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MORPHOLOGICAL CHANGES IN THE KIDNEYS IN ACUTE RENAL FAILURE INDUCED BY EXPERIMENTAL PANCREATITIS

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UDC 616.61-008.64-036.11-02:616.37-002.1]-091

KEY WORDS: acute pancreatitis; acute renal failure; renal biopsy

Clinical data indicate a high frequency (from 37 to 60%) of kidney involvement associated with acute pancreatitis [1, 3]. However, there is no unanimity regarding the explanation of the morphological nature of the lesion. Most investigators have relied on autopsy data, which do not rule out the possibility of obscuring of the morphological picture by postmortem changes. Moreover, the sequence of the morphological changes in the kidneys in acute pancreatitis has not been studied.

To determine the precise character of the kidney damage in acute pancreatitis, the writers have studied the time course of the morphological changes as revealed by punch biopsy of the kidneys.

EXPERIMENTAL METHOD

Experiments were carried out on 42 dogs with acute hemorrhagic pancreatitis. An enzymic-hypertensive model of pancreatitis, induced by injection of duodenal contents under pressure into the system of pancreatic ducts, followed by ligation of the ducts, was used [2]. Open renal biopsy was performed with a needle (I-118, technical specification 64-1-2702-73) from the All-Union Research Institute of Instruments every hour for 8 h. The kidneys removed immediately after sacrifice or death of the animal were subjected to histological investigation. The following staining methods were used: hematoxylin and eosin, Van Giesons' PAS reaction, Weigert's, and Mallory's method for fibrin. An electron-microscopic investigation was also undertaken.

EXPERIMENTAL RESULTS

Light microscopy of biopsy material taken 60 min after the beginning of the disease revealed moderate congestion of the glomerular vessels. Electron microscopy revealed thickening and coarsening of the material of the basement membrane of the glomeruli, increased density of the material of the podocytes, the appearance of a fibrous structure in them, and proliferation of mesangial cells (Fig. 1).

By the 2nd hour of the experiment the congestion appeared more severe under the light microscope. Electron microscopy revealed a fibrous structure of the basement membrane and a picture of lysis of the podocyte nuclei; a fibrous structure also was observed in the feet of the podocytes. The picture described above, 3 h after the beginning of the experiment, was supplemented by stasis in the capillaries, and solitary thrombi in the glomerular capillaries. Filling of the glomeruli with exudate and proliferation of mesangial cells without any significant increase in size of the mesangial matrix were observed on the electron micrograph. After 4 h, besides the changes mentioned above, a mild degree of focal segmental glomerulitis was observed under the light microscope in single glomeruli: segmental focal mesenangial proliferation without enlargement of the mesangial matrix (Fig. 2). By the end of the 4th hour isolated regions of splitting of the basement membrane of the glomerulus could be seen on the electron micrograph.

First Department of Internal Medicine, S. M. Kirov Leningrad Postgraduate Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 10, pp. 489-491, October, 1986. Original article submitted January 13, 1986.

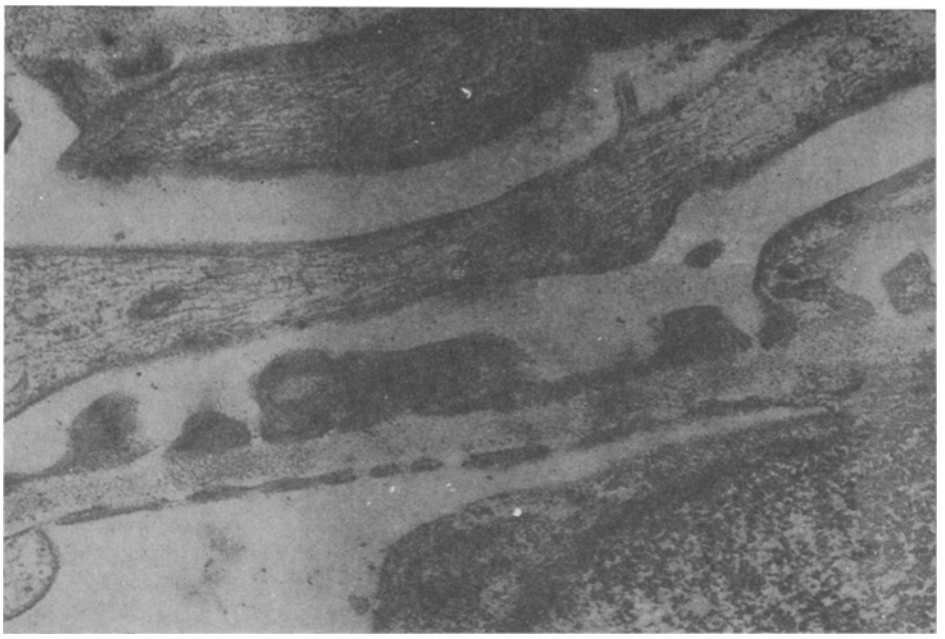

Fig. 1. Morphological changes in the kidneys 1 h after beginning of experiment. Fusion of feet of podocytes, appearance of fibrous material in them, uneven thickening and coarsening of material of basement membrane. 63,000×.

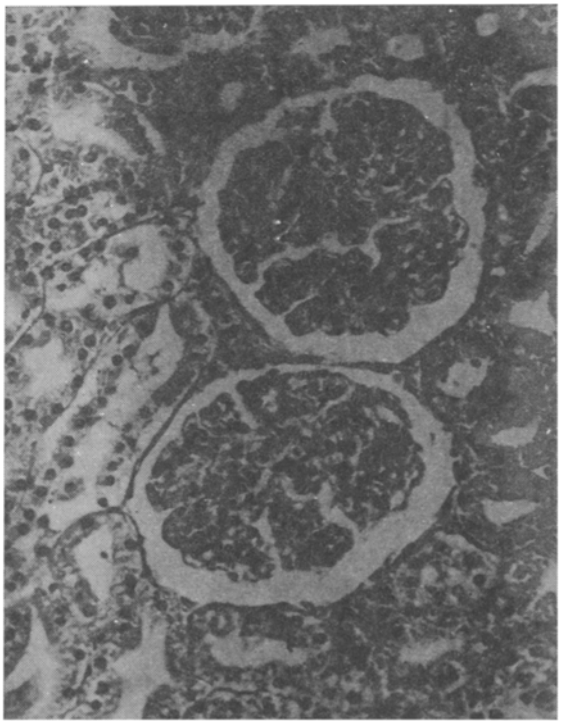

Fig. 2. Morphological changes in kidneys 4 h after beginning of experiment. Segmental mesangial-proliferative glomerulitis, marked mesangial proliferation of glomeruli. Hematoxylin and eosin. 250×.

Toward the end of the 5th hour the signs of focal segmental glomerulitis, seen under the light microscope, increased in severity and were accompanied by moderate proliferation of the mesangial matrix and congestion of the glomerular capillaries. Splitting of the basement membrane of the glomerulus and tubule was observed on the electron micrograph during this period.

TABLE 1. Parameters of Renal Function in the Early Period of Acute Experimental Pancreatitis

Stage of investigation	Diuresis, ml/min	Blood creatinine, mmoles/liter	Glomerular filtration, ml/min
Before acute pancreatitis	0,73±0,13	0,05±0,02	84,68±4,33
After creation of acute experimental pancreatitis			
1-h	0,66±0,03	0,058±0,006	66,1±3,26
2-h	0,62±0,09	0,06±0,005	60,24±4,2
3-h	0,45±0,02	0,15±0,03	32,07±3,29
4-h	0,2±0,008	0,407±0,05	28,53±2,02
6-h	0,22±0,001	0,48±0,02	17,0±2,68
8-h	0,28±0,002	0,58±0,03	25,6±3,3

By the end of the 6th hour the signs of focal segmental glomerulitis were further advanced: widening of the mesangial matrix and proliferation of the mesangial cells were more severe in character. Deposits of fibrin and PAS-positive material appeared on the glomerular membranes at this period. Considerable widening of the venous network and capillaries and their congestion with blood were observed in the stroma of the cortical layer. Most of the proximal tubules were unchanged. Fusion of the feet of the podocytes, condensation of their cytoplasm, and considerable condensation and irregular thickening of the basement membrane were observed on the electron micrograph. Degeneratively changed cells, difficult to differentiate morphologically, were seen. Toward the end of the 7th hour of the experiment, a mild degree of edema of the stroma of the medulla was added to the changes described above.

Profound changes took place toward the end of the 8th hour of the experiment: marked congestion of the glomerular capillaries, focal segmental glomerulitis, deposition of fibrin, diapedetic hemorrhages in the cortical layer, infiltration of the stroma of the cortical layer with lymphocytes and plasma cells, and edema of the medullary stroma. Besides the changes described above, congestion of the lumen of the glomeruli with blood was revealed by electron-microscopic investigation. Under the light microscope examination of autopsy material revealed mesangial-proliferative diffuse glomerulitis, approaching the generalized degree. Erythrocytes could be seen in the capsular spaces, and small foci with desquamated epithelium, moderate infiltration of the stroma mainly by lymphocytes, and edema of the stroma in the region of the collecting tubules, were observed in the lumen of the tubules. In two cases a small infarct could be seen, with mild inflammatory infiltration at the periphery. At autopsy hyperplasia of the juxtaglomerular apparatus of the glomeruli was found in all cases. In sections stained by Weigert's method a delicate network of fibrin could be seen in single loops.

The morphological changes in the kidneys thus revealed a definite time course corresponding to the development of diffuse mesangial-proliferative glomerulitis, accompanied by deposition of fibrin between the endothelium and the basement membrane. Changes in the form of microcirculatory disorders began to appear during the first hour of the experiment. According to the results of electron microscopy, by the end of the first hour of the experiment condensation and coarsening of the material of the basement membrane and podocytes were observed. The most important changes, expressed as widening of the mesangial matrix and proliferation of the mesangial cells, developed 4 h after the beginning of experimental pancreatitis. Comparison of the morphological data with the results of kidney function tests under experimental conditions indicated that the morphological changes arising as early as during the first hour of experimental pancreatitis were much earlier than disturbances of kidney function, which did not appear until the 4th hour (Table 1). Morphological and functional changes in the kidneys are thus an early and important stage in the pathogenesis of acute pancreatitis.

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IMMUNOHISTOCHEMICAL AND ELECTRON-MICROSCOPIC IDENTIFICATION OF SEROTONIN, MELATONIN, AND β-ENDORPHIN IN GRANULES OF NATURAL KILLER CELLS

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UDC 612.112.94.017.4:612.112.94.018:577.175.823]-086.3

KEY WORDS: natural killer cells; endocrine function; cytotoxic effect

The attention of immunologists, oncologists, and specialists in other disciplines is currently being drawn to natural killer (NK) cells, which are large granular lymphocytes, whose function is linked with high cytotoxicity against cells of various types and, in particular, tumor cells [10, 11, 14]. Despite many investigations of NK cells, the problem of their origin and mechanisms of the cytotoxic action of NK cells on target cells has not yet been solved. Investigations of the functional significance of a characteristic ultrastructural feature of these cells, namely the presence of electron-dense granules, distinguishing them from other types of lymphocytes, have been few in number [11]. The discovery of the nature of these cytoplasmic organelles is a key approach to the uncoding of the mechanism of the cytotoxic effect of NK cells. The solution to this problem raises many questions: how is the presence of granules linked with the cytotoxic properties of NK cells, what biologically active substances are synthesized in the granules, and how is their activity realized? During analysis of the literature, attention is drawn to an investigation into the use of Leu-7 antigen as a marker of NK cells. It has been shown that Leu-7 antigen reacts positively not only with NK cells, but also with the secretory granules of many cells of the APUD system [17, 18] and it correlates with a property so characteristic of apudocytes as argyrophilia [15].

These data suggest that peptide hormones and biogenic amines, which are usually produced by cells of the APUD system [4, 12, 16], may be synthesized in the cytoplasmic granules of NK cells.

The aim of this investigation was to identify biologically active substances in the granules of NK cells and to evaluate their participation in the realization of their cytotoxic action.

EXPERIMENTAL METHOD

Peripheral blood lymphocytes from healthy blood donors and from the spleens of Wistar rats were used as the test objects. A lymphocyte suspension was obtained by centrifugation of the samples in a Ficoll–Verografin density gradient (d = 1.077 g/ml) by the method [10, 11]. Intact lymphocytes from separate donors and lymphocytes from different donors incubated together for 4 h at 37°C were studied. The aim of combined incubation was to provoke contact interaction between NK cells belonging to different donors. The material was stained with azure-eosin and by Grimelius' argyrophilic method. The immunohistochemical investigation was carried out by the immunoperoxidase method [13]. Antisera against serotonin and metatonin, prepared in the appropriate way, and antisera against β-endorphin, insulin, somatotrophic hormone (STH), chorionic gonadotrophin, ACTH, and C-peptide (from Amersham Corpora-

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