Pathomorphological Study of the Pancreas during Phospholipase A2-Induced Experimental Pancreatic Necrosis

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The pathomorphogenesis of experimental acute pancreatic necrosis induced by administration of phospholipase A2 into the pancreatic tissue is characterized by pathomorphological signs of hemorrhagic pancreatic necrosis (total necrosis and purulent fusion of some acini and massive hemorrhages in the interlobular and intralobular interstitial tissue) and fatty pancreatic necrosis (necrobiotic and necrotic changes in acinar cells that spread from the peripheral area and form the demarcation line).

Key Words: *pancreas; experimental pancreatitis; phospholipase A2*

Over the last 20 years, acute pancreatitis (AP) ranked third among acute surgical diseases of abdominal organs [2,4,5]. AP is a multifactorial disease. The development of this pathological process is mainly associated with blockade of the hepatopancreatic ampulla and major pancreatic duct. These disturbances are typical of cholelithiasis and ulcer disease of the duodenum [7,8]. The exposure to toxic or allergic factors and infectious agents plays an important role in the pathogenesis of AP. Recent medical and biological studies showed that carriers of some genetic mutations (*e.g.*, mutations in genes of trypsin inhibitor and cationic trypsinogen) are characterized by high risk of pancreatitides [9].

One of the major clinical symptoms of AP is the development of typical injury to the pancreas and progression of local and systemic complications. These disturbances require conservative and surgical treatment [1]. Morphofunctional changes in organs of the pancreaticoduodenal region were evaluated only in some investigations with pancreatic subjects. Little is known about reparative and compensatory-adaptive reactions in various structural and functional compartments of the pancreas under conditions of severe destructive injury. It is partly related to the absence of reproducible models for pancreatic necrosis [3,6] that simulate the most common types of this disease in humans.

Here we studied morphogenesis of experimental AP, which was induced by ductal hypertension and administration of phospholipase A2.

MATERIALS AND METHODS

AP in 30 Wistar rats was induced by administration of phospholipase A2 (5 mg/kg, activation of phospholipids) in 1 ml distilled water into the pancreas. Midline laparotomy in ether-anesthetized animals was performed under sterile conditions. The duodenum was delivered through the wound. Phospholipase A2 (EC 3.1.4, from the venom *Naja naja oxiana* snake) was administered along the common bile duct (0.5 ml) and into the tail of the pancreas (0.5 ml). The animals were examined in various periods of the study. The rats were divided into 2 subgroups of 15 specimens

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each. Subgroup 1 animals were examined for 3 h to evaluate the type of changes in abdominal organs. They were euthanized by ether overdose. The samples were taken for histological examination. None of these animals died during the experiment. Twelve rats of subgroup 2 (80%) survived after 24 h. The state of abdominal organs was evaluated by means of repeated laparotomy. The samples of visceral organs were taken for a pathohistological study.

The samples of the pancreas, duodenum, liver, heart, lung, and spleen were fixed in 10% neutral formalin, dehydrated in alcohols of increasing concentrations, and embedded in paraffin. Histological sections (5-7 μ) were stained with hematoxylin and eosin. Pearl's reaction was conducted. Tissue samples were fixed in 4% paraformaldehyde, postfixed in OsO_4, and embedded in a mixture of Epon and araldite to prepare the semithin and ultrathin sections. Semithin sections were stained with azure II. The paraffin and semithin sections were examined under a Leica DM 4000B universal microscope. Microphotographs were obtained using a Leica DFC 320 digital camera (Leica Qwin V3.0 software).

RESULTS

Severe edema of the head and tail of the pancreas was observed 5 min after administration of phospholipase A2 into the pancreatic tissue. Circulatory hyperemia was revealed in the proximal portion of the duodenum (segment 0.5-1 cm). Serous edema and duodenal hyperemia were shown to persist after 10-15 min. The degree of these changes tended to increase in time. Serous edema of the pancreas was accompanied by the appearance of a gelatinous hyperemic segment (0.3 cm in width) by the 20th minute after treatment. The severity of diffuse edema was shown to increase in the distal direction. The gelatinous crimson edema spread over the head of the pancreas and along the tail (area of phospholipase administration). The severity of diffuse duodenal edema increased in the distal direction. These changes were accompanied by paresis of the proximal portion of the duodenum. Abdominal effusion was not found.

The progression of pathological changes was revealed after 1 h. A gelatinous rose-colored edema of the duodenal segment appeared as hemorrhagic imbibition. Diffuse hyperemia was typical of the duodenum and small intestine (segment 10-12 cm). Severe plethora was observed the liver and kidneys. Hemorrhagic imbibition in the pancreas was not found after 3 h. Diffuse hyperemia, edema, and paresis of the proximal portion of the duodenum were shown to persist in this period. Moderate interlobular and intralobular edema and regions of focal micronecrosis of the acinar portion were revealed during microscopic examination of the pancreas. The regions of focal necrosis were infiltrated by neutrophils (demarcation infiltration). The duodenal mucosa was infiltrated by lymphocytes, macrophages and, to a lesser extent, by neutrophils. Plethora was found in the liver and spleen. Hypertrophy of lymphoid follicles was observed in the spleen.

Twelve specimens (80%) survived after 24 h. These animals were sluggish and "slovenly". Locomotor activity of rats was suppressed. They refused water and food. The signs of local peritonitis (peripancreatic infiltration) were found after laparotomy. The greater omentum was "soldered" to the pancreas and anterior and lateral wall of the abdomen. It was covered by numerous fatty plaques with the fibrin layers. Macroscopic examination showed that the pancreas has a crimson-rose color and includes regions of hemorrhagic imbibition. The duodenum and small intestine were strongly hyperemic and edematous (20-cm segment). Some regions of the transverse colon were necrotized. Severe plethora of the liver and kidneys persisted in these specimens.

Large areas of necrosis were found in the acinar part during a pathohistological study of the pancreas. The presence of microfocal and macrofocal abscesses reflected the development of diffuse and focal pancreatic necrosis (Fig. 1, *a*). These changes in the acinar compartment were accompanied by severe edema of the interlobular and intralobular stroma, deposition of fibrin, and diffuse infiltration by neutrophils and small amounts of macrophages. Massive hemorrhages (hemorrhagic imbibition) were found in the interlobular and intralobular stroma of many animals. Severe subcapsular hemorrhages were revealed in some rats (Fig. 1, *b*). These zones were characterized by deposition of fibrin and infiltration by inflammatory cells. Necrosis of acinar cells was observed in the head and, particularly, in the tail of the pancreas. Necrotic changes were shown to spread from the peripheral to the central region of the acini. The demarcation line consisted of neutrophils (Fig. 2, *a*). Acinar cells in the peripheral region of the acini were characterized by proteolytic changes (autolysis). They contained a homogenous mass and small fragments of the non-lysed cytoplasm, nuclei, or nuclear ghosts (Fig. 2, *b*). Agglomerates of neutrophils, macrophages, and lymphocytes were identified around these cells.

Acinar cells in the intact acini were reduced in size and contained a small amount of secretory granules (in the apical zone). Acinar cells were extremely heterogeneous in relation to staining agents. They were polymorphic (Fig. 2, *c*). We revealed the appearance of numerous "lamellar" cells (Fig. 2, *d*). These cells did not contain secretory granules. The "lamellar" structure of cells was related to an irregular

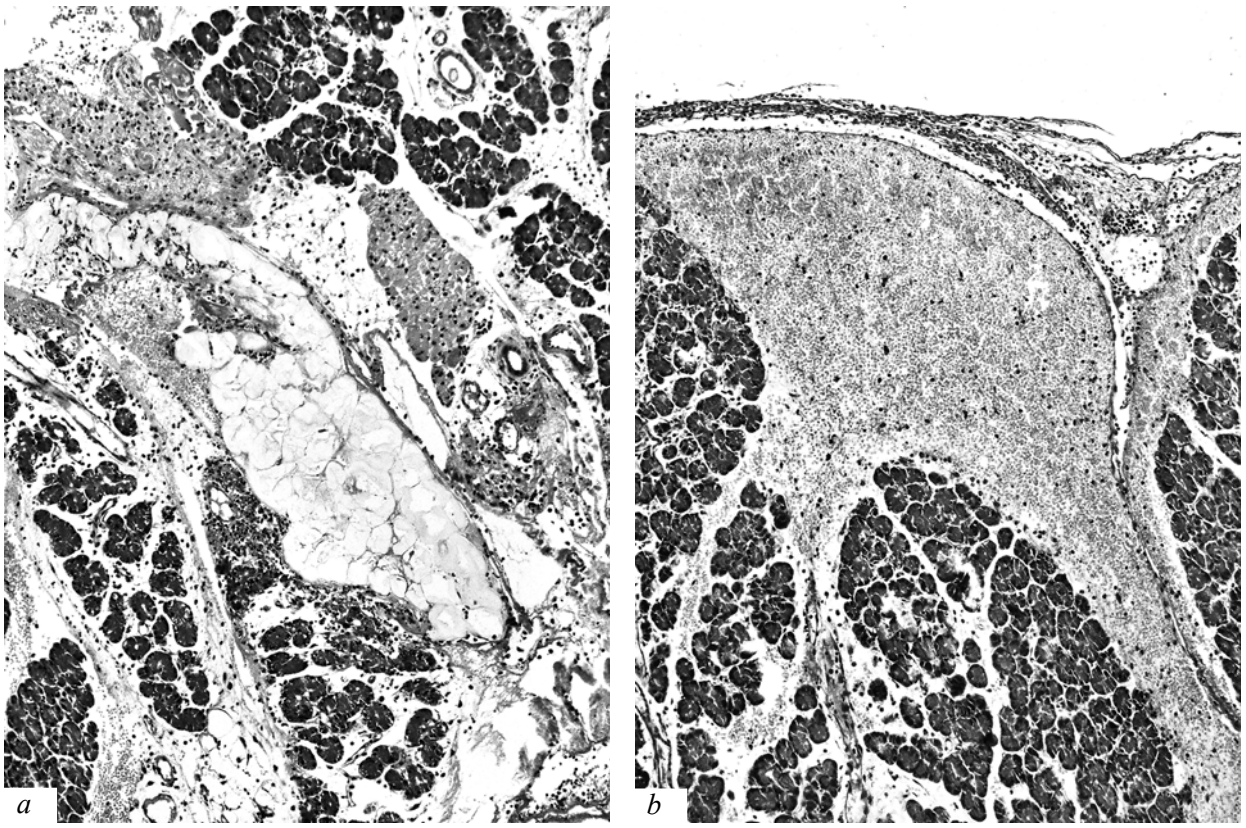

Fig. 1. Rat pancreas 24 h after administration of phospholipase A2. Staining by hematoxylin and eosin (×100). Focal necroses and fusion of the acini; edema and hemorrhages (*a*); massive subcapsular hemorrhages (*b*).

enlargement of the granular cytoplasmic reticulum. They were visualized by light microscopy. Among the small acinar cells with optically dense cytoplasm, we often revealed binucleated cells. Decomplexation of acinar cells was found near large vessels and necrotic focuses. They did not form typical rosettes and were characterized by severe atrophy and resorption by mononuclear cells. Necrotic changes were not identified in Langerhans islets. The islets were formed by insular cells that contained a small granular substance.

Hepatocytes were arranged in a beam-like structures. In some lobules, the cells were decomplexated and dystrophic. The sinusoids and central and portal veins were irregularly plethoric. The kidneys were characterized by necrobiotic changes of glomeruli and epitheliocytes in the distal and proximal tubules. Moderate edema and infiltration of the lamina propria by inflammatory cells were shown to persist in the duodenum. The degree of pathological changes under these conditions depended on the development of purulent omentitis.

Pathomorphological study showed that administration of phospholipase into the pancreatic tissue is followed by the appearance of typical pathomorphological signs for hemorrhagic pancreatic necrosis (total necrosis and purulent fusion of some acini, massive hemorrhages in the interlobular and intralobular interstitial tissue) and fatty pancreatic necrosis (necrobiotic and necrotic changes in acinar cells that spread from the peripheral area and form the demarcation line). Administration of phospholipase A2 into the pancreatic tissue causes injury to cell membranes, activation of lipase, and release and preterm activation of pancreatic enzymes. Among these enzymes, trypsin produces the greatest proteolytic effect. Trypsin activates not only zymogenic enzymes of the pancreas (elastase, carboxypeptidase, chymotrypsin, *etc.*), but also lysosomal enzymes and proteinases [10]. These changes potentiate proteolytic destruction of pancreatic cells (*i.e.*, progressive necrosis of the acinar tissue).

In this model of AP, the development of phospholipase A2-induced mechanical damage to the pancreas is accompanied by transformation of fatty pancreatic necrosis and hemorrhages into hemorrhagic pancreatic necrosis. This form of AP is characterized by a progressive course, severe pancreatogenic intoxication, and early development of polyorgan insufficiency. This disorder determines poor state (sluggishness and refusal of water and food) and mortality of experimental animals. Three rats died 24 h after administration of phospholipase. However, all animals survived 24 h after common bile duct ligation.

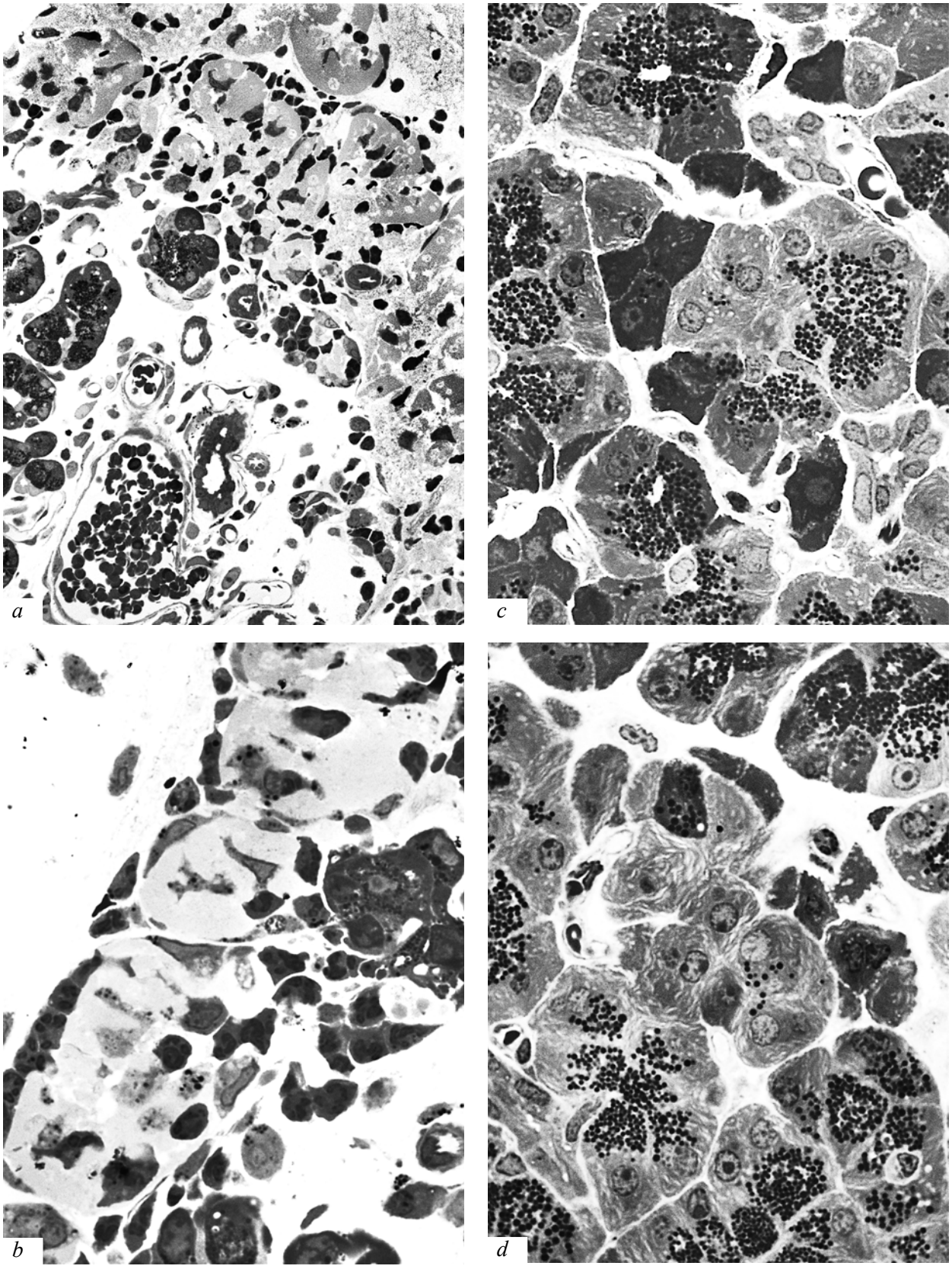

Fig. 2. Morphological changes in the rat pancreatic acini 24 h after administration of phospholipase A2. Semithin sections. Azure II staining. Acinar necrosis (proteolytic changes) in the peripheral region of the lobule (*a*, ×400); autolysis (self-digestion) of acinar cells (*b*, ×1000); significant heterogeneity of acinar cells (*c*, ×1000); increase in the count of "lamellar" cells in the acini (*d*, ×1000).

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