MORPHOLOGY OF THE PANCREAS AFTER INTRA-AORTIC INJECTION OF 5-FLUOROURACIL

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The most effective blocker of pancreatic synthesis at present is 5-fluorouracil (5-FU), a pyrimidine analog of uracil. In 1969 it was concluded from an electron-microscopic investigation of the pancreas that 5-FU may be extremely useful in the study of cell secretion [5, 6]. It was found during the treatment of experimental pancreatitis that 5-FU inhibits protein synthesis. The desire to improve 5-FU transport to the pancreas led many surgeons to suggest various ways of injecting it: into the retropancreatic space [2], intravenously [1], and into the celiac trunk [3]. No information could be found in the literature on optimal therapeutic doses of 5-FU for intra-aortic injection in acute pancreatitis. Existing data on its intravascular administration were highly contradictory [3].

The aim of the present investigation was to study the dynamics of morphological changes in the intact pancreas after intra-aortic injection of various doses of 5-FU with a view to selecting the optimal dose of the compound during treatment of pancreatitis.

EXPERIMENTAL METHODS

Experiments were carried out on 44 mongrel dogs weighing 5-12 kg. For intra-aortic injection of 5-FU the profunda femoris artery was exposed, its peripheral end ligated, and a catheter 1.5 mm in diameter was passed through an incision in the wall through the proximal end, and through the femoral artery into the aorta 2-3 cm above the origin of the celiac trunk. The precise location of the catheter was identified by palpation during laparotomy. There were two series of experiments. In series I the dogs received 5-FU in a dose of 10 mg/kg (24 animals), in series II a dose of 15 mg/kg (20 animals). For morphologic study, tissue was taken from different parts of the pancreas (head, body, and tail) during the operation or after autopsy on the animals, 15 min, and 2, 6, and 24 h after a single injection and on the 2nd and 3rd days after daily injection of 5-FU. Material for histologic study was fixed in 4.5% formalin solution in phosphate buffer and kept in formalin. Sections were stained with hematoxylin and eosin and by Mallory's method. Biopsy material was fixed for electron microscopy with osmic acid by Palade's method, then embedded in Araldite. Ultrathin sections were cut on the LKB 8800 III Ultratome after preliminary study of semithin sections stained with methylene blue and azure. Electron micrographs were obtained on the Soviet ÉMV-100B electron microscope.

RESULTS

In the animals used in the experiments of series I the usual histologic structure of the pancreatic lobes was preserved: The acini were compactly arranged and showed no signs of injury to the acinar cells (AC). Evidence of delayed release of secretion into the lumen of the ducts, manifested by excess accumulation of zymogen granules (ZG), was observed in the cytoplasm of AC, histologically and electron-microscopically only 15 min after injection of 5-FU.

Asynchronous accumulation of secretion in the cytoplasm of AC was observed histologically, 2, 6, and 24 h after a single injection of 5-FU: Accumulation was reduced in the tissue of the heat and body of the pancreas, whereas background accumulation with delayed release of secretion into the duct system was observed in the tail. After 2 and 6 h single

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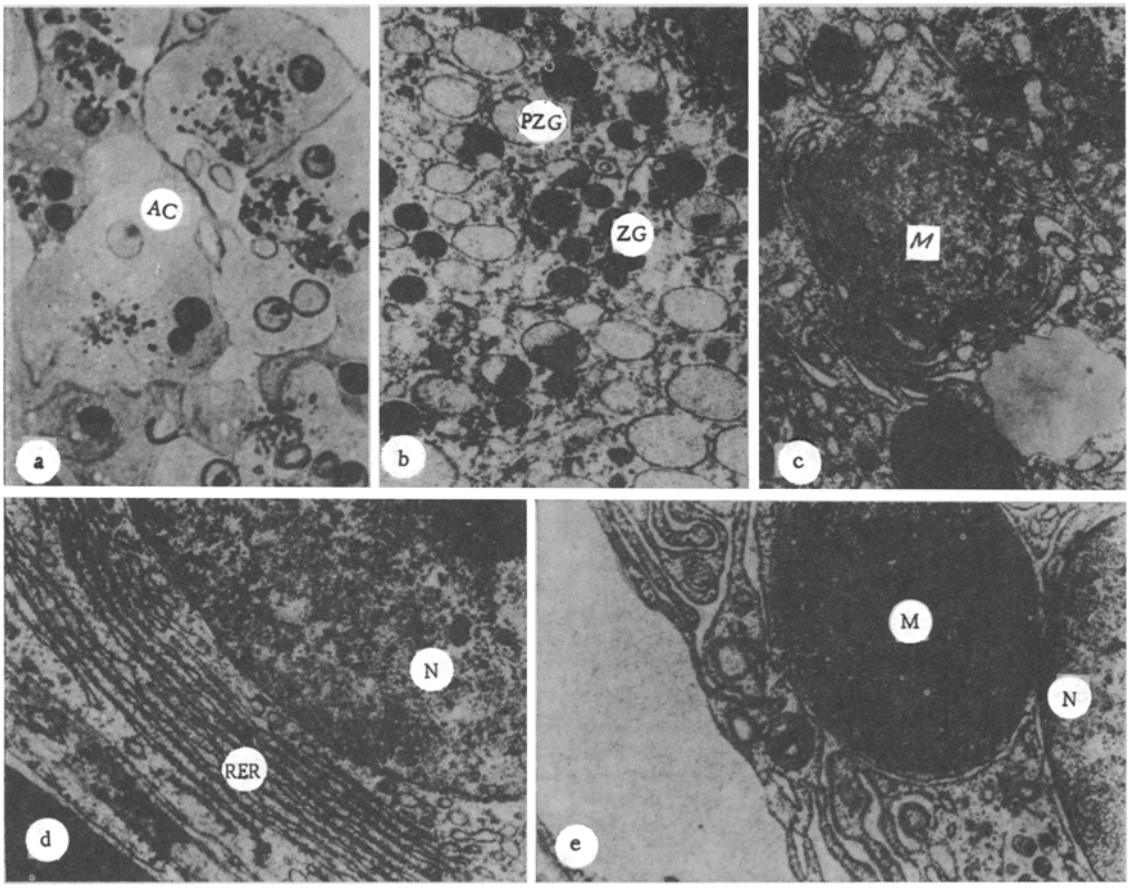

Fig. 1. Histologic (a) and electron-microscopic (b-e) changes in pacnreas 1, 6, and 24 h after injection of 5-FU. a) Moderate accumulation of ZG of varied density in cytoplasm of AC; semithin section, methylene blue—azure, 900×. b) Single mature ZG and prozymogen granules (PZG) in cytoplasm of AC; 18,000×. c) Mitochondria (M) with shortened cristae and rarefied matrix; 20,000×. d) Collapse of lumen of cisterns of rough endoplasmic reticulum (RER), reduced density of chromatin in nucleus (N), 18,000×. e) Increase in density of matrix, reduction of cristae of mitochondrion (M), 20,000×.

ZG were detected by the apical membranes around the centroacinar ducts. The basophilia of the cytoplasm of AC was reduced, the nuclei were centrally situated, preserved their round shape, and contained thinly distributed chromatin and tiny nucleoli. The ultrastructural study of AC showed reduced accumulation of maturing secretory granules. Their release into the lumen of the ducts was delayed. The ducts appeared collapsed and secretory granules did not come into contact with the apical membrane. Elements of the lamellar network occupied a small area and consisted of collapsed smooth cisterns of the endoplasmic reticulum and single microvesicles. Cisterns of the rough endoplasmic reticulum showed fragmentation and vacuolation, and regular parallel profiles still remained only in individual cells. The mitochondria had marginally arranged cristae and a translucent matrix (Fig. 1a-d). Changes discovered in the structure of the organelles of AC indicated a reduction of their secretory activity.

After 24 h signs of reduced accumulation and maturation of the secretory granules in the cytoplasm of AC were still found histologically and electron-microscopically. Submembranous edema of the cytoplasm could be seen in single AC, and ultrastructural study of these tissues revealed marginal dilatation of the cisterns of the rough endoplasmic reticulum. Under these circumstances the integrity of the plasma membranes of AC and of the basement membrane of the acinus were preserved. Individual hypertrophied mitochondria with a diffusely condensed matrix and with reduced cristae could be seen in the cytoplasm of the perinuclear zones of AC (Fig. 1e). The changes discovered in the structure of the mitochrondria were evidence of increased functional strain on the energy metabolim of AC.

After injection of 5-FU daily for 2-3 days signs of a sharp decline in the accumulation of secretory granules and of the basophilia of the cytoplasm of AC were found histologically

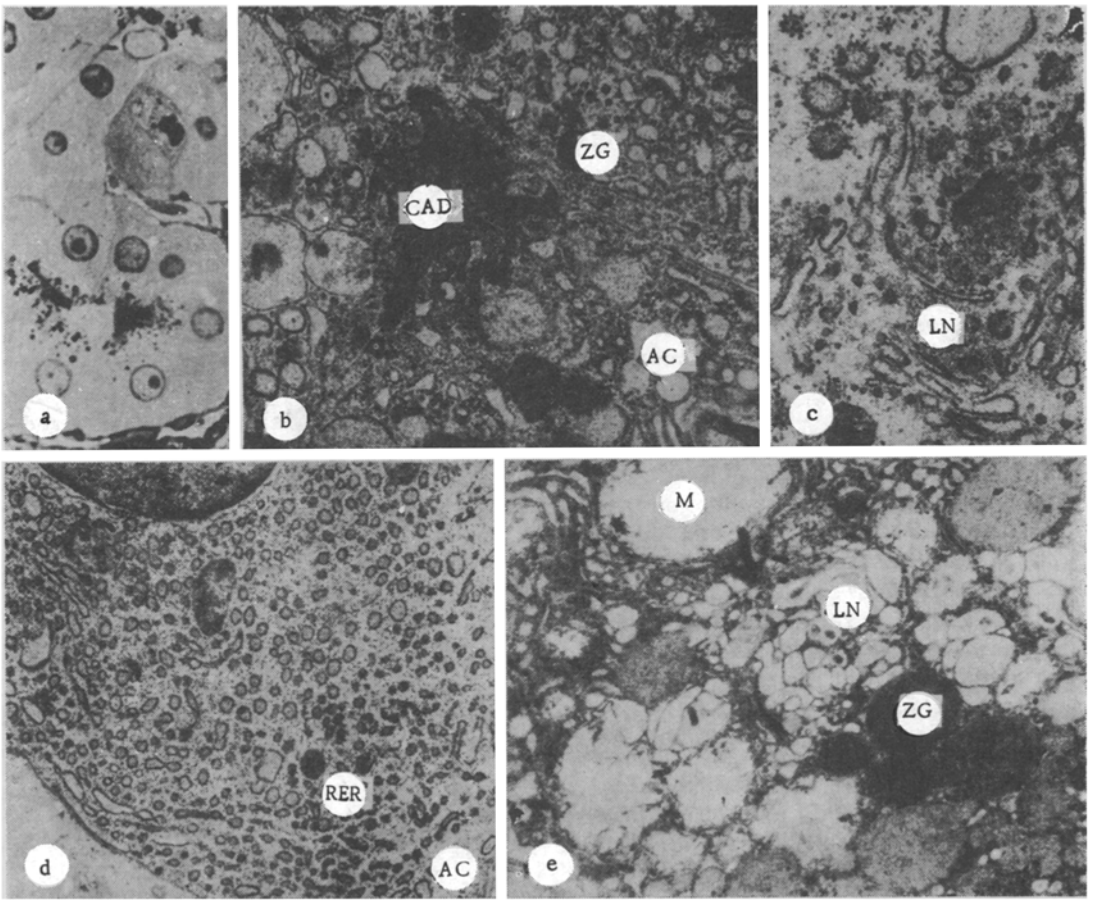

Fig. 2. Histologic (a) and electron-microscopic (b-e) changes in pancreas of animals in experiments of series I (a-d) and II (e). a) Small ZG around centroacinar ducts (CAD); semithin section, stained with methylene blue and azure; 900×. b) Collapse of lumen of CAD, single ZG in cytoplasm of AC; arrow indicates zones of tight junctions; 18,000×. c) Collapse of lumen of cisterns of lamellar network (LN); 38.000×. d) Microvesiculation of rough endoplasmic reticulum (RER); 20,000×. e) Swelling of mitochondria (M) with lysis of cristae and vacuolation of elements of lamellar network; 18,000×.

in all parts of the pancreas. The histologic pattern of the acini remained clear in the lobules. The centroacinar ducts were not outlined. The small nuclei of AC were central and basal in position. Electron-microscopically, tiny ZG, with different degrees of maturity, could still be seen near the apical plasmalemma of AC after two daily injections of 5-FU. Meanwhile the lumen of the centroacinar ducts were filled with secretion of low electron density. After three daily injections of 5-FU the cytoplasm of AC was completely emptied of ZG, and solitary prozymogen granules (PZG) were seen only in individual AC. The centroacinar ducts were in a collapsed state and were outlined only in zones of condensation of the contacting lateral membranes. Elements of the lamellar network stretched out toward the apical membranes and had the appearance of tightly packed cisterns. The rough endoplasmic reticulum, undergoing fragmentation, acquired the appearance of short collapsed cisterns or of granular microvesicles. The nuclear chromatin was diffusely rarefied and the nuclear pores were poorly distinguishable. The number of free ribosomes and polysomes in the cytoplasm was reduced in all parts of AC (Fig. 2a-d).

The structural changes discovered in AC (nuclei, rough and smooth endoplasmic reticulum) indicated inhibition of synthetic activity of the pancreatic cells in all parts of the pancreas.

The histologic and electron-microscopic study of the pancreatic acinar tissue thus showed that a single intra-arterial injection of 5-FU in a dose of 10 mg/kg reduced the secretory activity of AC after only 2 h, and these changes continued until 24 h after the beginning of the experiment. Single daily injection of 5-FU for 2-3 days lead to reduction

of synthesis and accumulation of secreted enzymes in all parts of the pancreas. The acinar parenchyma was undamaged under these circumstances. Consequently, the suggested methods of injection of 5-FU and its dosage satisfy the main demands for goal-directed action on the pancreas — to inhibit the synthetic activity of the acinar tissue.

In the experiments of series II histologic and electron-microscopic investigation of the pancreas of the animals 2 h after the beginning of the experiment and subsequently during the period of observation revealed progressive changes in the organelles of the acinar and duct cells, as well as in the endotheliocytes of the blood vessels, which were accompanied both by reduced secretory activity of AC and by degenerative changes, in the form of vacuolar swelling of the mitochrondria and of the smooth and rough endoplasmic reticulum. The appearance of lipid and fat inclusions in AC was evidently associated with the direct toxic action of 5-FU on the pancreas (Fig. 2e). Toward the end of the 2nd day after the 2nd injection of 5-FU all the dogs died. The pancreas of the dead animals showed congestion of the vessels, plasmorrhagia with intravascular aggregation of erythrocytes, and intracinar and interlobular edema. Necrotic foci could be seen in the cytoplasm of AC, in the form of accumulation of autophagosomes. Signs of reduction of the drainage function of the ducts, connected with dystrophic changes in the duct cells, which were swollen or showed diffuse fatty degeneration, were observed. Injection of 5-FU in a dose of 15 mg/kg was accompanied morphologically not only by reduction of the secretory activity of the acinar parenchyma, but also by long-lasting dystrophic and necrobiotic changes in the pancreas, with disturbance of its principal functions. Morphologic study of the pancreas after a single intra-aortic injection of 5-FU into animals in a dose of 10 mg/kg daily for 3 days revealed absence of pathological changes in AC, accompanied by marked depression of synthesis of the secretion of the acinar tissue.

Thus the dose and method of injection of 5-FU chosen completely satisfy the demands of pathogenetically targeted action of the compound on the pancreas in cases of pancreatic necrosis, and they can be recommended for use in clinical practice.

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