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Tetrapeptide Stimulates Functional Activity of Pancreatic Cells in Aging

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Abstract—In this study, the molecular mechanisms of pancreoprotective action of the tetrapeptide H-Lys-Glu-Asp-Trp-NH_2 in aging human pancreatic cells have been investigated. It has been established that the tetrapeptide under study increases the expression of matrix metallopreteinases MMP2 and MMP9, serotonin, glycoprotein CD79α, antiapoptotic protein Mcl1, and proliferation markers PCNA and Ki67, as well as decreases the expression of proapoptotic protein p53 in aged pancreatic cell cultures. Thus, the clinical effect of the tetrapeptide observed in elderly patients with type-2 diabetes mellitus and pancreatitis may be due to its ability to activate the expression of signaling molecules, i.e., markers of functional activity of pancreatic cells.

Key words: tetrapeptide, signaling molecules, pancreas, aging

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INTRODUCTION

The prevalence of type-2 diabetes and chronic pancreatitis is steadily growing [17]. According to the data of the WHO expert committee, more than 60 million people suffer from diabetes worldwide. This value increases by 6–10% annually. The frequency of diabetes is observed primarily in people of aged 40 and over [1]. The importance of this disease falls just after cardiac and oncological diseases. Chronic pancreatitis is also a frequent disease of the pancreatic gland. Pancreatitis prevalence is five to seven new cases per 100000 people in various countries [1]. Moreover, the incidence of the disease has doubled over last 50 years. This fact is connected not only with better diagnostics, but also an increase in alcohol consumption in some countries, which has increased the influence of negative environmental factors that weaken nocifensor, and the aging of the populations of developed countries [2].

Age-associated type-2 diabetes and chronic pancreatitis are caused by a decrease in pancreatic acinar and β-cells. The pathology of the pancreas on the subcellular level arises from the abnormal expression of signaling molecules, i.e., markers of the functional activity of cells. Serotonin stimulates the release of insulin from granules of pancreatic β-cells. The absence of serotonin causes diabetes mellitus [15]. Matrix metalloproteinases participate in the remodeling of the pancreatic intercellular matrix. The MMP2 and MMP9 metalloproteinases are supposed to participate in morphogenesis of pancreatic endocrine cells and regulate the migration of islet cells from epithelial tissue to intercellular space and formation of islets of Langerhans [14, 16]. P53 activates apoptosis in all tissues of the organism [5], and the antiapoptotic protein Mcl1 regulates cell survival. A decrease in Mcl-1 in β-cells is known to be responsible for their death and the origin of diabetes. Enhanced expression of Mcl-1 in β-cells increases their viability [3, 11].

The nuclear protein PCNA controls DNA reparation in proliferating cells. The reduction in the amount of $PCNA^+$ cells decreases the physiological regeneration of the pancreas [11, 13]. Ki67 is connected with cell division and can be an important marker of the decrease in the proliferation activity of cells and the involutive processes in pancreas. The CD79α transmembrane protein participates in the formation of autoimmune processes [7] and is a marker of type-1 diabetes.

Tetrapeptide H-Lys-Glu-Asp-Trp-NH_2, which was synthesized at the St. Petersburg Institute of Bioregulation and Gerontology, decreases the blood sugar level in experiments with animals and in elderly diabetics [8]. It was revealed that, in the case of alloxan diabetes in rats, the tetrapeptide reliably provides a 35% decrease in blood sugar level, which correlates with the decrease in fatal cases [10]. Clinical trials of the tetrapeptide showed its efficiency in diabetics [4, 12]. Thus, the tetrapeptide treatment caused a decrease in fasting plasma glucose and standard glucose tolerance test. The decrease in insulin concentration in blood plasma and insulin resistance index were

also marked. The tetrapeptide stimulated the expression of differentiation factors Hoxa3, CXCL12 and WEGC1 in human pancreatic cell lines. Furthermore, the inducing effect of the tetrapeptide was more pronounced in old cultures, which may be one of the mechanisms of its geroprotective effect [9]. Moreover, it was revealed that the tetrapetide is capable of penetrating the nucleus and nucleolus of cells and affects the action of endonucleases on DNA [6].

The purpose of the work is to explore the effect of the tetrapeptide on the expression of signaling molecules, i.e., markers of the functional activity of pancreatic cells at aging.

MATERIALS AND METHODS

Pancreatic cell lines MIA PaCa-2 of passages 1, 7, and 14 obtained at the Institute of Cytology, Russian Academy of Sciences (St. Petersburg) were used in the work. Passage 1 was considered as young culture, while passages 7 and 14 were considered to be mature and old cultures, respectively, according to the recommendation of the International Association for Cell Culture Research (United States, San Francisco, 2007). Cultures were divided into three groups. The first (control) group was treated with physiological saline. The second group was treated with the control tetrapeptide (H-Ala-Glu-Asp-Leu-OH, 20 ng/mL). The tetrapeptide under study (H-Lys-Glu-Asp-Trp-NH_2, 20 ng/mL) was added into the third group. Cells were cultured in Jet Biofil matrasses (Biolot) with the surface of 25 cm^2 in 5 mL of DMEM medium with the addition of L-glutamine (Biolot), 15% SC-BIOL fetal calf serum (Biolot) and 1% penicillin-streptomycin at 37°C. The initial concentration was 10^6 cells/mL. Immunocytochemistry was performed using primary monoclonal antibodies to metalloproteinases MMP2 and MMP9 (1 : 40, Novocastra); proapoptotic protein p53 (1 : 50, Dako); antiapoptotic protein Mcl1 (1 : 40, Novocastra); transmembrane protein CD79α (1 : 50, Dako); proliferation markers Ki67 (1 : 100, Novocastra) and PCNA (1 : 100, Novocastra); and serotonin (1 : 50, Dako). Biotinylated anti-mouse immunoglobulins (Novocastra) were used as secondary antibodies. Permeabilization was performed using 0.1% Triton X100. The reaction was visualized using horseradish peroxidase and diaminobenzidine (EnVision Detection System, Peroxidase/DAB, Rabbit, Mouse). The results of immunocytochemical staining were tested morphometrically on a Nikon Eclipse E400 microscope using a Nikon DXM1200 digital camera and Videotest Morphology 5.0 software. Five fields of vision with magnifications of ×200 were analyzed for each case. The expression area was calculated as the ratio between the area occupied by immunopositive cells and the total area of cells in the field of vision. The result was expressed as a percentage. This parameter characterizes the number of cells that express the studied signaling protein.

Table 1. Expression areas of signaling molecules in young pancreatic cell cultures

Marker	Control group	Control tetrapeptide	Tetrapeptide under study
*MMP*2	4.7 ± 0.3	5.2 ± 0.2	5.6 ± 0.2*
*MMP*9	4.3 ± 0.5	5.0 ± 0.4	5.7 ± 0.2*
Serotonin	2.3 ± 0.2	2.6 ± 0.1	2.9 ± 0.1*
*CD*79α	0.3 ± 0.1	0.6 ± 0.1*	0.9 ± 0.2*
*p*53	4.9 ± 0.2	5.2 ± 0.1	3.3 ± 0.2
*Mcl*1	5.0 ± 0.3	4.6 ± 0.2	4.4 ± 0.3
PCNA	3.3 ± 0.1	3.2 ± 0.1	3.4 ± 0.1
*Ki*67	3.0 ± 0.2	3.3 ± 0.1	4.1 ± 0.2*

Note: Here and in Tables 2 and 3, * means $p < 0.05$ compared to the analogous value in the control group.

Table 2. Expression areas of signaling molecules in mature pancreatic cell cultures

Marker	Control group	Control tetrapeptide	Tetrapeptide under study
*MMP*2	5.1 ± 0.1	5.0 ± 0.2	7.9 ± 0.2*
*MMP*9	5.0 ± 0.1	5.2 ± 0.2	8.6 ± 0.3*
Serotonin	2.1 ± 0.2	2.5 ± 0.4	3.3 ± 0.2*
*CD*79α	0.6 ± 0.1	0.9 ± 0.1*	1.3 ± 0.2*
*p*53	11.9 ± 0.3	9.1 ± 0.2*	5.1 ± 0.2*
*Mcl*1	4.9 ± 0.2	4.8 ± 0.1	6.1 ± 0.1*
PCNA	2.9 ± 0.2	3.0 ± 0.1	4.3 ± 0.2*
*Ki*67	1.9 ± 0.1	3.7 ± 0.1	5.3 ± 0.2*

Statistical treatment of the data included calculation of average, standard deviation and confidence interval for each sample and was performed using Statistica 7.0 software. The distribution was analyzed using the Shapiro-Wilk test. The statistical homogeneity of several samples was tested using the nonparametric method of the single-factor analysis of variance (Kruskal-Wallis test). The difference was considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

A significant increase in the expression area of metalloproteinases MMP2 and MMP9 by 1.2 and

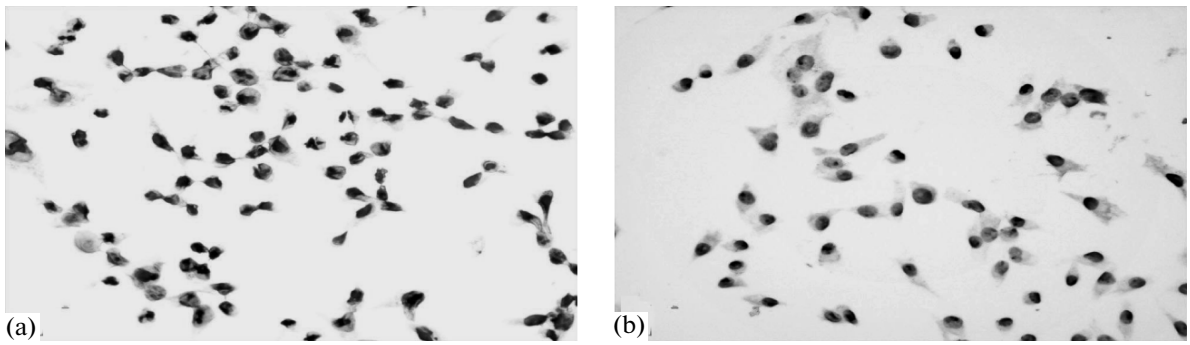

Fig. 1. Expression of proapoptotic marker p53 in pancreatic cell culture (passage 7), immunocytochemistry: (a) control group, (b) tetrapeptide under study. 200× magnification.

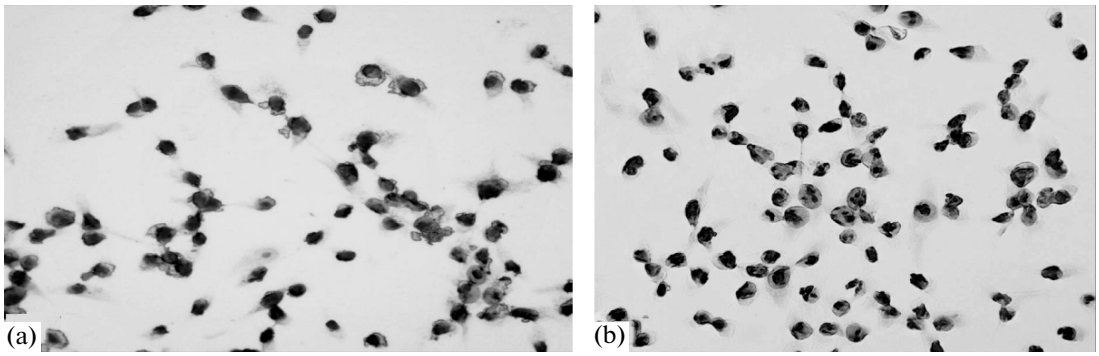

Fig. 2. Expression of transmembrane protein CD79α in pancreatic cell culture (passage 14), immunocytochemistry: (a) control tetrapeptide, (b) tetrapeptide under study. 200× magnification.

1.3 times, respectively, was observed in young cultures treated by the studied tetrapeptide. The corresponding values for serotonin, CD79α, and Ki67 were 1.3, 3.0, and 1.4 times, respectively (Table 1). The control tetrapeptide did not affect the expression of the studied markers except CD79α. The CD79α expression area was doubled under the action of the control tetrapeptide compared to the control group.

Mature cultures treated with the tetrapeptide under study demonstrated a reliable increase in the expression of all markers with the exception of p53 (Table 2). Thus, the expression of metalloproteinases MMP2 and MMP9 increased by a factor of 1.5 and 1.7, respectively. The expression of proliferation factors PCNA and Ki67 increased by 1.5 and 2.8 times. The corresponding values for serotonin, transmembrane protein CD79α, and antiapoptotic protein Mcl1 were 1.6, 2.2, and 1.2 fold, respectively (Table 2). The addition of the investigated tetrapeptide caused 2.3-fold decrease in the expression of p53 compared with the control group (Fig. 1; Table 2).

The control tetrapeptide increased the expression area of CD79α by two times, decreased the expression area of p53 by 1.3 times, and did not affect the expression of other markers.

The addition of the tetrapeptide under study into old cultures caused the significant increase in expression areas of MMP2, serotonin, CD79α, Mcl1, PCNA and Ki67. These values were 1.1, 2.3, 1.7, 1.3, 1.5, and 2.1-fold higher in comparison with the control group, respectively (Figs. 2, 3; Table 3). The expression of p53 decreased by 1.5 times, and the expression of MMP9 did not change significantly in comparison with the control group in the presence of the tetrapeptide under investigation (Table 3).

The control tetrapeptide did not influence the expression of the majority of markers. However its

Table 3. Expression areas of signaling molecules in old pancreatic cell cultures

Marker	Control group	Control tetrapeptide	Tetrapeptide under study
*MMP*2	7.6 ± 0.2	2.9 ± 0.3*	8.3 ± 0.1*
*MMP*9	8.6 ± 0.2	3.2 ± 0.2*	8.8 ± 0.1
Serotonin	1.6 ± 0.2	1.9 ± 0.2	3.7 ± 0.3*
*CD*79α	0.9 ± 0.1	0.8 ± 0.3	1.6 ± 0.2*
*p*53	4.7 ± 0.4	5.5 ± 0.4	3.1 ± 0.3*
*Mcl*1	4.7 ± 0.2	4.4 ± 0.2	6.3 ± 0.1*
PCNA	3.0 ± 0.3	2.7 ± 0.2	4.6 ± 0.2*
*Ki*67	2.2 ± 0.2	3.2 ± 0.3*	4.6 ± 0.1*

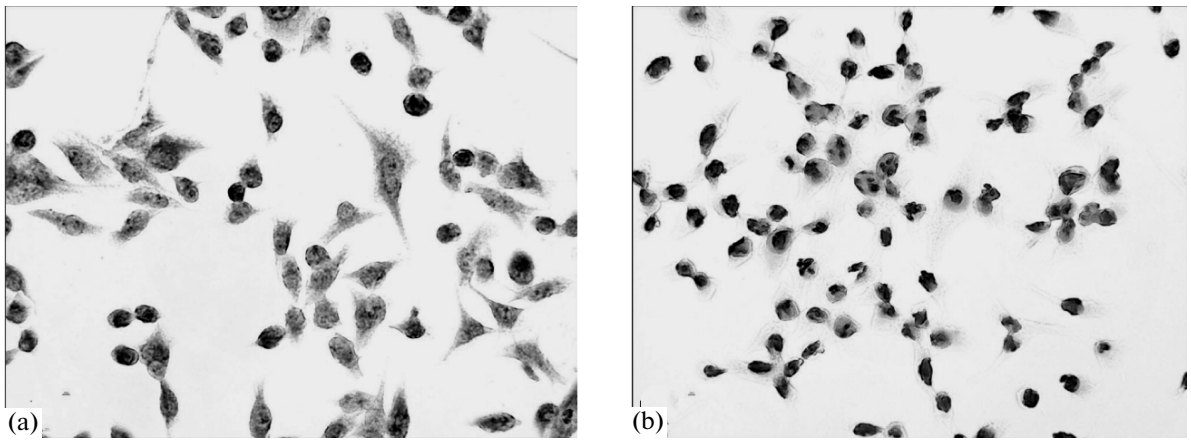

Fig. 3. Expression of Mcl-1 antiapoptotic marker in pancreatic cell culture (passage 14), immunocytochemistry: (a) control group, (b) tetrapeptide under study. 200× magnification.

addition caused 2.6 and 2.7-fold decreases in MMP2 and MMP9 expressions, respectively, and a 1.45-fold increase in Ki67 expression (Table 3).

It was revealed that the age-specific involution of the pancreas is accompanied by a decrease in the expression of the following signaling molecules: serotonin, Mcl1, PCNA, and Ki67. The tetrapeptide under study can enhance the expression of these signaling molecules, thereby increasing the proliferative capacity of cells. The most pronounced increase in expression was observed for the addition of the tetrapeptide under study to mature cell cultures. It is most likely that this effect can be explained by the influence of the investigated tetrapeptide on cells that are at the peak of their functional activity. The stimulating effect of the investigated tetrapeptide in old cell cultures is also significant, but less pronounced compared to mature cell cultures. This may be connected with the decrease in reserve capacity of cells at aging. The tetrapeptide stimulates insulin release from β-cells [14] by increasing the expression of serotonin. This can be the reason for its antihyperglycemic effect in elderly people. The increase in expression of the transmembrane protein CD79α lowers the risk of immunodeficiency associated with age-specific involution of pancreatic tissue, as well as neutralizes the risk of type-1 diabetes [7]. The stimulation of the expression of the Mcl-1 antiapoptotic marker and the suppression of the synthesis of proapoptotic p53 in old pancreatic cell cultures is indicative of the increment of functional activity of pancreatic endocrine cells at aging.

CONCLUSIONS

Consequently, the clinical effects of the investigated tetrapeptide are based on its ability to stimulate the synthesis of serotonin, activate proliferation, and decrease the apoptosis of pancreatic cells.

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SPELL: 1. pancreatitis, 2. oncological