7. I. L. Medkova, K. V. Smirnov, N. M. Nikolaeva, et al., Fiziol. Zh. SSSR, No. 11, 1504 (1983).
8. A. A. Polyakov, in: Morphology of Epithelium of the Proximal Part of the Digestive and Respiratory Systems [in Russian], Moscow (1971), pp. 78-83.
9. M. V. Rudenskaya, Byull. Éksp. Biol. Med., No. 9, 1133 (1976).
10. M. V. Rudenskaya, Byull. Éksp. Biol. Med., No. 5, 492 (1979).
11. M. V. Rudenskaya, Byull. Éksp. Biol. Med., No. 4, 472 (1980).
12. K. V. Smirnov, I. L. Medkova, N. M. Nikolaeva, et al., Dokl. Akad. Nauk SSSR, 271, No. 4, 1006 (1982).
13. N. G. Khlopin, General Biological and Experimental Bases of Histology [in Russian], Moscow (1964).
14. W. J. A. Patrick, D. Denham, W. Hawkins, and A. P. M. Forrest, Brit. J. Exp. Path., 56, 510 (1975).

PATHOMORPHOLOGY OF EXPERIMENTAL PANCREATIC NECROSIS AFTER ENDOLYMPHATIC INJECTION OF CONTRYKAL

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UDC 616.37-002.4-092.9-091/-092-07

KEY WORDS: experimental pancreatic necrosis; contrykal; electron microscopy.

Antienzyme treatment of acute pancreatitis (AP) is regarded as pathogenetically one of the soundest ways of combined treatment of this disease. Practical experience of the use of antienzymes in the treatment of AP has shown that their intravenous and intra-arterial administration do not arrest the course of pancreatic necrosis (PN) [6, 11]. Antienzyme treatment has proved to be most effective in the edematous form of AP [2, 5, 9]. However, mortality from destructive pancreatitis still remains high — from 30 to 86% [1, 3, 4].

The lymphatic system is a powerful collector of activated pancreatic enzymes and toxic breakdown products in AP [10]. The use of the lymphatic system, not only for the removal of toxic waste products by external drainage of the thoracic duct (TD), but also for endolymphatic administration of protease inhibitors in order to inactivate enzymes in the lymph, and thereby delay proteolysis in the pancreas, is a promising approach at the present time [7, 9].

The aim of this investigation was to study the trend of the morphological changes in the pancreas in experimental PN, treated by endolymphatic injection of contrykal, with simultaneous monitoring of pancreatic enzyme activity in the blood and lymph.

EXPERIMENTAL METHOD

Experimental PN was produced in mongrel dogs under general anesthesia by injection of infected bile under pressure into the pancreatic duct in a dose of 0.5 ml/kg body weight, with stimulation of pancreatic secretion by secretin (1 clinical unit/kg body weight, from Boots, England). Lymph for enzyme investigation was obtained by external drainage of TD. There were two series of experiments: I) control (five dogs), II) experimental (16 dogs). Every hour for 4 h, 10,000 antitrypsin units of contrykal was injected through a catheter into a lymphatic vessel of the hind limb of animals of the experimental series 2 h after production of experimental PN. The animals were withdrawn from the experiment by the usual method 9-10 h after its beginning. Pancreatic enzymes in the blood serum and lymph from TD were studied every hour (2 h after production of AP) in dogs of both series. Amylase activ-

Laboratory of Pathomorphology with Electron Microscopy Group, N. V. Sklifosovskii Moscow Emergency Aid Research Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Bylleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 11, pp. 622-626, November, 1985. Original article submitted January 18, 1985.

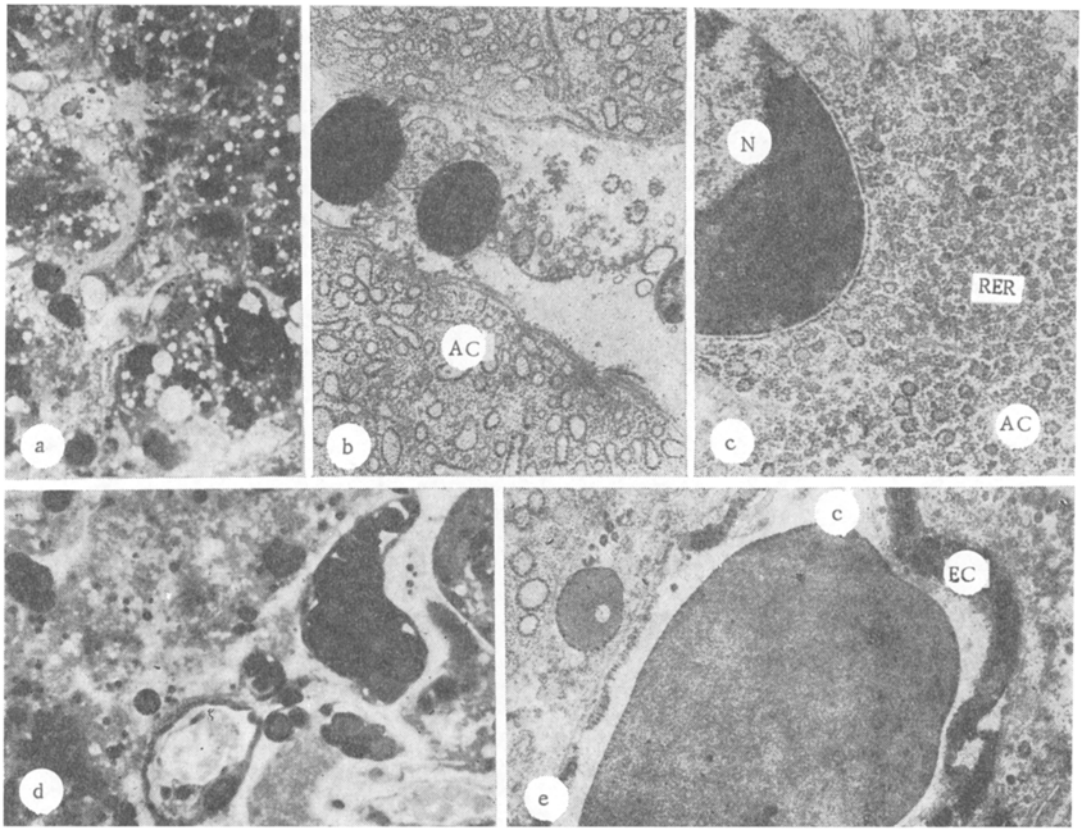

Fig. 1. Histological (a, d) and ultrastructural (b, c, e) changes in experimental PN. Semithin section. Methylene blue and azure, 900×; b) ZG in interacinar space. Electron micrograph. 18,000×; c) microvesiculation of rough endoplasmic reticulum (RER), condensation of chromatin in nucleus (N). Electron micrograph. 16,000×; d) microfocal acinar necrosis, congestion, and erythrostasis of interacinar capillaries. Semithin section. 900×; e) fibrinoid necrosis of endotheliocytes (EC) of a capillary (C). Electron micrograph. 16,000×.

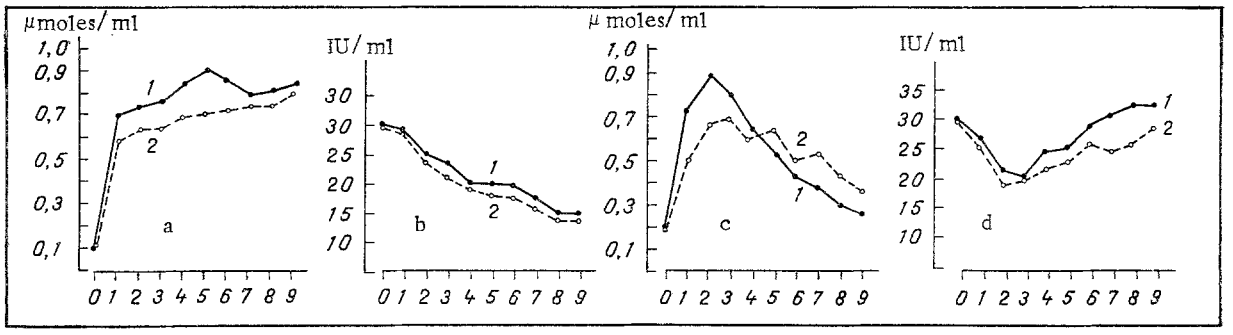

Fig. 2. Graph showing time course of changes in antitrypsin (b, d) and total BAEE-esterase (a, c) activity in central lymph (1) and blood serum (2) during development of experimental PN (a, b) and after treatment with contrykal (c, d). Abscissa, time (in h); ordinate, enzyme activity.

ity was determined by Caraway's method, lipase activity by Natelson's method, total BAEE*-esterase activity by the method in [12], and antitrypsin activity (ATA) by the method of Nartikova and Paskhina. Biopsy specimens from the head, body, and tail of the pancreas were taken for histological and histochemical investigation 30 and 60 min later, and thereafter every 2 h until the end of the experiment, fixed in 10% formalin buffered by Lillie's method, and embedded in paraffin wax. Histological sections were stained with hematoxylin and eosin,

*N-Benzene-1-arginine ethyl ester.

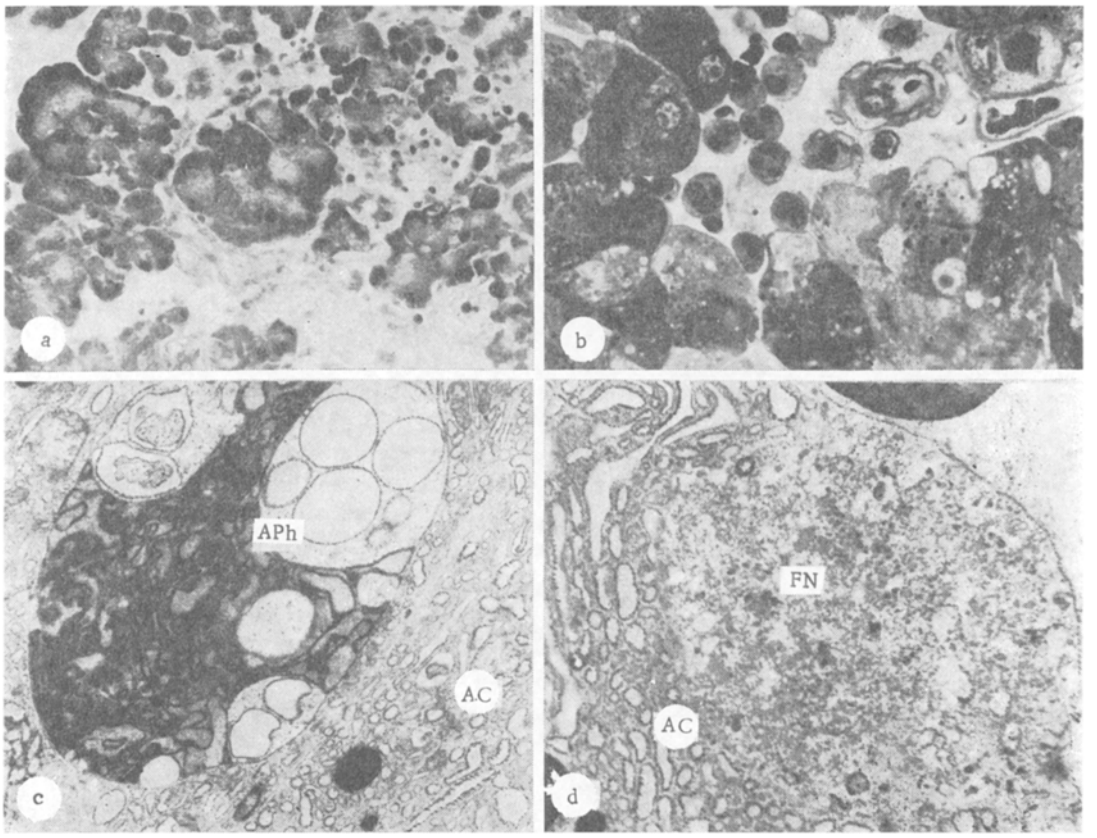

Fig. 3. Histological (a, b) and ultrastructural (c, d) changes in pancreas after treatment of experimental PN. a) Demarcation of injured, necrotically changed acini from uninjured. Hematoxylin and eosin. 400×; b) AC stands out against necrotic debris. Semithin section, methylene blue and azure. 900×; c, d) autophagosome (APH) and area of focal necrosis (FN) in cytoplasm of AC. Electron micrographs. 16,000×.

toluidine blue, and methyl green and pyronine by Brachet's method. Fibrin and zymogen granules (ZG) were stained by Mallory's method. Pancreatic material for electron-microscopic investigation was fixed by Palade's method and embedded in Araldite. Semithin sections were stained with methylene blue and azure, ultrathin sections by Reynolds' method, and examined in the ÉVM-100B electron microscope.

EXPERIMENTAL RESULTS

In the control series macroscopically visible changes in the pancreas were noted 30-60 min after injection of bile into Wirsung's duct, in the form of subcapsular, interlobular, and serous edema and centrilobular hemorrhages. Histologically, the acinar cells (AC) in the acini of the centrilobular regions appeared swollen, their boundaries were indistinct, and the distribution of ZG was diffuse throughout the cytoplasm (Fig. 1a). Against the background of interstitial edema microcirculatory disorders were observed: congestion and stasis in the capillaries of the centrilobular zones. Electron-microscopically, complexes of ZG in the cytoplasm of AC were disintegrating, with disturbance of their extrusion into the lumen of the ducts and secretion into the interstices through the damaged basement membranes. The rough endoplasmic reticulum showed vacuolation or microvesiculation with degranulation. Circumscribed focal intracellular necroses or necrosis of whole acini were formed (Fig. 1b, c).

Thus 1 h after production of experimental PN, intracellular necrobiotic changes could be observed in AC with destruction of cell membranes, allowing secretory granules to escape into the interstices, followed by activation of the proenzymes contained in them by cytosomes of the destroyed cells, and their internal secretion into the blood and lymphatic microcirculatory systems of the pancreas.

Diffuse centrilobular foci of necrosis with hemorrhages and aggregation of single polymorphonuclear leukocytes (PMN) were observed in the pancreas 2 h after the experiment began.

AC which had undergone colliquative necrosis, hemorrhages in the interstices and into the acini, and also progressive necrotic changes in AC of boundary zones due to progressive intracellular proteolysis, without restriction of the foci of necrosis, were discovered in the centers of the lobules. Signs of aggregation of platelets and erythrocytes and also erythrostasis with swelling of the endotheliocytes and pericytes of the vessel walls were observed in the lumen of the capillaries and venules (Fig. 1d). Meanwhile no morphological changes were seen in the lymphatics, which preserved their drainage function.

In the course of the observations on animals of the control series a picture of macrofocal or subtotal hemorrhagic PN developed macroscopically. The pattern of the lobules was completely obliterated and the pancreas became velvet-red in color with solitary foci of steatonecrosis in the cellular tissue of the subcapsular and interlobular spaces. Histologically, large-scale areas of necrosis were found in the acinar parenchyma, permeated with blood, and accompanied by interstitial hemorrhages. Signs of erythrostasis with hemolysis of erythrocytes were still present in the lumen of the capillaries and venules. Thrombi formed of fibrin and erythrocytes were found in individual vessels, with fibrinoid necrosis of the vessel walls (Fig. 1e). After 6-8 h lymphostasis was replaced by lymphothrombosis with destruction of the walls of the lymphatic vessels.

The morphological changes in experimental PN thus point to the progressive character of enzymic proteolysis of the acinar parenchyma of the pancreas, with disturbances of the blood and lymphatic circulation and they correlate completely, chronologically, with high enzyme levels in the blood and chyle. α-Amylase and BAEE-esterase activity (total activity of kallikrein, trypsin, and plasmin) rose progressively after the first hours of the experiment, to reach a maximum after 3 h, and remained high until the end of the experiment. There was a particularly marked tendency for ATA to fall. Lipase activity, however, unlike that of the other enzymes, rose slowly to reach a maximum after 4 h, which was maintained during the subsequent periods of observation (Fig. 2a, c).

Endolymphatic treatment of experimental PN, which began 2 h after injection of infected bile, at a time when the morphologic and enzymologic pictures of the disease were fully developed, showed a marked effect on morphogenesis of the disease. By 4-6 h after the beginning of treatment edema of the pancreas was reduced although the intralobular hemorrhages still remained. Histologically the scale of necrotic lesions of the pancreas was reduced, with demarcation of these zones by PMN (Fig. 3a, b). Electron-microscopically, signs of breakdown of the structure of the acini were not observed in areas adjoining the zones of necrosis, and focal necroses in the form of autophagosomes predominated in the pancreatic cells, with a tendency toward exocytosis into the interstices, where sequestration of cell debris also took place (Fig. 1d, e). Penetrating into injured AC, some PMN undertook the task of removing necrotic debris from the cells by phagocytosis. Unlike in untreated animals, in the dogs in this series of experiments neither erythrocytes nor fibrin were present in the lumen of the dilated lymphatics. Both in zones of injury to the pancreas and outside them congestion of the capillaries was observed but without injury to the endotheliocytes, which, under the electron microscope, differed in the density of their cytoplasm and exhibited features of increased pinocytosis. Peripheral regions of the basal plasmalemma of AC formed multiple folds, reflecting intensification of tissue-capillary exchange.

The most demonstrative enzymologic criterion of the efficacy of endolymphatic treatment of experimental PN was an increase in ATA, in either the lymph or the blood serum, after only the second injection of contrykal (Fig. 2b, d). These data are in agreement with ultrastructural features of stabilization of the cell membranes, with demarcation of foci of necrosis in AC. The fall in BAEE-esterase activity indicates both the direct inactivating action of the antienzymes and a decrease in the internal secretion of activated enzymes into the blood and lymph streams, due to regression of tissue destruction and restoration of extrusion of the secreted enzymes into the system of pancreatic ducts.

Endolymphatic injection of contrykal in the early stages of experimental PN, with diffuse and microfocal hemorrhagic lesions in the pancreas, thus has a very beneficial effect, as shown by the trends of the morphologic and enzymologic parameters. This method of treatment can be used under clinical conditions as part of a combination of measures for the care of patients with destructive forms of pancreatic necrosis.

LITERATURE CITED

1. I. S. Belyi and V. I. Desyateri, Khirugiya, No. 6, 51 (1980).
2. V. K. Gostishchev, V. G. Vladimirov, and A. G. Khuravlev, Khirurgiya, No. 1, 66 (1983).
3. V. G. Zaitsev, R. G. Ambartsumyan, and N. I. Markin, Khirurgiya, No. 10, 27 (1971).

4. V. S. Mayat, Yu. P. Atanov, and G. A. Buromskaya, Khirurgiya, No. 10, 5 (1983).
5. Yu. A. Nesterenko and Yu. P. Atanov, Khirurgiya, No. 10, 84 (1981).
6. Yu. V. Ognev and S. M. Kolodii, Klin. Khir., No. 11, 38 (1982).
7. R. T. Panchenkov, V. A. Pepin, and I. V. Yarema, Vest. Khir., No. 4, 33 (1977).
8. V. A. Penin, V. V. Petrov, G. P. Titova, et al., Farmakol. i Toksikol., No. 5, 83 (1982).
9. V. S. Savel'ev, V. M. Buyanov, and Yu. V. Ognev, Acute Pancreatitis [in Russian], Moscow (1983).
10. D. N. Sim, A. Duprez, and M. G. Anderson, Acta Gastroent. Belg., 29, 235 (1966).
11. J. E. Trapnell, Gastroenterology, 21, 5 (1978).
12. E. Werle and T. Trautschold, Münch. Med. Wschr., 103, No. 14, 773 (1961).

AUTORADIOGRAPHIC STUDY OF DNA SYNTHESIS IN RENAL TUBULAR EPITHELIAL CELLS OF ALBINO RATS WITH MERCURIC CHLORIDE NEPHROSIS

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UDC 616.61-002.4-099-092.9-07:616.612-008.939.633.2-073.916

KEY WORDS: mercuric chloride nephrosis; DNA synthesis.

Regeneration of the epithelium of the proximal convoluted renal tubules after damage by mercuric chloride ($HgCl_2$) has now been studied adequately by light-optical methods [3, 4, 7, 9]. Histological investigations have shown that the scale and volume of necrotic changes in the epithelium of the urinary tubules depend on the dose and mode of administration of $HgCl_2$ to the animals. A small dose of $HgCl_2$ causes loss of microvilli and of small areas of apical cytoplasm by the epithelial cells of the straight part of the proximal renal tubule. After injection of $HgCl_2$ into the animals in a dose of 0.4 mg/100 g body weight or more, partial necrosis of the nephrocytes of many urinary tubules is severe in character and is accompanied by sequestration of large volumes of cell cytoplasm into the lumen of the tubules.

Data on the character of reproduction of the renal epithelium, damaged by $HgCl_2$, have been obtained by ^3H-thymidine autoradiography [5, 6, 8]. The use of this method has shown that damaged epithelial cells, preserved in the zone of necrosis, can synthesize DNA, and the duration of periods of the cell cycle of regenerating nephrocytes has been determined. The duration of the cell cycle was found to be 14 h, the period of DNA synthesis 9 h, the presynthetic period 3 h 45 min, and the postsynthetic period 45 min.

In $HgCl_2$ nephrosis the renal tubules are filled with debris, which disturbs the flow of urine and causes dilatation of the lumen of the tubules and marked flattening of damaged epithelial cells, undergoing partial necrosis. The ability of these cells to regain their normal structure and, later, their specific function is a problem of great interest, which is not yet settled.

Since autoradiography with paraffin sections has inadequate resolving power for the detailed study of the structure of damaged nephrocytes and the character of distribution of radioactive label in them, in the investigation described below, to evaluate the structure and DNA-synthetic activity of epithelial cells damaged by $HgCl_2$ it was decided to use semithin (0.5 μ) sections, cut from blocks embedded for electron microscopy.

EXPERIMENTAL METHOD

Noninbred male rats (6 animals) weighing 170-210 g were given a subcutaneous injection of $HgCl_2$ in a dose of 0.5 mg/100 g body weight, and 72 h later an intraperitoneal injection

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(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 11, pp. 626-629, November, 1985. Original article submitted March 21, 1985.

0007-4888/85/0011-1603$09.50