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Proteolytic enzymes in the treatment of acute pancreatitis

Enzymy proteolityczne w leczeniu ostrego zapalenia trzustki

Acute pancreatitis (a.p.) is a severe disease causing multiorgan insufficiency including the impairment of cardiac, liver, brain, and kidney function, as well as the generation of microembolics. The essence of disturbances in a.p. is the breaking of a natural tissue inhibitor and plasmatic barriers, leading to the activation of trypsinogen. Lysosomal cathepsins and enterokinase play a decisive role in these processes. For many years, it was believed that activation of enzymes started inside the glandule in the case of disturbances with drainage via the pancreatic duct. Recent studies (Watanabe et al.) indicate intracellular mechanisms of activation (20). The activation of proelastase and of prophospho--lipase A2 plays an influential role in the pathogenic process of a.p. Phospholipase A2 causes liberation of histamine from mascots and it activates the hydrolysis of lecithin which leads to the lysis of pulmonary alveoli and to the destruction of surfactant (8, 12, 21). The notable activation of lipase causes increase in the free fatty acid level leading to lung damage. The free radical scavengers are also involved in the pathogenesis of respiration insufficiency. The activation of leucogins and macrophages causes the formation of free radical scavengers.

The main pathologic process of the a.p. is necrosis of the pancreatic gland and the neighbouring organs. It leads to production of peritoneal exudate and resorbtion of the pancreatic enzymes to the portal and general circulatory system. These enzymes start the activation of the enzymatic cascade, which begins the multiorgans disturbances.

Acute pancreatitis is a serious therapeutic and clinical problem. The effects of treatment of this disease are not satisfying, so the new therapeutic methods are still examined.

One of the methods of the treatment of acute pancreatitis is the "peritoneal lavage" (6, 7, 8, 10). Patients are treated intraperitoneally with physiological salt solution. The peritoneal cavity is filled with solution in the aim to remove necrotic tissues of the pancreatic gland. Some authors tried to use inhibitors of protease (3, 4).

In our experiment a combination of two lytic enzymes including fibrinolysin and bovine deoxyribonuclease was examined. (Fibrolan was used. It is a preparation of Parke–Davis Drug Company). Fibrinolysin is produced during the process of extraction and activation of plasminogen in bovine serum. Fibrinolysin is euglobulin which has got autogenic properties. It decomposes into fibrin and albumin of blood serum and inactivates coagulation factors like fibrinogen and factor V (proaccelerin) and factor VII (proconvertin). Fibrinolysin also causes dilation of blood vessels as well as corrects perfusion of tissues. According to Connell et al. fibrinolysin demonstrates bacteriostatic features (3).

Deoxyribonuclease is isolated from bovine pancreas. This enzyme catalyses hydrolytic cleavage of internal bonds of DNA and of DNA–albumin complex. It causes reduction of viscosity: for example of purulent secretion or prevention adhesions. Proteolytic enzymes cause removal of necrotic material and purulent exvoluate as well as blood coagulums from wounds, burns and from inflamed places. These enzymes influence only denature protein, which is present in necrotic tissues. Alive cells and healthy tissues are intact. Proteolytic enzymes are widely used in different medical disciplines i.a. in surgery, dermatology, gynaecology, otolaryngology, and urology. Satisfactory therapeutic results of the application of proteolytic enzymes were observed in different pathological cases such as complicated wounds caused by the effects of ischemia or trauma or burn or bedsore (decubitus ulcer). In such situations these substances cause very fast and atoxic and automatic removal of necrotic debris.

MATERIAL AND METHODS

The study was carried out on Wistar strain rats. The average weight of rats was 250–350 g. The animals lived in cages. The diet was standard. The rats were subjected to starvation. Rats were divided randomly into four experimental groups:

GROUP 0: The group of healthy rats, which were subjected to estimate initial values of biochemical parameters which occurred in the experiment. They were healthy and did not undergo surgery. There were 30 animals.

GROUP T: The group of animals with a.p. Acute pancreatitis was induced according to Heinkel and Aho method (1, 2). A.p. developed by injection of solution of sodium taurocholate (Sigma Chemical Co–St. Louis MO, USA) into the common hepatopancreatic duct. During the course of experiment, animals were administered intraperitoneally a solution of proteolytic enzymes. The animals were operated and sick and treated by enzymes. The group contained 100 rats.

GROUP S: The control group of rats with experimental a.p. We administered intraperitoneally a solution of 0.9% sodium chlorate. The animals were operated and sick and treated with sodium chlorate. The group included 100 rats.

GROUP K: The group of animals used to control mortality (death rate). We developed a.p. and did not interfere with the course of our experiment (150 animals).

In this trial 350 rats were subjects to surgery (groups T, S and K). On the contrary, 30 animals were not operated (group 0).

BIOCHEMICAL ANALYSES

Blood and urine analyses were carried out in the following manner. After anaesthesia with diethyl ether and thoracotomy, the blood from the left ventricle of the heart was taken in 24, 48, 72, 96, 120, 144 hr after the operation. After coagulation for 30 min., the blood was centrifuged at 2,500 rotations per minute for 10 min.

The urine was taken once per day. The activity of amylase was determined in the blood and urine using the reagents of the Cormay Company and by using the biochemical analyser produced by the Cobas Mira Plus Company. In this method the 2–chloro–4–nitrofenylo–α–maltotriozyd (CNP–G3) is the direct substrate for α–amylase. Using this substrate enables measuring the activity of enzyme without application of adjuvant enzymes. The product of this reaction is 2–chloro–4–nitrofenol (CNP) which causes the increase of absorbance at 405 nm. The intensity of colour of CNP is proportional to the activity of amylase.

STATISTICAL ANALYSES

The obtained results concerning levels of amylase activity in blood plasma were statistically analysed. The statistic analysis of amylase level in urine was performed (14, 19). In each experimental group of operated animals standard deviation (SD) and arithmetical average (M) as well as mean error of arithmetical average (SE) and variation coefficient (V%) were estimated for biochemical data.

RESULTS

Acute pancreatitis was developed in 350 rats, 79 animals dead. In some cases we did not have any possibility to measure enzyme activity, because the haemolysis process and destruction of samples occurred. 47 of the 150 untreated animals (group K) dead (31.3%). 19 of the 100 rats of group S dead (19%) and 13 of the 100 rats of T group dead (13%). However, the mortality rate was significantly higher in group K than in group S and significantly higher than in T group.

PLASMA ACTIVITY OF AMYLASE

The amylase activity in 24 hr of experiment was 769 ± 289.1 U/dl (M ± SD) in group K and 784 ± 171.3U/dl in S group and 681.0 ± 137.8 U/dl in T group. Between 24 and 144 hour of experiments we did not find any significant differences between these experimental groups. After 48 hr of our study the activity was 880.7 ± 231.1 U/dl in group S and 571.6 ± 93.2 U/dl in T group. After 72 hr amylase activity was 950.9 ± 261.8 U/dl in group K and 814.2 ± 176.9 U/dl in group S and 528.4 ± ± 92.0 U/dl in T group. After 48 hr and 72 hr after surgery this activity was more markedly decreased in T group than in group S and in group K. After 96 hr the activity was 721.7 ± 285.3 U/dl in K group, 516.4 ± 61.5 U/dl in S group and 385.4 ± 79.7 in T group. 120 hr after the start of experiment the measured activity was 486.2 ± 125.3 U/dl in group K and 385.3 ± 136.5 U/dl in group S and 85.8 ± ± 35.6 U/dl in T group, respectively. Thus, the plasma activity of amylase in T group was significantly lower than the one in group S; furthermore, this activity was markedly lower in group S than in group

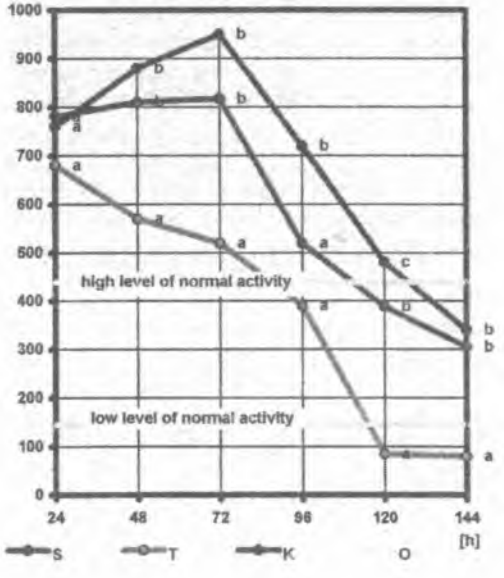
K. After 144 hr enzyme activity was 341.2 ± 58.1 U/dl in group K and 303.7 ± 78.5 U/dl in group S and 81.3 ± 37.4 U/dl in T group.

On the basis of results of amylase plasma activity in 0 group we determined the standard activity. It was 145.3 – 438.8 U/dl.

In each experimental group of the operated animals, the levels of amylase were increased between 24 and 72 hr of the study. 96 hr after surgery the enzyme activity was increased in group K and group S and in T group the enzyme level was normal. At 144 hr the increased activity was not noticed in any group. Worth our attention is the fact that animals in T group (86% of rats) at 120 and 144 hr had a decreased level of amylase activity compared with standard activity.

The levels of activity of amylase in blood serum were showed in Figure 1.

Fig. 1. Activity of serum



AMYLASE ACTIVITY IN URINE

24 hr after the start of the experiment, amylase activity was 1433 ± 784 U/l in group K and 1242 ± 385 U/l in group S and 1138 ± 223 U/l in T group. The enzyme activity in urine was similar in experimental groups. After 48 hr this activity was 1924 ± 869 U/l in group K and 1574 ± 728 U/l in group S and 753 ± 344 U/l in T group. The amylase activity was markedly lower in T group than in group K and in group S. After 72 hr enzyme activity was 2116 ± 891 U/l in group K and 1227 ± 518 U/l in group S and 160 ± 133 U/1 in T group. After 96 hr the amylase activity was 2374 ± 644 U/l in group K and 938 ± 383 U/l in group S and 38 ± 20 U/l in T group. After 120 hr the measured activity was 365 ± 171 U/l in group K and 133 ± 42 U/l in group S and 38 ± 16 U/l in T group. After 144 hr this activity was 116 ± 37 U/l in group K and 72 ± 26 U/l in group S and 31 ± 18 U/l in T group.

After 72, 96 and 144 hours of experiment significant differences were observed between experimental groups. The highest activity was measured in group K and the markedly lower one in T group.

On the basis of results of amylase urine activity in 0 group we determined the standard activity. It was 19.8 to 471.5 U/l. The levels of amylase activity in urine were showed in Figure 2. The levels of amylase were twice increased in group K between 24 and 96 hour of testing. Twice increased level of amylase activity in group S was between 24 and 72 hour of our study. Furthermore, this level was still increased at 96 hour. In T group the increased levels of amylase in urine were at 24 hour but at 72 hour of testing increased levels were not noticed. In groups K and S the enzyme activity was standard starting from 120 hour.

In T group of rats compared to group K the plasma activity as well as urine activity of amylase were decreased from 48 to 144 hour of testing. In T group compared to group S plasma activity of amylase and the amylase activity in urine were significantly decreased from 72 to 144 hour of experiment and also the amylase activity in urine and in plasma was decreased from 48 to 144 hour of research. The plasma activity of amylase at 96 and in 120 hour was decreased as well as this activity in urine from 72 to 144 hour of testing.

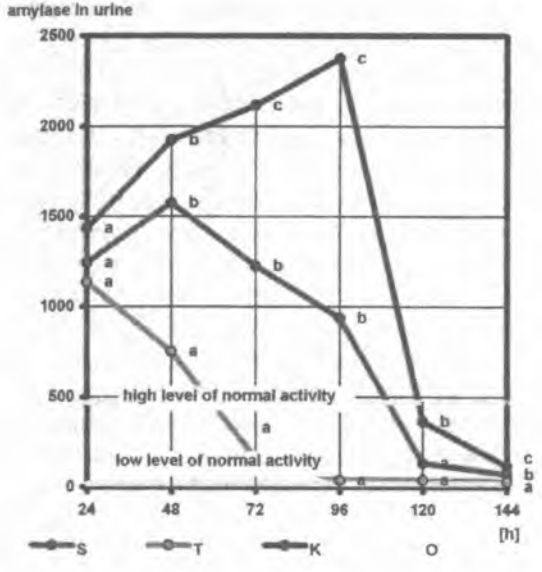


Fig. 2. Amylase activity in urine

DISCUSSION

Ulbrich et al. administered proteolytic enzymes intraperitoneally for treatment of patients with necrotic a.p. and cases with inflammation of the peritoneal cavity and with abdominal abscesses which can be located in Douglas cavity (17). Peritoneal lavage and washing out of cavities of abscesses, appear to inhibit formation of postoperative infiltration and of adhesives (5). It is interesting to note that Fibrolan is administered in gynaecology for the treatment of cervical erosion, postpartum injury

of reproductive organ, non–specific and severe vaginitis as well as cervicitis. It may have a useful role in therapy of inflamed condition after mastectomy or in treatment of abscesses of the breast. Urologic indications for the use of proteolytic enzymes are blood clots, and fibrinous exudate. They are the most common in pathological conditions of urinary bladder and of renal pelvises (3).

The estimation of the usefulness of new therapeutic agents, which are used in the treatment of definite disease, entails many complications. The first stages of experiments are usually researched on animals.

The studies were carried out on male Wistar strain rats whose course of a.p. according to recent publications, most resembles that of humans'. The experimental model of a.p. we chose was in conformity with Büchler (2). According to Heinkel and Aho models of administration of taurocholate sodium cause chronic a.p. (1). Worth our attention is the fact that the authenticity of experiment and its conformity with clinical investigation result from the adequate experimental method (9).

For many years we can observe the interest of enzymatic substances which cause the demarcation of necrotic tissues (5, 13). The application of enzymatic substances is routine procedure in the wounds with a lot of necrotic tissues and with purulent secretion. In this way we can shorten the term of the treatment of a.p.

In our experiment proteolytic enzymes were used intraperitoneally. There was a possibility of direct and local activity of drug on necrotic and inflamed tissues. By fast removal of the necrosis we prevented infection which can be the main reason of mortality in a.p. The administration of solution in this way causes the dilution of toxic substances in peritoneal fluid (4, 7). The neutralisation of this substance through peritoneum is easier. The active substances of Fibrolan, which are absorbed into the blood stream, have influence on systemic processes. Fibrinolysin is the main component, which transits to blood. It inactivates coagulation factors like factor I (fibrinogen) and factor V (proaccelerin) and factor VIII (antihemophilic globulin – AHG). This enzyme inhibits the haemostats and prevents consumption coagulopathy (DIC). In this way the first stage of a.p., which involves the activation of coagulation and inhibition of fibrinolysis, becomes interrupted. On the other hand, in the second stage of a.p, the haemorrhagic diathesis occurs.

Monitoring of the intensity of the pathologic processes and estimation of the therapeutic results were carried out by measurement of amylase activity in blood serum and in urine. These markers are commonly used in clinical routine although their usefulness is sometimes doubtful (11, 18). In our experiment we observed the lower level of this enzyme in the case of animals treated with Fibrolan. This fact may determine that this kind of treatment reduces pathological processes in the course of acute pancreatitis.

CONCLUSIONS

1. The intraperitoneal administration of the proteolytic enzymes during experimental a.p. have a palliative effect.

2. The activation of amylase in blood serum and of amylase in urine within 48 hours of experiment was significantly lower in the group of animals treated with proteolytic enzymes when compared to untreated rats.

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STRESZCZENIE

W doświadczeniu badano wpływ dootrzewnowo podawanego Fibrolanu na przebieg eksperymentalnego ostrego zapalenia trzustki. Fibrolan jest preparatem złożonym z dwóch enzymów proteolitycznych, których istotą działania jest demarkacja tkanek martwiczych. Wiedząc, że podstawowymi procesami patologicznymi w ostrym zapaleniu trzustki są oprócz zmian ogólnoustrojowych zmiany nekrotyczne gruczołu, podjęto próbę oceny terapeutycznego działania tego preparatu. Ciężkość przebiegu ostrego zapalenia trzustki koreluje często z poziomem amylazy w surowicy krwi i w moczu, enzymy te są rutynowo oznaczane w praktyce klinicznej podczas rozpoznawania i monitorowania tej choroby, dlatego też w naszym doświadczeniu oznaczaliśmy ich stężenia. Na podstawie przeprowadzonych badań można stwierdzić, że dootrzewnowe podawanie enzymów proteolitycznych łagodzi przebieg doświadczalnego ostrego zapalenia trzustki oraz powoduje istotne statystycznie obniżenie poziomu amylazy w surowicy krwi i w moczu.