4. S. D. Brown and T. C. Montie, Infect. Immun., 18, No. 1, 85 (1977).
5. J. P. Filkins, Circulat. Shock, 5, No. 4, 347 (1978).
6. J. M. Friedland and L. S. Dietrich, Anal. Biochem., 2, 390 (1961).
7. S. Kopprasch, U. Schaper, H. Orlik, and D. W. Scheuch, Z. Med. Lab.-Diagn., 25, No. 7, 360 (1984).
8. C. H. Lang, G. J. Bagby, and J. J. Spitrer, Am. J. Physiol., 248, No. 4, R471 (1985).
9. T. C. Montie, Pharmarcol. Ther., 12, No. 3, 491 (1981).
10. J. M. Naylor and D. S. Kronfeld, Am. J. Physiol., 248, No. 3, E309 (1985).
11. R. V. Walker, J. Infect. Dis., 118, No. 2, 188 (1968).
12. D. E. Wennerstrom, S. D. Brown, and T. C. Montie, Proc. Soc. Exp. Biol. (New York), 154, No. 1, 78 (1977).
13. L. Witek-Janusek and J. P. Filkins, Circulat. Shock, 8, No. 5, 573 (1981).
14. R. R. Wolff and J. F. Burke, J. Physiol. (London), 235, No. 4, R219 (1978).

ESTIMATION OF EFFECTIVENESS OF SPECIFIC PLASMA PERFUSION WHEN USED IN EXPERIMENTAL ACUTE PANCREATITIS

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Acute pancreatitis is a serious disease of the pancreas which is difficult to diagnose and treat. The overall mortality from acute pancreatitis is 8.3%, and in destructive forms it may reach 30-60% [9]. An important role in the pathogenesis of acute pancreatitis is played by proteolytic enzymes, which enter the blood stream as a result of activation of zymogens of proteinases, synthesized in the pancreas (trypsin, chymotrypsin, elastase), as a result of accumulation of granulocytes in the region of the affected organ (elastase of granulocytes etc.), and of tissue destruction (cellular cathepsins).

In the blood stream these proteinases, by activating the most important proteolytic systems, lead to changes in hemostasis and to the development of a disseminated clotting syndrome and a state of collapse and shock [2, 4]. A method of removing proteinases from the blood stream by specific hemoperfusion in experiments on animals is known. A soy bean inhibitor immobilized on silica-gel [5], a proteinase inhibitor from bovine organs of the Kunitz type (BPTI), and duck ovomucoid, covalently cross-linked with a polyacrylamide carrier [3], has been suggested as specific sorbents for proteinases.

The aim of this investigation was to develop a more effective sorbent of proteinases from the blood plasma of animals with acute pancreatitis, consisting of an acid-stable inhibitor (ASI) of proteolytic enzymes from human urine, immobilized on sepharose.

EXPERIMENTAL METHOD

The ASI was isolated by a modified method [6, 11] from the urine of patients with nephritis. The ASI was immobilized (virtually 100%) on sepharose by the cyanogen bromide method, with the addition of 2 mg of ASI to 1 ml of swelling sorbent to the reaction mixture. Granylocytic elastase was isolated from the buffy coat by a modified method [7].

Experiments were carried out on mongrel dogs of both sexes weighing 10-16 kg. In the animals (9) of group 1 (control), blood was taken under adequate anesthesia into heparin from

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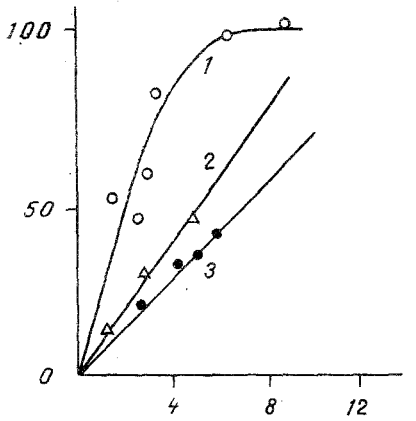

Fig. 1. Binding of human granulocytic elastase with ASI from human urine. Abscissa, ASI ($\times 10^6$ M); ordinate, inhibition (%). 1) ASI-sepharose; 2, 3) ASI with mol. wt. of 22,000 and 44,000 respectively. Substrate — MeOs and AlaAlaProValNA.

a superficial subcutaneous vein of the animal's forelimb. Acute pancreatitis was induced in the animals of groups 2 and 3 (each with 13 dogs) by injection of medicinal bile (1 ml/kg) into the ligated accessory pancreatic duct under a pressure of 200 mm Hg. All the animals with acute pancreatitis received maintenance therapy. In group 3 (5 animals) treatment by plasma perfusion was given on an apparatus from "Gambro" (Sweden), connected to an arteriovenous shunt, 3-23 h after the creation of experimental pancreatitis. A column with ASI-sepharose with a volume of 4-5 ml (capacity of sorbent: 8 mg trypsin and 7 mg chymotrypsin) was connected in series with a plasma filter. The procedure took 15-60 min, with detoxication of 1-3 volumes of the dog's circulating blood. The total blood flow rate was 50 ± 3 ml/min and the plasma flow rate was 20 ± 2 ml/min (20°C). The treated dogs were sacrificed 3.5 days after the creation of acute pancreatitis. The untreated dogs died naturally after 10-24 h (10.6 ± 4.9 h). Changes in the pancreas were evaluated histologically. The following parameters were measured in heparinized plasma from dogs of the three groups: α-amylase activity [1], trypsin (as BzArgNA) by a modified method [10], elastase (as BocALaONp) [6], chymotrypsin (as BzTyrOEt) [14], and antitryptic activity (as inhibition of hydrolysis of BzArgOEt by trypsin) [6]. All values of activity obtained were expressed per unit of optical density of blood plasma at 280 nm. Granulocytic elastase activity, as the rate of hydrolysis of MeOSuc-AlaAlaProValNA, was measured spectrophotometrically at 410 nm (pH 7.5) and at 37°C, as was described previously [13]. The antielastase activity of ASI-sepharose was determined as inhibition of hydrolysis of MeOSucAlaAlaProValNA by granulocytic elastase.

EXPERIMENTAL RESULTS

Two molecular forms of ASI were obtained from human urine (mol. wt. 20,000 and 44,000), which inhibited not only trypsin and chymotrypsin, but also granulocytic elastase. The bimolecular constant of pseudoirreversible inhibition by these forms of granulocytic elastase (k_{-i}), measured as indicated in [7], was about 10^4 $M^{-1} \cdot min^{-1}$. It follows from Fig. 1 that ASI-sepharose binds granulocytic elastase more effectively than unimmobilized ASI.

Urinary ASI was chosen as the basis of the specific proteinase sorbent in acute pancreatitis because of its unique enzyme specificity, namely — its property of inactivating pancreatic trypsin, chymotrypsin, and elastase [6], and also the elastase granulocytic plasminogen activator [7]. Incidentally, Japanese investigators have demonstrated a more marked therapeutic effect of intravenous injection of human urinary ASI by comparison with BPTI in experimental acute pancreatitis [12]. ASI is a human protein, and that reduces the possibility of anaphylactic shock (which is often observed when BPTI is used [8]), should leakage of the ligand take place (which is probable) from the sorbent during plasma perfusion.

Treatment of the dogs of group 3 began in the early period after creation of experimental pancreatitis, namely after 3 and 23 h (Tables 1 and 2). It will be clear from Table 1 that immediately after the end of plasma perfusion (commenced 3 h after creation of pancreatitis) the levels of α-amylase, trypsin, and elastase fell by 93, 95, and 12% respectively. After

TABLE 1. Effectiveness of Plasma Perfusion on ASI-Sepharose (17 IU), Started 3 h after Creation of Acute Pancreatitis in Dog

Blood plasma	Enzyme			
	α-amylase, King units	trypsin, BzArgNA	elastase, BocAlaONp	antitryptic activity, mIU/min
		nmoles/min (25°C)		
Before plasma perfusion	0,12	0,62	2,3	26
After plasma perfusion				
0 h	0,009	0,03	2,0	39
$2\frac{1}{2}$ h	0,14	0,03	1,3	48

TABLE 2. Effectiveness of Plasma Perfusion on ASI-Sepharose (17 IU) Started 23 h after Creation of Pancreatitis in Dog

Blood plasma	Enzyme				
	α-amylase, King units	trypsin, BzArgNA	elastase, BocAlaONp	chymotrypsin BzTyrOEt	antitryptic activity, mIU/min
		nmoles/min (25°C)			
Before plasma perfusion	0,82	8,0	0,8	20,8	74,0
After plasma perfusion (1h)	0,75	5,1	0,8	15,3	85,8

TABLE 3. Effectiveness of Plasma Perfusion on ASI-Sepharose in Experimental Acute Pancreatitis (M ± m)

Group of animals	Enzyme			
	α-amylase, King units	trypsin, BzArgNA	chymotrypsin, BzTyrOEt	elastase, BocAlaONp
		nmoles/min (25°C)		
1: healthy dogs (n = 9)	0,09±0,02	1,3±0,2	17,0±2,7	0,95±0,1
2: experimental pancreatitis (n = 8)	0,62±0,11	6,6±1,1	66,0±28,0	1,4±0,5
p	<0,05	<0,05	<0,05	>0,05
3: treatment by plasma perf. (n = 5)	0,33±0,06	3,7±0,9	19,0±3,3	0,9±0,2
p	>0,05	<0,05	>0,05	>0,05
Reduction of activity, %	67	44	70	35

Legend. Significance of change in value in group 2 relative to group 1 and in group 3 relative to group 2 is given. All parameters for dogs of group 3 correspond to 24 h after creation of experimental acute pancreatitis.

2.5 h some increase in α-amylase activity, a fixed low trypsin level, and a further decline in elastase activity (to 43%) compared with the initial levels, was observed in the blood plasma of the experimental animal. The antitryptic activity of the blood plasma after plasma perfusion was increased, evidence of mobilization of the defensive measures of the body [10] (Table 1).

During plasma perfusion at the stage of more advanced development of acute pancreatitis (Table 2), a less marked decline of α-amylase (10%) and trypsin (37%) activity was observed

1 h after the procedure, together with the initial level of elastase activity and a very small increase in the antitryptic activity of the blood plasma.

It follows from Table 3 that, on average, enzyme activity in the blood plasma of dogs with acute pancreatitis was significantly ($p < 0.05$) higher than the initial values (group 1). The plasma perfusion procedure leads to a substantial (in some cases significant) decline of enzyme activity by 35-70%, with the result that the survival of the experimental animals may be lengthened eightfold (see: Experimental Method), an effect which is greater than that obtained from the use of other sorbents [3]. The data of histological analysis of sections through the pancreas of the experimental dogs showed that the pancreatic tissue of the animals of group 2 was completely liquefied, whereas in the animals of group 3 (treated by plasma perfusion) there were isolated foci of necrosis in the tissue.

The results thus indicate that the specific proteinase sorbent — ASI-sepharose — used in the investigation is highly effective in the treatment of experimental acute pancreatitis.

LITERATURE CITED

1. Biochemical Methods of Investigation in Clinical Medicine. Handbook [in Russian], Moscow (1969), pp. 131-132.
2. P. A. Banks, Pancreatitis [Russian translation], Moscow (1982).
3. T. A. Valueva, I. A. Maklakova, V. A. Sinani, et al., Abstracts of Proceedings of the 5th All-Union Symposium on Engineering Enzymology [in Russian], Olaine (1985), p. 348.
4. K. N. Veremeenko, Enzymes of Proteolysis and Their Inhibitors in Medical Practice [in Russian], Kiev (1971).
5. V. G. Vladimirov, A. G. Zhuravlev, N. N. Larionova, et al., Author's Certificate 1123377 USSR.
6. O. G. Ogloblina, N. A. Galstyan, V. V. Karpitskii, et al., Vopr. Med. Khimii, 28, No. 4, 86 (1982).
7. O. G. Ogloblina, L. V. Platonova, and T. S. Paskhina, Dokl. Akad. Nauk SSSR, 265, No. 4, 982 (1982).
8. V. V. Fedotov, V. I. Kosyakov, and E. E. Agaponov, Khirurgiya, No. 1, 59 (1982).
9. V. I. Filin, G. P. Gidirim, R. V. Vashetko, et al., Khirurgiya, No. 6, 92 (1982).
10. H. Fritz and G. Wunderer, Arzneim.-Forsch., 33, No. 4, 479 (1983).
11. B. M. Jonsson, G. Loffler, and K. Ohlsson, Hoppe-Seyler's Z. Physiol. Chem., 363, No. 10, 1167 (1982).
12. H. Ohnishi, H. Kosuzume, J. Ashida, et al., Dig. Dis. Sci., 29, No. 1, 24 (1984).
13. K. Nakajima, J. C. Powers, B. M. Asche, and M. Zimmerman, J. Biol. Chem., 254, No. 10, 4027 (1979).
14. P. M. Starkey and A. J. Barrett, Biochem. J., 155, No. 1, 273 (1976).