Changes in Pancreahepatoduodenal Organs during Experimental Acute Lipogenic Pancreatitis

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 8, pp. 232-236, August, 2000
Original article submitted March 23, 2000

Experiments on animals with experimental lipogenic pancreatitis showed that disturbances in lipid metabolism stimulate destructive processes in the pancreas, liver, and spleen. Acute experimental lipogenic pancreatitis is characterized by extensive destructive changes in the pancreatic parenchyma accompanied with fatty necrobiosis of hepatocytes, fatty transformation of the gallbladder wall, and pronounced reaction of the reticuloendothelial stroma in the spleen.

Key Words: *dyslipoproteinemia; acute pancreatitis*

Experimental modeling of acute pancreatitis (AP) is an actual problems of clinical physiology, because detailed study of its pathogenesis is possible only under artificial etiological conditions. The models of AP do not encompass all etiopathogenetic mechanisms of this disease.

The first model of acute hemorrhagic pancreatitis was developed in 1856 by Claude Bernard, the founder of experimental pathology of the pancreas, who introduced mixture of bile and olive oil into ligated Wirsung's duct [2]. These studies substantiated the hypothesis that bile reflux into the pancreatic duct always causes AP. Only in 1931 Wangensteen *et al.* proved that obturation of the pancreatic duct and stimulation of pancreatic function are prerequisite for acute pancreatitis [3]. The models of AP can be subdivided into canalicular-hypertensive, vascular-allergic, traumatic and toxoinfectious, and alimentary [6].

When AP is modeled by restriction of secret outflow to the major bile duct, replacement of exocrine pancreatic parenchyma with connective tissue starts after 3-4-week mild AP and culminates in fibrosis. The endocrine pancreatic parenchyma is preserved for a longer period [5,10]. The acute phase of pancreatitis can be aggravated by stimulation of pancreatic secretion by pilocarpine, neostigmine, vitamin A, magnesium sulfate, cholecystokinin, alcohol, and other agents [7,9].

Modeling of AP by injection of various agents into Wirsung's duct is based on drastic pressure rise in the intercalated ducts and acinus lumens due to large volume of introduced agent. It leads to parenchymatous edema, which can develop into destructive pancreatitis depending on the pressure, cytotoxicity of applied preparation, and severity of damage to excretory duct epithelium [10]. The extra pressure is produced by infusion of fluid in a volume surpassing physiological capacity of the ducts [4].

The destructive process in the pancreas is accelerated when bile, trypsin, and other substances in combination with intestinal masses are infused against the background of stimulated pancreatic secretion. These changes in the pancreas appear during 24 h, and the animal usually dies after 3 days [10].

It should be noted that modern AP models do not take into account such important factor of the disease development as reactivity of the pancreas and organs of the pancreatobiliar system to experimental stress with acute inflammation. According to modern views, the pathogenesis of all diseases is a combination of structural (morphogenesis) and functional (functional genesis) abnormalities. Therefore, the general meta-

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0007-4888/00/0008-813$25.00

bolic background for the development of a disease is of primary importance for its complex assessment and designing the therapeutic strategy.

Dyslipoproteinemia as a peculiar state of the pancreatobiliary system providing metabolic prerequisites for the realization of the basic pathogenetic pathways of AP [8] is little studied. However, we found no the published data on modeled lipogenic pancreatitis.

Our aim was to study disturbances in the pancreas and organs of the hepatobiliary system under conditions of AP against the background of experimental dyslipoproteinemia (EDLP).

MATERIALS AND METHODS

Experiments were performed on 16 Chinchilla rabbits weighing 3.1±0.1 kg. In experimental rabbits (n=8) EDLP was modeled by addition of crystalline cholesterol (0.3 g/kg/day) to granulated food [1]. The control rabbits (n=8) fed normal diet including unrestricted fresh grass, vegetables, and tuberous root crops.

After 1 and 2 months of atherogenic diet, plasma content of total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, VLDL cholesterol, and dyslipoproteinemia coefficient were determined.

After 2 months, AP was modeled by the method of Pavlov [6] in control and experimental (with verified persistent EDLP) rabbits. Then median laparotomy and ligation of Wirsung's duct were performed under intravenous barbiturate narcosis. After 3 days all animals were sacrificed by barbiturate overdose, and the specimens of the liver, gallbladder, pancreas, and spleen were taken. For morphological analysis, small fragments of the examined organs were fixed in 10% neutral formalin. Some specimens were embedded in paraffin, and 5-7-μ slices were stained with hematoxylin and eosin. Other fixed specimens (excluding spleen specimens) were used for qualitative histochemical analysis: the content of lipids and cholesterol was determined on the cryostat slices stained with Sudan III and by the method of Schultz, respectively.

TABLE 1. Indices of Lipid Metabolism in Rabbits with EDLP (mg/dl, $M\pm m$, n=8)

Index	Control	EDLP	
		initial	on month 2
Cholesterol	30.6±1.4	39.5±6.9	683.2±171.5
Triglycerides	61.1±1.3	58.4±12.6	496.4±135.1
HDL	14.0±0.6	14.3±3.2	126.2±33.4
LDL	6.7±2.3	14.9±6.9	464.8±101.2
VLDL	12.3±0.4	12.5±0.7	95.7±25.8
Coefficient of dyslipoproteinemia	1.2±0.2	1.8±0.5	4.5±0.3

Note. *p<0.05 compared to the initial and control values.

RESULTS

In experimental group, EDLP was verified before AP modeling. It was characterized by increased levels of cholesterol (20-22 times), triglycerides (10-11 times), LDL (22-25 times), VLDL (8-10 times), and dyslipoproteinemia coefficient by 4-5 times in comparison with the control or initial values (Table 1).

Morphological analysis of liver, pancreas, gallbladder, and spleen specimens revealed different reactions of visceral organs depending on the presence and absence of EDLP and experimental pancreatic inflammation. In rabbits with EDLP, structural and functional alterations in the exocrine parenchyma were more severe and extensive. While focal necrobiotic and necrotic alterations prevailed in the control group (Fig. 1, *a*), experimental rabbits with AP developed against background EDLP demonstrated typical signs of pancreonecrosis. Large foci of coagulation parenchymatous necrosis with pronounced vascular reaction and perifocal polynuclear infiltration were observed in the majority of pancreatic lobules (Fig. 1, *b*). Dilation of microvessels, hemostasis, stromal edema, and cell infiltrates were regular signs of acute phase of pancreatic inflammation. Outside the necrotic foci, tissue structure was also significantly altered, which was manifested in dyscomplexation of acini, weak basophilia of exocrinocyte cytoplasm of, and hydration of intra- and interlobular tissue. In some lobules focal replacement of the parenchyma with adipose tissue was observed (Fig. 1, *c*). Adipose cells could appear in acini due to local necroses.

Comparison of the pathomorphological picture of the pancreas in the test groups showed that dystrophy of the pancreas caused by disturbances in lipid metabolism under conditions of additional load was manifested by extensive destructive lesion of the parenchyma and accompanied by accelerated necrotic process.

Histological analysis of liver specimens revealed both general trends and peculiarities in the reaction of this organ during AP modeling. Ligation of pancreatic excretory ducts in the control group induced dilation of bile ducts, changes in the configuration of bile capillaries, and focal (predominantly centrolobular) degeneration of the liver parenchyma. In histological preparations it was manifested by karyopyknoses, homogenization or swelling of the hepatocyte cytoplasm against the background of their small-drop fatty degeneration (Fig. 2, *a*). Focal vascular disorders in the portal system, such as hemostasis in interlobular veins, dilation of sinus capillaries, and diapedesis were also observed.

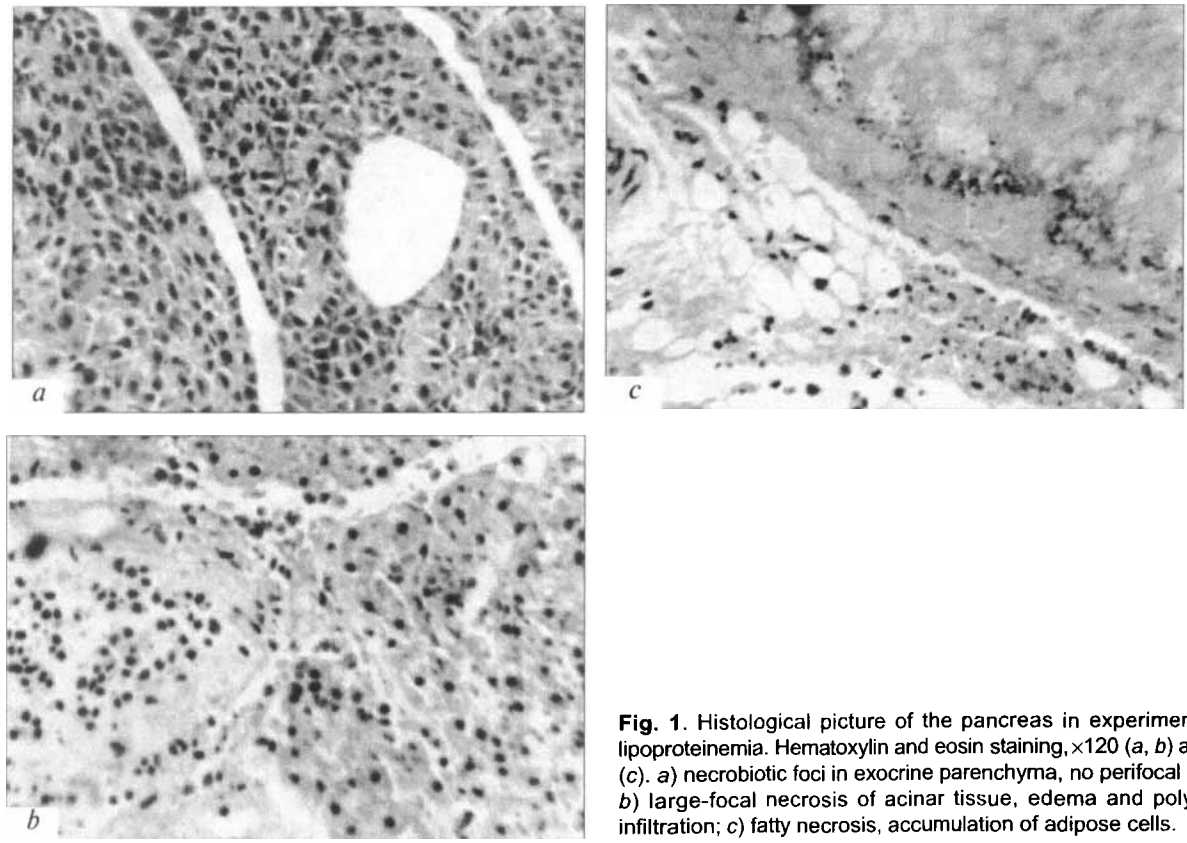

Fig. 1. Histological picture of the pancreas in experimental dyslipoproteinemia. Hematoxylin and eosin staining, ×120 (*a*, *b*) and ×480 (*c*). *a*) necrobiotic foci in exocrine parenchyma, no perifocal reaction; *b*) large-focal necrosis of acinar tissue, edema and polynuclear infiltration; *c*) fatty necrosis, accumulation of adipose cells.

AP modeled against the background of EDLP revealed the diffuse character of lipid infiltration of the liver and increased number of hepatocytes with histological signs of lipid necrobiosis. In addition, large destruction foci were observed in the hepatic parenchyma (Fig. 2, *b, c*). Frequently, the intralobular part of the biliary system looked like distended convoluted tubules with local varicosities filled with homogenous substance (evidently, thickened bile). Histiolymphocytic infiltrates were found both around the portal tracts and in the intralobular zones.

Vascular disorders in liver manifested in dilated sinusoids alternating with capillary-free areas (probably due to compression of blood capillaries by degenerative and destructed hepatocytes).

Disturbances of vascular permeability were observed not only in capillaries, but also in veins. They were manifested by characteristic peripheral lymphocyte stasis, sudanophilic inclusions in lympho- and monocyte cytoplasm, and perivascular intersticial hydration of the hepatic parenchyma.

In some specimens, centrolobular depositions of eosinophilic substance were revealed around the central veins; the nature of this substance was difficult to identify by the routine morphological analysis. This phenomenon probably marks the foci of "bile apoplexy", *i.e.*, bile release from destroyed liver trabecules into the intersticial space.

The revealed peculiarities of tissue reaction in AP developed against the background of EDLP corroborate the modern view that disturbances in lipid metabolism aggravate the course of all pathological processes [11]. It can also be assumed that hypoxia combined with destructive-degenerative cell damage can paralyze metabolic processes such as utilization of hepatic lipids and stimulate decomposition of lipoprotein complexes. These mechanisms may play a significant role in the pathogenesis of progressive hepatic steatosis with focal necroses of the parenchyma after lipogenic AP.

Functional morphology of the gallbladder in the examined groups did not differ so clearly as in the pancreas and liver, regional ambiguity of structural transformations being noteworthy. The gallbladder mucosa retained folded relief or demonstrated smoothening of the external contour (Fig. 3, *a*) with signs of epithelium degeneration, focal disintegration, and edema of the lamina propria partially infiltrated with hematogenic elements. In the EDLP-rabbits Sudan III staining revealed macrophages with ample lipid inclusions in edematous gallbladder stroma. In addition, some individual epitheliocytes underwent fatty degeneration (Fig. 3, *b*). In this group, the destructive and degenerative processes in the gallbladder wall (disappearance of folds, epithelium desquamation, and focal necrobiosis of cells) were most pronounced.

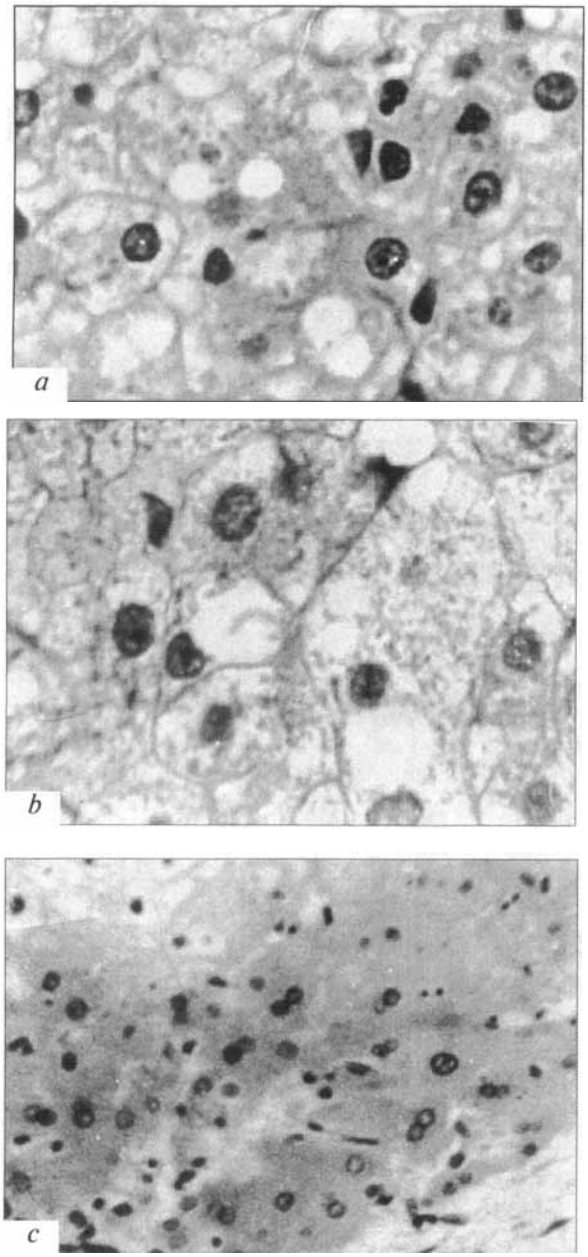

Fig. 2. Structure of the liver in experimental dyslipoproteinemia. Staining with hematoxylin and eosin (*a*, *b*) and Sudan III (*c*), ×480. *a*) hepatocyte degeneration and dilation of bile capillaries; *b*) balloon hepatocyte degeneration; *c*) focal fatty necrosis in the parenchyma.

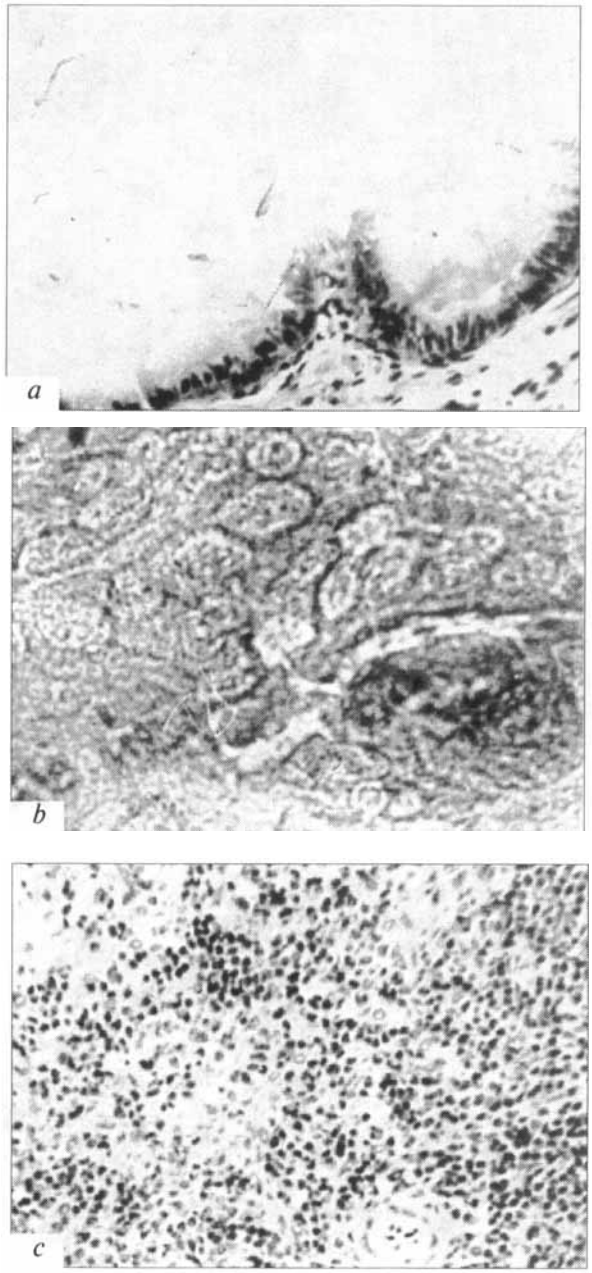

Fig. 3. Structure of the gallbladder and spleen in experimental dyslipoproteinemia. Staining with hematoxylin and eosin (*a*, *c*) and Sudan III (*b*), ×120 (*a*, *c*) and ×480 (*b*). *a*) destructive and degenerative lesions of gallbladder mucosa; *b*) large focus of lipid accumulation in gallbladder epithelium against the background of dust-like lipid epitheliocyte infiltration; *c*) spleen volume enlargement, extended reactive foci, and reticuloendothelial hyperplasia.

In both groups, the general morphologic study revealed no essential differences in the structural and functional status of the spleen during AP modeling. However, there were signs of antigenic stimulation: enlargement of folliculi, reactive centers, plasmatization and reticular hyperplasia of the white and red pulp more pronounced against the background of the EDLP (Fig. 3, *c*).

Therefore, disturbances in lipid metabolism during AP modeling stimulate the development of destructive processes in the pancreas, liver, gallbladder, and spleen. Peculiarities of experimental pancreatitis against the background of EDLP attest to correctness of isolation of a peculiar form of acute pancreatic inflammation — lipogenic pancreatitis, characterized by extensive destructive lesions of the pancreatic parenchyma, accompanied by fatty necrobiosis of hepatocytes, fatty degeneration of the gallbladder wall, and pronounced reaction of the reticuloendothelial stroma in the spleen.

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