Effects of α_1-Acid Glycoprotein on Free Radical Oxidation Processes in Experimental Liver Failure

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The effects of α_1-acid glycoprotein (2 intraperitoneal injections in a total dose of 300 mg/kg) on free radical oxidation during liver failure were studied in 53 outbred rats. On day 3 of liver failure, glycoprotein reduced plasma concentrations of free radical oxidation products, elevated the antioxidant potential of the plasma and liver and kidney homogenates, and restored functional reserve and capacity of leukocytes to generation of oxygen radicals.

Key Words: *liver failure; free radical oxidation; α_1-acid glycoprotein*

Injury to any tissue, including the liver, is associated with intensification of free radical oxidation (FRO) processes paralleled by induction of the synthesis of acute phase reactants in the liver. One of them, α_1-acid glycoprotein (AGP), is a plasma protein with a molecular weight of 44,100 Da characterized by a wide spectrum of functional activity and low toxicity. This protein modulates the immune response, the functions of the endothelium and blood cells, exhibits antiapoptotic and detoxifying properties [2-5,12]. The expression of AGP gene in hepatocytes is changed in experimental liver failure (LF) [13]. Many acute phase reactants modulate FRO processes [1], but no data on these properties were reported for AGP.

We evaluated the effect of AGP on FRO processes in blood plasma and cells and some visceral organs in experimental HP.

MATERIALS AND METHODS

The study was carried out on 53 outbred male albino rats (200-240 g) divided at random into 3 groups: 1) controls (n=18), 2) animals with LF (n=20), and 3) animals with LF, injected with AGP (n=15). Liver failure was induced by a single subcutaneous injection of CCl_4 (0.4 ml/100 g) [7]. Controls received an equivalent volume of saline. Liver injury was verified by morphological and biochemical methods. Serum total protein, bilirubin, AST and ALT were measured using Bio-La-Test kits (PLIVA-Lachema). Morphological signs included necrotic foci of centrolobular location, sites of dyscomplectation of hepatocyte cords, vacuolation and lipid infiltration of hepatocytes.

AGP (Orozine; Chelyabinsk Regional Station for Blood Transfusion) was injected intraperitoneally in a dose of 150 mg/kg twice: simultaneously with LF induction and 24 h later (this period was chosen with consideration for drug half-life period) [2-5]. The total dose of AGP was 300 mg/kg.

The study was carried out 48 h after LF induction (at the peak of changes in liver tissue) [7]. The rats were narcotized with diethyl ether, the blood was collected by heart puncture and stabilized with 3.8% sodium citrate (1:9 weight/volume ratio). Plasma, erythrocytes, leukocytes, and liver and kidney tissue homogenates were analyzed. The plasma was obtained by centrifugation of blood at 1200g. Erythrocytes were washed 3 times in phosphate saline and cell suspension (10^{11}/liter) was prepared. Liver and kidney tissues (1 g) were homogenized immediately after isolation and suspended in 10 ml PBS (pH 7.4). FRO processes were evaluated by

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chemiluminescence on a CL-003 device [6]. The chemiluminescent response of each sample was recorded twice and the means were analyzed. Cell chemiluminescence was stimulated with luminol in a final concentration of 10^{-6} M. Chemiluminescence of the plasma and liver and kidney cells was induced by adding 25 mM Fe^{2+}, leukocyte chemiluminescence was induced by adhesion of these cells to glass. Fast flash amplitude reflecting hydroperoxide concentrations in the medium, slow flash amplitude indicating maximum intensity of Fe^{2+}-induced LPO in the medium, and latency reflecting the pro- to antioxidant balance in the medium and its summary antioxidant activity were analyzed on chemiluminograms. Parameters of chemiluminescence of the plasma, liver and kidney homogenates were standardized for lipid weight in substrate volume. Total lipids were measured using Bio-La-Test kits (PLIVA-Lachema). Leukocyte fluorescence was evaluated by the total yield and maximum fluorescence in conversion to 10^5 phagocytes.

The results were statistically processed using Statistica 6.0 for Windows applied software. The type of data distribution was evaluated using the Shapiro—Wilk test. Statistical hypotheses in the groups were verified with consideration for the type of distribution, using parametric (Student's *t* test) and nonparametric (Mann—Whitney *U* and Wald—Wolfowitz) tests.

RESULTS

The development of LF was paralleled by statistically significant accumulation of FRO products (hydroperoxides) in the plasma and reduction of the plasma, erythrocyte, and hepatocyte antioxidant potential (Table 1). Functional activity of peripheral blood leukocytes decreased in LF (Table 2), the generation of oxygen radicals was limited and the

TABLE 1. Effect of AGP on Parameters of Chemiluminescence in LF (*M*±*m*)

Parameter		Group		
		1	2	3
Plasma	fast flash amplitude, arb. units	0.86±0.11	1.23±0.12*	0.80±0.03^{+}
	latency, min	0.99±0.23^{o}	0.71±0.09^{o*}	1.35±0.41^{*++o}
	slow flash amplitude, arb. units	2.06±0.76	1.83±0.23	1.21±0.38^{*+}
Erythrocytes	fast flash amplitude, arb. units	0.63±0.21	0.59±0.16	0.65±0.08
	latency, min	0.88±0.48^{o}	0.54±0.19*	0.68±0.21
	slow flash amplitude, arb. units	0.91±0.41	1.29±0.52	0.89±0.19
Liver	fast flash amplitude, arb. units	1.76±0.25^{o}	1.41±0.20	1.34±0.29
	latency, min	0.84±0.19	0.66±0.11*	1.11±0.18^{*+}
	slow flash amplitude, arb. units	16.55±6.15	14.39±2.85	14.92±3.28
Kidneys	fast flash amplitude, arb. units	1.04±0.21	0.97±0.25	0.77±0.16*
	latency, min	1.26±0.44	1.20±0.37	1.84±0.43^{*+}
	slow flash amplitude, arb. units	9.59±2.07^{o}	8.45±1.09^{o}	8.40±0.54^{o}

Note. Here and in Table 2: **p*<0.05 compared to group 1 (Mann—Whitney test); *p*<0.05 compared to group 2: $^{+}$Mann—Whitney test, $^{++}$Wald—Wolfowitz test; ^{o}samplings with irregular distribution.

TABLE 2. Effect of AGP on Parameters of Leukocyte Chemiluminescence in LF (*M*±*m*)

Chemiluminescence value (×10^5 phagocytes)	Group		
	1	2	3
Total yield, arb. units×min			
spontaneous	2.76±2.10^{o}	0.45±0.20*	3.44±2.71^{++}
induced	13.79±10.63^{o}	1.99±0.86*	17.72±12.40^{*++}
Maximum chemiluminescence, arb. units			
spontaneous	0.88±1.01^{o}	0.12±0.03*	1.51±1.55^{++}
induced	1.68±1.19^{o}	0.29±0.10*	2.42±1.77^{*++}

functional reserve of phagocytes was suppressed to a much greater degree. These results reflect the effect of $CCl_3^{\bullet}$ (an active metabolite of CCl_4), accumulation of FRO products in the plasma and exhaustion of the systems involved in their neutralization [7]. Reduced phagocyte capacity to generation of active oxygen forms seems to be caused by "liver" toxins circulating in the blood.

Treatment with AGP reducted plasma hydroperoxide concentration in rats with LF (Table 1). Interestingly, this parameter in nephrocytes dropped below the level in the control group. Presumably, the effect of AGP is nonspecific and does not depend on the initial level of FRO intensity in the cells. AGP increased the antioxidant potential of the plasma, liver, and kidneys and virtually did not modify FRO processes in erythrocytes. AGP restored oxygen radical generation by peripheral blood leukocytes and increased their functional reserve.

These effects of AGP in LF can be due to intrinsic antioxidant effect towards FRO metabolites (hydroperoxides, lipoperoxides), irrespective of the site of their hyperproduction. Moreover, the presence of AGP receptors on hepatocytes and leukocytes [4,8,12] suggests its involvement in intracellular metabolic processes, including those linked with the function of antioxidant systems. These data contribute to our knowledge on the mechanism of the protective effect of AGP in LF. The detoxifying effect of AGP demonstrated by us [10] can also play a role. AGP decreased the number of necroses in hepatocytes in animals with concanavalin A-induced LF [9], exhibited antiapoptotic effect towards hepatocytes on the model of galactosamine-induced LF [11], and protected mice from the lethal effect of galactosamine [8].

Hence, AGP in a total dose of 300 mg/kg normalized FRO processes by reducing plasma concentrations of FRO products and increasing the antioxidant potential of the plasma, liver, and kidneys in experimental LF induced by CCl_4. AGP normalized the function of leukocytes and increased their functional reserve.

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