Liver Damages during Experimental Acute Pancreatitis

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Acute pancreatitis in rats caused by cooling of the pancreas with chloroethyl is characterized by the development of focal necrosis and activation of lipid peroxidation in the liver, activation of blood aminotransferase and acid and alkaline phosphatase, and increase in total and indirect bilirubin concentrations.

Key Words: *experimental pancreatitis; liver*

Clinical symptoms of liver damage are present in 18-30% patients with acute pancreatitis (AP). Liver insufficiency accompanies pancreonecrosis in 25% cases [1]. Pancreatogenic damages to the liver are characterized by hypoalbuminemia, hypofibrinogenemia, increase in aminotransferase and lactate dehydrogenase activities and unconjugated bilirubin content in the blood [8].

To refine present notions about the pathogenesis of systemic inflammatory reactions accompanying AP, we studied the type and severity of metabolic disturbances in the pancreas and liver during this disease.

MATERIALS AND METHODS

Experiments were performed on 130 male outbred albino rats weighing 180-200 g and kept in a vivarium under natural light-dark regimen and free access to food and water. AP was induced by 1-min rinsing of the splenic lobe of the pancreas with chloroethyl [6]. Sham-operated animals served as the control. The rats were decapitated 3, 14, 21, and 28 days after surgery. Surgery and decapitation were performed under ether anesthesia. The survival of rats, duration of hexenal-induced sleep (60 mg/kg intraperitoneally), and retention of sulfobromophthalein (SBP, 5 mg/kg intravenously) were estimated. Serum activities of α-amylase, lipase, alanine transaminase (ALT), aspartate transaminase (AST), acid phosphatase (ACP), and alkaline phosphatase (ALP) and serum contents of glucose, lipids, protein, urea, creatinine, and bilirubin fractions were measured. Urinary creatinine and α-amylase activity were determined [2,4]. The contents of conjugated dienes and the rate of ascorbate- and NADPH-dependent formation of malonic dialdehyde (MDA) were measured in homogenates of the pancreas and liver [3]. Deparaffinized sections of the pancreas and liver were stained with hematoxylin and eosin and by the methods of Mallory and Einarson (gallocyanine) [5]. The results were analyzed by Student's *t* test.

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RESULTS

Animal mortality 3 days after cooling of the pancreas was 15%; 7 and 5% rats died after 14 and 21 days, respectively. All sham-operated animals survived. The structure and biochemical parameters of the pancreas and liver in these rats were normal.

Activity of enzymes, acinar destruction markers, and the content of creatinine and glucose in the blood increased in rats with experimental pancreatitis. The maximum increase in α-amylase and lipase activities (by 2.9-3.4 times), creatinine content (by 1.3-1.5 times), and glucose concentration (by 1.8-2.3 times) occurred 3-14 days after modeling of pancreatitis. Enzyme activities and glucose and creatinine contents remained high 21 days after treatment. On day 28 after injury α-amylase and lipase activities decreased, but remained above the control, while the contents of glucose and creatinine returned to normal. Urinary excretion of α-amylase underwent similar changes. Urinary creatinine content 3 and 14 days after modeling of AP

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decreased by 1.4-1.8 times, but then returned to normal (Table 1).

Biochemical tests characterizing membrane permeability and metabolism in hepatocytes confirmed early involvement of the liver into the pathological process during AP. Acute period of pancreatitis (3-14 days) was characterized by the development of hyperlipidemia, hypoproteinemia, cytolysis of hepatocytes, and 4-6-fold increase in blood transaminase and ACP activities. Blood urea increased by 1.7 times. Symptoms of cholestasis appeared: total and indirect bilirubin increased by 1.9-2.2 and 3.7-4.4 times, respectively, ALP activity increased by 4.7 times. Parameters of SBP test peaked 14 days after AP modeling. The duration of hexenal-induced sleep on days 3 and 14 after injury increased to 45.3±2.4 and 47.5±2.4 min; respectively (*vs*. 22.3±1.2 min in the control). The degree of bilirubin glucuronidation decreased from 83 to 56-66% (Table 1).

Twenty-one days after cooling of the pancreas, hyperlipidemia and hyperenzymemia became less pronounced, protein content increased, and blood urea level decreased. Bilirubin concentration remained high, and SBP retention decreased. The duration of hexenal-induced sleep was 35.4±3.1 min. The ratio of bilirubin glucuronides increased to 70%. Four weeks after AP modeling transaminase and ALP activities, contents of lipids, protein, and urea in the blood, and duration of hexenal-induced sleep (25.4±1.7 min) returned to normal. ACP activity, bilirubin concentra-

TABLE 1. Biochemical Parameters in Rats with AP (*M*±*m*)

Material	Intact	Time after AP modeling, days			
		3	14	21	28
Plasma					
α-Amylase, μcat/liter	7.2±0.7	24.2±1.6*	21.4±1.6*	18.9±1.4*+	12.4±1.2*+ox
Lipase, μcat/liter	14.3±1.1	45.6±3.0*	41.2±2.9*	32.1±2.3*+o	25.6±2.2*+o
ALT, μcat/liter	0.50±0.02	2.96±0.07*	2.94±0.06*	1.04±0.03*+o	0.48±0.03+ox
AST, μcat/liter	0.62±0.02	2.84±0.09*	2.92±0.08*	1.46±0.03*+o	0.68±0.04+ox
ACP, U/liter	10.8±0.5	44.6±1.4*	42.8±1.6*	20.8±1.7*+o	15.7±1.2*+ox
ALP, U/liter	209.7±7.8	982.5±19.8*	977.6±18.7*	242.7±8.5*+o	202.7±6.4+ox
Bilirubin, μmol/liter					
total	11.0±0.7	21.1±1.0*	24.5±1.6*	20.2±1.2*	16.4±0.9*+ox
idirect	1.9±0.1	7.1±0.4*	8.3±0.3*	6.1±0.3*+	4.2±0.2*+ox
Glucose, mmol/liter	6.2±0.6	11.3±1.0*	14.5±1.4*	11.0±1.1*	8.1±0.9o
Lipids, g/liter	3.8±0.2	13.8±0.7*	14.1±0.5*	7.2±0.5*+o	3.9±0.4+ox
Protein, g/liter	79.7±2.8	52.9±2.5*	53.2±2.3*	68.4±2.6*+o	75.4±1.7+o
Urea, mmol/liter	7.8±0.5	13.0±0.9*	13.3±0.7*	9.3±0.6+o	8.0±0.4+o
Creatinine, μmol/liter	89.1±2.4	114.6±5.6*	131.2±6.3*	108.6±4.6*	86.1±4.4+ox
SBP retention, %	2.2±0.2	7.8±0.6*	13.4±1.3*+	6.5±0.4*o	3.4±0.3*+o
Urine					
α-Amylase, μcat/liter	21.4±0.9	36.4±1.3*	38.5±1.4*	28.3±1.5*+o	25.7±1.7+o
Creatinine, μmol/liter	4.3±0.6	2.4±0.4*	3.0±0.4*	3.6±0.6	4.0±0.4+
Pancreas					
Conjugated dienes, U/mg lipids	0.81±0.07	1.98±0.14*	2.31±0.17*	1.72±0.16*o	1.35±0.12*+o
MDA, nmol/mg protein/min					
ascorbate-dependent	0.26±0.01	1.03±0.09*	1.11±0.09*	0.72±0.06*+o	0.47±0.03*+ox
NADPH-dependent	0.45±0.04	1.63±0.18*	1.82±0.16*	1.07±0.11*+o	0.65±0.05*+ox
Liver					
Conjugated dienes, U/mg lipids	2.64±0.23	6.34±0.48*	8.13±0.63*	4.27±0.32*+o	3.23±0.21*+ox
MDA, nmol/mg protein/min					
ascorbate-dependent	1.07±0.07	2.61±0.24*	3.72±0.26*+	2.31±0.20*o	1.54±0.09*+ox
NADPH-dependent	1.33±0.09	2.91±0.27*	4.02±0.33*+	2.46±0.25*o	1.95±0.12*+o

Note. $p<0.05$: *compared to intact animals; compared to pancreatitis +3, o14, and x21 days after treatment.

tion, and SBP retention in the liver tended to normal. The degree of bilirubin glucuronidation increased to 74% (Table 1).

In rats with AP, the contents of conjugated dienes in homogenates of the pancreas and liver increased by 1.7-2.8 and 1.2-3.5 times, respectively. Ascorbate- and NADPH-dependent MDA production in the pancreas and liver increased by 1.4-4.3 and 1.2-3.5 times, respectively (Table 1). LPO in the liver can be stimulated by prostaglandins, kinins, tumor necrosis factor-α, and interleukins 1, 6, 10, and 11 formed in damaged pancreas [7]. The intensity of LPO peaked 14 days after AP modeling, slightly decreased 21 and 28 days after injury, but far surpassed the corresponding parameter in intact and sham-operated rats.

Acute pancreatitis (3-14 days after injury) was characterized by severe morphological disturbances in the pancreas, including edema and inflammatory infiltration of the stroma, hyperemia, arterial thromboses, focal acinar necroses, spasm of pancreatic ducts filled with cell detritus, and degeneration of insular cells. Disarrangement of hepatic laminae, dilatation of sinusoids, perisinusoidal edema, hemorrhages, and focal necroses of hepatocytes were found in the liver. Signs of regeneration appeared on day 28 after AP modeling and included proliferation of ductal epitheliocytes and centroacinar cells in the pancreatic exocrine parenchyma and accumulation hypertrophic and binuclear hepatocytes with high RNA content.

Our experiments demonstrated that acute pancreonecrosis developed 3-14 days after cooling of the pancreas with chloroethyl was accompanied by pronounced pathological changes in the liver, including focal necrosis in the parenchyma, activation of LPO, increase in blood transaminase, ACP, and ALP activities, and increase in total and indirect bilirubin concentrations. The structure and biochemical parameters of the pancreas were partially restored 28 days after injury. Normalization of membrane permeability and detoxification and excretory functions of the liver started 21 days after treatment.

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