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Contractile Function of the Heart and the State of Some Stages of Lipid Metabolism during Acute Diphtheritic Intoxication

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Acute diphtheritic intoxication was modeled in rabbits by intravenous administration of native diphtherin (0.3 minimal lethal dose per 1 kg body weight). The contractile function of the left and right ventricles (peak systolic pressure under conditions of basal hemodynamics and during 5-sec occlusion of the aorta and pulmonary artery, respectively) was estimated 1, 3 and 5 days after the start of the pathological process, the intensity of lipid peroxidation was evaluated by measuring the content of TBA- reactive products in the myocardium. Impairment of the contractile function of both ventricles was observed in the course of intoxication. The level of TBA-reactive products in the left ventricle significantly decreased on day 1, but then returned to normal. Thus, impairment of the contractile function of the left ventricle at the early stages of diphtheritic intoxication is not mediated by activation of lipid peroxidation in cardiomyocytes.

Key Words: *diphtheritic intoxication; myocardium; left ventricle; lipid peroxidation*

Diphtheria, a live-threatening infectious disease, is now extremely rare due to extensive use of diphtheria and tetanus toxoids and pertussis (DTP) vaccine. However, diphtheria outbreak arose in countries of the former Soviet Union in 1990s. Thus, 158,000 diphtheria cases were registered in this territory from 1990 to 1999, including 4000 fatal cases [3].

Diphtheritic myocarditis is the most serious diphtheria complication and the major cause of death in diphtheria infection. The "diphtheritic heart" syndrome, rather than post-diphtheritic paralysis should be regarded as serious cause of mortality [2].

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Corynebacterium diphtheriae toxin is a cytochrome B protein component of diphtheritic bacteria, it competitively inhibits the synthesis of the corresponding enzyme of the mitochondrial respiratory chain in affected cells [5], which is followed by inhibition of tissue respiration and protein synthesis [4].

Crude disturbances of lipid metabolism develop at the earliest stages of diphtheritic myocarditis (within 1-3 days after the onset of the pathological process): lipids are intensively released from disintegrated mitochondrial membranes and form massive intracellular lipid inclusions in the myocardium [2].

Although the mechanism of action of diphtheritic exotoxin was thoroughly studied, many aspects of the pathogenesis of diphtheritic myocarditis and particularly the association of the impairment of myocardial

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contractile function with some stages of lipid metabolism, such as lipid peroxidation in the myocardium, are still poorly understood.

Here we studied the dynamics of contractile function of the right and left ventricles (RV and LV) and its association with the development of oxidative stress in the myocardium at the early stages of diphtheritic intoxication.

MATERIALS AND METHODS

Twenty male Chinchilla rabbits weighing 3.0-3.5 kg were divided into 4 groups, 5 animals per group: group 1 comprised controls (intact animals), groups 2, 3, 4 consisted of rabbits with 1-, 3- and 5-day diphtheritic intoxication, respectively. Diphtheritic intoxication was modeled by single intravenous injection of native diphtherin (0.3 minimum lethal dose (MLD) per 1 kg body weight). Preliminary titration of diphtherin was conducted in guinea pigs: 1 MLD corresponded to the amount of diphtherin inducing death of >50% animals (with signs of adrenal hemorrhage) within 3 days after single intraperitoneal injection.

On days 1, 3 and 5, blood pressure in heart chambers was recorded using MIKARD hardware-software complex which consisted of analog-to-digital converter and electromagnetic sensors connected to a personal computer. Rabbit chest was opened under general anesthesia (2% rometar), catheters connected to pressure gauge sensors were introduced into the ventricles though the ventricular walls, and curves for actual (peak systolic) intraventricular blood pressure in LV and RV (aLVIP and aRVIP respectively) were recorded. For evaluation of potential working capacity of the myocardium, the maximum intraventricular pressure in LV and RV (mLVIP and mRVIP) was recorded during 5-sec occlusion of the ascending aorta (for LV) or pulmonary trunk (for RV) [1]. All curves were processed using special software.

The myocardium tissue from LV was homogenized in Potter homogenizer (1500 rpm) for 3 min in 2 volumes of 20 mM HEPES buffer (pH 7.4). For measuring the content of TBA-reactive products, an aliquot of the homogenate was diluted 1:2 with 20 mM HEPES buffer, pH 7.4, the remaining homogenate was centrifuged for 10 min (1500*g* at 4°C) and the supernatant was collected. The content of TBA-reactive products was measured as described previously [6]. To this end, 50 μl 8.1% sodium dodecyl sulfate, 0.375 ml of 30% CH_3COOH (pH 3.0-3.5) and 0.375 ml 0.8% TBA were added to 50 μl homogenate or supernatant (final protein concentration 1 mg/ml) or homogenization buffer (blank sample). The mixture was incubated for 60 min at 96-100°C in a water bath until a yellow-rose color appeared. Then the test tubes were cooled and denaturated proteins were removed by 10-min centrifugation at 1500*g*. Spectrometry of the supernatant was carried out using an Ultrospec II spectrometer (LKB) at 535 nm and 560 nm against blank probe.

RESULTS

Judging from LV and RV blood pressure values measured under conditions of actual circulatory dynamics and during occlusion of the ascending aorta or pulmonary trunk, the progress of diphtheritic intoxication was associated with impairment of the contractile function of the heart involving both LV and RV (Table 1). Thus, aLVIP and mLVIP significantly decreased by 20 and 23%, respectively, on day 1 of observation. On day 3 of diphtheritic intoxication, mLVIP reached its minimum (decreased by 37% from the control) and remained low until experimental day 5.

Similar dynamics was observed for aRVIP and mRVIP except that these indices returned to the initial level on day 5 of observation.

Changes in systemic circulatory dynamics are indicative of severe impairment of cardiac contractile function in the course of acute diphtheritic intoxication. Accumulation of TBA-reactive products (which reflects activation of lipid peroxidation in the myocardium accompanying the pathogenic effect of diph-

TABLE 1. LV and RV Myocardium Contractility in the Dynamics of Diphtheritic Intoxication (*M*±*m*)

Index	Control	Intoxication		
		Day 1	Day 3	Day 5
aLVIP, mm Hg	139.6±1.3	113.7±1.5*	114.2±1.7*	123.4±2.1*
mLVIP, mm Hg	231.4±6.5	178.3±4.7*	146.3±3.2*	187.3±4.4*
aRVIP, mm Hg	36.4±1.2	28.7±0.7*	29.7±0.8*	33.9±1.0
mRVIP, mm Hg	53.5±1.8	41.9±1.3*	35.4±1.0*	51.4±2.2

Note. Here and in Table 2: *$p \leq 0.05$ compared to the control.

TABLE 2. Content of TBA-Reactive Products in LV and RV Myocardium in the Dynamics of Diphtheritic Intoxication (*M*±*m*)

Index	Control	Intoxication		
		Day 1	Day 3	Day 5
Homogenate (optical density/g tissue)	58.3±3.4	49.68±3.31	63.82±4.95	56.76±5.4
Supernatant (optical density/mg protein)	2.44±0.11	1.8±0.2*	2.76±0.28	2.62±0.33

therin on the myocardium) was to be expected against the background of this depression of heart contractile function. However, our experiments revealed no such changes (Table 2). On the contrary, the content of TBA-reactive products in the supernatant significantly decreased 24 h after induction of diphtheritic intoxication and returned to the initial level by day 3 of the experiment. On day 1, this parameter also tended to decrease, but the difference was not statistically significant. At later terms, the content of TBA-reactive products returned to normal. These observations suggest that the development of lipid peroxidation during diphtheritic intoxication is most intense within the 1st day, which probably causes a sharp decrease in the content of lipid peroxidation substrates in the myocardium and, as a consequence, a decrease in lipid peroxidation intensity by the end of day 1. However, at later stages, the intensity of lipid peroxidation returned to normal irrespective of ongoing intoxication and impaired myocardium contractile function caused by these changes. Thus, it can be assumed that impairment of the contractile function of the heart at early stages of diphtheritic intoxication is not mediated by activation of lipid peroxidation in cardiomyocytes.

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