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STUDY OF MMP IN PATIENTS WITH CHRONIC PANCREATITIS AT RISK OF DEVELOPING OF PANCREATIC CANCER

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INTRODUCTION

Pancreatic cancer (PC) is one of the most lethal pathologies of the pancreas with an average patient survival of less than 6 months after diagnosis [1]. Numerous investigations results indicate that both acute and chronic pancreatitis (CP) are associated with the development of PC by common risk factors and the involvement of common mechanisms in the pathogenesis [2]. The MMP class has a great variability that provides a complex intervention in pathophysiological conditions. MMPs' roles in pathology may be grouped into the following main types: tissue destruction, as in cancer invasion and metastasis, rheumatoid arthritis, osteoarthritis, different types of ulcers, periodontal disease, brain injury and neuroinflammatory diseases; fibrosis, as in liver cirrhosis, fibrotic lung disease, otosclerosis, atherosclerosis, and multiple sclerosis; weakening of matrix, as in dilated cardiomyopathy, epidermolysis bullosa, aortic aneurysm, and restenotic lesions [3,4].

Herein, the current study analyzed different matrix metalloproteases (MPPs) and serine proteases activities, the redistribution of different molecular weight proteins and accumulation of low and middle molecular weight substances in the pancreas, and blood of the patients under CP and PC.

MATERIALS AND METHODS

The study was done on 40 patients with confirmed adenocarcinoma of pancreas (n=20) and pacreatitis (n=20) who underwent resection. Normal pancreatic tissue, there were analyzed the normal areas of the tissue resected in an autopsy from person who died not from pancreatic pathologies. The blood samples were gotten from a conditionally healthy person (n=20) and all the aforementioned patients.

Total proteolytic activity was evaluated using casein as a substrate. The low and middle molecular weight (LMMW) substance fractions were obtained according to [5]. The optical density of the samples was determined with a spectrophotometer Smart SpecTMPlus (BioRad, USA) at 254 nm in the case of low molecular weight substances determination, and at 210 nm, 238 nm in the case of middle molecular weight substances determination. SDS-Polyacrylamide gel electrophoresis (SDS-PAGE) was performed as reported (Laemmli, 1970), using 4% (w/v) stacking gel and 10 % (w/v)

separating gel. SDS-PAGE was conducted using Mini-Protean Tetra System (BioRad, USA) at 19 mA for stacking and 36 mA for separating gels.

Zymography was done according to the method Ostapchenko et al [6]. The protein concentration was determined according to the method described by [7], using crystalline bovine serum albumin as the standard protein, and the absorbance was measured at 595 nm.

RESULTS AND DISCUSSION

The analysis of the protein profile of pancreatic tissue revealed prevalence the proteins having 10-35 kDa molecular weight of the control and pathological samples. There were also detected polypeptides with a maximum molecular weight up to 67 kDa in the control pancreas. At the same time, the pathologically changed samples had significant amounts of protein fractions up to 100 kDa (18% in case of PC), and in the case of CP up to 150 kDa (11%). Attention should be also paid to the decrease in the content of low-molecular-weight peptides up to 10 kDa under CP and their absence under cancer conditions.

The homogenates of pancreatic tissues affected by cancer manifested both gelatinase and collagenase activities in a wider range of protein molecular weights (10-150 kDa) comparing with the control and CP. At the same time, attention should be paid to a significant concentration of proteolytic activity showed in the zone of low molecular weight proteins, which may be associated with the appearance of MMPs forms that are absent in conditionally healthy individuals. Among the candidates for such enzymes are the smallest members of the MMP family that are encoded by *MMP7* gene (matrilysin, 30 kDa) and *MMP28* (epilysin, 20 kDa). MMP7 is exclusively expressed in the metaplastic ductal epithelium of CP patients and has been shown to control acinar cell apoptosis through proteolytic release of its pro-apoptotic molecule Fas ligand. The numerous studies have also compared MMP7 expression in pancreatic ductal adenocarcinoma patients with pancreatitis patients and/or healthy controls. Results of these investigations have clearly shown that MMP7 levels are elevated in PC patients, therefore MMP7 can be considered as an important regulator of tumor formation.

CONCLUSIONS

In CP, the values of the majority of the studied parameters (content of Pg, PS, TM, IGF-1, MMP-1, -2, -3, -8, -10, TIMP-1, IL-1β, -4, -6, - 8, as well as TNF-α) at the level of systemic circulation increased markedly. Taking into account the fact that statistically significant deviations in blood plasma under the conditions of PC were detected only for PC and TM, other parameters, in particular, the content of MMPs and ILs, can serve as a promising diagnostic criterion for determining the transition of chronic inflammation in pancreas to malignant transformation. The conducted studies revealed the peculiarities of proteolytic processes taking place in blood plasma and pancreatic tissue under conditions of chronic pancreatitis and cancer. The detected changes were accompanied by the accumulation of low and middle molecular weight substances, including a significant number of proteins with pronounced regulatory functions. The latter undoubtedly is followed by the processes of extracellular matrix

turnover and modulation of matrix metalloproteinase composition, which in the presented study was manifested by the increase in the number of bands as well as expansion of the molecular masses range of enzymes that showed collagenolytic and gelatinolytic activity both in plasma and in diseased organ under the investigated pathologie.

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