Aggravation of Cyclophosphamide-Induced Acute Neurological Disorders under Conditions of Artificial Acidification of Chyme in Rats

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The effect of artificial acidification of the intestinal content on neurological manifestations of acute severe cyclophosphamide intoxication was studied in rats. The animals were gavaged with 20 ml/kg sulfuric (0.05 M), hydrochloric, boric, or lactic acids (0.1 M) 3 h before intraperitoneal injections of the cytostatic in doses of 0, 200, 600, or 1000 mg/kg. The decrease in pH (by.0) and ammonia-producing activity of the cecal chyme developed within 3 h after administration of acids. Cyclophosphamide caused hyperammonemia; glutamine/ammonia and urea/ammonia ratios in the blood decreased. These changes augmented after administration of acids (boric acid produced maximum and lactic acid minimum effects). Acid treatment resulted in greatest elevation of ammonia level in the portal venous blood and a lesser elevation in the vena cava posterior blood. Acid treatment promoted manifestation of cyclophosphamide neurotoxic effect and animal death. Hence, acidification of the chyme inhibited the formation of ammonia in it, while ammonia release from the gastrointestinal tract into the blood increased; the treatment augmented hyperammonemia and aggravated the neurological manifestations of cyclophosphamide intoxication.

Key Words: *rats; acids; cyclophosphamide; ammonia metabolism; neurological disorders*

Cyclophosphamide (CP) treatment aimed at myeloablation is accompanied by acute neurotoxic effects limiting its dose [5]. Simulation of these effects in rats showed intensification of ammonia release from the gastrointestinal tract (GIT) into the blood [3], which augmented manifestations of CP neurotoxicity [2]. One of approaches to hyperammonemia control in hepatic encephalopathy is to inhibit ammonia-producing activity of the chyme by reducing its pH [1] and inhibition of the urease reaction yielding up to $^1/_4$ of gastrointestinal ammonia [4]. On the other hand, the efficiency of this approach in acute CP intoxication is not at all obvious: deviation from enterocyte glutamine synthase optimum pH of 7-8 [6] paralleled by enzyme alkylation can disorder ammonia detoxification in the intestinal wall, thus facilitating its release into the blood.

We studied the effects of chyme acidification by lactic, sulfuric, hydrochloric acids, and by orthoboric acid, a competitive inhibitor of urease [7], on ammonia metabolism and severity of neurotoxic effect manifestations in acute CP intoxication in rats.

MATERIALS AND METHODS

The study was carried out on male albino rats (140-160 g) from Rappolovo Breeding Center. The animals received no food and water *ad libitum* for 24 h before experiment. Acids (chemically pure: 0.05 M sulfuric, 0.1 M boric, hydrochloric, or lactic) were administered intragastrically (20 ml/kg). Cyclophosphamide (Deco Company) was injected intraperitoneally (200, 600, or 1000 mg/kg; 10 ml/kg). Controls were injected

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with saline. Laparotomy was carried out under ether narcosis.

In experimental series I, the cecum contents was resuspended in 4-fold volume of 0.9% NaCl 0.5, 1.5, 3, or 6 h after administration of acids. In order to measure ammonia content, 0.1 ml 0.25 M H_2SO_4 was put into the central glass of Warburg's vessel and 1 ml suspension into the main section; after hermetic closure the suspension was mixed with 1 ml potassium carbonate. In order to evaluate the ammonia-producing activity of the chyme, potassium carbonate was added after 30-min incubation of samples at 37°C, which was carried out without urea or after addition of 400 μmol urea into the samples. After 18 h, ammonia was measured by titration. Measurements of pH in the chyme were carried out by EV-74 ionometer after centrifugation.

In experimental series II, the veins were punctured 3 h after injection of CP in a dose of 1000 mg/kg (6 h after acid administration): *v. portae* (cranially to *v. pylorica*) and *v. cava caud.* in two points: caudally to *vv. renalis* issue and cranially to *vv. hepaticae* issue at the level of the diaphragm. Blood samples (1 ml) were deproteinated and ammonia (with Nessler's reagent), glutamine (by ammonia, after 10-min hydrolysis at 100°C with 0.3 M H_2SO_4), and urea (with diacetyl monoxyme, Allwex Diagnosticum kit) were measured in the supernatant. Glutamine/ammonia and urea/ammonia coefficients were calculated, which were used for evaluating ammonia detoxication.

In experimental series III, we evaluated the effects of acids on CP acute neurotoxicity. The equilibrium and coordination function were evaluated by the time of rat's balance on a glass sphere 9 cm in diameter, muscle strength and endurance were evaluated by the duration of rat clutching (back down) to a wire lattice. Testing was carried out 1 h after CP injection in doses of 200, 600, or 1000 mg/kg and then hourly over 6 h or until animal death; the data for each animal were averaged. The time of animal death was recorded.

The significance of differences between the means for groups was evaluated by the Student *t* test, differ-

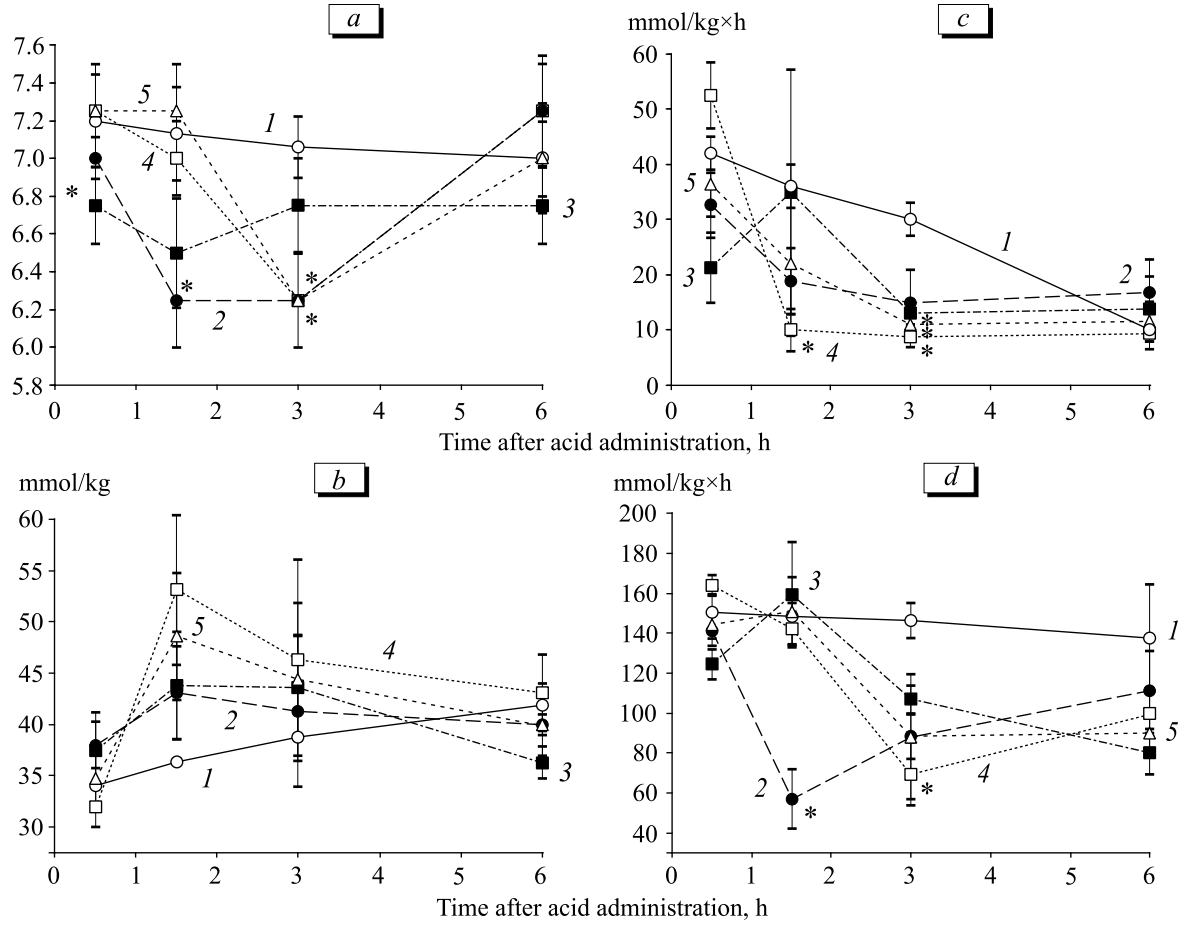

Fig. 1. Effects of intragastric acids on pH and ammonia metabolism parameters in the rat cecal chyme. Each point corresponds to the mean value for 4 animals (*M*±*m*). *$p<0.05$ in comparison with the control. *a*) pH of 20% chyme suspension after centrifugation; *b*) ammonia content in the chyme; *c*) ammonia-producing activity of the chyme; *d*) ammonia-producing activity of chyme in the presence of urea. *1*) saline (control); *2*) boric acid; *3*) lactic acid; 4) sulfuric acid; 5) hydrochloric acid.

ences in the mean lifespan (MLS) were assessed by the Mann–Whitney test.

RESULTS

The cecal chyme pH decreased (by 0.8-1.0) 1.5-3.0 h after administration of sulfuric, hydrochloric, or boric acid; ammonia content in the chyme virtually did not change. Accumulation of ammonia in the chyme was decelerated by 2.0-3.4 times during incubation without urea and by 1.7-2.6 times during incubation with urea in comparison with the control. After 6 h, all parameters approached the control level (Fig. 1).

In 3 h after CP injection, the level of ammonia was elevated by 2.6 times in the portal blood, by 5.5 times in the blood of the caudal segment of *v. cava*

TABLE 1. Effects of Intragastric (i/g) Administration of Acids and/or Intraperitoneal (i/p) Injection of CP to Rats on Ammonia Metabolism Values in the Blood from the Abdominal Cavity Veins ($M\pm m$, n=6)

Site of venipuncture	Substance received before venipuncture		Ammonia metabolism parameters		
	i/g, 6 h before	i/p, 3 h before	blood ammonia content, μM	glutamine/ ammonia ratio	urea/ ammonia ratio
v. portae	Saline	Saline	173±26	8.8±1.4	32.7±4.9
	Boric acid	Saline	230±46	6.5±2.0	37.2±11.2
	Lactic acid	Saline	182±13	6.5±0.7	33.6±3.2
	Sulfuric acid	Saline	167±12	7.6±0.8	38.6±5.2
	Hydrochloric acid	Saline	147±36	13.0±5.1	70.6±34.0
	Saline	CP	442±75*	5.1±1.0	27.0±5.7
	Boric acid	CP	970±70*+	2.1±0.2*+	10.6±1.0*+
	Lactic acid	CP	675±80*	2.7±0.4	16.2±2.2*+
	Sulfuric acid	CP	927±68*+	1.5±0.1*+	11.8±1.0*+
	Hydrochloric acid	CP	953±114*+	1.7±0.3*+	11.9±1.7*+
v. cava caudalis (cranial segment)	Saline	Saline	97±10	11.7±1.4	62.6±6.6
	Boric acid	Saline	207±16+	5.6±0.9+	33.7±5.7+
	Lactic acid	Saline	107±12	11.5±2.1	64.6±13.0
	Sulfuric acid	Saline	110±12	10.3±1.3	62.6±5.9
	Hydrochloric acid	Saline	126±16	9.1±1.1	51.5±7.0
	Saline	CP	428±60*	5.2±0.4*	27.9±3.0*+
	Boric acid	CP	827±57*+	2.4±0.2*+	12.5±1.0*+
	Lactic acid	CP	580±56*	2.6±0.2*+	19.1±1.8*+
	Sulfuric acid	CP	820±86*+	1.9±0.2*+	14.7±1.4*+
	Hydrochloric acid	CP	672±84*+	3.3±1.1	17.8±1.8*+
v. cava caudalis (caudal segment)	Saline	Saline	93±15	12.4±2.8	66.2±11.2
	Boric acid	Saline	267±45+	4.2±1.3+	26.1±5.2+
	Lactic acid	Saline	125±16	10.2±2.3	54.5±11.1
	Sulfuric acid	Saline	115±8	9.7±0.8	54.6±3.8
	Hydrochloric acid	Saline	127±49	18.4±5.9	108.2±42.9
	Saline	CP	507±63*	4.2±0.6*	23.2±2.7*
	Boric acid	CP	793±16*+	2.5±0.2*+	12.7±0.6*+
	Lactic acid	CP	625±63*	2.4±0.1*+	17.2±1.8
	Sulfuric acid	CP	820±66*+	1.8±0.2*+	13.6±0.9*+
	Hydrochloric acid	CP	733±53*+	2.4±0.2*+	14.8±1.4*+

Note. *p<0.01 in comparison with the animals receiving no CP; +p<0.05 in comparison with the animals administered no acids.

TABLE 2. Effects of Acids on the Severity of CP Intoxication in Rats (*M*±*m*, *n*=6)

Acid	CP dose, mg/kg	Time of balance on sphere, sec	Time of hanging on lattice, sec	Lifespan, h
Saline (control)	200	3.36±0.25	>30	223.6±27.7
Boric		2.24±0.13**	>30	115.0±11.0*
Lactic		2.33±0.08**	>30	158.3±18.6
Sulfuric		3.17±0.21	>30	146.2±13.0*
Hydrochloric acid		2.64±0.18*	>30	144.5±19.3*
Saline (control)	600	2.83±0.15	14.83±0.21	25.1±3.6
Boric		2.02±0.14**	11.90±0.58**	11.4±1.1*
Lactic		1.64±0.13**	12.38±0.55**	12.7±1.3*
Sulfuric		2.71±0.15	12.19±0.48**	16.5±1.4
Hydrochloric acid		2.10±0.23*	14.48±0.44	14.8±1.3*
Saline (control)	1000	1.61±0.20	2.68±0.38	6.2±0.3
Boric		0.83±0.15*	0.97±0.22*	5.0±0.4*
Lactic		0.96±0.08*	1.21±0.09*	5.3±0.2*
Sulfuric		0.99±0.21	1.35±0.26*	5.4±0.3
Hydrochloric acid		0.99±0.11*	1.32±0.22*	5.4±0.3

Note. *p<0.05, **p<0.01 in comparison with the control.

posterior, and by 4.4 times in its cranial segment. The glutamine/ammonia and urea/ammonia ratios in the blood decreased (maximally 3-fold, in the caudal segment of *v. cava posterior*). The levels of ammonia were higher after administration of acids and CP, while the coefficients were lower than in rats injected with CP alone. Acid treatment led to the most pronounced changes in the portal blood composition: ammonia content increased 2.1-2.2 times, glutamine/ammonia coefficient reduced 1.9-2.4 times, urea/ammonia coefficient reduced 1.7-2.5 times; the highest effect was recorded after boric acid administration. Isolated administration of the acids produced similar, but less pronounced changes, significant only for boric acid. Blood ammonia level in the cranial segment of *v. cava posterior* was 1.8 times lower (p<0.05) than in the portal blood. This difference reflected the ammonia-detoxifying function of the liver and was in fact completely canceled by injection of CP or by boric, sulfuric, or hydrochloric acid (Table 1).

Acid administration caused no changes in the appearance or behavior of intact rats, but augmented the acute neurotoxic effects of CP. The mean duration of keeping balance on the sphere reduced 1.3-1.9 times for animals receiving hydrochloric, lactic, or boric acid. Sulfuric, lactic, and boric acid reduced the duration of hanging on the lattice 1.2-2.8 times. The lifespan of animals after acid administration reduced 1.2-2.2 times (Table 2). Adynamia and tremor caused by CP dose of 1000 mg/kg were more pronounced in these animals.

Hence, intragastric administration of acids suppressed ammonia formation in the cecal chyme of rats, but under conditions of severe CP intoxication disordered ammonia detoxification in the intestinal wall and liver, augmented hyperammonemia and neurological disorders. These data did not confirm the initial hypothesis according to which the drugs reducing the intestinal chyme pH could have a favorable effect on the neurological status under conditions of myeloablational CP treatment.

REFERENCES

1. A. N. Okorokov, *Treatment of Visceral Diseases* [in Russian], Moscow (2001), Vol. 1.
2. V. L. Rejniuk, T. V. Schäfer, and Ju. Ju. Ivnitsky, *Byull. Eksp. Biol. Med.*, **149**, No. 6, 657-660 (2010).
3. T. V. Schäfer, V. L. Rejniuk, and Ju. Ju. Ivnitsky, *Ibid.*, **150**, No. 8, 170-176 (2010).
4. K. Kim, W. Lee, and N. J. Benevenga, *J. Nutr.*, **128**, No. 7, 1186-1191 (1998).
5. G. B. McDonald, J. T. Slattery, M. E. Bouvier, *et al.*, *Blood*, **101**, No. 5, 2043-2048 (2003).
6. P. J. Reeds and D. G. Burrin, *J. Nutr.*, **131**, No. 9, Suppl., 2505S-2508S (2001).
7. W. Zaborska, *Acta Biochim. Pol.*, **42**, No. 1, 15-118 (1995).