EFFECT OF EXCHANGE BLOOD TRANSFUSION ON SOME INDICES OF SKELETAL MUSCLE METABOLISM

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After replacement of 85% of the blood volume in healthy dogs and also in animals with transfusion shock the content of the nitrogenous fractions and activity of aspartate and alanine aminotransferases in the skeletal muscles were studied for 7 days. The exchange blood transfusion produced a good therapeutic effect on the animals with transfusion shock. However, the process of "flushing" of nonprotein substances from the tissues of these animals was much less complete than in healthy animals.

KEY WORDS: exchange blood transfusion; muscle metabolism; blood transfusion shock.

In the last decade much evidence has been obtained of the therapeutic value of exchange blood transfusion (EBT) in various pathological states [4, 7, 10, 11, 13]. However, in some organs under these circumstances the tissue response is productive of shock [8]. Injection of isologous blood in small doses (5 ml/kg) is accompanied by substantial changes in tissue metabolism [1-3, 9].

It was decided to study the dynamics of the concentrations of nitrogenous fractions and of transaminase activity in skeletal muscle after EBT.

EXPERIMENTAL METHOD

Altogether four series of experiments were carried out (on 6 or 7 dogs in each series). In the first two series the concentrations of nitrogenous fractions and transaminase activity were investigated in muscle before and 1, 3, and 7 days after EBT in healthy dogs. Isologous donor's blood, after the short period of keeping, was transfused simultaneously with exsanguination; up to 85% of the blood volume was exchanged. In the experiments of series III and IV EBT was carried out under conditions of transfusion shock. After preliminary bleeding (150-200 ml blood) the dogs were given a transfusion of heterologous (human) blood in a dose of 50-100 ml. During the development of transfusion shock (when the blood pressure fell to 20 mm Hg, accompanied by intravascular hemolysis, defecation, and micturition), EBT was carried out. The operation lasted 1-1.5 h. One group of dogs was transfused with isologous blood, preserved in accordance with the L'vov Blood Transfusion Institute L'vIPK 2 formula (the preservative contains 0.2% sodium citrate and 7% sodium lactate solution), the other group with blood prepared by using the Central Blood Transfusion Institute TsOLIPK 7b formula. The hind limb muscles were taken for investigation. Nitrogenous fractions were studied by the usual methods. Activity of aspartate amino transferase (AST; E.C. 2.6.1.1) and alanine aminotransferase (ALT; E.C. 2.6.1.2) was determined in extract of tissue homogenate and in the mitochondria. The incubation medium contained (in μmoles): phosphate buffer, pH 7.4, 50; alanine or L-aspartic acid 10; α-ketoglutaric acid 0.1; with 0.1 ml supernatant (diluted 1:50 or 1:100); total volume of mixture 0.6 ml, incubation for 30 min at 37°C. The protein content was determined by Lowry's method [12].

EXPERIMENTAL RESULTS

In the healthy dogs EBT led to a decrease in the total nitrogen concentration in the muscles. On the 7th day this index was 17.3% below its initial value ($P < 0.05$). The protein nitrogen concentration on the 3rd day after EBT was raised, whereas the concentration of low-molecular-weight nitrogen compounds in the muscle was low at all times of the investigation. When blood preserved with L'vIPK 2 and TsOLIPK 7b formulas was used the changes in concentration of the muscle nitrogen fractions were almost identical.

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TABLE 1. Effect of EBT on Transaminase Activity (in μg pyruvate/mg protein) in Skeletal Muscle of Healthy Dogs and in Blood Transfusion Shock (M ± m)

Index studied	Preservative	Initial values	After EBT		
			24 h	72 h	7 days
Healthy					
AST total	LIPK 2	11,50±1,89	+5,70±0,53*	+4,39±1,76*	+3,30±1,56†
ALT total		4,56±0,70	+0,33±0,50	−1,53±1,12	−0,73±0,27*
AST:					
total	TsOLIPK 7b	9,25±2,75	−1,23±0,89	+2,03±1,31	+1,84±1,37
mitochondrial		6,83±1,13	+1,03±0,72	+4,09±2,65	+3,33±2,04†
ALT:					
total		2,89±1,10	+0,55±0,41	+0,82±0,72	0
mitochondrial		1,49±0,34	+0,58±0,33	+0,03±0,03	+0,76±0,32*
Blood transfusion shock					
AST:					
total	LIPK 2	13,16±1,30	+0,14±0,71	+1,82±1,31†	+0,86±1,60
mitochondrial		11,18±1,21	−0,43±0,48	+0,87±1,36†	+2,08±1,80
ALT:					
total		3,50±1,34	−0,06±0,46	+0,54±0,30†	−0,18±0,54
mitochondrial		2,37±0,66	−0,15±0,40	+0,59±0,47†	+0,05±0,34
AST:					
total	TsOLIPK 7b	15,62±1,98	+0,55±1,49	+2,86±0,72*	+1,93±1,22
mitochondrial		13,39±1,97	−0,88±1,18	+0,78±1,08	+1,01±1,64
ALT:					
total		4,72±0,27	+0,13±1,17	+0,44±1,53	+1,14±0,88
mitochondrial		2,28±0,33	+0,57±0,37	+0,61±1,13	+0,78±0,50

*P < 0.05 by Student's criterion.
† P < 0.05 by the criterion of signs.

In the case of EBT preceded by transfusion shock the total nitrogen level in the muscle was raised after 24 h. No significant changes were found in the protein nitrogen concentration. The concentration of low-molecular-weight nitrogen compounds, including free amino acids, in the muscle was not significantly reduced ($P > 0.05$). Similar results were obtained when blood preserved with both solutions was used.

In healthy animals the total AST activity in skeletal muscle was increased at all times of the investigation (Table 1). More definite changes in transaminase activity were observed when the blood was preserved with L'vIPK2. ALT activity 1 and 24 h after EBT was unchanged; on the 7th day it was depressed. Activity of mitochondrial AST and ALT isozymes was increased at all times of the investigation, but the increase was statistically significant only after 7 days.

When EBT was carried out after transfusion shock the transaminase activity increased only on the 3rd day.

The action of a massive blood transfusion was thus shown to depend to a definite degree on the initial functional state of the recipient. Low-molecular-weight nitrogenous substances are flushed out of the skeletal muscle of healthy dogs during EBT, and the process continued for up to 7 days. Under these circumstances transaminase activity, especially mitochondrial, is increased. EBT is therapeutically highly effective in dogs with transfusion shock, but the process of flushing out of nonprotein nitrogenous substances from the tissues is considerably weakened.

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COLONY-FORMING ABILITY OF THE BONE MARROW IN MICE AFTER BURN TRAUMA

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The dynamics of the number of colony-forming units (CFU) in the bone marrow of CBA mice after receiving third-degree thermal burns covering 15% of the body surface was studied by the exogenous splenic colony method. The number of CFU in the bone marrow of the mice was reduced by 41-52% on the 4th and 16th days after burning. Thymus cells of intact mice, if injected simultaneously with bone marrow of the burned mice, increased the number of exogenous splenic colonies formed in the recipients. The results suggest that not only is the number of CFU reduced in the bone marrow after burns, but also the number of thymus-dependent cells necessary for normal colony formation.

KEY WORDS: colony-forming units; burns; thymus-dependent cells.

The question of the action of thermal burns on hematopoietic stem cells has received insufficient study. Yet it is evident that the principal pathogenetic factors of burns, namely extensive tissue destruction, infection, and toxemia of microbial and nonmicrobial origin, must affect the pool of stem cells. According to data in the literature [3, 6], a decrease in the number of lymphocytes in the thymus and in the peripheral population of T cells, as well as a disturbance of their function, are observed in burns. The T cells are known to play an important role not only in immunity, but also in hematopoiesis [4].

Under these circumstances it was decided to study the number of colony-forming units (CFU) in the bone marrow of mice at different times after burn trauma by the exogenous splenic colony method. The effect of thymocytes on proliferation of the stem cells in the bone marrow of burned mice in the spleen of lethally irradiated recipients also was studied.

EXPERIMENTAL METHOD

Male CBA mice aged 2.5 months were used. The effect of burns on the CFU population was studied by the exogenous splenic colony method [5]. A suspension of bone marrow cells (10^5 cells per mouse) from burned and control (intact) donors was injected intravenously into syngenetic recipients 24 h after they had been irradiated with ^{60}Co γ rays (Gamma-Cell 220 apparatus) in a dose of 900 rad (dose rate 1800 rad/min). A third-degree burn covering 15% of the body surface was obtained by immersing the dorsal region of the anesthetized mouse (0.7% pentobarbital solution, 0.15-0.20 ml intraperitoneally) in hot water (92°C) for 4 sec. In the experiments to study the effect of thymus cells on splenic colony formation by the bone marrow of the burned mice, syngenetic mice of the same age and strain were used as donors of the thymus. Thymus cells (10^7) were injected into the irradiated recipients 40 min before the injection of bone marrow cells. To prevent embolism, the mice were given 50 units heparin by intraperitoneal injection 10-20 min before receiving the injection of thymus cells. On the 9th day the recipients were killed, the spleen was removed and fixed in a mixture of acetic acid and ethanol (1:3), and the number of colonies was counted. The experimental results were analyzed with the aid of Student's criterion.

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