Increase of Ammonia Pool in the Gastrointestinal Tract of Rats Potentiates Acute Toxicity of Cyclophosphamide

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For evaluation of the impact of changes of ammonia pool in the gastrointestinal tract on acute toxicity of cyclophosphamide, the dynamics of blood levels of ammonia and urea of rats was studied after intraperitoneal injection of cyclophosphamide (600 mg/kg) and clinical manifestations of intoxication and lifespan of rate were studied after cyclophosphamide injections in doses of 200, 600, 1000, and 1400 mg/kg alone or in combination with ammonium acetate. Ammonium acetate stimulated the hyperammoniemic and uremic effects of cyclophosphamide. Combined effects of the toxicants were associated with symptoms characteristic of acute poisoning with ammonium salts; these symptoms were not observed under the effect of ammonium acetate alone. Ammonium acetate stimulated the lethal effect of cyclophosphamide injected in doses of 200, 600, 1000, or 1400 mg/kg: the mean lifespan of rats decreased by 1.5, 2.1, 2.8, or 6.1 times, respectively. These data indicate that ammonia redistribution from the gastrointestinal tract into circulating blood is one of the mechanisms of thanatogenesis in acute cyclophosphamide intoxication.

Key Words: *rats; cyclophosphamide; ammonium acetate; blood ammonia; blood urea*

In addition to local effects, yperite poisoning is associated with resorptive effects presenting by involvement of the nervous, gastrointestinal, and hemopoietic systems [2]. The manifestations of resorptive effect predominate in extensive contamination with yperites [1] and in therapy with cytostatics, analogs the nitrous yperite [4]. In extremely severe cases they are presented by anxiety and convulsions [1]. A possible mechanism of these neurological disorders can be stimulation of ammonia flow from the gastrointestinal tract into the CNS caused by yperite enterotoxicity [1] and hepatotoxicity [5]. If this hypothesis is true, the increase in the ammonia pool in the gastrointestinal tract aggravates the manifestations of the resorptive effects of yperites. In rats, this pool amounts to 1.1 mmol/kg (estimated by previous data [3]) and hence, intragastric administration of ammonium acetate (AA) in a dose of 12 mmol/kg (close to 0.4 LD_{50} for intact animals) increases this pool by an order of magnitude.

We evaluated the impact of this increase for the manifestations of the resorptive effect of cyclophosphamide (CP), a nitrous yperite analog.

MATERIALS AND METHODS

The study was carried out on male outbred albino rats (200-240 g) from Rappolovo Breeding Center. The animals received no fodder but had free access to water for 24 h before the experiment. Cyclophosphamide (Biokhimik) was injected intraperitoneally; AA or sodium acetate (SA; control) (KGaA Merck) were administered intragastrically. All agents were administered in water solutions (10 ml/kg). The doses of AA and SA were 12 mmol/kg, those of CP 200, 600, 1000, or 1400 mg/kg.

In experimental series I, the effects of CP and/or AA on the time course of blood ammonia and urea were studied. The blood was collected directly after

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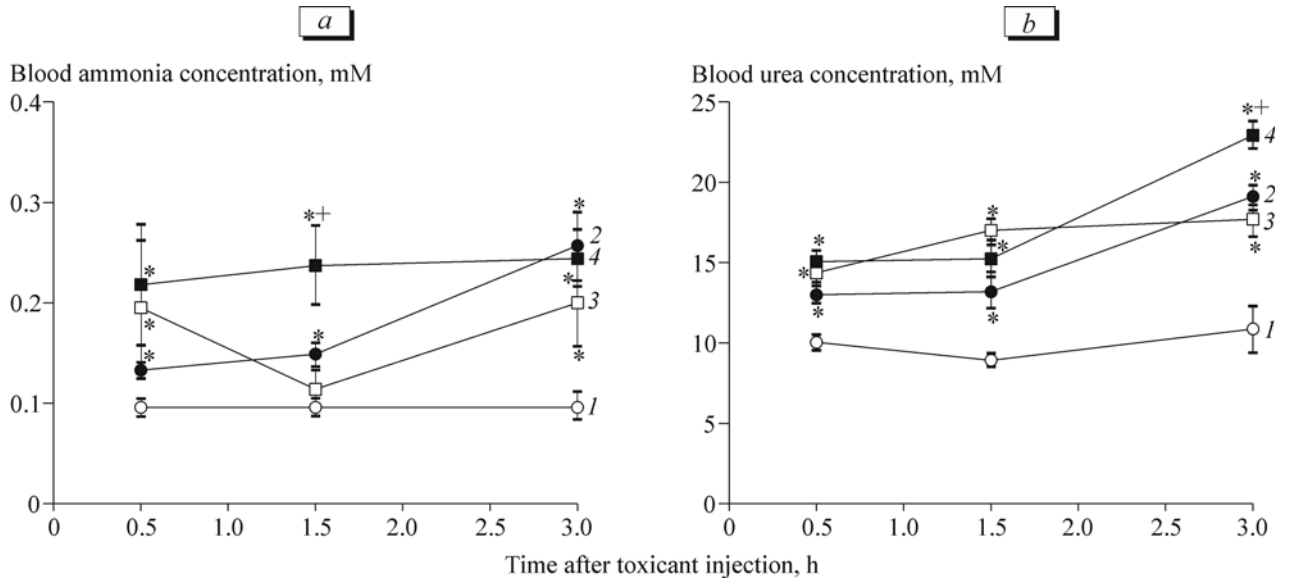

Fig. 1. Blood levels of ammonia (*a*) and urea (*b*) in rats (*M*±*m*; *n*=6) after intraperitoneal injection of CP in a dose of 600 mg/kg and/or intragastric AA (12 mmol/kg). *1*) control (SA); *2*) CP; *3*) AA; *4*) AA+CP. $p<0.05$ compared to: *control; +AA.

decapitation. Immediately after AA or SA injection, the animals received CP in a dose of 600 mg/kg. The blood was collected after 0.5, 1.5, or 3.0 h and deproteinized with trichloroacetic acid. Ammonia was measured using Nissl reagent, urea was measured with diacetyl monoxime (using Allwex Diagnosticum kit).

In experimental series II, the effects of AA on clinical manifestations of acute resorptive effect of CP in doses of 200, 600, 1000, or 1400 mg/kg and on the mean lifespan (MLS) of rats were studied. Controls received SA before CP.

The significance of differences in the mean values of blood metabolites in the groups was evaluated using heteroscedastic Student's *t* test, differences in MLS in the groups were evaluated using Mann–Whitney test.

RESULTS

Injection of CP in a dose of 200 mg/kg was not associated with appreciable clinical manifestations of intoxication over 18 h. The dose of 600 mg/kg led to slowly progressing somnolentia and stupor. The doses of 1000 and 1400 mg/kg led to tremor and loss of postural and audiomotor reflexes within the nearest 3 h; this condition was sometimes paralleled by tonic convulsions emerging during hours 1-6. These symptoms were not seen before 0.5 h after isolated injection of CP.

Hyperammoniemia was registered 0.5-3.0 h after injection of CP and/or AA. Ammonia level in the blood was 2.3 times higher 0.5 h after combined treatment with the toxicants. This increase was recorded 1.5-2.0 h later after isolated CP. Combined CP+AA treatment led to a 1.5-2.3-fold increase of blood ammonia level 0.5-1.5 h after injection of CP in comparison with CP alone. Blood urea level was elevated during all periods of the study, the highest values being recorded after combined use of the toxicants (Fig. 1).

Ammonium acetate potentiated the lethal effect of CP: the MLS reduced 1.5, 2.1, 2.8, and 6.1 times after CP doses of 200, 600, 1000, and 1400 mg/kg, respectively (Fig. 2). Combined treatment with the toxicants led to emergence of the signs characteristic of ammonium salt poisoning in lethal doses: exophthalmos, periodical excitation, tremor followed by opisthotonus and apnea. No clinical manifestations of intoxication were observed after AA alone.

Hence, an increase (by about an order of magnitude) of ammonia pool in the gastrointestinal tract of

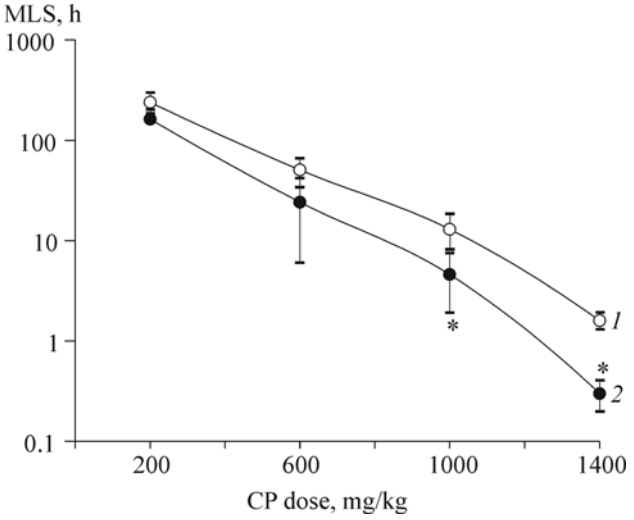

Fig. 2. Effects of intragastric administration of AA (12 mmol/kg) on MLS in rats (*M*±*m*; *n*=11) after subsequent intraperitoneal injection of CP in doses of 200-1400 mg/kg. *1*) control (SA); *2*) AA. *$p<0.05$ compared to the control (Mann–Whitney test).

rats after intragastric administration of AA aggravated acute CP intoxication, judging by sooner deaths and by the emergence of the symptoms of acute poisoning with ammonium salts. This effect was due to the CP potentiation of the hyperammoniemic effect of AA administered into the gastrointestinal tract; no signs of appreciable disorders in the urea formation were detected. These data indicate the involvement of ammonia present in the gastrointestinal tract of rats in the formation of neurological disorders and lethal outcome in acute CP intoxication.

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