CUMULATION OF 5-FLUOROURACIL INDUCED BY AN ELECTRIC FIELD IN THE TREATMENT OF ACUTE EXPERIMENTAL PANCREATITIS

V. I. Mironov and L. V. Zabrodina

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Favorable results have been reported in the literature from the use of psychostatics and, in particular, of 5-fluorouracil (5-FU) in the treatment of acute pancreatitis [2, 3, 13]. 5-FU, accumulating selectively in the pancreas, inhibits protein synthesis and is a most active inhibitor of synthesis of pancreatic enzymes [4]. However, a marked therapeutic effect is achieved only by the use of 5-FU in large, near-toxic doses [10]. There is thus an urgent need to develop ways of increasing the concentration of drugs in tissues of the pancreas in order to enhance their therapeutic action. Some workers have suggested increasing the concentration of drugs in the tissues selectively by their electrically induced elimination from the blood stream by means of a dc electric field. In the Soviet literature the method has been called electrocumulation and it is used in oncologic and stomatologic practice [5, 6, 8].

The aim of this investigation was to study the possibility of using targeted administration (electrocumulation) of 5-FU in the treatment of acute experimental pancreatitis.

EXPERIMENTAL METHOD

Six series of experiments were carried out on 283 albino rats of both sexes weighing 180-250 g. Acute pancreatitis was induced by the method in [9]: Under ether anesthesia laparotomy was performed and for 2 h the common bile duct was compressed by means of a tourniquet applied actually at the point where the duct enters the duodenum, with simultaneous intramuscular injection of pilocarpine (0.01 g/kg body weight). The animals of series I (67 rats) were untreated and served as the control. In series II 60 animals were given an injection of 5-FU, in series III (56 rats) injection of 5-FU was combined with exposure to a dc electric field, in series IV 63 rats were exposed to a dc electric field but did not receive 5-FU, series V consisted of 15 rats undergoing a mock operation, and series VI comprised 22 intact rats. 5-FU was injected intraperitoneally in a dose of 4.5 mg/100 g body weight at the moment when the tourniquet was removed from the common bile duct. The dc field was induced by means of a "Potok-1" galvanizing apparatus. The current density was 0.1-0.2 mA/cm^2 and the area of the electrodes was 4 cm^2. The electrodes were applied to the dorsal and ventral surfaces of the animal's body, corresponding to projection of the pancreas. The skin was shaved beforehand where the electrodes were applied. The dc field was applied 10-15 min after injection of 5-FU for 30 min. The rats were decapitated 3, 6, 12, 24, and 48 h after injection of 5-FU (series II) and exposure to the dc electric field (series III). Activity of L-amylase, lipase, trypsin, and total trypsin inhibitor in the blood serum and protease activity of pancreatic tissue homogenate were studied. L-amylase activity was expressed in milligrams of starch hydrolysed by 1 ml serum during incubation for 1 h at 37°C [14], serum trypsin activity in nanomoles p-nitroaniline formed by hydrolysis of substrate (BAPNA) by trypsin in 1 liter of blood serum during incubation for 1 sec at 37°C [7, 11], and trypsin inhibitor activity was expressed in nanomoles p-nitroaniline in 1 liter of serum during incubation for 1 sec at 37°C [7, 12]. The result of determination of lipase activity was expressed in lipase units [15]. Pancreatic protease activity was studied by a modified Sakaguchi's test [1] in tissue homogenate in a dilution of 1:100, made up in cold phosphate buffer, pH 7.4, and was expressed in micromoles of pepsin digest of oxidized lysozyme during incubation for 1 min at 37°C in 1 ml of tissue homogenate. The results were subjected to statistical analysis by Student's test.

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TABLE 1. Changes in Total Serum Trypsin Inhibitor Activity (in moles p-nitroaniline/liter serum during incubation for 1 sec at 37°C) (M ± m)

Series of experiments	Times of investigation, h				
	3	6	12	24	48
I (pancreatitis)	3602,2±252,2	4787,9±273,1	3408,8±152,0	3219,8±108,6	3356,7±152,0
II (pancreatitis+5-FU)	4763,5±202,6	6407,8±151,4	4228,8±247,2	4158,3±152,0[a]	4328,6±228,8
III (pancreatitis+5-FU+ electric field)	6515,5±81,9[a,b]	8196,4±101,9[a,b]	4908,2±152,0[a,b]	5584,5±168,7[a,b]	5587,8±126,9[a,b]

Legend. Total trypsin inhibitor activity of serum of intact animals was 4255.2 ± 263.9. a) $P < 0.001$ compared with data before treatment, b) $P < 0.001$ compared with corresponding data for series II, c) $P < 0.05$ compared with data before treatment, d) $P < 0.05$ compared with corresponding data for series II.

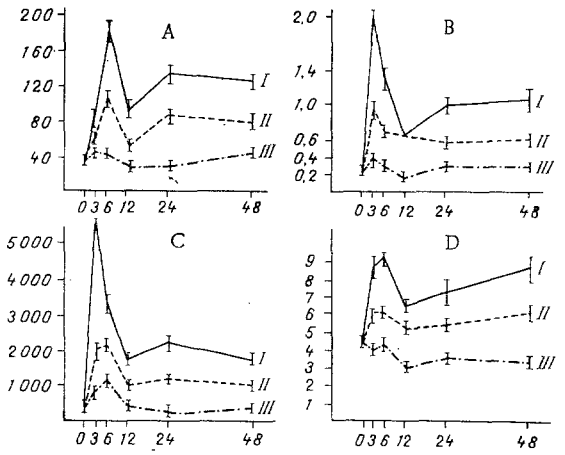

Fig. 1. Effect of 5-FU on serum and pancreatic enzyme activity in rats with acute experimental pancreatitis. Abscissa, time (in h); ordinate, enzyme activity. A) Serum trypsin, B) serum lipase, C) serum L-amylase, D) total pancreatic tissue protease activity. I) Without, II) with administration of 5-FU, III) injection of 5-FU + cumulative effect of dc electric field. Each point on graph corresponds to determination of enzyme activity in 6-12 animals.

EXPERIMENTAL RESULTS

No significant changes compared with the control were observed in any of the parameters studied in animals exposed to the dc electric field only (series IV) and animals undergoing the mock operation (series V) compared with intact rats (series VI).

In rats with acute experimental pancreatitis (series I) synchronous changes were observed in activity of the serum enzymes. The greatest increase in enzyme activity took place in the first hours (3-6) of development of acute pancreatitis. L-amylase, lipase, and serum trypsin levels then fell to reach minimal values after 12 h, followed by an increase after 24 h (Fig. 1, I).

Under the influence of 5-FU (series II) significant depression of enzyme activity compared with the control was observed. The maximal fall of serum enzyme activity was observed 3-6 h after the time of development of acute pancreatitis. In animals receiving 5-FU and subjected to the cumulative effect of the dc electric field (series III) inhibition of serum enzyme activity was more marked. For instance, in series II lipase activity after 3-6 h was 44.0%, L-amylase activity 37.2%, and trypsin activity 56.9% of the corresponding value in the control animals, whereas in series III the figures were 18.5, 16.3, and 24% respectively. As the data show, activity of these enzymes in series III was 2.4, 2.3, and 2.4 times lower respectively than that in the rats of series II (Fig. 1, II, III).

Changes in pancreatic tissue protease activity in acute experimental pancreatitis showed a similar trend to those of activity of the blood serum enzyme spectrum in animals of series I. Highest proteolytic activity of the pancreas was observed 6 h after the development of acute pancreatitis. Pancreatic protease activity of the animals was inhibited after injection of 5-FU at all times of acute pancreatitis, with a maximal fall of 32.4% after 6 h (Fig. 1D, I and II).

Cumulation of 5-FU by a dc electric field (series III) significantly reduced the protease activity of the pancreas compared with that of animals receiving 5-FU without application of the dc electric field (series II). Pancreatic tissue protease activity in rats of series III after 6 h was 51% lower than in the control, or 19% lower than in rats of series II (Fig. 1D, II, III).

Table 1 gives the results of a study of the time course of total serum trypsin inhibitor activity in animals of series I-III. Application of 5-FU evidently activated trypsin inhibitor in the early stage (2-6 h) of development of acute pancreatitis. A more marked increase in trypsin inhibitor activity with preservation of the effect until the end of the investigation was found after 5-FU administration in conjunction with the dc electric field.

The use of a dc electric field for selective accumulation of 5-FU in pancreatic tissue in acute experimental pancreatitis thus considerably reduces serum enzyme activity and proteolytic activity of the pancreas and activates serum trypsin inhibitor by a greater degree than intraperitoneal injection of the compound.

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