Comparative Efficiency of Succinate-Containing Antihypoxants in Traumatic Toxicosis

I. V. Zarubina, I. A. Yunusov, V. V. Marysheva, and P. D. Shabanov

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Experiments on Wistar rats showed the development of endotoxicosis 12 h after severe compression injury. Endotoxicosis manifested in disorders in bromosulfaleine excretion from the blood, increase of blood urea, uric acid, creatinine, and potassium levels and aminotransferase activities. Injection of succinate-containing antihypoxants (reamberine, cytoflavin, metaprot plus, succinamic acid 2-amino-4-acetylthiasolo[5,4-b]indole) directly after decompression promoted recovery of liver function, prevented the development of hyperfermentemia and renal failure as a result of reduced blood levels of potassium and non-protein nitrogen. The protective effect of the drugs in traumatic toxicosisdecreased in the following order: metaprot plus>cytoflavin>2-amino-4-acetylthiasolo[5,4-b]indole succinaminic acid>reamberine.

Key Words: *traumatic toxicosis; antihypoxants; succinate; metabolism; excretory function; liver*

Traumatic toxicosis develops as a result of soft tissue injury of ischemic nature caused by long-term compression (4-6 h and longer). The causes of toxicosis are toxic products released from ischemic muscles into the circulation: potassium, myoglobin, medium-molecular-weight molecules, urea, creatinine, peptides, and acid metabolites, this leading to hemodynamic and deep metabolic disorders [2]. The severity of acute renal failure, a specific feature of traumatic toxemia, depends on the volume of damaged muscles and length of their compression [1,7]. The development of renal insufficiency is paralleled by hepatic dysfunction, described in clinical practice as the hepatorenal syndrome, largely determining the course and outcome of traumatic toxicosis [9]. Numerous pathogenetic factors of traumatic toxicosis eventually cause structural and functional cell damage of hypoxic nature, which received the name of a "shock cell" [10]. The common regularities of cell injury development and course in traumatic toxicosis determine the universal approaches to its drug correction by classical antihypoxants. Of these, prospective cytoprotectors are succinate-containing drugs. Their high therapeutic efficiency was shown in metabolic disorders of different origin [3,4,6,11]. The armory of succinate-containing antihypoxants is not large. Reamberine and cytoflavin [3] are now used in toxicological practice. The most interesting of the new prospective compounds are 2-amino-4-acetylthiasolo[5,4-b]indole succinaminic acid (BM-616 compound) and 2-ethylthiobenzimidasole with succinic acid (Metaprot plus). Their extensive pharmacological activities were shown in many diseases, including toxic involvement of the liver [4].

We compared the efficiency of succinate-containing antihypoxants in traumatic toxicosis.

MATERIALS AND METHODS

Traumatic toxicosis was induced in 68 male Wistar rats by 4-h compression of soft tissues of the hip in a special clamp of 5 cm^2 area with a U-shaped incision to prevent the femoral bone fracture [4]. All the studied antihypoxants were intraperitoneally injected to animals (groups of 10-12 rats) directly after de-

Department of Pharmacology, S. M. Kirov Military Medical Academy, St. Petersburg, Russia. ***Address for correspondence:*** i.v.zarubina@inbox.ru. I. V. Zarubina

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compression in the optimal effective doses: 10 ml/kg reamberine, 1.5 ml/kg cytoflavin (both drugs manufactured by Polisan), 25 mg/ml metaprot plus (Antiviral), and 25 mg/kg compound BM-616 (synthesized at Department of Pharmacology, S. M. Kirov Military Medical Academy). Immobilized animals injected with an equivalent volume of saline served as the control. Material for the study was collected 12 h after decompression. The protective effect of antihypoxants in traumatic toxicosis was evaluated by blood levels of urea, uric acid, creatinine, and potassium ions, measured by standard kits, and by the rate of bromosulfaleine (BSF) elimination from circulating blood [4]. Retention of BSF was calculated by the formula:

$$R(\%)=C_8\times 100/C_2,$$

where C_2 is BSF concentration in the blood 2 min after its injection and C_8 its concentration in the blood 8 min after injections.

The data were statistically processed using Student's *t* test.

RESULTS

Severe injury to the skeletal muscles caused inhibition of BSF excretion from the blood in comparison with the control group of immobilized rats (Fig. 1). Retention of BSF in the blood of rats was 310%, this indicating inhibition of its excretion through the bile ducts and disorders in the excretory function of the liver in compression injury. Injection of antihypoxants directly after decompression led to recovery of BSF elimination pattern from the rat blood 12 h after the injury. Reamberine reduced the BSF retention coefficient by 86%, compound BM-616 by 153%, cytoflavin by 198%, and metaprot plus by 246% ($p<0.05$). Hence, injections of antihypoxants prevented disorders in the even distribution of BSF and inhibition of its elimination from the blood, this indicating recovery of the excretory function of the liver.

Severe compression injury was associated with a 3-fold increase in blood concentration of urea (Table 1). In addition, the content of uric acid in the blood increased 3.8 times 12 h after decompression, creatinine level increased 3-fold, and potassium level 4-fold (Table 2). These data indicate renal dysfunction and development of endotoxemia in severe decompression injury of soft tissues of the limb. Injections of antihypoxants reduced manifestation of traumatic toxicosis. Reamberine injection led to a 29% reduction of urea content in the blood, 50% reduction of uric acid, 32% reduction of creatinine, and 52% reduction of potassium level in the blood ($p<0.05$). The effects of compound BM-616 and cytoflavin were similar: blood urea levels reduced by 56%, uric acid by 66%, creatinine by 47%, and potassium by 65%. Metaprot plus exhibited the highest efficiency: urea level reduced by 63%, uric acid by 73%, creatinine by 66%, and potassium by 71% in comparison with immobilized animals.

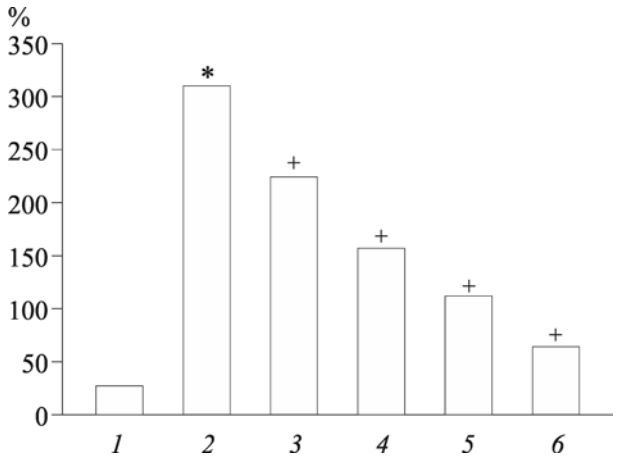

Fig. 1. Effects of antihypoxants on BSF retention coefficient. *1*) control; *2*) injury; *3*) injury+reamberine; *4*) trauma+BM-616; *5*) trauma+cytoflavin; *6*) trauma+metaprot plus. $p<0.05$ compared to: *control, +injury.

Traumatic toxicosis in rats was associated with the increase in blood levels of transamination enzymes ALT and AST. This indicated disorders in the enzyme-producing function of the liver and development of the cytolytic syndrome as a result of disorders in selective permeability of plasmatic membranes (Table 3). Injections of antihypoxants to animals after decompression led to reduction of hyperfermentemia intensity. The activity of ALT reduced by 26, of AST by 24% after reamberine injection. Compound BM-616 reduced ALT activity by 32%, AST activity by 34%. Cytoflavin reduced enzyme activities by 52 and 37%, metaprot plus by 57 and 45%, respectively ($p<0.05$).

Hence, severe compression injury involves extra work for the liver (detoxification of toxic products excess). Our study showed that as early as just 12 h after decompression the levels of toxic products (urea, uric acid, creatinine, potassium) increase in the blood of rats, while liver capacity to BSF excretion is disordered. This is paralled by an increase in activities of transamination enzymes in the blood involved in nitrous catabolism and ammonium detoxification. Aminotransferase hyperfermentemia can be also caused by disorders in their elimination from the blood, realized with participation of the reticulo-endothelial system. Central circulation and microcirculation insufficiency leads to disorders in liver functions, including the detoxification function. This fact confirms high sensitivity of the liver tissue to hypoxia caused by disorders in oxygen supply and emerging soon after soft tissue compression before disorders in systemic hemodynamics.

TABLE 1. Effects of Antihypoxants on Urea and Uric Acid Content in the Blood (*M*±*m*)

Animal group	Urea, mmol/liter	Uric acid, mg/100 ml
Control	7.1±6.0	3.2±0.5
Injury	21.8±11.0*	15.2±1.2*
Injury+reamberine	15.4±12.0^{+}	7.6±1.4^{*+}
Injury+BM-616	9.1±8.0^{+}	5.5±1.3^{+}
Injury+cytoflavin	10.1±9.0^{+}	4.9±0.8^{*+}
Injury+metaprot plus	8.0±5.5^{+}	4.1±0.7^{+}

Note. Here and in Tables 2, 3: $p<0.05$ compared to: *control, $^{+}$injury.

TABLE 2. Effects of Antihypoxants on Blood Levels of Creatinine and Potassium (*M*±*m*)

Animal group	Creatinine, μmol/liter	Potassium, mmol/liter
Control	57.21±12	4.2±0.8
Injury	178.4±15*	18.2±1.3*
Injury+reamberine	121.3±13^{*+}	8.7±1.2^{*+}
Injury+BM-616	97.6±14^{*+}	6.4±0.9^{*+}
Injury+cytoflavin	92.2±11^{*+}	6.1±0.7^{+}
Injury+metaprot plus	61.2±11^{+}	5.3±0.8^{+}

TABLE 3. Effects of Antihypoxants on ALT and AST activities (*M*±*m*)

Animal group	ALT, μmol/ml/h	AST, μmol/ml/h
Control	1.58±0.34	1.48±0.35
Injury	3.88±0.36*	2.96±0.33*
Injury+reamberine	2.84±0.32^{*+}	2.25±0.27^{*+}
Injury+BM-616	2.63±0.29^{*+}	1.96±0.28^{+}
Injury+cytoflavin	1.88±0.29^{+}	1.85±0.28^{+}
Injury+metaprot plus	1.67±0.27^{+}	1.62±0.33^{+}

The efficiency of the complex of therapeutic measures in traumatic toxicosis largely depends on the efficiency of restoration of the functional activity of cells and tissues, which can be attained by antihypoxic cytoprotectors. We showed that injections of succinate-containing antihypoxants in traumatic toxicosis promote recovery of the excretory function of the liver, prevent the development of hyperfermentemia and renal failure due to reduction of blood levels of urea, uric acid, creatinine, and potassium. By the efficiency of protective activity in traumatic toxicosis, the drugs rank as follows, from most to least effective: metaprot plus>cytoflavin> 2-amino-4-acetylthiasolo[5,4-b] indole succinaminic acid>reamberine. The efficiency of the studied antihypoxants in traumatic toxicosis is presumably due to high energotropic characteristics of succinate due to its thermodynamic advantages in oxidation rate in comparison with other cell respiration substrates. In addition, succinate is involved in the regulatory function of signal molecules participating in the maintenance of metabolic homeostasis at the total systems level [12]. These properties indicate good prospects of therapeutic use of succinate-containing antihypoxants in traumatic toxicosis for correction of the functional activity of the liver and improvement of resistance to endotoxicosis.

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