Ceruloplasmin Prevents Hemostatic Disorders during Experimental Hyperammonemia

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Experimental hyperammonemia in rats was accompanied by hemostatic disorders manifesting in coagulopathy (activation of the intrinsic pathway of blood coagulation) and suppression of platelet function. Ceruloplasmin in a total dose of 60 mg/kg effectively normalized coagulation hemostasis and functional activity of platelets by improving secretory processes in platelets and increasing aggregation rate.

Key Words: *hyperammonemia; hemostasis; coagulopathy; thrombocytopathy; ceruloplasmin*

Hyperammonemia (HA) is observed during massive hemorrhage and hyperthermia. This disorder accompanies various diseases of the hepatobiliary system, hepatic and renal failure, and hereditary enzymopathies of urea synthesis [7]. Prolonged physical exercises can also result in HA [11]. These states are usually accompanied by shifts in the coagulant and anticoagulant potential of the blood. These data suggest that HA is a risk factor for coagulopathy. Little is known about the state and methods for correction of coagulation hemostasis in HA. Ceruloplasmin (CP) was selected for pharmacological correction of hemostatic disorders during HA. CP is a polyfunctional blood protein of higher animals. CP regulates platelet component of hemostasis under normal conditions and during experimental disorders [4]. The effects of CP under conditions of coagulation hemostasis remain unknown. Here we studied hemostatic disorders during experimental HA and evaluated the efficiency of CP as a correcting agent.

MATERIALS AND METHODS

Experiments were performed on 55 male outbred albino rats weighing 200-220 g. The animals were divided into 3 groups. Group 1 included intact rats. Group 2 animals were subjected to ammonium load. Group 3 rats received CP under conditions of ammonium load. HA was produced by oral water load for 7 days. Drinking water was substituted for 0.28 M (1.5% w/v) aqueous solution of ammonium chloride *ad libitum* [14]. The mean daily dose of NH_4Cl was 650 mg/kg. Group 3 animals received intraperitoneal injections of CP (Immunopreparat) in a dose of 20 mg/kg on days 2, 4, and 6. The total dose of CP was 60 mg/kg. Group 2 animals received an equivalent volume of physiological saline. The measurements were performed on day 7 of the study. The blood was taken by heart puncture. Sodium citrate (3.8% w/v) served as anticoagulant. The blood/citrate ratio in study of coagulation hemostasis and platelet function was 9:1 and 5:1, respectively. Platelet-rich plasma and platelet-poor plasma were obtained by centrifugation at 150*g* and 1200*g*, respectively. Platelet aggregation was studied by the method [10] with modifications [6]. ADP served as the aggregation-inducing agent. The study of coagulation hemostasis included the measurements of the following parameters: recalcification time in platelet-rich plasma and platelet-poor plasma; activated recalcification time in platelet-rich plasma and platelet-poor plasma; activated partial thromboplastin time; prothrombin time; and thrombin time. Activity of factor P_3 was estimated [1].

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Plasma fibrinogen concentration was measured as described previously [3]. We used Teknologiya-standart kits and Zero-med reagents. The results were analyzed by means of Statistica 6.0 software [2].

RESULTS

Study of platelet function during experimental HA revealed a significant decrease in the rate and amplitude of cell aggregation (Table 1). The first wave of platelet aggregation underwent most significant changes. This wave reflects cell-cell interactions after addition of the inductor (ADP). The amplitude of aggregation decreased, while the time of aggregation remained unchanged. These changes contributed to a decrease in the rate of the first wave. We revealed a decrease in the time and amplitude of the second wave of platelet aggregation. Therefore, the aggregation rate of this wave remained unchanged. The second wave of platelet aggregation reflects secretion and response of cells to autoaggregants. Activity of factor P_3 significantly decreased under these conditions. We conclude that HA is accompanied by platelet dysfunction. Metabolic acidosis during HA probably results in impairment of intracellular metabolic processes in platelets under conditions of prolonged ammonium acidosis (receptor interaction; secretion and/or imbalance between platelet-derived pro- and antiaggregants). Previous studies showed that parenteral ammonium acetate load is followed by a decrease in platelet aggregation. It can be hypothesized that these changes are related to acceleration of NO synthesis from L-arginine in platelets [15]. CP administration during HA was accompanied by various changes in platelet function (Table 1). The amplitude and rate of platelet aggregation returned to normal. The aggregation rate increased due to an increase in the

TABLE 1. Effect of CP on Platelet Aggregation during Experimental HA ($M\pm m$)

Parameter	Group		
	1 (n=13)	2 (n=6)	3 (n=6)
Maximum amplitude, mm	60.50±4.74	40.67±3.20*	53.67±5.48
Aggregation time, min	6.88±0.49	6.40±0.47	4.62±0.42*+
Aggregation rate, mm/min	9.19±0.94	6.42±0.38*	11.59±1.46*+
Amplitude of the first wave, mm	41.33±3.58	28.50±2.38*	40.67±4.49
Time of the first wave, min	2.87±0.22	3.40±0.35	2.15±0.16*+
Rate of the first wave, mm/min	15.72±2.02	8.87±0.41*	18.71±1.08*
Amplitude of the second wave, mm	19.17±2.41	12.17±1.08*	13.00±1.21
Time of the second wave, min	4.03±0.46	3.17±0.24x	2.47±0.28*+
Rate of the second wave, mm/min	4.84±0.38	3.83±0.14	5.39±0.44*
Factor P_3, sec	63.92±4.76	47.50±4.98*	85.60±5.31*+

Note. Here and in Table 2: n, number of animals. $p<0.05$: *compared to group 1; +compared to group 2 (Mann—Whitney test); xcompared to group 1 (Wald—Wolfowitz test).

TABLE 2. Effect of CP on Coagulation Hemostasis during Experimental HA ($M\pm m$)

Parameter		Group		
		1 (n=17)	2 (n=8)	3 (n=5)
Recalcification time, sec	PRP	83.00±5.06	60.88±3.86*	80.00±5.39+
	PPP	159.42±8.03	120.75±10.74*	176.60±11.07+
Activated recalcification time, sec	PRP	48.00±3.72	34.75±2.21*	36.80±3.34
	PPP	111.92±5.09	82.25±6.97*	122.40±7.14+
Activated partial thromboplastin time, sec		23.17±0.71	20.38±0.99*	26.40±1.81+
Prothrombin time, sec		14.17±0.77	13.25±0.88x	15.40±0.40+
Thrombin time, sec		20.67±0.95	18.13±0.79*	23.60±1.60+
Fibrinogen, g/liter		4.73±0.33	4.60±0.25	5.08±0.29

Note. PRP: platelet-rich plasma; PPP: platelet-poor plasma.

rates of the first and second waves of aggregation. Hence, the aggregation rate in treated rats exceeded that in intact animals. Platelet thromboplastin activity (factor P_3) in CP-receiving rats was above the normal. CP modulated the ability of platelets to trigger the coagulation cascade. These effects were probably associated with antioxidant activity of CP [8] and protection of the platelet membrane. Similar results were obtained in experimental thrombinemias of different etiology [4]. CP improves renal function [5] and accelerates metabolic processes and ammonium cation excretion, plays a role in compensation for metabolic acidosis, and has an indirect positive effect on platelet function.

Experimental HA was followed by severe hypercoagulation in platelet-rich plasma and platelet-poor plasma (Table 2). The development of hypercoagulation was related to primary activation of coagulation factors, but not to the involvement of platelets. Most parameters, including activated partial thromboplastin time, reflect increased activity of factors for the intrinsic pathway of blood coagulation. The minor decrease in the prothrombin time illustrates the contribution of the extrinsic pathway of blood coagulation. Activation of the intrinsic pathway of blood coagulation is of greater importance under these conditions. The activated partial thromboplastin time and prothrombin time decreased by 12 and 6%, respectively, compared to the baseline levels. The decrease in the thrombin time reflects acceleration of the final stage of blood coagulation. Therefore, experimental HA is accompanied by an increase in the total coagulant potential of the plasma and decrease in functional activity of platelets. Hypercoagulation during HA serves as a nonspecific reaction of the hemostasis system to ammonium acidosis [13]. Endothelial dysfunction and changes in the production of prohemostatic and antihemostatic substances are the major pathogenetic factors for these changes [12]. Parameters of coagulation hemostasis in experimental animals with ammonium chloride load returned to normal after treatment with CP (except for activated recalcification time in platelet-rich plasma). Moreover, the test parameters in treated rats were higher compared to intact animals. This effect of CP was probably associated with normalization of platelet activity and endothelial function. It should be emphasized that CP recovers endothelial function via specific receptors on the surface of endotheliocytes [9].

Our results suggest that experimental HA is accompanied by changes in the hemostasis system. Functional activity of platelets decreases under these conditions, while changes in parameters of coagulation homeostasis attest to hypercoagulation. CP in a dose of 60 mg/kg effectively regulates the hemostasis system by improving functional activity of platelets above the normal level and preventing hypercoagulation. These data suggest that HA contributes to the development of hemorheological disorders under extreme conditions. CP holds much promise as an efficient correcting agent.

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