DOI 10.1007/s10517-018-4065-2

Effect of Natural Cytokine Complex on the Structure and Metabolism of the Cardiac Conduction System in the Myocardium under Normally and Increased Hemodynamic Load

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 164, No. 12, pp. 681-685, December, 2017
Original article submitted June 9, 2017

Effect of natural complex of cytokines with activity of IL-1, IL-2, IL-6, TNF, MIF, and GTFβ on the structure and metabolism of conduction cardiomyocytes was assessed in the control and under acute experimental aortic stenosis. After systemic administration of the cytokine complex in the control, structural abnormalities were revealed in a relatively low number of conduction cardiomyocytes; their relative number increased in the left ventricle and interventricular septum. When the complex was administered against the background of aortic stenosis, morphological changes in the conduction system were seen in a significant number of cells with their plasma imbibition, especially in the left ventricle and interventricular septum. Systemic administration of the natural cytokine complex inhibited the major metabolic processes in the conduction system, both in the control and under conditions of sharply increased hemodynamic load. In conduction cardiomyocytes, deceleration of glycolysis and citric acid cycle, inhibition of oxidation of free fatty acids and their metabolites, and suppression of shuttle mechanisms and biosynthetic reactions were observed. Increased blood levels of cytokines, primarily of the proinflammatory ones, can cause structural and metabolic disturbances in the cardiac conduction system and promote the development of arrhythmias, especially in case of sharply increased hemodynamic load.

Key Words: *cardiac conduction system; structure; metabolism; cytokines*

Clinical observations show that the risk of cardiac arrhythmias correlates with increased blood levels of some proinflammatory cytokines. In particular, the significantly increased levels of TNF, IL-6, and IL-18 were reported in patients with atrial fibrillation [9,13]. Experimental studies have demonstrated the effect of proinflammatory cytokines, including TNF, IL-1, IL-2, and IL-6, on the parameters of bioelectrical activity of cardiac myocytes and the development of rhythm disturbances [10-12,14,15]. However, these studies are usually confined to contractile myocardium. Much less is known about the effect of cytokines on the cardiac conduction system, whose pathological changes underlie the development of arrhythmia. In this context, it is important to assess the state of the conduction system in case of increased blood level of cytokines, especially against the background of sharply increased hemodynamic load, which, as it is known, provokes arrhythmia occurrence.

Superlimf is a natural cytokine complex (NCC) with activity of TNF, IL-1, IL-2, IL-6, MIF, and GTFβ [2]. We have previously assessed the effect of its systemic administration on the morphological state and metabolic processes in contractile cardiomyocytes un-

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der conditions of acute hemodynamic overload of the left ventricle (LV) and without it [5].

Here we studied the influence of NCC on the structure and metabolism of conduction cardiomyocytes in the control and under conditions of acute LV afterload elevation.

MATERIALS AND METHODS

The study was carried out on guinea pigs of both sexes weighing 500-700 g under conditions of open chest and artificial ventilation. To assess the functional state of the cardiovascular system, we recorded ECG, BP and blood flow in the aorta, pressure in cardiac ventricles and its first derivative.

Animals were divided into 3 groups. Groups 1 (n=8) and 2 (n=10) consisted of animals subjected to all instrumental and surgical interventions, including artificial ventilation, chest opening, and ventricular catheterization, except aortic stenosis. Group 3 (n=8) consisted of animals with increased hemodynamic load to the heart modelled by partial ligation of the ascending aorta 15 min after catheterization. The degree of narrowing was controlled by changes in systolic pressure in the LV, which was increased by 100% above the baseline level. The duration of aortic stenosis was 30 min.

To animals of groups 2 and 3, the preparation Superlimf was injected into the cavity of the LV in 15 min after catheterization and then 3 times every 10 min (single dose 12.5 μg in 1 ml 0.9% NaCl).

After completion of the experiment, the hearts were excised and cut longitudinally into two halves, one half was used for histological examination, and the other one for histoenzymological analysis. The walls of the LV and right ventricle (RV) and the interventricular septum (IVS) were used for microscopic examination.

The material for morphological study was fixed in 10% neutral formalin in Lilly buffer and embedded in paraffin. Serial 5-μ sections were stained with hematoxylin and eosin, Schiff's reagent according to McManus with amylase control, Regaud iron hematoxylin. The relative number of conduction cardiomyocytes with positive amylase-resistant PAS reaction of the sarcoplasm and the proportion of Regaud-positive cells in the conduction system were determined.

The material for histoenzymological study was frozen in petroleum ether, cooled with dry ice, and serial cryostat 10-μ sections were prepared. Activities of succinate dehydrogenase (SDH), isocitrate dehydrogenase (ICDH), malate dehydrogenase (MDH), α-glycerophosphate dehydrogenase (α-GPDH), lactate dehydrogenase (LDG), β-hydroxybutyrate dehydrogenase (β-HBDH), NAD-diaphorase, and NADP-diaphorase were detected by conventional methods and scored using a 4-point scale. In addition, enzyme activity was determined by computer-assisted densitometry.

The data obtained in morphological and histoenzymological studies of each preparation in 10 fields of view were processed using mathematical statistics (Student's t test). The data are presented as $M \pm m$.

RESULTS

In group 1 animals, cell damage detected by Regaud staining was observed in a small portion of conduction cardiomyocytes. The relative number of Regaud-positive cells in LV was 8.8±1.6%, in RV — 8.3±2.0%, and in IVS 9.7±2.0%. In preparations incubated with amylase and stained with Schiff's reagent, signs of plasma imbibition were detected in single conduction cardiomyocytes. The proportion of cells with PAS-positive amylase-resistant sarcoplasmic reaction was 0.6±0.3% in LV, 0.8±0.6% in RV, and 1.9±0.9% in IVS.

In group 2 animals, of relative number of damaged conduction cardiomyocytes detected by Regaud method was not increased. The proportion of Regaud-positive cells in LV was 7.8±2.7%, in RV 10.6±3.2%, in IVS 10.2±3.4%. In preparations incubated with amylase and stained with Schiff's reagent, signs of plasma imbibition were revealed in a small portion of conduction cardiomyocytes. When compared to group 1, the proportion of cells with PAS-positive amylase-resistant sarcoplasm reaction was increased in LV (4.6±1.6%, p<0.01) and IVS (7.9±2.2%, p<0.01), but was not in RV (2.6±1.3%).

Thus, systemic NCC administration to control animals did not significantly affect the morphology of the intraventricular conduction system. Signs of structural damage were found in a relatively small number of conduction cardiomyocytes, the proportion of these cardiomyocytes was increased in the LV and IVS.

In group 3 animals, the number of damaged conduction cardiomyocytes, revealed by both methods was significantly increased in comparison with groups 1 and 2 (p<0.01). The proportion of Regaud-positive cells in LV was 54.0±6.3%, in RV 22.8±5.8%, in IVS 37.3±5.1%. The proportion of cells with PAS-positive amylase-resistant sarcoplasmic reaction was 63.1±5.9% in LV, 27.6±5.2% in RV, and 34.9±4.9% in IVS.

Thus, in case of systemic NCC administration against the background of acute experimental aorta stenosis, a significant proportion of cells in cardiac conduction system was damaged, especially in the LV and IVS. Similar changes were detected previously under acute aortic stenosis in absence of NCC, but they were much less severe [7]. First of all, this concerns conduction cardiomyocytes with signs of plasma

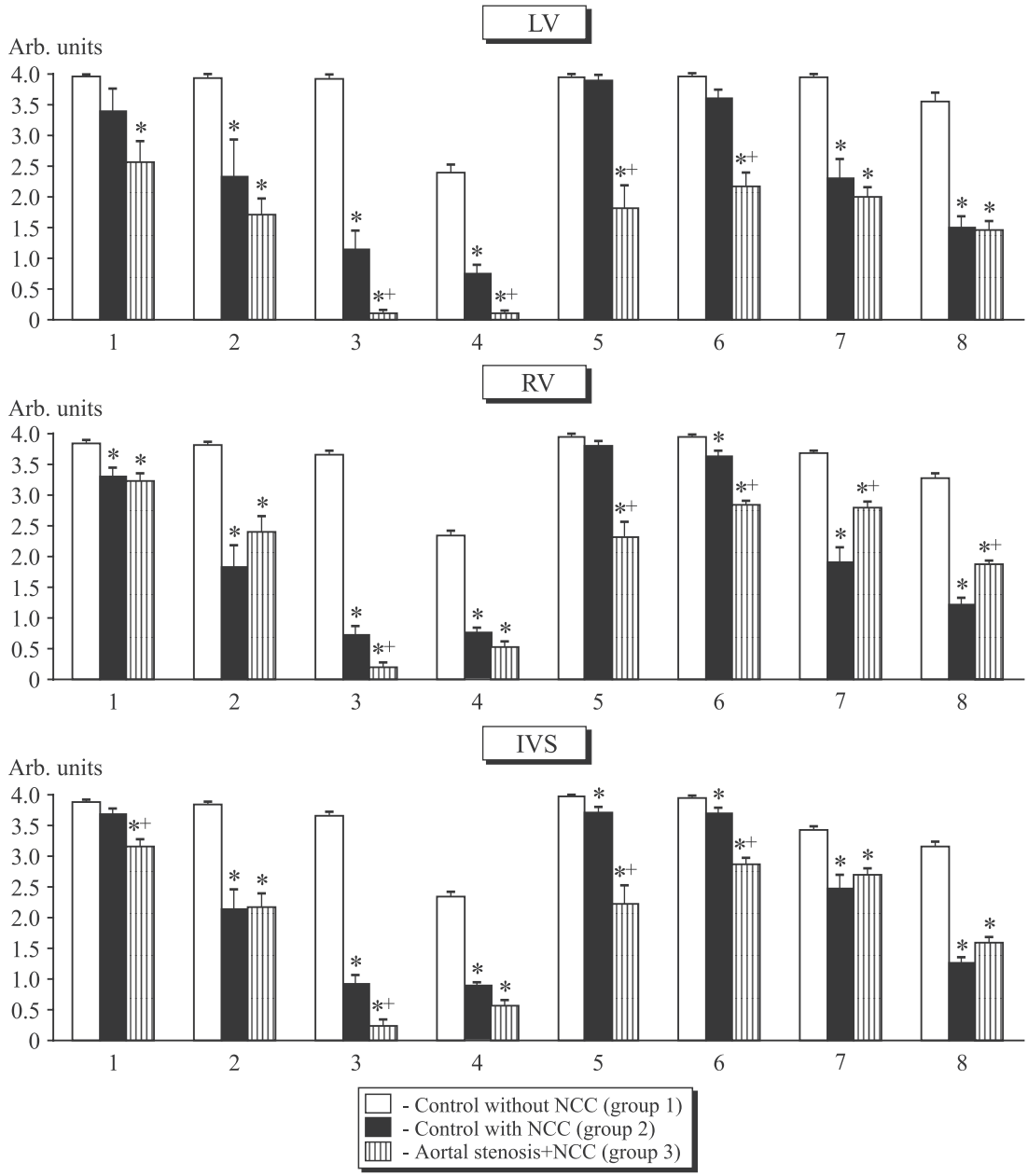

Fig. 1. Effect of NCC on the histoenzymological profiles of conduction cardiomyocytes in the control and under conditions of acute experimental aortic stenosis. Ordinate: activity of enzymes. *1*) SDH, *2*) ICDH, *3*) MDH, *4*) α-GPDH, *5*) LDH, *6*) NAD-diaphorase, *7*) NADP-diaphorase, *8*) β-HBDH. $p<0.01$ in comparison with *group 1, +group 2.

penetration, the proportion of which in case of aortic stenosis in absence of NCC was 33.4±6.9% in the LV, 2.4±1.0% in the RV, and 8.0±2.5% in the IVS ($p<0.01$ in comparison with group 3 in this study). Altogether, the results of this and previous studies suggest that systemic NCC administration against the background of sharply increased hemodynamic load has an additional alterative effect on the conduction system. The severity of heart damage was different: maximum damage was observed in LV, minimum damage in RV, and intermediate in IVS.

Systemic NCC administration to control animals (group 2) was followed by inhibition of practically all studied enzymes (Fig. 1). Reduced activity of SDH, ICDH, and MDH attested to deceleration of the citric acid cycle. Reduced activity of LDH and α-GPDH indicates glycolysis inhibition. The pronounced decrease in MDH and α-GPDH activities can also be associated with deceleration of shuttle mechanisms. Low β-HBDH activity reflects inhibition of oxidation of free fatty acids and their metabolic products. The decreased activity of NAD-diaphorase and NADP-

diaphorase reflects general inhibition of catabolic and anabolic processes in conduction cardiomyocytes. In the conduction system of LV, RV and IVS, histoenzymological changes were of similar nature and severity.

When NCC was administered to animals with acute aortic stenosis (group 3), activity of all studied enzymes was lower than in group 1 ($p<0.01$; Fig. 1). When compared to group 2, activity of the majority of enzymes was also lower or did not differ except NADP-diaphorase and β-HBDH in RV conduction cardiomyocytes, whose activity was higher ($p<0.01$). As for the rest, changes in the conduction system of LV, RV, and IVS were similar. These data attest to inhibition of the basic metabolic processes in conduction cardiomyocytes in case of systemic NCC administration against the background of increased hemodynamic load. Similar histoenzymological changes in conduction system cells were previously observed in acute aortic stenosis without NCC administration [6]. Comparison of the previous data and current results shows that against the background of aortic stenosis, activity of all the studied enzymes (with the exception of SDH) in the conduction cardiomyocytes of both ventricles and IVS was lower in case of NCC administration than without it ($p<0.01$).

In general, histoenzymological findings suggest that systemic NCC administration inhibits the main metabolic processes in the intraventricular conduction system cells both in the control and in case of sharply increased hemodynamic load. This effect can be induced by the proinflammatory cytokines, components of the test complex. Specific mechanisms of their effect on the metabolism of conduction cardiomyocytes have not been determined yet. In any case, inhibition of cytoplasmic and mitochondrial energy-generating reactions can lead to deficit in the energy supply of electrophysiological processes in the conduction system and contribute to emergence of cardiac arrhythmias.

Structural and metabolic changes in the conduction and contractile [5] cardiomyocytes in case of systemic NCC administration have both similarities and differences. In both types of myocytes, the inhibitory effect on activity of cell enzymes (especially for MDH and α-GPDH) was observed both in the control and under conditions of acute hemodynamic load increase. In the latter case, less pronounced metabolic disturbances and more structural changes were observed in the conduction system in comparison with contractile myocardium. This attests to a relatively minor role of metabolic shifts in cell damage in the cardiac conduction system and is consistent with the previous data [6]. Dyscirculatory disturbances are apparently more critical, which can be explained by structural and functional features of the conduction system: its components are immersed in the connective tissue matrix and have close contact with myocardial blood vessels and relatively high membrane permeability [1]. Increased blood concentration of proinflammatory cytokines leads to immune inflammation characterized by the presence of vascular exudative and cellular components closely related to the state of blood circulation in the organ [4]. It has been previously shown that in case of acute aortic stenosis the density of functioning capillaries, indicative of increased myocardial blood flow, is maximally increased in the LV and IVS, where more pronounced dyscirculatory disturbances are also present [8]. This is consistent with the results of our study, which revealed maximum number of conduction cardiomyocytes with signs of damage in LV and IVS. It is believed that mechanisms, underlying the alterative effect of cytokines on tissues, are associated with production of arachidonic acid metabolites, ROS, and nitric oxide and degranulation of leukocytes with release of proteolytic enzymes [4,11]. It is assumed that these mechanisms are to a certain extent caused by increased intracellular Ca^{2+} concentration [3,9,10,12,14]. It has been demonstrated that increased ROS production by phagocytes in response to stimulation after preliminary treatment with the natural cytokine complex Superlimf used in this study is a Ca^{2+}-dependent process [3].

Thus, increased blood cytokine level observed during systemic inflammation reactions or due to cytokine therapy can lead to structural damage and inhibition of the basic metabolic processes in the heart conduction system, thereby contributing to arrhythmia development. Systemic increase in the level of proinflammatory cytokines in combination with acute hemodynamic heart overload causes deeper impairment of the morphofunctional state of the conduction system.

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