Role of Orosomucoid in the Regulation of Plasma Proteolytic Systems during Experimental Renal Failure

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Activity of plasma proteolytic systems was studied in outbred albino rats with acute renal failure. The possibility of treating this disorder with acute phase protein α-1-acid glycoprotein was evaluated. Acute renal failure was induced by single subcutaneous injection of mercury chloride (II). The parameters were evaluated on day 5 postinjection. α-1-Acid glycoprotein in a dose of 150 mg/kg was administered 3 times. Acute renal failure was accompanied by activation of the complement system and fibrin formation (with factors for the intrinsic and common pathways of blood coagulation) and inhibition of the fibrinolytic system and antithrombin activity. Treatment with glycoprotein was followed by partial recovery of fibrin formation and complement system. These changes were probably related to accumulation of glycoprotein in the renal tissue and *in situ* protective effect.

Key Words: *α-1-acid glycoprotein; renal failure; plasma proteolytic systems*

Plasma systems with the cascade of stepwise proteolysis serve as a functional regulator of blood cells and endotheliocytes, determine cellular and humoral interactions, contribute to the defense response, and play a role in the pathogenesis of acute injuries of various types (*e.g.*, acute renal failure, ARF) [4]. The development of renal failure of different genesis is accompanied by changes in activity of the plasma proteolytic system [13]. α-1-Acid glycoprotein (AGP, orosomucoid, or seromucoid) is a polyfunctional acute-phase reactant of the second order, which belongs to the lipocalin family [8]. Increasing interest in AGP is due to a wide range of functional activities and low toxicity of this protein. Previous studies showed that AGP improves the course of experimental renal failure. However, the mechanism underlying the action of AGP remains unknown. The effect of AGP is probably associated with regulation of the plasma proteolytic systems.

Here we studied the effect of AGP on plasma proteolytic systems during experimental ARF.

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MATERIALS AND METHODS

Experiments were performed on 60 male outbred albino rats weighing 200-220 g. The animals were randomized into the following three groups: group 1, intact rats; group 2, rats with ARF; and group 3, rats with ARF receiving AGP. ARF was induced by subcutaneous injection of mercury chloride (II) in a single dose of 5 mg/kg (into the interscapular region). Intact animals received an equivalent volume of physiological saline. ARF was verified by morphological and biochemical tests. The concentrations of urea and creatinine in blood plasma were measured with Bio-La-Test kits (PLIVA-Lachema). Morphological signs included epithelial cell necrosis in the nephron. AGP (Orosin, Chelyabinsk Regional Blood Transfusion Station) in a dose of 150 mg/kg was administered 3 times (48, 72, and 96 h after the induction of ARF). This scheme of treatment was determined by AGP excretion half-life. The total dose of AGP was 450 mg/kg. The study was conducted on day 5, which corresponded to the development of severe changes in renal tissue. The blood was sampled from diethyl ether-anesthetized rats by puncture of the left ventricle. Blood samples were

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stabilized with 3.8% sodium citrate (1:9). Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were obtained by centrifugation at 150*g* and 1200*g*. The main proteolytic systems were examined (fibrin formation, fibrinolysis, anticoagulant system, and complement system). Activity of the coagulation cascade was estimated from the following parameters: recalcification time in PRP and PPP; activated recalcification time in PRP and PPP; activated partial thromboplastin time (APTT); prothrombin time; thrombin time; and plasma fibrinogen concentration [1]. Functions of the anticoagulant system were evaluated from antithrombin activity. Plasma fibrinolytic activity was determined from the time of XIIa-kallikrein-dependent fibrinolysis. Experiments were performed with Tekhnologiya-standart reagents and Zero-med reagents. Total complement activity of the plasma was estimated by titration (50% hemolysis) [3]. The results were expressed in arb. units of 50% hemolysis. AGP concentration in the plasma and kidney homogenate was measured on a Fluoroscan-II fluorometer (Labsystems) with a fluorescent dye quinaldine red (Sigma-Aldrich) [9]. The results were analyzed by Statistica 6.0 software.

RESULTS

ARF was accompanied by activation of the coagulation cascade and complement system, inhibition of fibrinolysis, and decrease in antithrombin activity (Table 1).

Activation of the coagulation cascade is mainly associated with factors of the intrinsic pathway. The decrease in the majority of parameters of fibrin formation (*e.g.*, thrombin time) attests to activation of final stages of blood coagulation. At first glance, hyperfibrinogenemia seems to contradict the observed changes. This state probably develops during the acute phase response to renal tissue injury, since fibrinogen is a positive acute-phase reactant. Activation of blood coagulation by the intrinsic pathway in ARF can be related to a contact with polyanion surfaces (*e.g.*, uremic toxins). Variations in antithrombin activity and fibrinolytic system are probably associated with hypercoagulation during ARF. The decrease in plasma antithrombin activity is probably a result of its consumption during inactivation of coagulation factors. Inhibition of the fibrinolytic system is usually related to plasminogen deficiency [1]. Streptokinase is extensively used for evaluation of the role of various components (factor XII, prekallikrein, high-molecular weight kininogen, plasminogen, and their inhibitors). Streptokinase does not normalize the process of lysis under conditions of plasminogen deficiency. This possibility was excluded, since rat plasminogen is practically insensitive to streptokinase [5]. Deceleration of XIIa-dependent euglobulin lysis due to consumption of fibrinolytic components during hypercoagulation in ARF is confirmed by published data on an increase in the concentrations of fibrin-monomer and D-dimer in blood plasma [10]. Inhibition of fibrinolysis is related to not only consumption of main components, but also increased activity of plasminogen activator inhibitors PAI-1 and PAI-2 during ARF [7]. Activation of the complement system during ARF is realized via the alternative pathway. It is related to accumulation of uremic toxins in the blood and results in the deposition of component C_3 along the nephron walls [14]. Moreover, the observed changes can be associated with mutual regulatory interactions of proteolytic systems. A negative correlation was revealed between activation

TABLE 1. Effect of AGP on Activity of the Plasma Proteolytic System during ARF (*M*±*m*)

Parameter	Group 1 (*n*=18)	Group 2 (*n*=12)	Group 3 (*n*=12)
TT, sec	20.67±0.95	18.58±0.47*	17.60±0.40
PTT, sec	14.17±0.77	13.58±0.65	14.80±0.37
APTT, sec	23.17±0.71	14.50±1.00*	20.20±1.49^{+}
ART (PRP), sec	48.00±3.72	32.08±2.38*	41.40±6.83
ART (PPP), sec	111.92±5.09	68.17±6.68*	93.40±10.57^{+}
RT (PRP), sec	83.00±5.06	63.67±3.61*	79.40±10.12
RT (PPP), sec	159.42±8.03	104.33±7.08*	129.00±9.48^{+}
Fibrinogen, g/liter	4.63±0.27	7.04±0.51*	5.36±0.23^{+}
Antithrombin activity, sec	200.13±10.27	134.33±4.33*	130.60±13.41*
Fibrinolysis, min	10.92±1.56	45.50±9.89*	48.20±4.26*
Activity of the complement system (CH_{50}, arb. units)	60.18±1.06	84.08±2.88*	73.20±0.60*$^{+}$

Note. TT, thrombin time; PTT, prothrombin time, ART, activated recalcification time; RT, recalcification time. Here and in Table 2: $p<0.05$: *compared to group 1; $^{+}$compared to group 2.

TABLE 2. AGP Concentration in the Plasma and Kidney Homogenate during ARF (*M*±*m*)

Parameter	Group 1 (*n*=18)	Group 2 (*n*=12)	Group 3 (*n*=12)
Plasma AGP concentration, mg/ml	0.17±0.02	0.41±0.03*	0.51±0.04*+
AGP concentration in the kidney homogenate, mg/ml	0.02±0.01	0.07±0.01*	0.11±0.01*+

of the intrinsic pathway of coagulation (APTT) and antithrombin activity (r=0.79, p=0.002), fibrinolysis time (r=-0.97, p=0.008), and complement activity (r=-0.65, p=0.022).

Administration of AGP to ARF animals was followed by a decrease in fibrin formation and activity of the complement system (Table 1). APTT, recalcification time and activated recalcification time in PPP, and plasma fibrinogen concentration were completely recovered after AGP treatment. AGP had little effect on the thrombin time, recalcification time, and activated recalcification time in PRP. However, these parameters did not differ in animals of the treatment group and intact specimens. The effect of AGP on coagulation is not mediated by changes in blood antithrombin activity.

We believe that AGP produces a multicomponent effect on functional activity of the plasma proteolytic system. AGP is a plasma protein, which has the highest negative charge. AGP directly interacts with coagulation factors (due to tropism for any polyanion surface) and, therefore, blocks active sites of these factors. AGP plays a role in the regulation of plasma proteolytic systems. It forms a stable complex with plasminogen activator inhibitor type I and stabilizes the inhibitory action of this agent, thus creating a "reserve" of plasminogen activator during inflammation or acute phase response [6]. The regulatory effect of AGP on plasma proteolytic systems can be related to the accumulation of this factor in injured organs (kidneys). The *in situ* protective effect of AGP is followed by suppression of the acute phase response. Previous studies showed that AGP exhibits a tropism and is selectively accumulated in abnormal structures [15]. The concentration of AGP in the site of injury is associated with changes in vascular permeability and selective transport of this agent into the pericapillary space (transcytosis) [12]. We showed that the concentration of AGP in blood plasma and kidney homogenate increases during ARF (Table 2). Administration of exogenous AGP during ARF is followed by an increase in AGP concentration in the plasma and kidney homogenate. The abnormal renal tissue is probably characterized by a functional deficiency of AGP. Protective activity of AGP may be also related to the actoxification and antioxidant properties [2].

Our results indicate that administration of AGP in a total dose of 450 mg/kg partially recovers the process of fibrin formation and activity of the complement system. Exogenous administration of AGP is probably followed by its concentration in the abnormal renal tissue and *in situ* protective action.

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