MECHANISMS OF PATHOGENETIC PREVENTION OF EXPERIMENTAL ACUTE PANCREATITIS

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The treatment of acute pancreatitis is one of the most important problems in abdominal surgery. This is because of the considerable complexity of the pathogenesis of development of acute pancreatitis, especially postoperative. This is based on activation of enzymes, which is aided by a number of factors such as increased pressure in the pancreatic duct, duodenostasis followed by regurgitation of the contents into the pancreatic duct, angiospasm, and a sharp increase in vascular permeability [1, 2, 4, 5, 8, 10].

The aim of this investigation was an experimental study of the time course of acute pancreatitis, and its prevention by 5-fluorouracil and Intralipid.

EXPERIMENTAL METHOD

Experiments were carried out on 60 noninbred male rats weighing 200-220 g. Acute pancreatitis was produced by our modification [3] of the method [6], by clamping the terminal portion of the common bile duct by means of a tourniquet for 2 h, accompanied by simultaneous stimulation of secretion by pilocarpine in a dose of 0.01 g/kg body weight. The experiments of series I served as the control. In series II, 5-fluorouracil was injected intraperitoneally at the end of the operation in a dose of 4.5 mg/100 g body weight. In series III Intralipid was given together with 5-fluorouracil. The animals were killed 5, 12, and 24 h after the operation. Activity of the following enzymes was studied in the blood serum at the times of sacrifice: amylase [7], trypsin, and the nonsecretory pancreatospecific enzyme transamidinase. Pancreatic tissue for histological investigation was fixed in a 20% solution of neutral formalin and embedded in celloidin. Sections were stained with hematoxylin and eosin. Some material was investigated electron-microscopically. Double fixation in paraform with postfixation by osmic acid by Palade's method followed by embedding in Araldite was used for electron microscopy. Ultrathin sections were cut on the LKB 8800 III ultratome in selected areas chosen after preliminary study of semithin sections. Electron micrographs were obtained on the EMV-100 L electron microscope.

EXPERIMENTAL RESULTS

In the experiments of series I, 6 h after the operation marked disturbances of the circulation in the pancreatic tissue were observed in the animals: congestion of the vessels and capillaries, capillary stasis, edema and focal hemorrhages in the interlobular and interacinar spaces, with leukopedesis in some places. Destruction of organelles was observed in some cells. After 12 h the circulatory disturbance, edema, and leukocytic infiltration of the interstitial tissue increased. Foci of necrosis, suppuration, and interacinar infiltration were observed, in the surrounding fatty areolar tissue, to reach a peak after 24 h (Fig. 1). Similar changes were observed in the interlobular and interacinar spaces. Multiple foci of degeneration and necrosis of cells of the exocrine parenchyma also were found. Those glands which remained were swollen and the cytoplasm of their cells contained much eosinophilic secretion. Signs of suppurative infiltration were seen in the surrounding areolar tissue.

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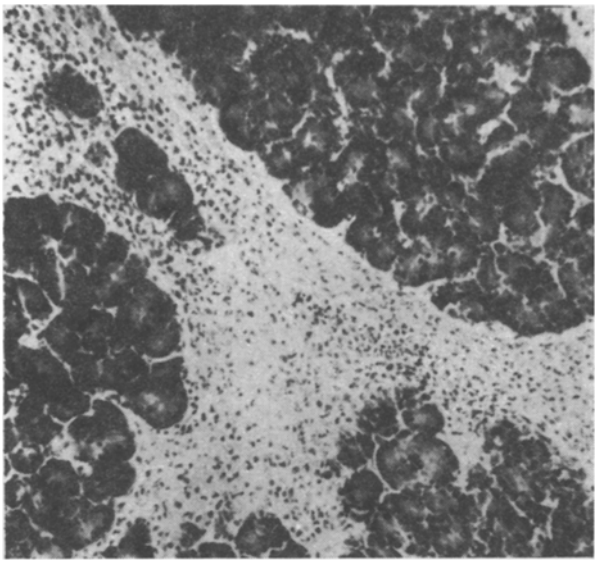

Fig. 1. Edema and diffuse leukocytic infiltration of interlobular connective tissue after 12 h of experiment. Here and in Fig. 3: a) stained with hematoxylin and eosin. 56 ×.

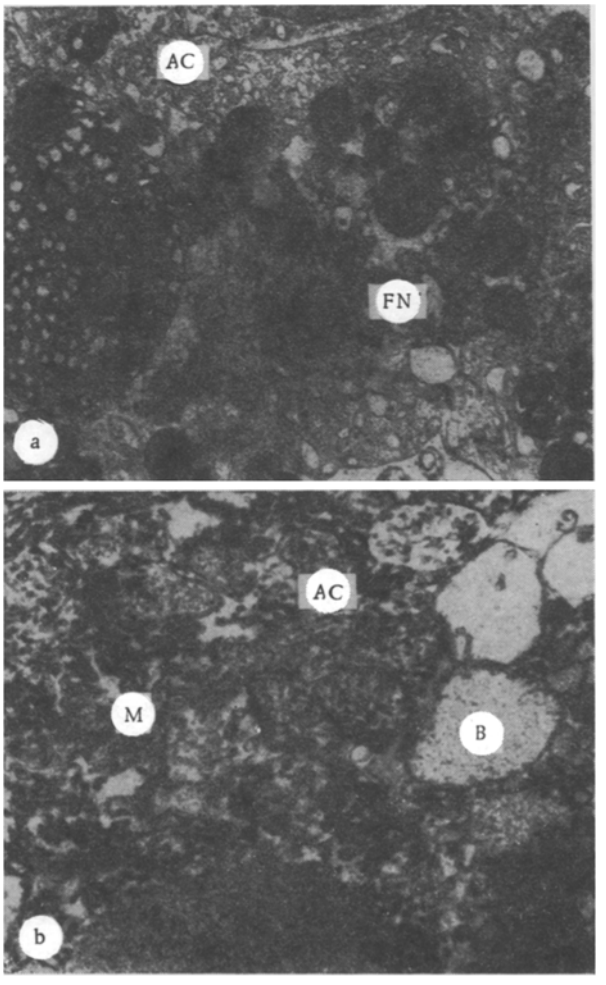

Fig. 2. Ultrastructural changes in acinar cells 12 h after beginning of experiment. a) Focal necroses in cytoplasm of acinar cells in experimental pancreatitis, 18,000 ×; b) necrosis of organelles of acinar cell in the form of vacuolar and membranous degeneration, 24,000 ×. AC) Acinar Cell; FN) focal necrosis, M) membranous, and V) vacuolar degeneration.

TABLE 1. Effect of 5-Fluorouracil and Intralipid on Serum Enzyme (α-amylase, trypsin, transamidinase) Activity in Experimental Acute Pancreatitis

Experimental conditions	α-amylase			Trypsin			Transmidinase		
	6 h	12 h	24 h	6 h	12 h	24 h	6 h	12 h	24 h
Acute pancreatitis	4018±146,8	5819±468,2	8798±325,6	6,66±0,42	9,18±0,95	12,32±0,95	0,047±0,005	0,06±0,01	0,23±0,018
Acute pancreatitis + 5-fluorouracil	3180±298,1	3974±368,9	3091±295,6	3,95±0,31	4,16±0,38	2,96±0,39	0,00±0,00	0,01±0,001	0,05±0,004
Acute pancreatitis + Intralipid	3312±181,4	4194±372,4	3816±275,7	4,15±0,43	6,03±0,61	5,04±0,88	0,024±0,003	0,04±0,003	0,003±0,003

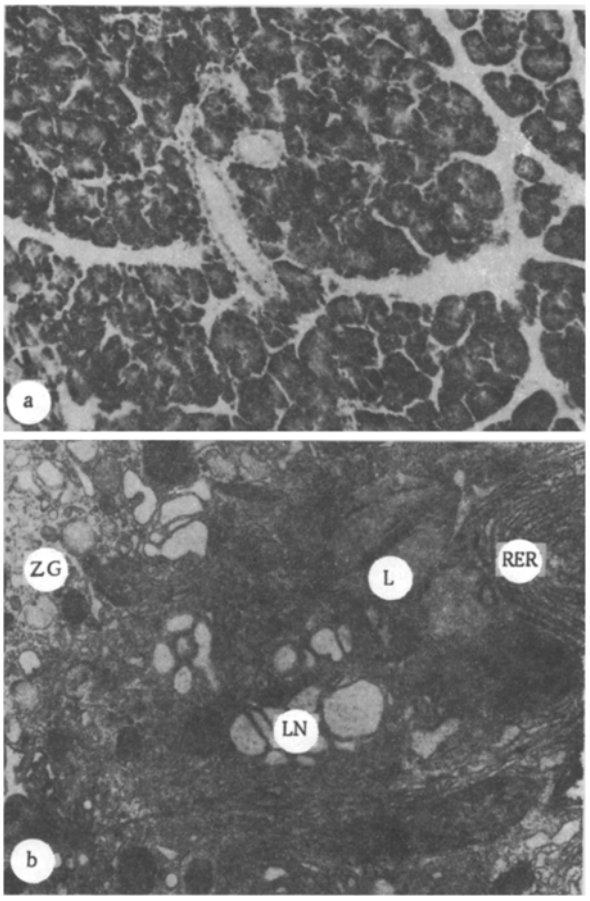

Fig. 3. Morphological picture of the pancreas 12 h after beginning of experiment with combined administration of 5-fluorouracil and Intralipid. a) Absence of necrosis of acinar cells in lobules of pancreas; b) reduction in number of zymogen granules (ZG) in cytoplasm of acinar cell, vacuolation of elements of the lamellar network (LN), collapse of cisterns of the rough endoplasmic reticulum (RER), and presence of secondary lysosomes (L). Magnification 16,000.

Electron-microscopic investigation revealed fatty degeneration in the cytoplasm of the exocrine cells with evidence of focal necrosis, a basal orientation of the zymogen granules (ZG), swelling of the cytoplasm of the endotheliocytes, and signs of plasmarrhagia and pericapillary edema were observed; later focal necrosis affected individual cells and whole groups. Increased release of secretion into the lumen of the duct also was observed (Fig. 2a, b). The increasingly severe morphological changes, typical of acute pancreatic necrosis correlated well with the rise in the levels of the serum enzymes α-amylase and trypsin. The discovery of serum transamidinase in the animals of this group is evidence of destruction of the acinar cells, correlating fully with the morphological data (Table 1).

In the experiments of series II moderate edema of the interlobular and interacinar spaces was observed 6-8 h after injection of 5-fluorouracil. Congestion and paralytic dilatation of the veins and capillaries were observed. Numerous cells — polymorphonuclear leukocytes, macrophages, and mononuclear cells — were found in the interlobular spaces.

After 12-24 h the signs of moderate congestion of vessels and capillaries were still preserved and the architectonics of the acini was normal. The cytoplasm of the exocrine cells was weakly eosinophilic. The number of ZG was reduced. Foci of destruction of the gland tissue were not present, and marked round-cell infiltration was observed in the interlobular bands.

In the experiments of series III moderate interstitial inflammatory edema with weak focal infiltration of the interlobular and perivascular tissue mainly by lymphoid cells was observed 6 h after the operation and injection of 5-fluorouracil together with Intralipid. The acini were equal in diameter, and the cytoplasm of the exocrine cells was weakly eosinophilic, evidence of a marked decrease in their zymogen content. Interstitial inflammatory edema was absent after 12-24 h. Moderate congestion of the veins in the adipose tissue surrounding the gland still remained. The acini of the gland were in close juxtaposition, and were mainly equal in diameter. The cytoplasm of the exocrine cells was weakly eosinophilic and its ZG content was sharply reduced. The efferent ducts were collapsed and contained no secretion. Foci of necrosis were absent in the gland tissue (Fig. 3a). Electron microscopy revealed absence of destruction of the cell membranes and membranes of the organelles. Besides a marked reduction in size of the organelles, their secretory activity was depressed. Evidence of vacuolation in the rough endoplasmic reticulum was more marked (Fig. 3b), and this was confirmed by a considerable decrease in serum trypsin and amylase activity of the animals (Table 1).

Administration of 5-fluorouracil and Intralipid thus prevents the development of suppurative-necrotic pancreatitis; 5-fluorouracil, an inhibitor of RNA and DNA synthesis, blocks release of the secretion. Intralipid, a stabilizer of the lipid component of cell and capillary membranes, prevents disturbance of permeability of the capillary walls, on the one hand, and diffusion of the enzyme and destruction of the intracellular organelles, on the other hand.

Complementary in their action, these drugs, characterized by a pathogenetic mode of action, when given together produce a marked therapeutic effect.

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