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Acid phosphatase activity in different organs as a marker of acute pancreatitis

Acute pancreatitis is a severe disease causing multiorgan insufficiency including impairment of the liver, kidney, cardiac and brain function, as well as the generation of microembolics. The essence of disturbances in pancreatitis is to break off a tissue inhibitors and plasmatic barriers, which leads to the activation of trypsinogen. Lysosomal enzymes play a decisive part in this process. Complications of acute pancreatitis may include not only local pancreatic necrosis with pseudocyst or abscess formation, but extrapancreatic manifestations such as pulmonary, renal, hepatic, endocrine, and coagulation abnormalities. Coagulation abnormalities associated with pancreatitis and with acute respiratory disease syndrome are potentially life threatening.

OBJECTIVE

The aim of the experiment was to establish and quantify the changes in the activity of acide phosphatase in the pancreas, liver, spleen and kidneys during the course of experimental pancreatitis.

MATERIAL AND METHODS

The experiment was carried out on 65 male rats of Wistar strain, whose weight varied from 250 to 350 g. The animals were standard fed. They drank only water 24 hours before operation. The rats were randomly divided into three groups: A – intact animals group which were not operated and were used to mark initial biochemical parameters (15 rats), B – the experimental group of animals which were injected by retrograde way with sodium taurocholate into the common bile-pancreatic duct to induce acute necrotic pancreatitis (50 rats).

Bioethical Committee of the Medical University of Lublin approved the experimental protocol. The animals were anaesthetised by ketamine injection in a dose of 4 mg/kg per weight mass. Cutting

in the medial line opened the peritoneal cavity. After laparotomy an injection needle 0.5x16 mm was inserted into the common bile-pancreatic duct via the proximal part of the duodenum (Aho's method – 1). The hepatic duct was closed near the hilus with soft, small surgical forceps. The lean ligature (2-0) was put around the bile-pancreatic ostium.

After 2, 6, 12, 24, 48 hours rats were anaesthetised again, and thoracotomy was performed by taking blood for amylase determination from the left ventricle of heart. Then the animals were given an overdose of ketamine, and the organs were removed during laparotomy and frozen at the temperature of –20°C.

Alpha-amylase (EC 3.2.1.1) activity in the blood serum was determined by the enzymatic method using the substrates of the Cormay Company and Cobas Mira Plus Company biochemical analyser. The activity of amylase was demonstrated in U/dl.

The above-mentioned organ samples were defrosted in 0.9% solution of NaCl at 4 °C. 1 g of the tissue of each sample was taken for biochemical investigation. They were placed in a 0.3 M sucrose solution at 4 °C in proportions of 1 g of tissue to 5.0 ml of sucrose and homogenised. The obtained homogenate was centrifuged for 10 minutes at 2,200 g at 4 °C. The supernatant was decanted and centrifuged for 20 minutes at 35,000 g. The obtained sample containing the free fraction of enzyme was assigned as supernatant 1 (13). The precipitate was placed into 5.0 ml of 0.3 M sucrose containing 0.1% Triton X-100 and stored for 24 hours at 4 °C. Triton was used to rupture the lysosomal membrane. Then the precipitate was centrifuged for 20 minutes at 35,000 g. The supernatant, containing the fraction of bound (membranous) enzyme, was decanted and assigned as supernatant 2.

Acid phosphatase (EC 3.1.3.2) activity was assayed by spectrophotometric methods using a substrate (Sigma) which releases 4-methyloumbeliferol reacting with the enzyme (3,14). 45 mg of sodium phosphate 4-methyloumbeliferol, methyloumbeliferol dissolved into 100 ml 0.1 M acetate buffer pH 5.0 were used as the substratum. 100 µl of 2nd supernatant were incubated with 500 µl of the above mentioned substratum for 18 hours at 37 °C. The reaction was inhibited by addition of 600 µl alkaline buffer, and after 5 min. extinction was read at the wave length 360 nm on the spectrophotometer.

The level of protein was determined by the method of Lowry et al. (8). Bound fraction of acid phosphatase activity was expressed in nmol/1mg of protein/1 hour of incubation.

The statistical analysis was made using the SAS system v. 6.11 (SAS Institute Inc., SAS Campus Drive, Carry, NC 27513, USA). Results are expressed as means ± SD. Differences between groups were analysed by ANOVA. The correlation coefficients (r) between analysed characters (X, Y) were counted. If $p < 0.05$, differences between the mean values were considered statistically significant.

RESULTS AND DISCUSSION

During the experiment 10 animals died (mortality rate 20%). The severity of AP was monitored in our experiment by measuring amylase activity in blood. The initial concentration was measured to have an average value of 293 U/dl (Fig. 1). Twelve hours after induction of AP the activity had increased to nearly 800 U/dl in experimental group. After 48 h the peak level of 950 U/dl in group B was observed. The analysis of variance showed that there were highly significant differences between the mean values of amylase activity of intact and in the animals belonging to B group ($p < 0.01$).

Results of the measurement of the acide phosphatase activity in different organs are presented in Figure 1. Differences in values of the enzyme activity were statistically significant only in pancreas and in liver ($p < 0.05$). There were no significant changes in activity in different periods of experiment in the spleen and kidneys.

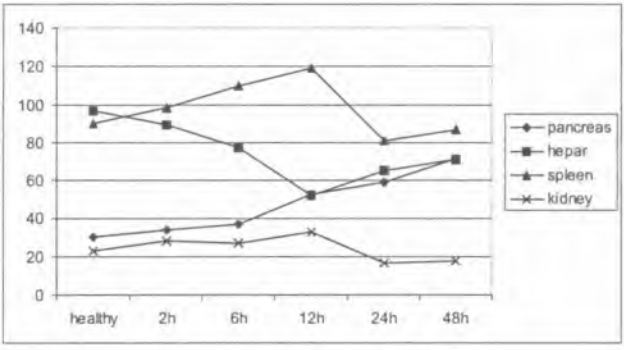

Fig. 1. The curves of changes in acid phosphatase activity in the pancreas, hepar, spleen and kidneys during acute pancreatitis. Values are expressed in nmol/mg of protein/1 hour of incubation

There are many mediators of the systemic complications associated with acute pancreatitis. The scientific research has proved that lysosomal enzymes are very important during initiation of pancreatitis and multiorgan complication occurrence (4, 5, 7, 15). We have found only few papers devoted to problem of multiorgan complications of experimental pancreatitis publishing during last few years (6, 10, 11, 12).

N o r m a n et al. have established that cytokine production is correlated with disease severity and occurs within the pancreas first and subsequently within distant organs (12). B a n k s et al. and Hughes with co-workers concluded that severe pancreatitis in the rat model is associated with significant up-regulation of TNF alpha mRNA in mononuclear cells (2). It seems that impairment in enzymes activity is dependent not only on inducing substance, but also on the increase in duct pressure. This pathomechanism, which resembles more biliary acute pancreatitis in humans, is also stressed in the works of L u t h e n et al. (9).

CONCLUSIONS

1. The activity of membranous fraction of acid phosphatase changed non specifically over the course of experimentally induced acute pancreatitis in rats.

2. Statistically significant difference was found in the enzyme's activity during different periods of pancreatitis only in the pancreas and in the liver.

REFERENCES

1. A h o H. J. et al.: Experimental pancreatitis in the rat. Sodium taurocholate-induced acute haemorrhagic pancreatitis. Scand. J. Gastroenterol., 15, 411, 1980.
2. B a n k s R. E. et al.: Is fatal pancreatitis a consequence of excessive leucocyte stimulation? The role of tumor necrosis factor alpha. Cytokine, 3, 12, 1991.
3. B a r r e t t A. J.: Lysosomal enzymes. In: Lysosomes. A Laboratory Handbook, eds. Dingle J.T., North-Holland Publ. Co., Amsterdam – London 1972.

4. Draus J. et al.: Polymorphonuclear elastase as a diagnostic marker in experimental acute pancreatitis. Pol. Przeg. Chir., 69, 889, 1997.
5. Grondin G., Beaudoin A. R.: Immunocytochemical and cytochemical demonstration of a novel selective lysosomal pathway /SLP/ of secretion in the exocrine pancreas. J. Histochem. Cytochem., 44, 357, 1996.
6. Ho H. S., Frey C. F.: Current approach to the surgical management of chronic pancreatitis. Gastroenterologist, 5, 128, 1997.
7. Lasson A., Ohlsson K.: Consumptive coagulopathy, fibrinolysis and protease-antiprotease interactions during acute human pancreatitis. Thrombosis Research, 41, 167, 1986.
8. Lowry O. H. et al.: Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193, 265, 1951.
9. Luthen R. et al.: Influence of ductal pressure and infusates on activity and subcellular distribution of lysosomal enzymes in the rat pancreas. Gastroenterology, 109, 573, 1995.
10. Maciejewski R. et al.: Effects of acute pancreatitis on activity of lysosomal enzymes in the liver. Hot topics in hepatology and hepatobiliary malignancies (Abstr.) Lake Buena Vista, Florida 1998.
11. Mahmoud A. E. et al.: Poor prognosis and limited therapeutic options in patients with Budd-Chiari syndrome and portal venous system thrombosis. Eur. J. Gastroenterol. Hepatol., 9, 485, 1997.
12. Norman J. G. et al.: Tissue-specific cytokine production during experimental acute pancreatitis. A probable mechanism for distant organ dysfunction. Dig. Dis. Sci., 42, 1783, 1997.
13. Novikoff A. B.: Lysosomes in the physiology and pathology of cells. Contributions of staining methods. In: Ciba Foundation Symposium on Lysosomes. Little, Brown and Co., Boston 1963.
14. Ruth R. C. et al.: A new technique for isolation of particulate lysosomal activity from canine and rat myocardium. J. Mol. Cell. Cardiol., 10, 739, 1978.
15. Wójtowicz Z. et al.: The activity of lysosomal enzymes of the wall of the pulmonary trunk in rabbits. Ann. Univ. M. Curie-Skłodowska, sectio D, vol. 49, Lublin 1994.

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SUMMARY

The aim of the experiment was to establish and quantify the changes in the activity of acid phosphatase in the pancreas, liver, spleen and kidneys during the course of experimental pancreatitis.The experiment was carried out on 65 male rats of Wistar strain, whose weight varied from 250 to 350 g. The animals were standard fed. They drank only water 24 hours before operation. The rats were randomly divided into three groups: A – intact animals group which were not operated and were used to mark initial biochemical parameters (15 rats), B – the experimental group of animals which were injected by retrograde way with sodium taurocholate into the common bile-pancreatic duct to induce acute necrotic pancreatitis (50 rats). After laparotomy an injection needle was inserted into the common bile-pancreatic duct via the proximal part of the duodenum (Aho's method). After 2, 6, 12, 24, 48 hours rats were anaesthetised again, and thoracotomy was performed by taking blood for amylase determination from the left ventricle of the heart. Then the animals were given an overdose of ketamine, and the organs were removed during laparotomy and frozen at the temp. of –20°C. Alpha-amylase activity in the blood serum was determined by the enzymatic method. Acid phosphatase activity was assayed by spectrophotometric methods using a substrate which releases 4-methyloumbeliferol reacting

with the enzyme. The authors concluded that the activity of membranous fraction of acid phosphatase changed non-specifically over the course of experimentally induced acute pancreatitis in rats, but statistically significant difference was found in the enzyme's activity during different periods of pancreatitis only in the pancreas and in the liver.

Aktywność fosfatazy kwaśnej w różnych narządach jako marker zapalenia trzustki

Celem pracy była ocena znaczenia fosfatazy kwaśnej jako wczesnego markera rozpoczynających się powikłań wielonarządowych w przebiegu doświadczalnego ostrego zapalenia trzustki u szczurów. Do pracy użyto 65 zwierząt, podzielonych na grupę kontrolną (15 szt) i doświadczalną (50 szt.). W czasie doświadczenia zwierzęta miały przez cały czas wolny dostęp do wody i stałą temperaturę otoczenia. Zapalenie trzustki wywoływano metodą Heinkela i Aho, używając 5% roztworu taurocholanu sodu jako czynnika inicjującego zapalenie. Zabieg wykonywano w warunkach jałowych, z przestrzeganiem procedur wymaganych przez Komisję ds. Badań na Zwierzętach AM w Lublinie. Następnie zwierzęta podzielono losowo na 5 podgrup, po 10 sztuk w każdej. Po 2, 6 , 12, 24 i 48 godz. od indukcji zapalenia trzustki zwierzęta usypiano ponownie, pobierano krew do oznaczenia poziomu amylazy i po otwarciu brzucha pobierano w całości trzustkę, wątrobę, nerki i śledzionę. Narządy zamrażano i przechowywano w temp. -20°C. Po rozmrożeniu pobierano 1 g tkanki z każdego narządu i poddawano procedurze obróbki biochemicznej, mającej na celu uwolnienie obu frakcji enzymów lizosomalnych – wolnej i związanej. Wyniki obliczano spektrofotometrycznie, a następnie metodą Lowry'ego oznaczano aktywność białka. Wyniki podano w nmol/1 mg białka/1 godz. inkubacji. Stwierdzono, że statystycznie istotne zmiany aktywności badanego enzymu w różnych okresach choroby dotyczyły jedynie wątroby i trzustki i mogą mieć zastosowanie diagnostyczne w warunkach doświadczalnych.