INFLUENCE OF INFLAMMATORY REACTION ON BLOOD CONCENTRATION OF CHOLESTEROL AND OTHER BIOCHEMICAL VALUES WITH REGARD TO CARDIAC MUSCLE DAMAGE IN RATS

IRENEUSZ CAŁKOSINSKI¹, ROBERT SKALIK¹, LUDMILA BORODULIN – NADZIEJA¹, URSZULA WASILEWSKA¹, ANNA JANOCHA¹, MAREK CEGIELSKI², BEATA PONIKOWSKA¹ AND ANNA GOŻDZIK³

¹Department of Physiology, ²Department of Histology and Embryology
³Department of Cardiac Surgery,
Medical University of Wrocław, 50-368 Wrocław, Poland

e-mail: m.calcosinska@xl.wp.pl

Received for publication July 12, 2004.

Abstract

The ultrastructure of myocardium during experimentally induced acute pleuritis and role of lipid metabolism in the protection of cardiac muscle were investigated. The study was performed on 24 rats from Buffalo strain. In the study group, the experimental acute pleuritis was induced and the scraps of heart muscle were taken at the 24th, 72nd and 120th h after the onset of inflammatory reaction. The measurements of total and fractionated (LDL, HDL) cholesterol, triglycerides, interleukin 4 (Il-4) and cortisol concentrations in blood were made at the same hours. The significant decrease in lipoprotein concentration and increase in Il-4 concentration during acute pleuritis were found in the group of rats with histopathologically confirmed myocardial cell damage.

Key words: rats, inflammation, cholesterol, interleukin 4, cortisol, myocardial damage.

In the course of inflammatory process, the cellular immunological response is accompanied by the activation of proteolytic processes, which may induce impairment of distant organs, e.g. cardiac muscle, (8, 16). The stimulation of the sympathetic nervous system in the course of inflammatory reaction brings on secretion of adrenal glucocorticoids, which was confirmed by previous investigations (5). The increased secretion of glucocorticoids can be also observed as a result of irritation of pain receptors by mechanical and humoral stimuli (bradykinin, decrease in pH) during inflammatory process (9, 21). The secreted glucocorticoids stimulate lymphocytes Th₂, enhance synthesis of acute phase proteins and modulate lipid metabolism. The significant role of endogenous glucocorticoids is also the inhibition of phagocytosis and stabilization of lisosomal membranes (26). Besides, the respectable production of proinflammatory cytokines such as: II-1β, II-6, TNF proceeds during inflammatory response, which was reported by Castella (10). The glucocorticoids and cytokines contribute significantly to the elimination of inflammatory factors and eradication of damaged cells. They cause platelet aggregation, activate macrophages and enhance adhesion of neutrophils, which in turn produce whole array of proteases such as collagenase and elastase (3, 4, 10). The enzymes contribute to dominance of destructive processes in inflammatory focus and increase concentration of collagen degradation products in blood (7, 14, 23). The increased concentration of proinflammatory cytokines activates fibroblasts to produce collagen, which replaces the damaged cells and promotes in this way fibrosis of postinflammatory tissue (2, 6, 13, 16, 17). The proinflammatory interleukins also stimulate synthesis of fibrinogen by hepatocytes and other acute phase proteins. The significant increase in blood concentration of lipoproteins and cholesterol, which is stimulated by adrenal glucocorticoids in response to inflammatory process, was reported by some investigators (1, 17, 21). It is hypothesised that the lipoproteins are bound to produce complexes with fragments of foreign antigens thus reducing inflammatory reaction. Hence, the increase in lipid metabolism is supposed to be a preventive reaction. Moreover, lipoproteins play very significant role in the reconstruction of cellular membranes which were damaged in the course of immunological response to foreign antigens (12, 19).

The study was supposed to answer the question whether inflammatory process in a distant organ (pleura) can contribute to the formation of destructive foci in the heart. At the same time, the hypothesised role of lipid metabolism in the protection of cardiac muscle against the adverse effects of immunological response to acute inflammatory process in distant organ was investigated.
Material and Methods

The study was performed on 24 rats from Buffalo strain, aged 16 weeks, with body weight of 180-200 g. Two groups of the rats were investigated: the study group – 18 rats and control group – 6 rats. The experimental pleuritis was induced by intrapleural injection of 0.15 ml of 1% solution of carragenin (Sigma). The scraps of heart muscle (left ventricle) were taken at the 24th, 72nd and 120th h after the onset of inflammatory reaction. Afterwards, the scraps were routinely prepared for electron microscope analysis (8). Besides, the measurements of total and fractionated LDL and HDL cholesterol, triglycerides, IL-4, and cortisol concentrations in blood were made at the 24th, 72nd and 120th h of acute pleuritis in all the rats (22). The blood samples were taken from the aorta of rats anaesthetised with tiopental (30 mg/kg of body weight). The concentration of total and HDL cholesterol and triglycerides was measured with use of biochemical analyser machine Olympus 560-U and tests from BioMérieux. The concentration of LDL cholesterol was calculated with use of Friedewald formula. The statistical analysis of the results was performed with t-Student test.

Results

The electron microscope analysis of the cardiac muscle scraps taken in the course of experimentally induced pleuritis showed the increase in mitochondria volume at the 24th h of the experiment, in comparison with the control group. Besides, the derangement of internal structure of mitochondria was observed at the 72nd h of the acute pleuritis. The further course of the inflammatory process (120th h) was characterised by enhanced activity of fibroblasts and increased collagen production.

The comparison of blood concentration of biochemical values between the control group and the study group (included in Table 1) was obtained at the 24th, 72nd, and 120th h of the experiment. In reference to the control group, the significant decrease in blood concentration of total and fractionated HDL cholesterol in the study group, was observed in case of total cholesterol the highest decrease was found at the 72nd (P≤0.05) and in case of fractionated HDL cholesterol at the 24th h (P<0.001) of the experiment. The blood concentration of fractionated LDL cholesterol and triglycerides transiently decreased at the 24th h (P<0.05) of the experiment and next came back to the baseline values at the further hours of the acute pleuritis (the cholesterol and triglyceride concentrations throughout the experiment are presented in Table 1).

In reference to the control group, the blood concentration of IL-4 in the study group, gradually increased and reached its maximal value at the 120th h of the experiment. These changes were statistically significant (P<0.001). The blood concentration of cortisol increased at the 24th h and was the highest at the 72nd h. Both changes were statistically significant (P<0.001). The blood concentration of cortisol came back to the baseline values at the 120th h of the experiment (IL-4 and cortisol concentrations throughout the experiment are presented in Table 1).

Table 1
Comparision of blood concentration of biochemical values between control and study group

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol mg/dl</th>
<th>HDL cholesterol mg/dl</th>
<th>LDL cholesterol mg/dl</th>
<th>Triglycerides mg/dl</th>
<th>Interleukin 4 pg/ml</th>
<th>Cortisol nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=6)</td>
<td>55.40±5.55</td>
<td>26.20±3.11</td>
<td>11.40±4.39</td>
<td>87.80±19.90</td>
<td>11.11±2.89</td>
<td>14.97±0.71</td>
</tr>
<tr>
<td>Study group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24h (n=6)</td>
<td>51.80±7.26</td>
<td>19.80±2.68</td>
<td>19.00±3.61</td>
<td>66.60±11.26</td>
<td>23.59±2.38</td>
<td>18.37±1.06</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>P≤0.001</td>
<td>P≤0.05</td>
<td>P≤0.05</td>
<td>P≤0.001</td>
<td>P≤0.001</td>
</tr>
<tr>
<td>Study group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72h (n=6)</td>
<td>48.40±1.80</td>
<td>21.40±2.41</td>
<td>10.00±2.74</td>
<td>85.20±15.99</td>
<td>44.45±3.32</td>
<td>19.00±2.15</td>
</tr>
<tr>
<td></td>
<td>P≤0.05</td>
<td>P≤0.05</td>
<td>NS</td>
<td>NS</td>
<td>P≤0.001</td>
<td>P≤0.001</td>
</tr>
<tr>
<td>Study group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120h (n=6)</td>
<td>49.40±1.67</td>
<td>22.20±1.30</td>
<td>10.80±2.49</td>
<td>82.00±17.65</td>
<td>54.34±3.80</td>
<td>15.93±1.09</td>
</tr>
<tr>
<td></td>
<td>P≤0.05</td>
<td>P≤0.05</td>
<td>NS</td>
<td>NS</td>
<td>P≤0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>
Discussion

The significant contribution of inflammatory processes to formation of destructive foci in cardiac muscle is considered in the pathogenesis of heart failure (8, 16). The appearance of such significant morphological changes in the myocardium during the experimentally induced non-bacterial pleuritis in rats indicates that inflammatory processes in a designated area (pleura) or system of organisms can affect distant organs (heart), which was previously confirmed only by few investigators (2, 17). The destructive postinflammatory foci in cardiac muscle are presumably caused by the proinflammatory cytokines IL-1β, IL-6 and TNF, which are secreted in the early stage of inflammatory reaction (2, 4). The noticed significant increase in blood concentration of cortisol during acute pleuritis can ward off to some degree the adverse effect of proinflammatory cytokines on the heart (18). The intensive release of adrenal glucocorticoids in response to foreign antigen during inflammatory reaction also enhances triglyceride and cholesterol metabolism. It is hypothesised that the significant increase in blood concentration of glucocorticoids in the very early phase of inflammation is related to the immediate stimulation of the hypothalamus-pituitary-adrenal axis and steroidogenesis in the adrenal glands by proinflammatory cytokines (TNF alfa) (21). According to some authors, the increase in blood concentration of lipoproteins both before and during the inflammatory process presumably exerts its protective effect on cardiomyocytes and prevents cardiomyocyte damage (19). Hence, the normal or too low blood concentration of cholesterol may contribute to deterioration of myocardial dysfunction in the course of inflammatory reaction (19). There are more and more literature reports on pleiotropic and immunomodulatory effects of statin therapy distinct from their cholesterol-lowering and LDL-lowering action (19). It is well known that statins stabilize atherosclerotic plaques through inhibition of inflammatory reaction. Some investigators argue that in the nearest future the anti-inflammatory effect of statins eg itself will be more important for the prevention of acute coronary syndromes than aggressive lowering of cholesterol concentration (11). Hence, some authors propose administration of non- lipid-lowering statins with their preserved anti-inflammatory properties in patients with severe heart failure (11). According to Rauchhaus and Coats (19), patients with coronary artery disease obviously benefit from lipid-lowering statin therapy. However, this positive effect of statins on survival is not warranted in patients with ischaemic or non-ischaemic chronic heart failure. It is assumed that patients with chronic heart failure can be affected by transient episodes of endotoxaemia, and lipid-lowering treatment with statin might enhance the activity of bacterial lipopolysaccharide in this case (19). Vredevoe et al. (24) confirmed the immediate relation between low LDL, HDL, and triglyceride concentrations and impaired survival in patients with symptomatic chronic heart failure. The perioperative mortality of patients with severe chronic heart failure supported by a left-ventricular-assist device was highly associated with low cholesterol concentrations (20). Rauchhaus et al. (19) showed that low total cholesterol was highly predictive for impaired 1-year event-free survival in patients with chronic heart failure, independent of cause (ischaemic or non-ischaemic heart failure).

The significant decrease in total cholesterol concentration during experimentally induced acute pleuritis in the group of rats with histopathologically validated cardiomyocyte damage was observed. The fall in cholesterol concentration accompanied by increased secretion of adrenal glucocorticoids during the experiment could be caused by surmounting utilization of lipoproteins over their synthesis (metabolic enhancement) in an efficacious response to advancing inflammatory process in spite of potential stimulative effect of cortisol on lipid metabolism (19). These results are in accordance with Kelley’s investigations, which validated decrease in cholesterol concentration concomitant with the reduction of inflammatory cells in the peripheral blood and atheromatous plaques (15). It is also proved by previous investigations that lipids are utilized by organisms to rebuild cellular membranes damaged by inflammatory process. Hence, significant reduction of total and HDL cholesterol concentration in our experiment was presumably associated with excessive lipoprotein turnover indispensable for reconstruction of lipid membranes of pleural and cardiac cells in the group of rats with foci of myocardial cell damage.

There are few studies discussing the role of anti-inflammatory IL-4 in immunological response to inflammation. In our experiment, IL-4 concentration gradually increased from the early stage of the investigation in order to reach its maximal value at the 120th h of the inflammatory process. However it must be stressed that previous investigations showed mainly increase in proinflammatory cytokines concentration such as IL-1β, IL-6 and TNFα in the early hours of inflammatory reaction (27). Then, the obtained results of our study confirmed complexity and intricacy of immunological response to foreign antigen. In our study the significant increase in IL-4 concentration was noted at the 24th h of inflammatory process, i.e in the very early stage of inflammatory reaction, when rise of proinflammatory cytokines is usually expected. It is also very intriguing that the gradual increase in anti-inflammatory IL-4 concentration was accompanied by gradual fall in total cholesterol concentration. It is highly probable that the reduction of total cholesterol concentration was induced by excessive utilization of lipoproteins for the inhibition of inflammatory reaction. It is validated in the experimental studies that IL-4 inhibits staphylococcal superantigen-induced apoptosis of helper lymphocytes CD4+ which are necessary for antiinflammatory response (25). The presented above observations and literature reports suggest that the process of myocardial damage and further course of chronic heart failure are influenced by infectious and non-infectious factors, which was proved by our
experiment. The overlap of chronic atherosclerotic myocardial ischaemia and destructive effects of the infectious and non-infectious factors (viruses, bacteria, chemical compounds) on the heart lead to the increased secretion of cardiotonic proinflammatory cytokines.

The performed experiment on the animal model confirmed the immediate relation between inflammatory process in pleura and impairment of cardiomyocytes. Hence, it can be assumed that inhibition of active inflammatory process in pleura and impairment of cardiomyocytes. The overlap of chronic atherosclerotic ischaemic myocardial damage and potentially contribute reduce the adverse effects of ischaemic or non-ischaemic myocardial damage and potentially contribute to the improvement of survival in chronic heart failure.

References