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DIAGNOSTIC VALUE OF LEUCINE-AMINOTRANSFERASE ASSAY IN ACUTE PANCREATITIS

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Leucine aminotransferase (LAT), which catalyzes the reaction transferring an amino group from leucine to α-ketoglutaric acid with the formation of α-ketoisocaproic and glutamic acids, is found in all tissues of the human and animal body and is located both in the cytoplasm and in the subcellular organelles [4-6]. High activity of this enzyme in the pancreas, salivary glands, and lactating mammary glands points to its important role in the function and metabolism of these organs [4, 7]. These facts suggest that destructive diseases of the pancreas may be accompanied by significant changes in LAT activity both in that organ and in the blood.

The aim of this investigation was to determine the differential diagnostic value of investigation of LAT activity in the blood serum and peritoneal exudate in different forms of acute pancreatitis (serous and destructive pancreatitis).

EXPERIMENTAL METHOD

LAT activity was determined in the blood serum of 10 clinically healthy individuals (blood donors) and in the serum and peritoneal exudate (if present) of 20 patients with acute pancreatitis. LAT activity was determined in the pancreatic tissue and blood serum of intact (15) rats and rats undergoing a mock operation (24), and in the blood serum and peritoneal exudate of rats with pancreatic necrosis (40 animals). Experimental subtotal pancreatic necrosis was induced by cooling the splenic part of the pancreas with ethyl chloride [3], and serous pancreatitis was induced by interference with the drainage of pancreatic juice [2]. LAT activity was determined in the cytoplasmic (soluble) fraction of pancreatic tissue obtained by differential centrifugation of a 10% tissue homogenate at 25,000g. The

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TABLE 1. LAT Activity in Blood Serum, Peritoneal Exudate (in μmoles α-ketoisocaproic acid/ml/h) and in Pancreatic Tissue (in μmoles α-ketoisocaproic acid/g tissue/h) in Intact Rats, Rats Undergoing Mock Operation (A), and Rats with Subtotal Pancreatic Necrosis (B) (M ± m)

Test object	Intact animals (n = 15)	Time of observation, h					
		1		3		24	
		A ($n=8$)	B ($n=8$)	A ($n=8$)	B ($n=8$)	A ($n=8$)	B ($n=8$)
Blood serum	0,092±0,012	0,070±0,018	0,332±0,016	0,096±0,016	0,800±0,016	0,117±0,030	0,330±0,020
	P	<0,01	<0,001	>0,1	<0,001	>0,5	<0,001
	P_1	—	<0,001	—	<0,001	—	<0,001
Splenic part of pancreas	108,17±7,61	105,26±5,68	72,22±4,39	72,87±5,27	35,86±2,93	80,21±2,75	18,40±2,22
	P	>0,5	<0,01	<0,01	<0,001	<0,01	<0,001
	P_1	—	<0,01	—	<0,001	—	<0,001
Peritoneal exudate	—	—	1,134±0,012	—	1,103±0,107	—	0,251 Fluid discovered in two cases

Legend. P) Compared with normal, P_1) compared with rats undergoing mock operations.

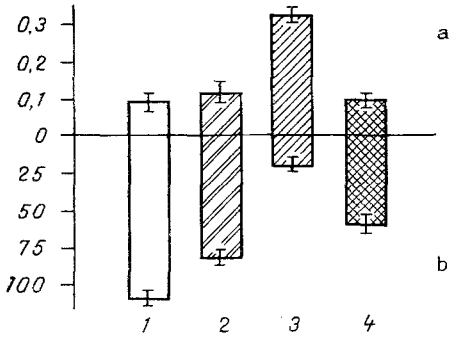

Fig. 1. LAT activity in blood serum (a) and pancreatic tissue (b) of normal rats, rats undergoing mock operations, and 24 h after induction of various forms of acute pancreatitis. Abscissa: 1) normal, 2) mock operation, 3) pancreatic necrosis, 4) serous pancreatitis; ordinate: above — LAT activity in micromoles α-ketoisocaproic acid/ml/h; below — the same, in micromoles α-ketoisocaproic acid/g tissue/h.

supernatant, in a volume of 0.15 ml, was incubated for 1 h at 37°C with a substrate mixture containing 0.3 ml of L-leucine (20 μmoles), 0.15 ml α-ketoglutaric acid (15 μmoles), and 0.15 ml pyridoxal-5-phosphate (0.02 μg); in the control group 0.3 ml of phosphate buffer, pH 7.8, replaced the leucine. The biological fluids (blood serum or peritoneal exudate) from human subjects and animals were taken in a volume of 0.15 ml and incubated with a substrate mixture of the same composition, except that the amount of α-ketoglutaric acid used was 2 μmoles. LAT activity was judged from the quantity of α-ketoisocaproic acid formed, which was determined by the authors' own method [1].

EXPERIMENTAL RESULTS

It will be clear from Table 1 that 1-3 h after induction of pancreatic necrosis in the rats (the hemorrhagic phase of pancreatic necrosis) a sharp decline in LAT activity was observed in the soluble fraction of cells in the damaged part of the pancreas. In the stage of parenchymatous necrosis (after 24 h) LAT activity was reduced by almost five-sixths. The opposite picture was observed in the blood serum: 1 h after the beginning of the disease activity rose to 4 times the normal level, to almost 10 times after 3 h, and 4 times after 24 h; later (after 3 and 7 days) it returned to normal, namely 0.085 ± 0.002 and 0.105 ± 0.028 μmoles α-ketoisocaproic acid/ml/h, respectively. LAT activity in the peritoneal exudate

climbed to even higher values, 2 or 3 times higher than in the blood of the animals with pancreatic necrosis. In animals undergoing mock operations, the serum LAT activity was unchanged compared with intact rats, but in the splenic part of the pancreas it showed a small decrease.

LAT activity in the pancreas in serous pancreatitis was reduced slightly, but no increase in LAT activity was observed in the blood (Fig. 1).

Comparison of the results of these two groups of experiments shows that in serous pancreatitis, when the component of frank tissue destruction is absent, LAT activity in the blood is unchanged, whereas in destructive pancreatitis, on the other hand, it is sharply increased. It follows from these results that in the early stages of acute destructive pancreatitis the enzyme is released from the focus of pancreatic necrosis into the blood stream and peritoneal cavity as a result of disturbance of the membrane structures of the cells and of cytolysis.

The pattern of change in LAT activity during the course of experimental pancreatic necrosis provided a basis for clinical trials of this test.

Analysis of the results of the trials showed that of 20 patients with acute pancreatitis studied, the serum LAT activity of ten patients with a clinical diagnosis of hemorrhagic pancreatic necrosis was raised to 0.150-0.487 μmoles/ml/h compared with 0.00-0.80 μmoles/ml/h normally. In 4 of the 10 cases the diagnosis of hemorrhagic pancreatic necrosis was confirmed on the operating table during emergency laparotomy. In two cases of pancreatic necrosis a hemorrhagic exudate (2 and 3.5 liters) with a high LAT content 59 and 65 μmoles/liter, respectively, was found in the peritoneal cavity.

LAT activity in ten patients with a clinical diagnosis of serous pancreatitis varied within normal limits: 0.00-0.100 μmole/ml/h. In 3 of 10 patients the diagnosis of serous pancreatitis was confirmed on the operating table during emergency laparotomy.

Determination of blood levels of activity of pancreatic excretion enzymes (α-amylase, trypsin) and trypsin inhibitors revealed no clear dependence of changes in their activity on the type of pancreatitis. In both edematous and destructive pancreatitis the trend of the change in these parameters was the same. The mean statistical range of blood α-amylase activity in serous pancreatitis was 72.0-228.5 U and in pancreatic necrosis 123.0-251.4 U, for trypsin it was 0.72-8.30 and 1.8-4.7 IU, and for trypsin inhibitors 463.8-811.6 and 394.2-802.9 IU, respectively. The wide range of variations in the activity of the pancreatic excretion enzymes and the low informativeness of these parameters for the differential diagnosis between different forms of acute pancreatitis have been noted by other workers also [2]. The significant increase (four-tenfold) in the LAT activity in the blood and peritoneal exudate is evidence of destruction of the pancreatic cells and of its release from the organ.

The experimental and clinical data suggest that determination of the serum LAT activity can be recommended as a very convenient additional test for the early differential diagnosis of two forms of acute pancreatitis, namely pancreatic necrosis and serous pancreatitis. The informativeness of this test for differentiation between these two forms of acute pancreatitis is particularly enhanced by frequent determinations of the blood enzyme level. An increase in LAT activity may be a signal revealing when serous pancreatitis changes into pancreatic necrosis and, consequently, may help to determine the therapeutic tactics.

The simplicity and high informativeness of the method enables it to be recommended for introduction into clinical practice as a reliable enzymic test for differentiation between edematous pancreatitis and pancreatic necrosis.

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