EFFECT OF 5-FLUOROURACIL ON THE MORPHOLOGY OF EXPERIMENTAL PANCREATIC NECROSIS

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A basic stage in the pathogenesis of acute pancreatic necrosis is autodigestion of the tissue of the gland by activated proteolytic enzymes. Destruction of the acinar cells (AC) of the gland, coupled with increased enzyme synthesis, leads to the internal secretion of proteolytic and lipolytic enzymes into the blood and lymphatic streams, and this is manifested clinically as high blood enzyme levels and enzyme shock [1, 4, 12-14]. The pathogenetic treatment of pancreatitis, based on inhibition of secretion of exocrine acini [2, 9, 10], has become widely adopted in recent years. AC of the pancreas possess high protein-synthesizing activity, which is performed on ribosomes of the rough endoplasmic reticulum (RER) under the genetic control of nuclear RNA [8].

The compound 5-fluorouracil (5-FU) is known to have an inhibitory action on secretory cells, due to inhibition of DNA synthesis in the nucleus and of RNA synthesis on the ribosomes [11].

The object of this investigation was an experimental study of changes in synthetic activity of pancreatic AC during treatment with 5-FU.

EXPERIMENTAL METHOD

Experiments were carried out on 100 noninbred male rats weighing 180-250 g. Acute pancreatic necrosis was induced by a modified Arai's method [5] by compressing the terminal portion of the common bile duct by means of a tourniquet for 2 h, and simultaneously stimulating secretion with pilocarpine in a dose of 0.01 g/kg body weight. There were two series of experiments (50 rats in each series): series I) untreated animals with experimental pancreatic necrosis; series II) animals receiving a single intraperitoneal injection of 5-FU in a dose of 4.5 mg/100 g body weight. The rats were decapitated 3, 6, 12, and 24 h after the operation and activity of the following enzymes in the blood serum was tested at the corresponding times: α-amylase by Caraway's method [6], trypsin by Erlanger's method in Shaternikov's modification [7], and nonsecretory specific pancreatic transamidinase by Karelin's method [3]. Pancreatic tissue for histological study was fixed in 4.5% paraform solution in phosphate buffer and embedded in paraffin wax; sections were stained with hematoxylin and eosin, by Mallory's method, and for RNA by Brachet's method. Double fixation in paraform with postfixation with osmic acid by Palade's method and embedding in Araldite were used for electron microscopy. Trimming of the blocks was carried out on the LKB 8800 III Ultratome after preliminary study of semithin sections stained with methylene blue—azure. Photographs were taken on the EMV-100L electron microscope.

EXPERIMENTAL RESULTS

In the group of untreated animals histological and electron-microscopic investigation 3 h after the operation revealed signs of increased liberation of secretion into the lumen of the ducts, fatty degeneration of AC, congestion of the capillaries, and evidence of plasmarrhagia and pericapillary edema. After 6 h signs of focal necrosis, a basal orientation of zymogen granules (ZG), increased secretory activity of components of the lamellar network,

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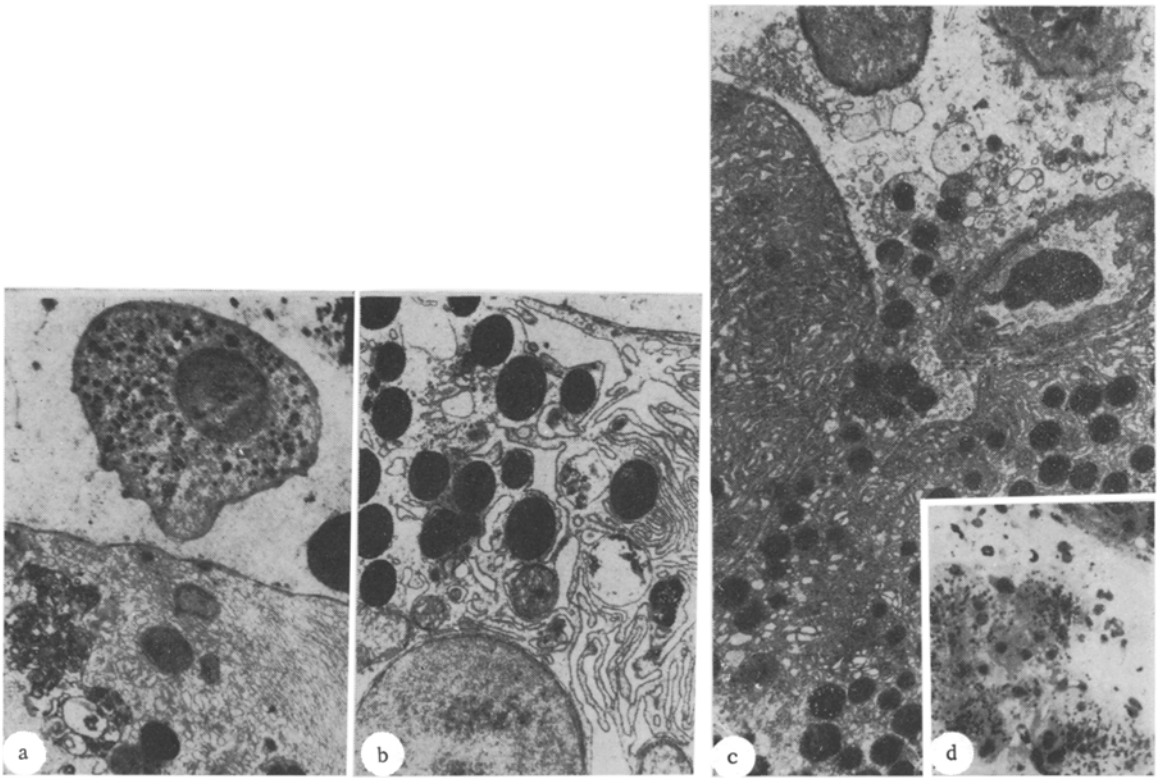

Fig. 1. Morphological changes in experimental pancreatic necrosis: a) electron micrograph (18,000 ×): large cytolysomes in cytoplasm of AC, erythrocytes (E), fibrin (F), and polymorphonuclear leukocyte (PML) in periacinar space (PAS); b) electron micrograph (24,000 ×): concentration of ZH in basal regions of cytoplasm of AC, dilatation of cisterns of RER; c) electron micrograph (14,000 ×): disturbance of integrity of basal plasma membrane of AC with ZG detectable in PAS, CL) capillary lumen; d) semithin section stained with methylene blue–azure (480 ×): disintegration of complex structure of pancreatic acini with diffuse arrangement of ZG, disturbance of integrity of plasma membranes, and signs of inflammation in PAS.

TABLE 1. Indices of Enzyme Activity (α-amylase, trypsin, transamidinase) in Blood of Untreated Animals with Experimental Pancreatic Necrosis and Animals Treated with 5-FU in Its Early Stages (M ± m)

Series of expts.	Blood enzymes	Control	Experimental pancreatic necrosis			
			3 h	6 h	12 h	24 h
I	Transamidinase	0,00±0,00	0,00±0,00	0,23±0,03	0,06±0,01	0,14±0,40
II			0,00±0,00	0,00±0,00	0,01±0,01	0,04±0,01
I	Trypsin	2,49±0,22	3,52±0,87	6,66±0,31	9,18±0,42	12,32±0,95
II			3,18±0,46	3,95±0,31	4,16±0,38	2,96±0,39
I			2805±171,5	4018±146,8	6819±468,4	8798±325,6
II	α-Amylase	2165±132,4	2615±312,6	3180±298,1	3974±368,9	3031±295,9

swelling of the cytoplasm of the endotheliocytes, and deposition of fibrin in the perivascular spaces were found in the cytoplasm of AC (Fig. 1a, b). After 12 h, focal necrosis of individual AC and of whole acini were clearly visible, integrity of the cell membranes of AC was disturbed, and diffusion of ZG in the intercellular spaces could be seen (Fig. 1c). In the capillaries, besides diapedesis of leukocytes and erythrocytes, degenerative changes in the endotheliocytes were more marked: swelling of their cytoplasm, a decrease in the density of their organelles, and fatty degeneration. Large areas of acinar and lobular necrosis with marked inflammatory infiltration of the stroma were observed 24 h after the operation (Fig. 1d). The increasingly severe morphological changes typical of acute pancreatic necrosis correlate

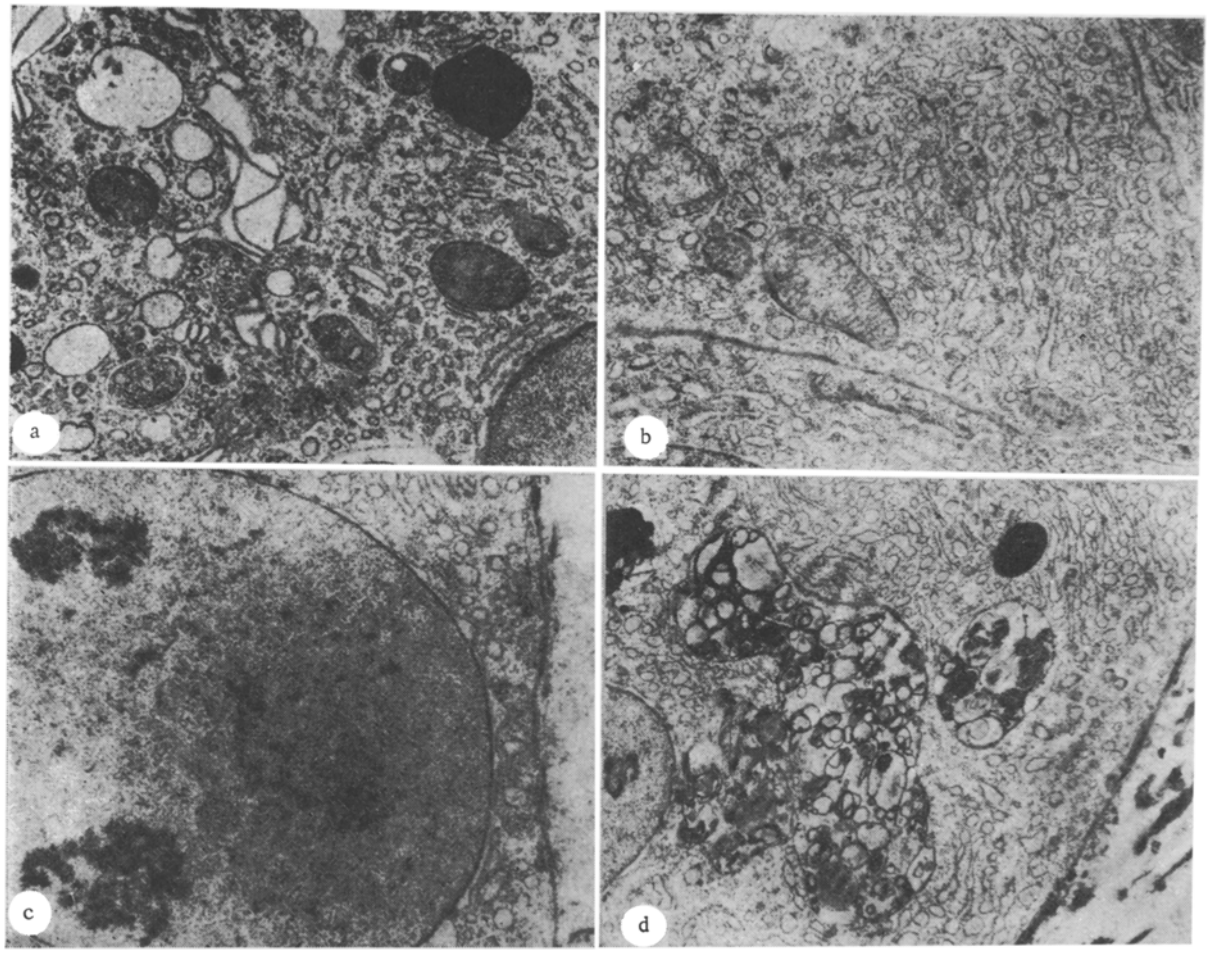

Fig. 2. Electron-microscopic changes in structure of AC after treatment of experimental pancreatic necrosis with 5-FU: a) vacuolation of structures of lamellar network (LN) of AC with no sign of maturation of secretory granules; b) fragmentation and vacuolation of RER of acinar cells; c) submembranous location of nucleus (N) of AC; d) single foci of focal necrosis (FN) in cytoplasm of AC, 24,000 ×.

closely with an increase in the blood 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 AC, in full agreement with the morphological data (Table 1).

Histological and electron-microscopic study of the pancreas of animals with pancreatic necrosis treated with 5-FU 3-6 h after the operation revealed synchronization of the secretory activity of AC, signs of accumulation of ZG in the cytoplasm, and a decrease in intracellular secretory activity. Elements of the lamellar network were represented by single dilated cisternae and mature ZG. The absence of the typical morphological picture of the phase of maturation of the secretion indicated delay in the formation of secretory products. The intensity of the reaction for RNA was reduced in the cytoplasm of AC and vacuolation of elements of RER was observed electron-microscopically (Fig. 2a).

A study of the pancreas 12 h after the operation showed signs of focal disruption of the complex structure of the acini, with diffuse distribution of ZG throughout the cytoplasm of AC, as far as the basal portions of the cells, thus explaining the high blood enzyme level still persisting at this period of the experiment. However, destruction of the cell membranes and acini had not occurred. Besides a considerable decrease in the degree of injury to AC, signs of depression of secretory activity were discovered. The nuclei occupied the basal part of the cytoplasm of the cells beneath the membrane, they had narrow nuclear pores, and had centrally situated nucleoli with homogenized nucleolonemma, and signs of vacuolation were more marked in RER (Fig. 2b, c). Structural changes of this sort in cytoplasmic components of protein-synthesizing cells are evidence of depression of nucleocytoplasmic exchange

and a disturbance of the primary phase of enzyme synthesis at the nucleus–RER level, accompanied by a marked decrease in trypsin and amylase activity in the animals' blood serum (Table 1).

On morphological investigation 24 h after the operation focal disruption of the complex structure of the acini was still present, with basal orientation of the granules, and foci of necrosis were present in the cytoplasm of AC, but with reduced accumulation of ZG. Slight polymorphocellular infiltration was discovered in the interlobular connective-tissue stroma.

5-FU thus inhibits secretory activity, reduces the degree of destruction of AC, and inhibits inflammatory changes in the gland and the rise in the blood enzyme levels. It can be concluded from the results of these biochemical and morphological investigations that 5-FU can be used as an additional aid in the combined pathogenetic treatment of patients with pancreatic necrosis.

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EFFECT OF THE HELIUM–NEON LASER ON ULTRASTRUCTURE AND PROLIFERATION OF THE EPITHELIUM OF THE GASTRIC MUCOSA

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Year by year lasers are increasingly being employed in medicine. They are used in surgery to divide tissues [7] and also to coagulate them to arrest bleeding [4]. These uses have made it necessary to study morphological changes in the tissues under the influence of laser irradiation [2, 5]. Comparatively powerful sources of laser radiation with high energy density are as a rule used for these purposes. There is information that radiation of low-power helium–neon lasers has a stimulating action on wound repair processes [1, 3].

The object of this investigation was to study the effect of the helium-neon laser on proliferation and ultrastructure of cells of the gastric mucosa.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male albino rats weighing 120-130 g. For autoradiographic investigation the animals were given an intraperitoneal injection of ^{3}H-thymidine at 10 a.m. in a dose of 0.5 μCi/g body weight. The peritoneal cavity was opened under ether anesthesia and the anterior wall of the stomach was irradiated with the LG-51-1 helium–neon

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