SUBCELLULAR CHANGES IN THE MYOCARDIUM OF RATS WITH NEPHROTOXIC GLOMERULONEPHRITIS

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Disorganization of the motochondria, myofibrils, and sarcolemma, intra-and intercellular edema, swelling and pycnotic changes in the endothelium, occlusion of the capillaries by blood cells, and thickening and condensation of the basement membranes were observed in the myocardium of rats with nephrotoxic glomerulonephritis induced by Masugi's method. The changes reached a maximum on the 10th-20th day after the beginning of production of glomerulonephritis. After the 20th day intracellular regenerative processes became intensified. Correlation was found between the subcellular, histological, and clinical manifestations of glomerulonephritis.

KEY WORDS: myocardium; experimental glomerulonephritis; subcellular changes.

Cardiac failure is a serious complication of acute glomerulonephritis [1-3,7,8]. However, a contradictory assessment of the character and severity of the myocardial lesion in this disease is to be found in published reports of morphological studies of the heart. The overwhelming majority of investigations have been carried out on autopsy material by the use of light-optical methods [4,5,9,10].

With these considerations in mind it was decided to study subcellular changes in the myocardium in experimental glomerulonephritis.

EXPERIMENTAL METHOD

Nephrotoxic glomerulonephritis was produced in rats by Masugi's method by injection of antikidney serum. The source of the heterologous nephrotoxic serum was rabbits immunized with a homogenate of rat renal cortex. The serum (titer 1:1200) was injected parenterally into the rats in a dose of 0.8-1 ml/100 g body weight on from 1 to 5 consecutive days.

For electron-microscopic investigation of the animals decapitated 1-75 days after injection of the serum, the myocardium was taken from the subedocardial portion of the left ventricle, fixed in glutaraldehyde, post-fixed in osmium tetroxide, and, after dehydration in alcohols of increasing concentration, embedded in a mixture of Araldite and Epon. Sections stained with lead and uranyl acetate were examined in the JEM-7A microscope.

Experiments were carried out on 46 rats, in 36 of which glomerulonephritis was produced, four animals received an injection of native rabbit serum, two received distilled water, and four rats were left intact.

EXPERIMENTAL RESULTS

The development of glomerulonephritis in the rats was characterized by worsening of their general state, loss of appetite, apathy, edema of the subcutaneous cellular tissue, marked albuminuria (up to 30%) and, frequently, diarrhea. In some animals the blood nonprotein nitorgen level was increased and hypo-and dysproteinemia were observed. On the ECG changes were observed in the R and T waves and the S-T interval, as well as other changes indicating myocardial damage.

Histologically changes characteristic of membrano-proliferative glomerulonephritis were found in the

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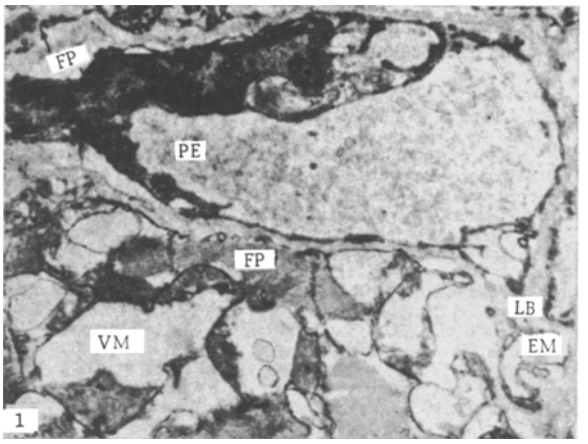

Fig. 1

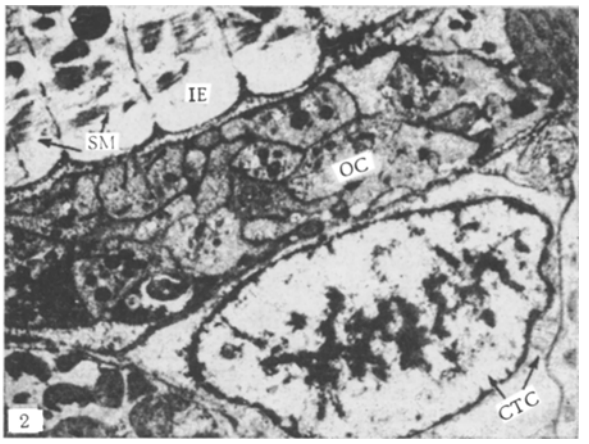

Fig. 2

Fig. 1. Destructive changes in myocardium of rat 5 days after production of glomerulonephritis. Fragmentation of plasmalemma (FP) and vacuolation (VM) and elimination (EM) of mitochondria of cardiomyocytes, pycnotic changes in endothelium with formation of myelin-like bodies (PE), and loosening of basement membrane (LB); 15,000 ×.

Fig. 2. Myocardium of rat 22 days after production of glomerulonephritis. Intracellular edema (IE). Stretching and rupture of sarcomeres (SM). Occlusion of capillary lumen by platelets (OC). Connective-tissue cell in interstitial tissue (CTC); 15,000 ×.

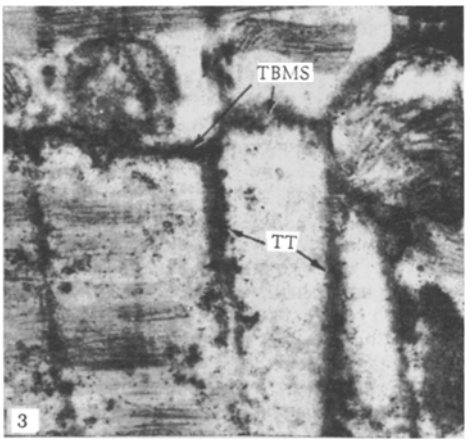

Fig. 3. Thickening of basement membranes in sarcolemma (TBMS) and T-system (TT) in myocardium of rat 36 days after production of glomerulonephritis; 30,000 ×.

kidneys of the rats at all times of the investigation. Examination of the heart with the light microscope revealed congestion of the capillaries and venules, perivascular edema, focal lympho-histiocytic infiltration of the interstitial tissue, and degeneration of individual muscle fibers in the subendothelial zones of the myocardium. The histological changes in the kidneys and heart were most marked on the 10th-20th day [4].

Comparison of electron micrographs of the experimental and control animals showed the presence of substantial changes in the cardiomyocytes, capillaries, and interstitial tissues in the myocardium of the rats with nephrotoxic glomerulonephritis. A definite dynamics of these changes could be traced depending on the duration of glomerulonephritis. In the early stages, from 1 to 10 days after the beginning of injection of the nephrotoxic serum (12 rats), when symptoms of glomerulonephritis were starting to appear but no regular changes were found on the ECG, the changes in the myocardium affected mainly the mitochondria, which in the overwhelming majority of myocardial cells were swollen, vacuolated, and deformed. Their cristae were

appreciably reduced in number and their matrix was translucent. Meanwhile, however, signs of hyperplasia and hypertrophy of the mitochondria also were observed. The remaining organelles had no destructive changes. Sings of intracellular edema were observed in many cardiomyocytes. The myocardial capillaries of most animals at this time had a normal ultrastructure. However, in four rats with the most marked changes in the myocardial cells, the capillaries were profoundly altered. Swelling or condensation of the endothelial cells and loosening and thickening of the basement membranes were observed in their walls and an accumulation of electron-dense material, leukocytes, and erythrocytes in their lumen (Fig. 1).

On the 11th-20th day after injection of nephrotoxic serum (15 rats) the clinical features of glomerulonephritis were at their maximum. On the ECG of 80% of the rats the heart rate was slowed, the R wave was depressed, the voltage of the other waves was reduced, changes were observed in the terminal part of the ventricular complex (the T wave and S–T interval), and the systolic index was increased. During this period intracellular, intercellular, and pericapillary edema were conspicuous features of the myocardium of nearly all the anumals, coupled with severe edema of the subcutaneous cellular tissue of the rats. Large vacuoles with structureless or fine-grained contents, formed at the site of groups of destroyed mitochondria, were found in the cardiomyocytes. The tubules of the sarcoplasmic reticulum were dilated. Focal injury to the myofibrils was observed, in the form of fragmentation, lysis, and overstretching of individual sarcomeres. In addition, marked disorganization of the sarcolemma was observed in many myocardial cells. In some parts its plasma layer was broken into fragments, diffuse in structure or absent altogether, but the basal layer was condensed and thickened. Appreciable condensation and thickening of the basement membranes also were observed in tubules of the T-system exposed as a result of intracellular edema. At the same time, it must be pointed out that the nuclei of the myocardial cells as a rule preserved their normal ultrastructure, and often showed signs of increased functional activity (twisted outlines, large nucleoli, numerous pores in the karyolemma). Large clusters of mitochondria were frequently observed around the nuclei. The capillary walls in most cases consisted of swollen and pycnotic endothelial cells, frequently containing myelin-like structures. Separation of the endotheliocytes with disturbance of intercellular contacts was observed. The basement membranes of the blood capillaries were nearly always thickened and condensed and were frequently in line with the sarcolemma of the adjacent cardiomyocytes. More often than previously, accumulation and stasis of erythrocytes, leukocytes, fibrinoid masses, and groups of platelets were discovered in the lumen of the microvessels, sometimes completely blocking the lumen of the capillary. Leukocytes, erythrocytes, fibrocytes, plasma cells, and mast cells, as well as mitochondria of damaged myocytes were observed in the intercellular and perivascular spaces (Fig. 2).

In the later stages (up to 75 days), when the manifestations of glomerulonephritis were diminishing or had cleared up, slight changes in the R wave and in the terminal part of the ventricular complex were observed on the ECG of only 50% of the rats. Examination of the myocardium of nine rats showed that most of the ultrastructural changes described above were less commonly found. However, disorganization of the basement membranes in the myocytes and capillaries continued to progress (Fig. 3). Meanwhile muscle and endothelial cells, larger than previously and with organelles of normal structure, were detected. In addition, intracellular regenerative processes were intensified in the myocardial cells: activation of nuclei and nucleoli, accumulation of ribosomes and polysomes in the sarcoplasm, new myofibril formation, and division and hypertrophy of mitochondria.

In the rats studied on the 15th and 22nd days after injection of native rabbit serum or of distilled water, subcellular changes in the myocardium were manifested only as swelling of some mitochondria and moderate intracellular edema of individual myocytes, and also by the presence of single platelets and clusters of erythrocytes in some capillaries.

Destructive and reparative subcellular changes in the myocardium of rats thus appeared after the first days of development of nephrotoxic glomerulonephritis and affected both its muscle fibers and its capillary system. Definite correlation was observed between the dynamics of the morphological and clinical manifestations of glomerulonephritis.

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SPECIFICITY OF THE EFFECT OF GROWTH HORMONE ON THE DNA CONTENT IN LYMPHOCYTE NUCLEI OF HYPOPHYSECTOMIZED RATS

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The effect of growth hormone on the DNA content in lymphocyte nuclei in the thymus, spleen, and lymph nodes was investigated cytophotometrically. In hypophysectomized rats growth hormone was shown to increase the DNA content in nuclei of medium lymphocytes of these organs but did not change its content in small lymphocytes. Lymphocytes of the thymus were most sensitive to the action of growth hormone. The DNA content in the nuclei of these cells increased as early as 1 h after injection of the hormone and reached its maximum after 4 h. Other hormones with anabolic action (insulin, thyroxine, testosterone) caused no increase in DNA in the thymocyte nuclei during this period. It is concluded that growth hormone has high affinity for cells of the lymphoid organs and, in particular, for thymocytes (medium lymphocytes of the thymus).

KEY WORDS: growth hormone; nuclei of lymphocytes; thymocytes; hypophysectomy.

Investigations in the writers' laboratory have shown that a few months after hypophysectomy in rats only growth hormone and, to a far lesser degree, thyrotropic hormone can increase the weight of the thymus significantly when administered daily for 10 days [2,3]. No other hormones had this property. The special role of growth hormone in relation to lymphoid tissue is relfected in the stimulation of incorporation of labeled precursors into proteins and nucleic acids of lymphocytes [8] and also the increase in the mitotic activity of lymphocytes [9]. It has recently been shown that thymocytes specifically bind growth hormone [4].

In this investigation a comparative cytophotometric analysis was made of the specificity of the early effect of growth hormone on DNA synthesis in different types of cells in several lymphoid organs.

EXPERIMENTAL METHOD

Female Wistar rats weighing 65-70 g were hypophysectomized by the transauricular route [1]. The animals were used in the experiments 14 days after the operation. In the experiments of series I the rats were divided into four groups with 10 animals in each group. Group 1 served as the control, the animals of groups 2 and 3 received a single intraperitoneal injection of growth hormone in a dose of 200 μg, dissolved in 0.25 ml physiological saline and were killed 4 and 6 h later, respectively, and the rats of group 4 were intact animals of

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