

Influence of Preparation Nuclex on the Cytokine Profile of the Patients with Diabetes Type 2 and Neuropathic Form of Diabetic Foot

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Abstract Its objective was to study the effectiveness of the drug Nuclex in the treatment of patients with diabetes and neuropathic form of diabetic foot (NFDF). The studies included 32 patients with DT 2 and NFDF. 16 patients received 2 capsules of Nuclex through 3 times a day for 21 days. To address this goal were used the following methods: general clinical examination, biochemical methods (insulin level in the blood), instrumental methods (Doppler ultrasonography, vascular flowmetry) and ELISA (TNF α , IL-1 β , IL-2, IL-4, IL-6, IL-10). Usage of Nuclex for treatment of patients with diabetes and NFDF is accompanied by a reduction of proinflammatory cytokines on a background of moderate reduction of inflammatory cytokines, which correlates with positive clinical dynamics and tendency of decreasing the insulin daily dose. It is rather prospective to continue to study the distinct mechanisms of Nuclex efficiency in the regulation of expression of genes of insulin system and cytokine cooperation.

Keywords Diabetes, Diabetic Foot Syndrome, Nuclex, Cytokines, Insulin, Treatment

1. Introduction

According to WHO, nowadays over 120 million people are ill with diabetes and every 12-15 years the number doubles[1]. The most frequent complication of diabetes is the development of suppurative – necrotic lesions (SNL) of the lower extremities[2-4]. High susceptibility of diabetic patients to the development of SNL is caused by the symptom complex of the anatomic – functional changes, connected with diabetic micro-, macroangiopathy, neuropathy, and osteoarthropathy, and it is called diabetic foot (DF) syndrome[5-8]. However, it may sound paradoxically, but it is a fact that the correction of metabolism, strict glycemia control and the usage of different means and methods, which influence the normalization of microcirculation, haven't made a significant impact on the prevention of suppurative – necrotic complications (SNC), which are presented in the category of such patients in general, and especially after amputations of the extremities. The usage of antibiotics to prevent SNC and to stimulate the healing process of postoperative wounds has fallen short of expectations as well [9]. Moreover, many researchers have mentioned the decreasing of general immunological resistance of the

organism under the influence of antibiotics, which causes the increasing of percentage of suppuration of postoperative wounds of the patients with diabetes[3]. The problem of choosing the best methods of treatment the patients with diabetes and SNL of feet is rather relevant and not sufficiently studied[11].

Terms of treatment the patients with such diseases are 1, 5-2 times longer, then the recovery time of the patients with the same process, but without diabetes, which is caused not only by the abnormalities of all kinds of metabolism, but also by immunological abnormalities[12]. SNL of feet in case diabetes should be based not only on the active drainage of the wound along with the usage of different antibiotics, but also on the usage of the medical preparations, which have a positive influence on the patient's immune system[13-15]. It helps to renew the absorbing and the bactericidal functions of the neutrophils, the activity of the enzymatic systems of the phagocytes, complement titer and the level of the circulating immune complexes. It also helps to normalize cellular and humoral immunity. The immunoactive drugs is perspective[16, 17]. Our attention was attracted by the modern medicine – Nuclex, which shows cardiovascular and specific antiviral activity, which is based on the mechanism of immunomodulation, anti-inflammatory effect and on the influence on the conformation of surface antigens and receptors of the viruses[18-23]. This medicine is used during the complex therapy of the cardiovascular diseases, chronic viral diseases and secondary immunodeficiencies[24-26].

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The purpose of this research is to study the effectiveness of the drug Nuclex in the treatment of patients with diabetes type 2 (DT 2) and NFDF and Nuclex influence on the indexes of cytokine profile of blood.

2. Materials and Methods

This clinical study was held as an open, controlled, comparative, randomized, parallel. The study involved 32 patients (men - 8 women - 24) aged from 40 to 65 years (average age 46.3 ± 1.2 years) suffering from diabetes and NFDF. The depth of spreading corresponded the II degree, according to the Wagner's classification[23]. All the patients were undergoing hospital treatment in the surgical department of Lugansk multi – field hospital № 2 (the base of general surgery department of Lugansk state medical university). Patients were divided into 2 groups (basic and comparison) in a ratio of 1:1. Dividing into groups was performed randomly. The scheme of randomized dividing was formed on the basis of random numbers, generated by MS Excel function of random numbers. All the patients had clinical, biochemical and instrumental examination. The state of peripheral arterial blood flow was assessed according to the data received by means of ultrasonic Doppler auscultation (Logidop-2 Kranzbühler, Germany) along with the counting of the ankle-brachial index (ABI). The intensity of microcirculation was measured by laser flowmeter BZF-21 Transonic Systems Inc. (USA), using type R sensor - for surface measurements. The patients in both groups were receiving basic therapy during their treatment. Taking into account the presence of purulent wounds, all the patients during their hospital treatment were transferred to the introduction of simple insulin (Aktropid, 5 times a day). The dosage depended on the level of glycemia. In addition, the patients of the basic group additionally received Nuclex (2 capsules 3 times a day for 21 days). Under testing medicine "Nuclex" contains ribonucleic acid – 250 mg, the excipient is mannitol. Manufacturer: LLC «Pharma Start», Ukraine, PLC «Kiev medpreparat», Ukraine[18]. All the supervised patients had special tests, including the study of the concentration of pro-and anti-inflammatory cytokines in the patient's blood serum, using enzyme-linked immunosorbent assay (ELISA) with the help of equipment manufactured by Sanofi Diagnostics Pasteur (France), including immunoenzyme analyzer PR 2100. The concentration of cytokines (TNF α , IL-1 β , IL-2, IL-4, IL-6, IL-10)[27-29] in blood was identified by certified reagents produced by LLC "Proteinovyy contour" (ProCon) (Russia - St. Petersburg) [30]. To rate the efficiency during the analysis of this experiment the following criteria were used:

- Positive changes of the results according to the clinical examination (disappearance of local and general signs of disease);
- Positive changes of the results according to the laboratory testing (includes general clinical laboratory tests and positive changes of the immune status).

Statistical analysis of the results of the research was carried out using PC Intel Core 2 Duo 3, 0 with the help of multivariate dispersive analysis of the licensed software packages Microsoft Office 2007, Microsoft Excel Stadia 6.1/prof i Statistica[31]. The basic principles of the usage of statistical methods during the clinical testing of medical preparations were taken into account too[32].

3. Results and Discussion

During the patients' undergoing of the hospital therapy they were complaining about the swelling of their feet and legs, the presence of SNC on the feet, general weakness, the absence of pronounced pain syndrome, feet's functions disorders. 15% of the patients were suffering from diabetes less than 5 years, 19% - from 5 to 10 years, 19% from 10 to 20 years, 47% - more than 20 years. Suppurative – necrotic feet's changes had round or oval shape, different size and were located on the plantar surface (12 cases) and on the dorsal surfaces of toes, very seldom in the place of metatarsal joints. There was an inflammation around the wounds, the width of inflammation was 1.5 - 2 cm, and there were no signs of granulation and epithelialization in the wounds. In 6 cases there were some signs of reactive inflammation of the feet with a pronounced hyperemia of the foot's tissues and lymphadenitis in the area of the patients' shin. All the patients were overweight (body mass index, BMI – 31.2 ± 0.8 kg/m², normal index - 19-25 kg/m²). The level of the compensation of carbohydrate metabolism was assessed by glycosylated hemoglobin content (HbA1c) $8.7 \pm 0.2\%$, normal content - 6.4%. 60% of the patients were diagnosed with diabetic retinopathy. Average systolic blood pressure was 160 ± 4 mm Hg. According to the results of instrumental examinations, 29 patients in both groups had high rates of ankle-brachial index (ABI) (> 1.2); it was the evidence of medial calcification. During the researches of arterial pressure in the posterior tibial artery, in both groups of the patients the average result was 05 ± 10 mmHg and it didn't change significantly in the end of treatment. Average indexes of microcirculation according to the LDF in the patients' feet were the following: 0.77 ± 0.07 ml. per min/100g. of tissue, the first finger - 0.66 ± 0.07 ml. per min./100g. of tissue. It should be mentioned that we did not measure the level of microcirculation when the reactive inflammation of the foot and the inflammation of the wounds in the area of the first toe were present. The indexes of the level of microcirculation in the end of the treatment in both groups of the patients did not change significantly and were the following: on the foot – 0.85 ± 0.09 ml. per min/100g. of tissue, on the first toe – 0.72 ± 0.07 ml. per min/100g.

Assessing the local status, we mentioned that after the start of treatment in the group, where the patients were receiving Nuclex, there was much more rapid improvement in the area of SNCs on the foot. Thus, on the 12-14th day after the start of treatment, we observed the reducing of fibrin in the wound and the formation of the initial granulation in the basic group.

At the same time, the patients from the second group had the same improvements on the 15-16th day. The macroscopic data was confirmed by histological studies, which noted a concentration of mast cells with the subsequent decreasing of macrophages and fibroblasts. Moreover, the patients who received basic therapy along with Nuclex had a smaller inflammatory area before the process of the granulation, the width was less than 0.5 cm, while the patient from the second group had the inflammatory area with the width of more than

0, 5 cm. On the 4 – 6th day of the treatment the patients, who received Nuclex had the decreasing or practically had no signs of reactive inflammation of the feet and the manifestations of lymphadenitis in the area of the patients' shin. As a result of special immunological studies, it was found the presence of some abnormalities in the parameters of cytokine profile which had been of the same type before the starting of the treatment in both groups.

Table 1. Indexes of cytokine profile of the patients before treatment (M ± m)

Parameters	Normal index	Groups of the patients		P
		First group (n=16)	Second group (n=16)	
TNFα, pg / ml	39.6±2.2	73.6±2.3***	72.7±2.1***	>0.05
IL-1β, pg / ml	18.8±1.2	40.1±1.5***	39.7±1.7***	>0.05
IL-2, pg / ml	20.8±2.1	43.6±2.2***	43.0±2.0***	>0.05
IL-4, pg / ml	47.2±1.6	56.5±1.3*	56.0±1.3*	>0.05
IL-6, pg / ml	22.7±1.6	40.2±1.7***	39.9±2.0***	>0.05
IL-10, pg / ml	17.2±1.3	25.2±1.4**	25.0±1.1**	>0.05
IL-1β/IL-10	1.09±0.04	1.59±0.06**	1.59±0.04**	>0.05
TNFα/IL-10	2.3±0.03	2.92±0.05*	2.91±0.09*	>0.05
IL-2/IL-4	0.44±0.03	0.77±0.04***	0.77±0.05***	>0.05

Notes. The accuracy of the difference according to the normal index in the 1st and second tables: if P < 0.05, ** - P < 0.01, *** - p < 0.001; column P – the probability of differences between the indices of the first and the second group

Table 2. Indexes of cytokine profile of the patients at the end of treatment (M ± m)

Parameters	Normal index	Groups of the patients		P
		First group (n=16)	Second group (n=16)	
TNFα, pg / ml	39.6±2.2	41.1±1.7	62.9±1.9***	<0.001
IL-1β, pg / ml	18.8±1.2	19.4±1.3	35.1±1.7***	<0.001
IL-2, pg / ml	20.8±2.1	21.4±1.9	35.2±2.0***	<0.001
IL-4, pg / ml	47.2±1.6	47.8±1.5	54.4±1.7*	<0.05
IL-6, pg / ml	22.7±1.6	23.0±1.5	33.7±1.4**	<0.01
IL-10, pg / ml	17.2±1.3	17.7±1.2	23.4±1.1*	<0.01
IL-1β/IL-10	1.09±0.04	1.1±0.03	1.50±0.03*	<0.01
TNFα/IL-10	2.3±0.03	2.32±0.04	2.69±0.04*	<0.05
IL-2/IL-4	0.44±0.03	0.45±0.04	0.65±0.02**	<0.01

Table 3. The dynamics of daily dose of insulin of the patients from the basic group (M ± m)

The severity of diabetes	Number of patients	The initial dosage of insulin	The dosage of insulin on the seventh day	The dosage of insulin on the 14 th day
1 type	3 (18.75%)	18±1.25*	14±1.15*	-
2 type	11 (68.75%)	36±1.56**	30±1.44**	28±1.32**
3 type	2 (12.5%)	56±2***	48±1.6***	44±1.4***

Note: reliability * - if P < 0.01, ** - P < 0.05, *** - P > 0.05

The above abnormalities were characterized by a significant increase of the level of pro-inflammatory cytokines along with a slight increase in the concentration of inflammatory cytokines. There were no significant differences between the content of the studied indices of CC in the serum of the patient's blood in two groups. Thus, the level of pro-inflammatory cytokine – TNF α in the first (basic) group was higher than the normal index in 1.86 times ($P < 0.001$), it reached an average index of (73.6 ± 2.3) pg / ml, the second group indexes were the following: higher than the normal index in 1.84 times ($P < 0.001$) with the average index of (72.7 ± 2.1) pg / ml. The level of another pro-inflammatory cytokine IL-1 β in the basic group in the beginning of the treatment was higher than the normal index in 2.13 times ($P < 0.001$) (40.1 ± 1.5) pg / ml. and in the second group - in 2.11 times ($P < 0.001$). with the average index of (39.7 ± 1.7) pg / ml. The concentration of IL-6 was increased in 1.77 times according to the normal index in the basic group and was equal to (40.2 ± 1.7) pg / ml, while in the second group it was increased in 1.76 times and was equal to (39.9 ± 2.0) pg / ml ($P < 0.001$). The concentration of IL-2 in the serum of the patients from the second group before the treatment was 43.6 ± 2.2 pg / ml, which was higher than the normal index in 2.1 times ($P < 0.001$). In the second group this index increased the normal one in 2.06 times ($P < 0.001$) and the average 43.0 ± 2.0 pg / ml. Before the treatment. the concentration of anti-inflammatory IL-4 in serum of the patients from the basic group was higher than the normal index in 1.2 times ($P < 0.05$) and was the following: (56.5 ± 1.3) pg / ml. In the second group the concentration was in 1.2 times higher than the normal index too ($P < 0.05$). the average index was the following: (56.0 ± 1.3) pg / ml. The concentration of IL-10 during the period of treatment was increased in 1.47 times according to the normal index in the basic group. in the second group the concentration was increased in 1.45 times ($P < 0.01$). The average indexes were the following: 25.2 ± 1.4 pg / ml (25.0 ± 1.1) pg / ml. Before the treatment. the indexes, which show the ratio between cytokines with pro-inflammatory and anti-inflammatory activity, were increased. Thus, indexes IL-1 β /IL-10 in both groups were higher than the normal index in 1.46 times ($P < 0.01$), TNF α /IL-10 - in 1.27 times ($P < 0.05$), IL-2/IL-4 - in 1.75 times ($P < 0.01$). After re-examination of the concentration of cytokines in the patient's serum. it was found that, in the end of the treatment of the patients from the basic group, who were receiving Nuclex, there were positive changes. ELISA showed the decreasing of pro-inflammatory cytokines along with the reduction of inflammatory cytokines, that's why indexes IL-1 β /IL-10 and TNF α /IL-10 raised to the upper bound of the normal condition (table 2).

The patients from the second group only had a slight tendency of normalization of the indexes of cytokine, that's why the indexes from this group were different from the normal indexes and the indexes of the patients from the basic group. Thus, the concentration of IL-1 β in the patient's serum from the second group in the end of the therapy was (35.1 ± 1.7) pg / ml, but it remained in 1.87 times higher than

normal index ($P < 0.001$), the concentration of TNF α was (62.9 ± 1.9) pg / ml and in 1.59 times higher than normal index ($P < 0.001$), the concentration of IL-2 decreased to (35.2 ± 2.0) pg / ml, at the same time it increased normal index in 1.69 times ($P < 0.001$), the concentration of IL-4 was higher in 1.15 times ($P < 0.05$), the concentration of IL-6 was equal to (33.7 ± 1.4) pg / ml, and was higher than normal index in 1.48 times ($P < 0.01$), the concentration of IL-10 was (23.4 ± 1.1) pg / ml, and was in 1.36 times higher than normal ($P < 0.05$), IL-1 β /IL-10 indexes were in 1.38 times higher than normal index ($P < 0.05$), TNF α /IL-10 was in 1.16 times higher than normal index ($P < 0.05$), IL-2/IL-4 - in 1.48 times higher than normal index ($P < 0.05$). Thus, as we can see from the data, despite positive changes, the patients from the second group didn't have the normalization of tested parameters. It is known that Nuclex has immunomodulatory activity. It normalizes the level of T- and B-cells with reduced content, their functional activity, stimulates the production of T-helper and T-suppressor cells, eliminates the imbalance of lymphocytes, normalizes the concentration of antigen-reactive cells, serum immunoglobulin classes G, A, M, reduces the number of zero lymphocytes, induces the production of endogenous interferon [18]. Nuclex increases the phagocytic activity of peritoneal phagocytes and leukocytes, activates poly- and mononuclear cells, increases chemotaxis. Along with the immunomodulatory activity Nuclex has anti-inflammatory properties, inhibits oxidative processes in cell membranes, stabilizes cell membranes, improves oxygen-hydrogen processes in tissues [18]. Described immunomodulatory and anti-inflammatory properties of Nuclex were clearly seen during the research of cytokines profile of the patients from the basic group. The reduction of pro-inflammatory cytokines along with moderate decreasing of inflammatory cytokines were clearly observed. These figures correlate with clinical manifestations of recovery, which couldn't be found on the basis of the second group. Ribonucleotide preparations are recommended for healing wounds by many famous international patents, but the wounds which are described in these works are cutting wounds, which are not accompanied by the complications, which can occur during diabetes. Thus, the ability of ribonucleotide preparation Nuclex to regulate cytokine profile of patients with diabetes, which was discovered by our research, reveals some features of the mechanism of anti-inflammatory action of the preparation and its influence on the general system of inborn protection, which undoubtedly includes cytokines [3]. If in case of the treatment of the controlled group of patients we don't see normalization of cytokine profile, it means that the preparations don't influence the general system of inborn protection and as the result, the clinical recovery is less obvious.

During the treatment we drew attention on the fact that in the basic group, patients who were receiving Nuclex along with the basic therapy (2 capsules 3 times a day for 21 days), had a tendency of decreasing the daily dose of insulin. The data is presented in the third table.

As it is seen from the third table 3 patients (18.75%) were diagnosed with the 1 type of diabetes, 11(68.75%) with the second type and 2 (12.5%) with the third type[8]. In one week the patients with the first type of diabetes had the reduction of dosage of insulin $14 \pm 1,15$ ($P < 0.01$) units per day, in two weeks insulin was replaced with hypoglycemic preparations. In case of the 2 type the dosage was decreased by 6 units and was the following: $30 \pm 1,44$ ($P < 0.01$) units. In 2 weeks it was decreased by 8 units $28 \pm 1,32$ ($P < 0,01$) units per day. In case of the third type the reduction was the following: in 1 week the dosage was decreased to 8 units $8 \pm 1,6$ ($P > 0,05$), in 2 weeks the dosage was decreased to 10 units $44 \pm 1,4$. It is known that, in case of diabetes adrenergic and cholinergic regulation of heart, coronary vessels and the system of blood circulation can change. During the experiments on animals, provided the integrity of organism and the preservation of natural breathing and blood circulation in the chest cavity, it was shown that the injection of ribonucleotide preparations, which were the substances for Nuclex, caused dose-dependent dilatation of coronary vessels[33].

Nuclex either single – injected or its infusion into the coronary perfusion flow can enhance potential opportunities of adaptive and protective mechanisms of adrenergic and cholinergic regulation of the heart's activity. It can also enhance coronary and systemic circulation, which are realized with the participation of the sympathetic and parasympathetic nervous systems[33].

Moreover, it is known that in case of some abnormalities of endothelium-dependent mechanisms of blood regulation which can occur in case of diabetes, insulin has a negative influence on vasodilatation. Similar situation can be seen in case of nitric oxide system blocking[34].

This negative influence of insulin on coronary blood flow, which can be observed after blocking NO-synthetase by its inhibitor L-NAME, can be easily removed by yeast RNA containing preparations. The results of research are designed to search for the mechanisms of influence of RNA preparations on cardiovascular system. We can say that nitric oxide system takes part in the process of vaso- and cardiotropic actions of RNA preparations. In case of RNA preparations intracoronary infusion there is an increasing of cholinergic receptors' sensitivity to their stimulation with acetylcholine[35].

The ability of yeast RNA containing preparations, which is the substance of Nuclex, to change the conformation of proteins and receptor is shown for other body systems too[36].

In our view, the reduction of daily insulin dose can be caused by Nuclex influence on conformation of proteins and receptors, involved in the regulation of insulin. There seems to be a participation of adrenergic and cholinergic systems and the system of nitric oxide regulation in Nuclex mechanism of influence on the cardiovascular system and the realization of its vaso- cardiotropic actions in case of diabetes.

However, the explanation of distinct mechanisms of

Nuclex influence on the course of diabetes is possible with further study of signaling systems such as "PI3 and ERK" which take part in the regulation of expression of genes of insulin system. This aspect will be studied in the near future.

Thus, this data shows a positive influence of modern preparation Nuclex on cytokine profile, which is accompanied by more distinct positive clinical symptomatology of the patient from the basic group.

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