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Peptide Correction of Tissue Hypoxia in Diabetic Foot Syndrome

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Abstract

The problem of treating complications of diabetes mellitus continues to be a pressing issue in modern medicine. One of the complications of diabetes is diabetic foot syndrome. With this complication, a complex of anatomical and functional changes is observed, leading to the development of tissue ischemia (hypoxia), accompanied by increased trauma and infection of the soft tissues of the foot. It should be noted that such complications lead to early disability in patients, up to amputation of the foot and death. The use of the dipeptide bioregulator L-glutamic-L-tryptophanic acid (L-Glu-L-Trp) contributes to the activation of reparative processes in ischemic tissues of patients with diabetic foot syndrome by inhibiting the synthesis of the HIF 1α factor and, as a result, oxygenation of soft tissues.

Keywords: *Diabetes Mellitus; Diabetic Foot; HIF 1 Factor, Dipeptide*

Introduction

Treatment of complications of diabetes mellitus remains an urgent problem in modern medicine. According to the WHO, more than 285 million people in the world currently suffer from diabetes mellitus (DM). Forecasts show that by 2025 the number of patients with diabetes mellitus will increase to 380 million people, and 60% of patients with this disease will have various complications, among which vascular complications will be the leading ones.

Diabetic foot syndrome holds the leading position among all complications of DM. The prevalence of diabetic foot syndrome among such patients can be up to 20% [9,10]. The main mechanism for the development of tissue ischemia in diabetes mellitus is the activation of HIF 1 factor (specific regulatory protein – hypoxia-inducible factor). HIF-1 is a dimeric protein complex involved in the regulation of tissue functions upon a decreased oxygen concentration [27]. For the first time, this transcription factor was visualized by scientist Johns Hopkins University in Baltimore, United States, in 1992 as the regulator of erythropoietin expression [16]. HIF-1 regulates the expression of the genes enhancing cell adaptation and survival in hypoxia [4]. This protein is among the key factors involved in the immune response and the homeostatic processes increasing vascularization in hypoxic tissues. HIF-1 can be regarded as a major component for the antihypoxic therapeutics [25]. As is known, HIF-1α can be a predictor of diabetes mellitus at a rate of 70%. Defects in hypoxia-mediated (hypoxia - mediated) neovascularization in various organs are characteristic of diabetes mellitus [12]. An inadequate formation of the compensatory collateral vessels in response to ischemia increases the risk for complications and mortality in diabetes mellitus patients [18]. Such diabetic microvascular defects may result from an insufficient production of angiogenic cytokines and VEGF [11,18]. Many

researchers believe that a decrease in the hypoxia-induced VEGF expression in diabetes mellitus patients results from disturbed HIF-1α transactivation [17-19]. HIF-1α expression increases in patients at stages 3 and 4 as compared with stages 1 and 2 [15]. This agrees with the data from Yan, H.T. and Su, G.F. (2014), who demonstrated that HIF-1α expression increases with the diabetes progression, since it is accompanied by hypoxia. In addition, a direct correlation is observed between the expression of HIF-1α and VEGF at different diabetes mellitus stages.

Thus, the pathogenetic role of the HIF 1α factor opens up the possibility not only to correct hypoxia itself, but also to treat complications of diabetes mellitus, such as diabetic foot.

Since 1973, prof. V. Khavinson and his team of scientists have been conducting continuous scientific research in the field of creating and studying peptide bioregulators, the most popular innovative products on the modern pharmaceutical market. Over the years, more than 20 complexes of physiologically active peptides have been isolated and synthesized from amino acids of 17 short di-, tri-, tetrapeptides, for which patents have been obtained in many countries of the world (USA, Canada, Australia, Europe, Japan, Korea, Israel, Russia, etc.). After many years of experimental and clinical studies, six medicinal peptide preparations were approved for medical use in the USSR, Russia, and then in the CIS countries. These include: Timalin®, a drug from the thymus, a regulator of cellular immunity; Epithalamin®, a drug from the pineal gland of the brain, a regulator of the endocrine system, which restores the level of melatonin during aging, has no analogue in world medical practice; Cortexin®, a drug from the cerebral cortex, a regulator of brain functions; Prostatilen®, a drug from the prostate gland, a regulator of prostate function; Retinalamin®, a drug from the retina that restores retinal functional activity in various degenerative diseases and has no analogue in world medical practice; Timogen®, a dipeptide L-Glu-L-Trp (EW), first isolated from Timalin® and then synthesized from amino acids, an immunity regulator [6,14,21]. Since 1990 Timogen® has been registered on the territory of the Russian Federation as an immunomodulatory drug (approved by the Ministry of Health of the USSR for medical use by order No. 250 dated 19.06.1990 (registration No. 90.250.1)). According to the literature, Timogen® stimulates the reactions of cellular and humoral immunity, as well as nonspecific reactivity. Timogen® normalizes the number of T-helpers, T-suppressors, restores the ratio of subpopulations of T-lymphocytes (CD4+/CD8+) in patients with various immunodeficiency diseases. In addition, Timogen® leads to normal biochemical, hematological blood parameters in immunodeficiency states. At the same time, it should be noted that the dipeptide does not have a significant effect on subpopulations of lymphocytes in healthy individuals. In addition, at a dose 10,000 times higher than the therapeutic one, the dipeptide does not cause toxic reactions, does not accumulate in the body, does not have chronic toxicity, does not have allergenic, locally irritating, carcinogenic, mutagenic and teratogenic effects. Timogen® is compatible with almost all drugs of various pharmacological groups and can be used in combination with conventional methods of therapy. In clinical studies in the United States, this peptide was codenamed IM862 [6,8,13, 23]. In addition, during many years of studying the biological properties of bioregulatory peptides, it was found that peptides are directly involved in the processes of tissue-specific regulation of gene expression and biosynthesis. As a result of peptide regulation in cells, the rate of accumulation of pathological changes (DNA damage, mutations, malignant transformation, etc.) decreases and the activity of reparative processes aimed at restoring cellular homeostasis increases [7,24].

Numerous experimental and clinical studies of the last 30 years have revealed new biological activities in Timogen®. In particular, the ability of the dipeptide L-Glu-L-Trp to enhance tissue oxygenation in diabetes mellitus due to inhibition of the HIF 1α factor. Patents were obtained for a new biological activity in the USA, Australia, Canada, the European Union and the Russian Federation [20]. This biological activity made it possible to use this peptide drug Timogen® (Epitemp®) in the treatment of such a severe complication of the disease as diabetic foot. The basis for this was successful preclinical studies on the model of soft tissue wounds in rats against the background of streptozotocin-induced diabetes mellitus [3]. The influence of the dipeptide on the activation of tissue oxygenation was assessed using an immunohistochemical study. This method made it possible to analyze the content of the HIF-1α protein in the tissues of the animals of the control and experimental groups.

Against the background of the clinical manifestations of diabetes in all animals of the control and experimental groups, an increase in the expression of the HIF-1α factor was registered. As is known, the activity of the HIF 1α factor increases with a decrease in oxygen tension in the blood and in the tissues of the body. Therefore, a significant accumulation of HIF-1α in tissues indicates tissue ischemia in experimental animals. However, under the action of the dipeptide in the animals of the experimental group, the expression level of the HIF-1α factor was significantly lower than in the control group. The data obtained prove the ability of the L-Glu-L-Trp dipeptide to enhance tissue oxygenation. The positive effect of the dipeptide on oxygenation processes in tissues contributed to faster healing of soft tissue wounds, which led to a reduction in the regeneration period, the appearance of granulation tissue in the wound, the onset of marginal epithelialization or complete epithelialization in animals in the experimental group. Moreover, it should be noted that the rate of complete epithelialization of wounds with the introduction of dipeptide was 3.2 times higher than that in the control group.

However, it should be noted that the cause of the formation of non-healing wounds in patients with diabetes mellitus is also impaired local and general immunological reactivity, leading to the development of chronic inflammation, accompanied by increased secretion of pro-inflammatory cytokines, changes in the activity of matrix metalloproteases (MMPs), and a decrease in the production of growth factors, which in general inhibits the processes of regeneration and wound healing [5]. Therefore, the ability of the L-Glu-L-Trp dipeptide to have a modulating effect on metabolic processes, stimulate the functional activity of immune system cells, have an antioxidant effect, activate the functions of connective tissue cells, endotheliocytes, macrophages and leukocytes in the lesion, and inhibit the production of histamine and serotonin in inflammation, contributed to the stimulation of tissue regeneration processes and accelerated wound healing against the background of diabetes mellitus [Petlenko I.S.].

Successful preclinical studies served as the basis for further clinical use of the medicinal dipeptide L-Glu-L-Trp in the treatment of diabetic foot in patients with non-insulin-dependent diabetes mellitus.

Materials and Methods of Research

A single-center, open, prospective, randomized, controlled study included patients treated at the Medical Center of the St. Petersburg Institute of Bioregulation and Gerontology with a clinical diagnosis of E11.5 “diabetic foot” in patients with non-insulin-dependent diabetes mellitus. Patients were randomized using the envelope method. The exclusion criteria from the study were the following: decompensated diabetes mellitus; trophic wounds involving bone tissue; exacerbation of any somatic, including infectious, diseases that may affect the course of the disease; patients taking corticosteroids; presence of hepatic, renal, cardiovascular, adrenocortical insufficiency, tuberculosis in the active stage, hepatitis, HIV infection, oncological diseases and mental disorders; pregnant and lactating women; patients for whom, according to the physician, any of the procedures in the protocol may pose a risk that outweighs the potential benefit of participating in the study. Patients’ participation in the study was voluntary and was confirmed by signing an informed consent form. Informed consent was signed with each participant of the study in accordance with protocol No. 27 of December 15, 2016, approved by the ethical committee of the St. Petersburg Institute of Bioregulation and Gerontology (St. Petersburg, Russian Federation).

29 patients with non-insulin-dependent diabetes mellitus were under observation. All patients had been diabetic for 5 - 23 years and were between 51 and 82 years of age. At the time of examination, diabetes mellitus was compensated, all patients received hypoglycemic drugs in the dosage they needed.

In all patients, during the examination, trophic wounds were revealed involving the skin, subcutaneous fat, muscle tissue, without damage to the bone tissue. The wounds were clean and not infected. Patients complained of edema, moderate pain in the wound area, fatigue in the leg area. When examining the skin, pigmentation, dryness of the skin, hyperkeratosis, and a significant decrease in tactile sensitivity were noted. All patients underwent a visual assessment of the wound surface, the nature and stage of the wound process were determined, and standard treatment of the wound surface with antiseptic preparations was performed.

Patients were randomly divided into two groups. The control group (14 people) received basic therapy of hypoglycemic drugs and standard treatment of the wound surface, the main group (15 people) in addition to their similar main therapy received dipeptide L-Glu-L-Trp intramuscularly daily at 200.0 mcg 2 times a day for 20 days (8.0 mg per course of treatment).

The impact of the L-Glu-L-Trp dipeptide on the clinical course of the diabetic foot complication of diabetes mellitus, as well as its effect on the level of tissue oxygenation (by the level of the HIF-1a protein concentration in human blood plasma) was assessed twice: at the beginning and on the day after the end of the observation (on the 21st day). The level of HIF-1a was assessed using enzyme-linked immunosorbent assay (ELISA) according to the method of A. Levina., *et al.,* 2009. As an additional control, 10 healthy volunteers without diabetes mellitus participated in the study; on the first day of the study and on day 21, they had venous blood taken to determine the level of HIF-1a protein concentration in blood plasma.

Statistical processing of the results was carried out using the SPSS Statistics 17.0 software. Differences between samples were assessed using Student's parametric t-test. The critical level of significance of the null hypothesis (about the absence of significant differences) was taken equal to 0.01 or 0.05. The arithmetic mean ($\bar{X}$) and standard error of the arithmetic mean (SE) were calculated for each sample. The data in the tables are presented as $\bar{X} \pm SE$.

Research Results

All patients with diabetes mellitus, as can be seen from table 1, had a significantly higher HIF-1a protein concentration in blood plasma, which indicates the presence of tissue ischemia. Initially, there was no significant difference in this indicator in the control and main groups.

Group	HIF-1α (pg/ml)	
	Baseline	Indicator on the 21st day
Healthy volunteers (n= 10)	3,8 ± 0,3	3,9 ± 0,4
Control (n= 14)	6,7 ± 0,7#	7,1 ± 0,5#
Main (n= 15)	6,9 ± 0,5#	4,8 ± 0,4*#

Table 1: *The influence of dipeptide Timogen L-Glu-L-Trp on the level of HIF-1α in blood plasma.*

#p < 0.05 – differences are statistically significant compared to healthy ones.

**p < 0.05 – differences are statistically significant compared to the baseline.*

However, under the action of the dipeptide, a significant decrease by 30% in the concentration of the HIF-1a protein in the blood plasma was found in patients of the main group compared with the control. The data obtained indicate that the dipeptide has the property of enhancing tissue oxygenation in complications of diabetes mellitus, in particular, in diabetic foot.

Signs of clinical manifestations

1. Point - none
2. Points - weakly expressed
3. Points - expressed
4. Points – strongly expressed (pronounced).

As can be seen in table 2, under the action of the dipeptide L-Glu-L-Trp, the clinical manifestations of the diabetic foot syndrome significantly decreased. Improved oxygenation of tissues contributed to the improvement of trophic processes in them, which was reflected in the improvement of the structure of the skin: reduced dryness, restored tactile sensitivity, and reduced fatigue. These processes correlated with the rate of healing of the wound surface in patients of the main group. Thus, by the end of the study, in 73.3% of patients, the improvement in tissue oxygenation processes contributed to the complete epithelialization of the wound surface, which is 5 times higher than in the control group (Table 3).

Clinical manifestations	Control (n = 14)		Main (n = 15)	
	Before Therapy	After Therapy	Before Therapy	After Therapy
Pigmentation	3,3 ± 0,22	3,3 ± 0,22	3,0 ± 0,25	3,0 ± 0,25
Dry skin	3,8 ± 0,33	3,7 ± 0,29	3,9 ± 0,30	2,9 ± 0,29*
Leg fatigue	3,8 ± 0,25	3,8 ± 0,29	3,9 ± 0,31	2,7 ± 0,35*
Tactile sensitivity (reduced)	3,6 ± 0,22	3,7 ± 0,25	3,7 ± 0,22	2,5 ± 0,20*

Table 2: *Clinical manifestations of the diabetic foot syndrome.*

**p < 0.05 - differences are statistically significant compared to the baseline in the control and experimental groups.*

Groups	Number of patients		
	With signs of granulation	With initial signs of epithelialization	With complete epithelialization
Control (n = 14)	5 (35,7%)	7 (50,0%)	2 (14,3)
Main (n = 15)	1 (6,7%)	3 (20,0%)	11 (73,3)

Table 3: *Stage of healing of the wound surface on the 21st day of the study.*

Figure 1 and 2 show a patient's M. clinical example, demonstrating the effect of dipeptide on the healing process of the wound surface.

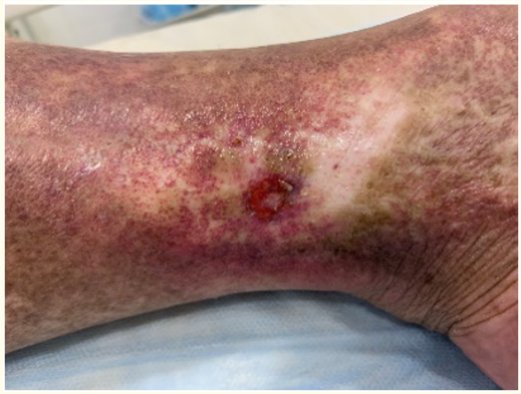

Figure 1: *Clinical manifestations of "diabetic foot" syndrome in patient M. (main group), before treatment.*

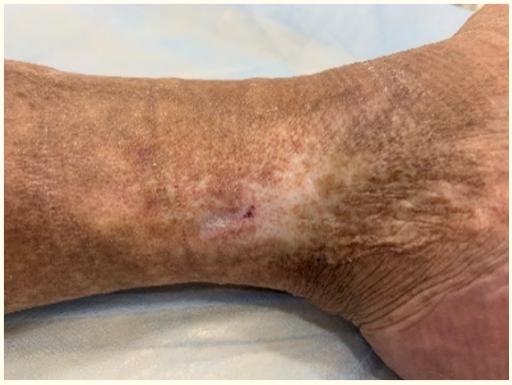

Figure 2: *The healing of the wound surface in the same patient M. (main group), on 21st day of treatment.*

Thus, the dipeptide n L-Glu-L-Trp has a pronounced wound healing property and reduces the period of wound healing in comparison with standard therapy.

Conclusions

As is known, tissue ischemia that accompanies diabetes mellitus negatively affects the course of the wound process, slowing down wound healing, and rehabilitation processes take a long, recurrent form. Therefore, development of pharmacological therapy aimed at activating soft tissue oxygenation by inhibiting the synthesis of the HIF 1α factor is a pathogenetic approach to the treatment of complications of diabetes mellitus.

According to experimental and clinical studies, the use of the dipeptide L-Glu-L-Trp, due to its ability to restore oxygenation processes in tissues, contributes to faster rehabilitation of patients with diabetic foot.

Thus, the use of the dipeptide bioregulator L-Glu-L-Trp promotes the activation of reparative processes in ischemic tissues of patients with diabetic foot syndrome due to inhibition of the synthesis of the HIF 1α factor and, as a result, increased oxygenation of soft tissues, which makes the drug promising for various diseases and conditions accompanied by ischemia and impaired oxygenation.

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