{but}$, where FI_{but} and FI_{SA} are the fluorescence intensities of the same concentrations of ANS in butanol and SA at the maximum binding. The significance of differences was evaluated using Student's *t* test. Correlation and regression analyses were performed using special software [7,9].

RESULTS

Typical manifestations of acute intoxication (hypodynamia, sanguineous discharge from the nose, and disheveled wool) were observed 24 h after administration of TCM.

The total blood content of SA decreased 13.8% (Table 1), which may be due to increased clearance of damaged albumin molecules from the circulation. However, there were no considerable changes in the total concentration of binding sites, since the mean number of binding sites per albumin molecule increased. The binding constant decreased by 16.9%, reflecting a noticeable reduction in the mean binding ability of blood serum. An increase in the binding reserve calculated as the ratio between the mean number of binding sites per albumin molecule to the total blood albumin content suggests that the population of SA molecules is partially renewed by mobilization from the intracellular reserves and/or intensification of *de novo* synthesis (compensatory reaction).

It should be noted that during intoxication the quantum efficiency of the SA—ANS complex increased more than 3-fold. The quantum efficiency of ANS is determined by the accessibility of ANS to water; the ANS fluorescence is quenched due to clusterization of water molecules around ANS molecules [3]. Presumably, this phenomenon reflects changes in the interaction between SA and ANS during TCM intoxication: albumin molecules which had not been modified by TCM more effectively prevent the interaction between ANS and water molecules.

The mean binding ability of blood serum, which reflects the affinity of serum for a foreign ligand, was chosen as an indicator of preventive and therapeutic activities of the studied preparations. This parameter is calculated as a product of the serum concentration

of binding sites by K_b. It decreases in TCM intoxication. As Table 1 shows, sodium γ-oxybutyrate and tomerzole, but not dibunol, normalize serum binding ability. We believe that these preparations act by different mechanisms: sodium γ-oxybutyrate increases the number of binding sites on SA molecule, while tomerzole increases the SA concentration. The low therapeutic effectiveness of dibunol may be associated with the decline of serum albumin concentration (probably due to intense degradation of this protein and inhibition of its synthesis in hepatocytes) and a decrease in K_b (presumably, as a result of direct hydrophobic interaction with SA).

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