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EFFECT OF 5-FLUOROURACIL ON ACTIVITY OF SOME PANCREATIC AND SERUM ENZYMES IN RATS WITH ACUTE PANCREATITIS

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A considerable increase in the incidence of acute pancreatitis (AP) has been recorded everywhere in recent years, and the mortality in this disease still remains high [4, 5]. Methods of conservative and operative treatment of AP have not been finally settled. In the pathogenesis of AP a basic role is played by activation of pancreatic enzymes, followed by injury to the organ by activated proteinases [1, 2].

The main structural feature distinguishing the exocrine cell of the pancreas is the well-marked development of its endoplasmic reticulum and the high density of its ribosome populations [10], essential for enzyme production and secretion. This is responsible for the high rate of protein synthesis in the acinar cells of the pancreas, directly proportional to the rate of RNA synthesis [13].

It was accordingly decided to study the effect of 5-fluorouracil (5-FU) on the activity of some pancreatic enzymes in rats with AP. The molecular mechanism of the action of 5-FU is based on the fact that, first, it inhibits DNA synthesis by depressing activity of thymidylate synthetase, an enzyme which methylates deoxyuridylic acid into deoxythymidylic acid, and second, that it blocks protein synthesis by incorporating a metabolite of uracil instead of normal uracil into the newly synthesized RNA.

It has been shown [9] that treatment of animals with acute experimental pancreatitis by 5-FU inhibits enzyme secretion by the pancreatic acinar cells and that in small doses 5-FU prevents digestion of pancreatic tissue by activated pancreatic proteinases. The authors cited [9] tested the effect of 5-FU on the course of AP only as reflected in the change in the serum α-amylase level and they did not measure the activity of other secretory, as well as nonsecretory, intracellular pancreatic enzymes.

To obtain a deeper insight into the mechanism of action of 5-FU on the course of AP, its effect on the activity of several serum and pancreatic enzymes was studied, including an enzyme specific for the pancreas, namely transaminase (TA).

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TABLE 1. Effect of 5-FU on Activity of Pancreatic and Serum Enzymes of Rats with AP (M ± m)

Experimental conditions	TA in tissue	Serum TA	Serum α-amylase	Serum trypsin	Trypsin inhibitor
Control	1,90±0,02	0	2165±132	2,49±0,22	409,8±31,9
Pancreatitis	1,07±0,06	0,066±0,001	6819±468	9,18±0,61	370,9±36,5
Pancreatitis + 5-FU	0,440±0,006	0,014±0,008	3973±370	4,16±0,38	520,2±59,7

Legend. Asterisk indicates cases in which AP was induced by the first method (see Experimental Method). Serum TA activity was expressed in micromoles arginine per hour of incubation at 37°C per milliliter of serum.

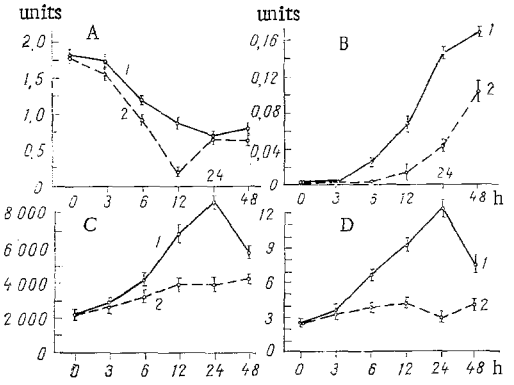

Fig. 1

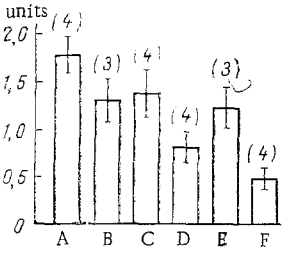

Fig. 2

Fig. 1. Effect of 5-FU on activity of pancreatic and serum enzymes of rats with AP. A) TA in tissue; B) serum TA; C) serum α-amylase; D) serum trypsin. 1) Without 5-FU; 2) with 5-FU. Each point on graph corresponds to determination of enzyme activity in six to 10 animals. Abscissa: time (in h), ordinate: activity of enzymes (in units).

Fig. 2. Effect of actinomycin D, an inhibitor of RNA synthesis, and of cycloheximide, an inhibitor of protein synthesis, on pancreatic TA activity. A) Normal; B) normal + 5-FU (4.5 mg/100 g); C) normal + actinomycin D (100 μg/100 g); D) normal + actinomycin D + 5-FU; E) normal + cycloheximide (75 μg/100 g); F) normal + cycloheximide + 5-FU. Number of determinations in parentheses.

EXPERIMENTAL METHOD

Experiments were carried out on 120 rats of both sexes weighing from 180 to 250 g. AP was produced in two ways: 1) Under pentobarbital anesthesia the common bile duct was compressed immediately above the point where it empties into the duodenum (pilocarpine in a dose of 0.01 g/kg body weight was injected intramuscularly at the same time); 2) 1 ml of a 0.2% solution of human bile was injected into the common bile duct, which was then ligated in its distal part [6]. 5-FU was injected intraperitoneally immediately after ligation of the common bile duct in a dose of 4.5 mg/100 g body weight. The rats were decapitated 3, 6, 12, 24, and 48 h after the operation and their serum α-amylase [7], trypsin and trypsin inhibitor [8], and TA [3] activity was determined.

α-Amylase activity was expressed in milligrams of glucose formed per hour of incubation at 37°C per milliliter blood serum. Serum trypsin activity was expressed in micromoles p-nitroaniline formed as a result of hydrolysis of the substrate (BAPNA) by trypsin, per milliliter blood serum during incubation for 1 min at 37°C. Trypsin inhibitor activity was expressed in milliunits of trypsin inhibited per milliliter blood serum during incubation for 1 min at 37°C.

TA activity in the pancreatic tissue was determined as in [12]. The reaction product, arginine, was measured by the Sakaguchi reaction in the modification in [9]. TA activity was expressed in micromoles arginine formed from canavanine and ornithine during incubation for 1 h at 37°C per gram wet weight of pancreatic tissue.

EXPERIMENTAL RESULTS

The level of TA activity in the pancreas was reduced 6 h after induction of AP and reached a minimum after 12 h (Fig. 1A). The degree of the decrease in TA activity in the pancreas of rats treated with 5-FU was much greater. After 12 h the TA activity in the pancreas of rats with AP, treated with 5-FU, was only 21% of the level of its activity in the pancreas of animals not treated with 5-FU. Corresponding to this fall in TA activity in the pancreas of the rats, TA appeared in the blood serum of animals treated with 5-FU only after 12 h, and later, after 24-48 h, the curve of TA activity followed a much lower course than that of serum TA activity of rats with AP not treated with 5-FU (Fig. 1B). The presence of microdestruction in the pancreas was confirmed histologically.

Under the influence of 5-FU a sharp drop was observed in the level of serum α-amylase activity and, what is particularly important, in serum trypsin activity (Fig. 1, C and D). 5-FU caused a decrease in the activity of these enzymes in the blood serum as early as 3 h after the beginning of AP, to reach a minimum after 24 h. The results of investigation of the effect of 5-FU on the activity of these enzymes in the pancreas of rats with AP 12 h after the beginning of the disease are given in Table 1. For instance, serum α-amylase and trypsin rose in AP to 6819 ± 468 and 9.18 ± 0.61 units respectively compared with 2165.4 ± 132 and 2.49 ± 0.22 units in the control. After treatment of the rats with AP with 5-FU, the serum α-amylase activity was 3937 ± 370 units, and the serum trypsin activity 4.16 ± 0.38 units.

To prove that 5-FU inhibits the synthesis of pancreatic enzymes, a comparative study was made of the effect of 5-FU and a known inhibitor of DNA-dependent RNA synthesis, namely actinomycin D, and also of an inhibitor of protein synthesis, cycloheximide, on pancreatic TA activity. The results of this investigation are given in Fig. 2.

It can thus be concluded that 5-FU, injected into rats with AP, inhibits DNA and RNA synthesis and thus probably blocks the synthesis and, consequently, the liberation into the blood stream of several specific pancreatic secretory and nonsecretory intracellular enzymes.

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