Disturbances in Energy Metabolism during Traumatic Shock and Their Pharmacological Correction

L. D. Luk'yanova, N. N. Mikhailova*, D. V. Fomenko*, N. V. Kizichenko, and E. N. Dushina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 132, No. 9, pp. 263-267, September, 2001
Original article submitted March 26, 2001

In compression traumatic shock caused by mechanical crushing of the lower limbs and eventuating in death of 80% animals, hemodynamic disorders by the end of day 1 lead to the development of energy deficiency most pronounced in skeletal muscles and less pronounced in the liver and kidneys. Energy production in the brain and heart was not impaired. Inhibition of ATP synthesis correlated with decreased SOD activity in organs, but not always with activation of lipid peroxidation, which can occur without concomitant disorders in energy metabolism (in the heart and brain). Therapy with succinate-containing hydroxypyridine derivative decreased animal mortality to 10%; this treatment did not modify hemodynamic parameters, but normalized energy metabolism in organs and activity of the antioxidant and prooxidant systems. These findings suggest that tissue (bioenergetic) hypoxia plays an important role in the pathogenesis of compression traumatic shock and that antihypoxic drugs are essential components of therapy of this condition.

Key Words: *traumatic shock; tissue (bioenergetic) hypoxia; hemodynamics; energy metabolism; antihypoxants; antioxidants*

Traumatic shock (TS) is one of the most severe consequences of mechanical impacts on the body. The main pathogenetic factors of TS are pain, decreased volume of circulating blood, endogenous intoxication, and polyorganic insufficiency. The latter is caused by metabolic disorders related to microcirculatory disturbances, tissue (or bioenergetic) hypoxia, and energy deficiency [2]. However, the role of hypoxic factor in the pathogenesis of TS is still discussed. There are no comparative data on the development of tissue hypoxia in different organs and tissues during TS. This problem is important for elaboration of principles of drug therapy for TS, because the presence of hypoxia requires addition of antihypoxants to combined therapy. The aim of the present study was to detect tissue hypoxia in various organs during compression TS and to verify the efficiency of antihypoxant therapy in this condition [2-5].

Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow; *Affiliated Department of Institute of General Resuscitation, Russian Academy of Medical Sciences, Novokuznetsk

MATERIALS AND METHODS

Experiments were carried out on random-bred male albino rats (180-200 g). Compression TS was induced by 6-h compression of soft tissues on the thigh (compression force 6 kg/cm^2). The procedure was performed under light ether narcosis and induced severe TS with high mortality rate (up to 70-80% within 24 h).

Blood oxygenation was measured with an Optim-420 pulsoxymeter. Arterial pressure and heart rate were recorded on an MKh-03 surgical monitor after catheterization of the femoral artery.

The effect of TS on energy metabolism was studied in the blood and tissue homogenates (skeletal muscles, liver, kidneys, brain, heart). The blood and tissues were collected 24 h after the trauma and immediately frozen in liquid nitrogen.

0007-4888/01/1323-0837$25.00

For determination of ATP content, the organs were frozen, weighed, powdered with 5% trichloroacetic acid 1:10. The homogenates were centrifuged at 8,000 rpm for 15 min. The supernatants were analyzed. All operations were carried out on the cold (0-4°C). The contents of inorganic phosphate (P_i), ATP, lactate, pyruvate, and SOD activity were measured routinely on an FP-901 M analyzer; the content of lipid peroxidation (LPO) products was measured on an SF-26 spectrophotometer.

A succinate-containing hydroxypyridine derivative GB-288 (GB) correcting energy metabolism under conditions of hypoxia [2-5] was injected intraperitoneally in a single dose of 10 mg/kg to animals with TS immediately after decompression. The effect of antihypoxant therapy was evaluated by the survival rate, hemodynamic parameters, blood and visceral biochemical indices.

The results were statistically processed by Student's *t* test using Excel software, the differences were considered significant at $p<0.05$.

RESULTS

Animal mortality 24 h after compression injury was 70-80%. In survivors TS led to hemodynamic disorders typical of this condition: blood pressure 24 h after the injury decreased by 39%, heart rate by 7%, and blood saturation by 11%, which attested to the presence of hypoxemia. Blood concentrations of lactate and pyruvate increased 3- and 1.5-fold, respectively, compared to the control, which attested to compensatory activation of anaerobic glycolysis. Blood ATP significantly decreased, which is characteristic of the initial stage of tissue hypoxia, and P_i concentration increased 2-fold (Table 1).

Decreased ATP content in organs of experimental animals directly confirms the presence of tissue hypoxia in TS.

Skeletal muscles of the hind paws (target tissue for this condition) were most sensitive to compression: 24 h after compression the concentration of ATP drop-

TABLE 1. Effect of TS (after 24 h) on Metabolic Parameters in Rat Blood and Tissues (*M*±*m*)

Parameter		Control	TS	TS+GB
Blood	Lactate, mmol/liter	1.20±0.01	3.6±0.3*	1.20±0.01+
	P_i, mmol/liter	2.3±0.1	3.0±0.1	2.4±0.1+
	ATP, μmol/liter	425.6±6.4	374.2±19.4	546.3±10.0+
	SOD, arb. units/mg protein	849.8±36.4	789.6±6.0	1141.3±49.8+
	Pyruvate, mmol/liter	0.25±0.03	0.38±0.01*	0.35±0.02
Heart	Lactate, mmol/liter	23.7±0.8	25.9±2.5	26.6±1.0
	P_i, mmol/liter	11.2±0.8	11.0±0.7	13.7±0.9
	ATP, μmol/liter	2.1±0.5	2.1±0.2	1.9±0.2
	SOD, arb. units/mg protein	10.2±0.7	8.5±0.5	10.3±0.5
Brain	Lactate, mmol/liter	16.9±1.5	7.4±0.9*	18.2±1.0+
	P_i, mmol/liter	9.6±0.3	10.7±0.4	6.8±0.4+
	ATP, μmol/liter	1.3±0.2	1.8±0.2	1.5±0.2
	SOD, arb. units/mg protein	8.7±0.8	12.6±0.4*	8.5±0.6+
Liver	Lactate, mmol/liter	11.3±0.8	6.0±0.7*	11.9±0.8+
	P_i, mmol/liter	9.4±0.3	14.3±0.3*	6.3±0.4+
	ATP, μmol/liter	1.7±0.2	1.5±0.1	1.7±0.2
	SOD, arb. units/mg protein	15.3±1.3	10.8±0.5*	16.6±1.1+
Kidneys	Lactate, mmol/liter	5.9±0.9	7.5±1.4	4.9±0.9
	P_i, mmol/liter	13.3±0.2	16.0±0.5	12.0±0.2
	ATP, μmol/liter	1.9±0.1	1.40±0.08	1.9±0.1
	SOD, arb. units/mg protein	15.5±1.0	9.5±0.1*	15.3±1.0+
Skeletal muscles	Lactate, mmol/liter	21.9±3.5	10.1±0.7*	25.5±3.1+
	P_i, mmol/liter	19.6±0.1	17.2±1.0	22.0±0.7
	ATP, μmol/liter	3.7±0.3	2.0±0.1*	3.7±0.5+
	SOD, arb. units/mg protein	14.3±2.5	4.9±0.5*	12.7±2.0+

Note. $p<0.05$: *compared to the control; +compared to TS group.

ped by 46%, glycolysis was also inhibited (lactate concentration decreased 2-fold) (Table 1).

Resumption of the blood circulation in ischemic limbs after TS leads to redistribution of blood in other organs directed at the maintenance of the blood flow in vital organs (brain and heart) at the expense of blood supply to the liver and kidneys. This results in depletion of protein stores and disturbances in energy metabolism; hypoxia and ischemia induce degenerative changes in cells and functional insufficiency of these organs [7-9]. Therefore hepatorenal dysfunctions can determine not only the course, but also the outcome of shock.

In our experiments ATP content in the liver decreased by 22%, which correlated with a 1.5-fold increase in P_i content (reflecting dephosphorylation rate) and a 2-fold suppression of glycolysis (Table 1).

Significant disorders were revealed 24 h after TS in the kidneys: the content of ATP decreased by 26% and P_i content increased 1.2-fold, despite activation of glycolysis (Table 1).

Energy production in the brain and heart during compression TS was virtually unchanged. Twenty-four hours after the trauma ATP content in the brain did not decrease, but significantly increased compared to the control; P_i content was virtually the same, while lactate content decreased more than 2-fold (Table 1). These changes attested to sharp activation of aerobic energy production in the brain. In the heart all three parameters did not differ from the control (Table 1).

Hence, in organs with preserved circulation (brain and heart), whose function was maintained at the maximum physiological level, energy metabolism 24 h after TS was virtually unchanged, whereas in organs with pronounced circulatory disturbances and impaired function (skeletal muscles, liver, kidneys) changes in energy metabolism were most pronounced.

Injury to hind paw muscles initiated free radical oxidation processes. The intensity of LPO varied in different organs and tissues. The most intense production of intermediate LPO products was noted in skeletal muscles, heart, and liver, while in the brain and kidneys these processes were less pronounced (Fig. 1).

Activity of SOD, the main marker of the antioxidant system, decreased in the majority of organs 24 h

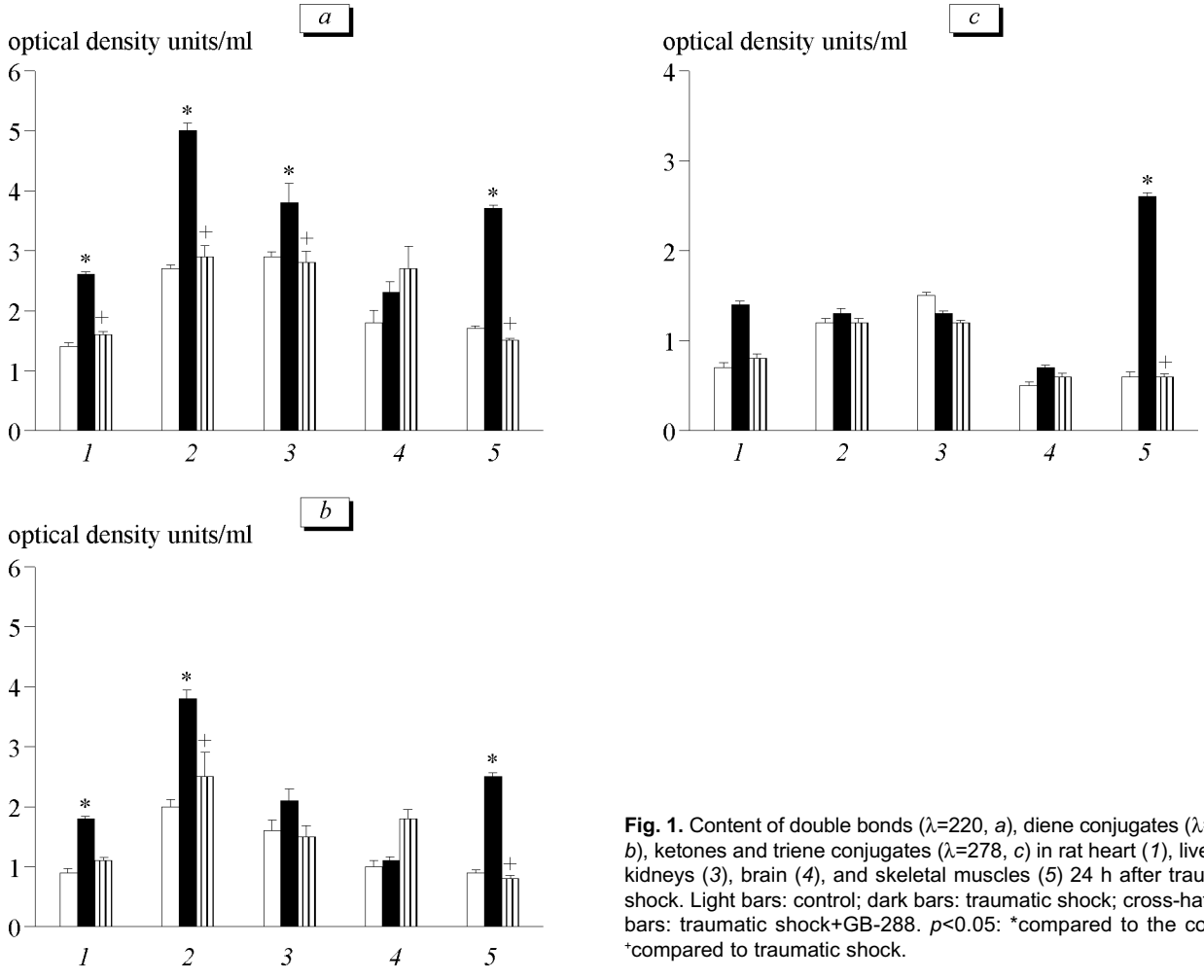

Fig. 1. Content of double bonds (λ=220, *a*), diene conjugates (λ=232, *b*), ketones and triene conjugates (λ=278, *c*) in rat heart (*1*), liver (*2*), kidneys (*3*), brain (*4*), and skeletal muscles (*5*) 24 h after traumatic shock. Light bars: control; dark bars: traumatic shock; cross-hatched bars: traumatic shock+GB-288. $p<0.05$: *compared to the control, +compared to traumatic shock.

after TS. This decrease was maximum (64%) in skeletal muscles, less pronounced in the liver and kidneys (39 and 29%, respectively), and minimum in the myocardium (17%). In the brain, SOD activity increased by 45% (Table 1).

Hence, activities of the antioxidant and prooxidant systems not always correlated with changes in energy metabolism during TS. In the heart free radical processes were sharply activated despite the absence of changes in ATP, P_i, and lactate concentrations. Activation of LPO in the brain was sometimes more pronounced than in the liver or kidneys, despite increased SOD activity and enhanced ATP production (Table 1, Fig. 1). This fact can play a role in the development of delayed functional metabolic disorders.

Administration of GB modulated the course of the pathological process induced by TS: animal mortality dropped to 10%. The drug had no effect on hemodynamic parameters (heart rate, blood pressure, and oxygenation), which did not differ from those in untreated animals with TS. Hence, GB specifically and directly stimulated aerobic energy production and corrected TS-induced disturbances in energy metabolism in skeletal muscles, liver, and kidneys (Table 1). GB elevated the content of ATP to the normal or even above normal (in the skeletal muscles) and normalized P_i and lactate concentrations (Table 1). GB had virtually no effect on energy metabolism in the brain and heart (Table 1).

Thus, a single injection of GB to rats with compression TS normalized energy metabolism and prevented the development of energy deficiency in target tissues, without changing hemodynamic parameters.

In animals receiving GB activity of SOD in the heart, liver and kidneys did not differ from that in intact animals, while in skeletal muscles it constituted 90% of normal. The content of diene conjugates and other LPO products also returned to normal (Table 1, Fig. 1). Hence, GB produced not only antihypoxic, but also antioxidant effects (suppression of excessive LPO activation and positive regulation of antioxidant enzymes), which is in line with other reports [1,6].

Hence, a single injection of GB to animals immediately after decompression eliminated energy deficiency developing during acute period of TS and corrected the imbalance in free radical processes by preventing accumulation of LPO products in tissues and by activating antioxidant defense enzymes.

REFERENCES

1. B. I. Krivoruchko, *Fundamental Studies as the Basis for Drug Creation* [in Russian], St. Petersburg (1995), p. 211.
2. L. D. Luk'yanova, *Byull. Eksp. Biol. Med.*, **124**, No. 9, 244-254 (1997).
3. L. D. Luk'yanova, *Vestn. Ross. Akad. Med. Nauk*, No. 3, 18-25 (1999).
4. L. D. Luk'yanova, *Ibid.*, No. 9, 3-12 (2000).
5. N. N. Mikhailova, D. V. Fomenko, N. V. Kizichenko, *et al., Fundamental Problems of Resuscitation* [in Russian], Vol. 1, Novokuznetsk (2000), pp. 429-450.
6. E. A. Mutuskina, *Hypoxia: Mechanisms, Adaptation, Correction* [in Russian], Moscow (1997), p. 84.
7. B. S. Myslovatyi, *Byull. Eksp. Biol. Med.*, **94**, No. 7, 19-22 (1982).
8. E. A. Nechaev, *Long-Term Compression Syndrome* [in Russian], Moscow (1993).
9. V. A. Shkurupii, *Byull. Eksp. Biol. Med.*, **126**, No. 7, 104-107 (1998).