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Morphofunctional Fundamentals for Peptide Regulation of Aging

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Abstract—Processes of aging related to different morphological and functional changes due to pathological or compensatory reactions are considered. Long-term studies of the use of peptide bioregulators showed their efficiency in the prophylaxis and treatment of pathologies characteristic for aged persons. The basis of the geroprotective action of peptides is revealed to be their capacity for active protein synthesis at the molecular level, cytodifferentiation activation, and proliferation on the cell level. At the same time, they prevent the development of mitochondrial apoptosis and at tissue level restore morphofunctional interactions that are weakened in aging.

Keywords: peptides, protein synthesis, differentiation, proliferation, apoptosis, aging.

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INTRODUCTION

Study of the problem of aging is one of the most challenging areas of contemporary biology and medicine. Presently, the proportion of the elderly generation in the world population is continuously growing [9]. The increase in life expectancy and, subsequently, progressive aging of the population [2, 17, 18] are expected to lead in the near future to the necessity of solving a series of medical, social, and economic problems. Therefore, the prophylaxis of age-related pathologies is considered the main task of contemporary geriatrics and becomes the most important medical problem.

Aging is a complex multilevel process that occurs not only as hypoplastic alterations of cells and tissues but also as the decrease in their functional activity. It is connected with a myriad of morphological and functional modifications, which are due to pathological or compensatory reactions of an organism [1, 3, 7, 11].

The aging process is characterized by the involution of a variety organs and regulatory systems such as the liver, pancreas, intestine, thymus, pineal gland, and retina, as well as the nervous, immune, and endocrine systems [4, 5, 8, 10, 12, 24]. For all of these organs and systems, the general principles of aging and some specific mechanisms that are due to their structural and functional features are defined.

At the cell level, the aging process occurs as the disorder of synthesis and secretion of a variety of peptides and proteins involved in cell signaling processes [15, 25, 26]. It is suggested that the rate of aging of an organism depends on the ratio of proliferating cells and cells undergoing apoptosis. This ratio is, in turn, determined by the relative abundance of pro- and antiapoptotic proteins [6]. At the molecular level, the decrease in peptide and protein biosynthesis in the organisms of elderly people is due to the accumulation of DNA damage at reparation, telomere shortening, and an increase in the relative abundance of heterochromatin in the nucleus [22]. Therefore, the activation of apoptosis takes place in the organisms of elderly people. At the intercellular and tissue levels, the decrease in abundance of regulatory proteins and peptides involved in cell signaling processes leads to desynchronization of nervous, immune, and endocrine system functions.

Regulatory systems control the relative abundance of different cell populations, their differentiation, proliferation, and apoptosis. The diversity of membrane receptors, neuromediators, cytokines, and other factors of intercellular regulation that integrate biosynthesis processes at the cell and organism level are characterized by the general term "bioregulation." It unites all extracellular, intercellular, and intracellular mechanisms controlling biosynthesis, metabolism, and reproduction of genetic information in multicellular organisms.

The development and improvement of molecular biological approaches in the second half of the twentieth century, which provided the tools for subcellular studies of regulatory systems, have led to the possibility of verification of the myriad of low-molecular weight hormone peptides, which were united into the group of biologically active regulatory peptides [20].

Owing to the development of scientific methodology in recent decades, it has become possible to study intracellular processes, which allowed confirmation of the fact that, in the diversity of information molecules, regulatory peptides play the role of universal information transporters at all levels of life organization, i.e., from the cell level to the organism level.

Regulatory peptides occupy a special position among vitally important bioregulators. Detailed study of the role of peptides in the biological regulation system of multicellular organisms is considered to be the most challenging task of contemporary physiology. It appears that the transduction and effect of any incoming information in an organism are regulated by peptides, whose main function is aimed at the protection of functional stability of the genome [21].

At the same time, information about changes in the inner and outer environment appears to be the main factor initiating necessary modifications in the bioregulation system that help sustain the required level of functional activity of cells. Some evidence suggests that these peptides normally fulfill their functions mostly at the cell level via induction of synthesis of a variety of regulatory proteins [16]. Peptide biosynthesis disorders are observed during aging at the cellular and subcellular levels [16, 19, 23]. Moreover, age-related organism involution occurs due to the loss of sensitivity to regulatory peptides by target cells in different tissues and organs [23].

At present, a series of synthetic peptides have been obtained at the St. Petersburg Institute of Bioregulation and Gerontology of the Russian Academy of Medical Sciences. The most promising preparations with respect to geroprotector efficiency are *AT–0* (testagen, *H–Lys–Glu–Asp–Gly–OH*), *AKS–P* (pankragen, *H–Lys–Glu–Asp–Trp–NH$_2$*), *AB–9* (*Nα–(γ–L–Glu)–L–Lys*), *AB–17* (normophthal, *H–Lys(H–Glu–OH)–OH*), *T–31* (katalacs, *H–Ala–Glu–Asp–OH*), *T–33* (pinealon, *H–Glu–Asp–Arg–OH*), *T–34* (honluten, *H–Glu–Asp–Gly–OH*), and *T–38* (vesugen, *H–Lys–Glu–Asp–OH*).

Long-term studies revealed that these peptides can recover functions of tissues and organs undergoing age-related involution. However, it has not been possible thus far to follow the mechanisms of protein functions from the organ and tissue levels to the cellular and intracellular systems.

Therefore, it appears interesting to study the morphological and functional characteristics of peptide-dependent regulation of aging at the organ, tissue, cellular, and molecular levels.

In order to reach this goal, we carried out a series of experiments allowing us to assess the regulatory effects of the peptides and, based on the data obtained, consider the most important mechanisms of their action at the cellular and molecular levels.

At the first stage of the study, we conducted a comparative analysis of the influence of 20 ng/ml of *T–34* and *T–38* peptides on the duodenum, spleen, and thymus of rats undergoing radiation aging. It was shown that irradiation leads to a series of pathological processes that occur at the organ level as a decrease in organ mass; at the tissue level as the destruction of the vascular bed, edema, and decrease in cell number; and at the cellular level as changes in the nucleus structure that may possibly lead to a decrease in protein synthesis.

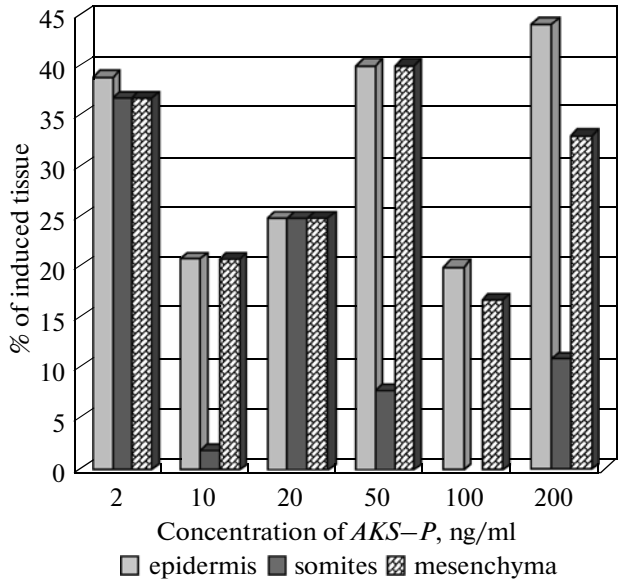
The administration of *T–34* peptide, which possesses an affinity to respiratory system tissues, did not affect the morphological and functional state of irradiated organs. However, the administration of *T–38* peptide, which possesses an affinity to vascular tissue, led to a significant increase in proliferation of all studied tissue cells, though the irradiation-induced pathology of the vascular bed was not observed.

Indeed, an increase in body weight and absence of signs of anemia were observed in experiments with irradiated animals treated with *T–38*. The *T–38* peptide induced proliferation in the tissues of the intestine, spleen, and thymus. By the structural and functional "intestinal crypt–villus" complex of the duodenum, the mucosal tunic did not differ from the control, and the cellular composition of the *lamina propria* was recovered. The effects of T–38 also led to normalization of microcirculation as well as recovery of the submucous layer and neural ganglion structure. In the spleen, treatment with *T–38* after irradiation led to a relative increase in white pulp abundance and the occurrence of large hematopoietic islets. In lymphatic follicles and the parafollicular zone, the abundance of huge lymphoblasts increased. Many of them underwent mitotic division, which indirectly suggests activation of reparation regeneration in the spleen. In the thymus, treatment with *T–38* peptide resulted in division into cortical and medulla zones, which completely disappeared after irradiation, suggesting geroprotector effects of *T–38* with respect to the thymus. At the same time, the proliferation potentials of all these tissues were higher than those in both the control group and irradiated animals.

Apparently, the synthetic peptide *T–38* affects the vascular bed in a tissue-specific manner, thus improving the tissue trophism and activating proliferation. However, the *T–34* peptide of the respiratory system was ineffective. The obtained results confirmed previously obtained data about the tissue-specific manner of peptide-mediated effects [14], which occur not only at the tissue and organ levels but also at the cellular level. Moreover, our study revealed that the geroprotector effects of the peptides are based on their capacity to activate intracellular processes, the most significant of which is protein synthesis.

The main goal of the second stage of our study was exploration of molecular mechanisms of the peptide effects at the level of protein synthesis and regulation of apoptosis.

Studies of mouse fibroblast cultures revealed that peptides fulfill their roles with respect to protein synthesis in a tissue-specific manner. Indeed, *T–31* peptide, which possesses an affinity to connective tissue, up-regulated cytoskeleton proteins (actin, vimentin, and tubulin) and karyoskeleton proteins (lamin *A* and *C*) when taken in the concentration 10 mM. On the other hand, *T–34* and *T–38* peptides, which are specific to the respiratory and vascular systems, did not produce any effect.



Tissues originating from the ectoderm of early stage gastrula of *Xenopus laevis* treated with different concentrations of the *AKS–P* peptide.

Peptide-induced activation of cytoskeleton protein synthesis suggests that these peptides affect intracellular signaling cascades, which are regulated via peptide-mediated cytoskeleton protein remodeling. It also appears that the peptide-dependent regulation of intranuclear protein synthesis may lead to alteration of the heterochromatin and euchromatin ratio, which in turn facilitates changes in the gene expression pattern.

Fibroblast apoptosis was induced by administration of *Helicobacter pylori* into the cell culture, which led to mitochondria destruction, outer and inner mitochondrial membrane damage, and a decrease in mito-GFP complex fluorescence in fibroblasts. Administration of either *T–31* or *T–34* peptides into the fibroblast cell culture prior to *Helicobacter pylori* resulted in increased mito-GFP complex fluorescence and the absence of mitochondrial membrane damage. The obtained data suggest that both *T–31* and *T–34* peptides obviously possess antiapoptotic activity with respect to fibroblasts, which occurs as the increase in mitochondria resistance to damaging factors and tolerance to the initiation of mitochondria-dependent programmed cell death.

Similar antiapoptotic effects of *T–34* were shown in the culture of human stomach epithelial cells. Interestingly, the revealed antiapoptotic activity of *T–34* was higher than that of clarithromycin, which is used as an antimicrobial agent for the treatment of *Helicobacter pylori*-associated stomach diseases.

At this stage of our research, we revealed that the geroprotector effects of the studied peptides are based on protein synthesis stimulation and prevention of abnormal forms of mitochondrial-dependent apoptosis. This conclusion is consistent with the previously obtained data about the antiapoptotic effects of bioregulators [13].

Moreover, the stimulatory effects of the peptides on protein synthesis are known to be realized in a tissue-specific manner. Indeed, the *T–31* peptide, which possesses an affinity to connective tissue, up-regulated cytoskeleton proteins (actin, vimentin, and tubulin) and karyoskeleton (lamin *A* and *C*) in fibroblasts. At the same time, *T–34* and *T–38* peptides, which are specific to the respiratory and vascular systems, did not produce such effects.

The differentiation potential of cells was shown to decrease in both the model conditions of radiation-induced aging and the natural age-related involution of organs. Therefore, the third stage of our research dealt with the study of the influence of *AKS–P* peptide on a pluripotential embryonic tissue, using the early gastrula ectoderm of *Xenopus laevis*.

In all control cultures of the embryonic pluripotential tissue, only atypical epidermis developed. Administration of 2, 10, 20, 50, 100, and 200 ng/ml of *AKS–P* peptide into the tissue culture induced the development of epidermis, somites, and mesenchyma in a dose-dependent manner. In other words, it was shown that induction of one or the other pathway of pluripotential tissue differentiation in culture depended on the concentration of the peptide studied.

The maximal differentiation-inducing activity of *AKS–P* peptide with respect to pluripotential cells was observed when its concentration in the culture medium was 2 ng/ml. It was observed that, under such conditions, more than 35% of ectoderm differentiated in the direction of the epidermis, mesenchyma, and somites. A less obvious effect was observed when the concentration of *AKS–P* peptide in the culture medium was 20 ng/ml (figure).

The obtained data suggest that the pluripotential-tissue differentiation-induction effect of *AKS–P* peptide is realized in a dose-dependent manner. Apparently, the capacity of *AKS–P* peptide to stimulate pluripotential cell differentiation is based on its ability to activate protein synthesis, in particular, the biosynthesis of α-actin, which is known to be one of the major cytoskeleton proteins.

Therefore, at the first three stages of our study, we found that the geroprotector effects of the studied peptide were realized in the model system of accelerated aging in a tissue-specific manner, and they are based on the induction of cytoskeleton and karyoskeleton protein synthesis and inhibition of apoptosis. Moreover, it was shown that stem cells, which can differentiate to form a variety of tissues of an organism owing to peptide-mediated regulation, can also be the targets for these peptides.

At the final stage of our study, we summed up all the effects of the peptides studied, which were revealed in former experiments on the immune system. The influence of *AT–0*, *AB–9*, *AB–17,* and *T–31* peptides

Effects of the peptides on cells of human hematopoietic organs, the thymus, and lymphocytes

Peptide	Embryonic hematopoietic organs	Thymus of embryos and children	Blood
AT–0	Expression of *CD4,8* by BM cells	↓ Quantity of DP ↓ Expression of *CD3* by SP ↑ Expression of HLA-DR by TEC	↓ Expression of *CD19* Expression of *HLA-DR* by *T*-cells ↑ T-cell proliferation
AB–17	Expression of *CD3* by EL cells	↓ DP Loss of *CD4* or *CD8* by DP Loss of *CD3* by DP and SP cells ↓ thymus epithelium cell apoptosis	Expression of *HLA-DR* by *T*-cells
AB–9	Expression of *CD4,8* by BM cells	↓ Quantity of DP ↑ Quantity of $CD4^+3^-$ and $CD8^+3^+$	Expression of *HLA-DR* by *T*-cells
T–31	Expression of *CD4,8* by BM cells, Expression of *CD3* by EL cells	↓ Expression of *CD3* by DP ↑ Quantity of $CD4^+CD8^-$ due to loss of *CD8* cells DP	–

Note: BM–bone marrow, EL–embryonic liver cells, ↓–decrease, ↑–increase, DN–double negative ($CD4^-CD8^-$) thymocytes, DP–double positive ($CD4^+CD8^+$) thymocytes, SP–single positive ($CD4^+CD8^-$ and $CD4^-CD8^+$) thymocytes, TEC–thymus epithelium cells.

taken in concentrations of 2, 20, and 200 ng/ml on cultures of immune cells of bone marrow, thymus, and liver of human embryos, as well as immune cells of adult peripheral blood, was studied.

It was revealed that the studied peptides produce multiple, mostly stimulatory, effects on differentiation, activation, proliferation, and apoptosis of human immune cells in the peripheral blood, bone marrow, liver, and thymus of embryos, as well as in the thymus of 1.5-year-old children (table). It should be noted that the character of these effects depends on both type and concentration of the peptide, as well as on the type of tissue the peptide is targeted to.

For several peptides, a stimulatory effect on human mature immune cell differentiation was shown.

Maximal effects on the differentiation capacity of $CD34^+$ stem cell precursors of embryonic bone marrow and liver were provided by *AT–0* and *AB–17* peptides in the concentration 2 ng/ml and 20 ng/ml. Bone marrow stem cells treated with these peptides were shown to differentiate in the direction of myeloid $CD14^+$ cells and immature $CD3^+$ *T*-lymphocytes, whereas the liver stem cells tend to differentiate in the direction of mature *T*-helpers and cytotoxic *T*-cells.

It was shown that *AT–0* peptide in the concentration 20 ng/ml and 200 ng/ml provided the most powerful stimulatory effect on cell differentiation in the direction of *T*-lymphocytes and NK-cells.

Moreover, the studied peptides were shown to modify the phenotype of differentiated human *T*-cells. It was shown that mature *T*-lymphocytes treated with the studied peptides, especially *AT–0*, in the concentrations 20 ng/ml and 200 ng/ml changed their phenotypes from $CD4^-CD8^+$ and $CD4^+CD8^-$ to $CD4^+CD8^+$. In other words, the *AT–0* peptide induced coexpression of two receptors on the membrane of this subpopulation of immune cells.

The majority of the peptides studied were shown to activate human immune cells, up-regulate their proliferation, and suppress apoptosis.

In the blood, *AT–0* peptide in the concentrations 20 ng/ml and 200 ng/ml up-regulated expression of the *HLA-DR* marker of late activation on the membranes of cytotoxic *T*-lynphocytes. Moreover, this peptide was shown to stimulate the mitotic potential and decrease the level of apoptosis in the population of *T*-lymphocytes in blood. The same concentrations of *AB–9* peptide provided similar effects on the activation, proliferation, and apoptosis of thymocytes.

The results obtained allow us to formulate some hypotheses about possible mechanisms of peptide-mediated effects on the morphological and functional characteristics of human immune cells, which may be proven by studies of other organs and systems.

The stimulatory effects of the studied peptides on differentiation and proliferation of immune cells are based on the capacity of these peptides to up-regulate gene expression and *de novo* protein biosynthesis.

This suggestion is based on data about the effects of peptides on the phenotypes of *T*-lymphocytes of blood and thymocytes. *T*-cells exhibited coexpression of two receptors in both the thymus and peripheral blood. Initially, this effect could have been explained either by peptide-induced stimulation of the biosynthesis of this receptor or by initiation of its biosynthesis due to up-regulation of previously silent genes. Experiments with a protein synthesis blocker revealed that the first suggestion was false. Therefore, it was concluded that the mechanism of peptide-induced modification of the mature *T*-cell phenotype in the thymus and blood is based on protein synthesis activation via up-regulation of previously silent genes rather than direct stimulation. Moreover, the studied peptides were shown to be tissue-specific. Indeed, the *AT–0* and *AB–17* peptides provided the most obvious effects on stem cell differentiation in embryonic bone marrow, brain, and liver.

The studied properties of peptides (*de novo* protein synthesis stimulation and tissue specificity) may be considered not only in relation to immune cells but also in relation to other tissues, which will likely allow fuller understanding of peptide-dependent regulation mechanisms of the morphological and functional conditions of aging organism tissues.

It is known that age-related organ and tissues involution is based on the decreased capacity of cells for activation, differentiation, and proliferation, and, conversely, on the activation of apoptosis. At the molecular level, these events are determined by the relative abundance of heterochromatin and inhibition of protein synthesis. As tissue-specific substances, peptides can directionally activate cell proliferation and differentiation, as well as inhibit the abnormal initiation of mitochondrial apoptosis. The geroprotector effects of the peptides are based on their capacity to stimulate protein synthesis, particularly of proteins involved in cell signaling mechanisms (cytoskeleton proteins) and regulation of chromatin structure (karyoskeleton proteins). Use of the radiation-induced aging model revealed that molecular and cellular effects of the peptides occur at the tissue level, which manifests as tissue structure recovery.

The first stage of our study revealed that, at the tissue level, peptides can recover the structure and function of organs that underwent accelerated aging. At the second stage of our research, we found that, at the cellular level, the peptides stimulate cytoskeleton and karyoskeleton protein synthesis and, conversely, inhibit abnormal forms of apoptosis, which occurs via a mitochondria-dependent mechanism. The third stage of the study allowed us to reveal that the peptides stimulate cell differentiation and proliferation, which are inhibited during aging. Finally, it was shown that all observed peptide-mediated effects were realized in immune cells, which are the most sensitive to age-related involution. Therefore, our study revealed the geroprotector effect of the peptides at all levels, beginning from organs and tissues to intracellular signaling molecules.

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