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Efficiency of pyrimidine nucleotides in the complex treatment of patients with type 2 diabetes mellitus complicated by diabetic polyneuropathy

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Abstract
This article represents and analyzes the data from a study of the immune status and the role of immune factors in the progression of polyneuropathy in patients with type 2 diabetes mellitus. Certain peculiarities of the disorders of the immune status of the studied patients depending on the severity of disability were revealed: activation of autoimmune processes, increased immunoreactivity, marked reduction of NK-natural killer cells (CD16+), which would cause a decrease in the stability of examined patients to infections. The relationship between electrophysiological markers of the functional state of myelin and some parameters of the immune system are determined. The most sensitive data are tibial nerve characteristics and in most cases – the residual latency period, that is the functional state index of myelin of the most dystal nerves' segments. It was found that the progression of the polyneuropathy severity is accompanied by the secondary demyelination which is a result of the deepening axonopathy process and immune disorders. Therefore, disorders of immune homeostasis can be considered as one of the pathogenetic links of occurrence and progression of polyneuropathy. Also the complex impact on the course of polyneuropathy in patients with diabetes mellitus type 2 medication containing pyrimidine nucleotides (Keltican) was analyzed. This medication applied during one month leads to acceleration of myelination of peripheral nerve fibers and promotes their regeneration.

Keywords: Type 2 diabetes, diabetic polyneuropathy, electron euromyo graphic study, immune homeostasis imbalance

1. Introduction
Diabetic polyneuropathy (DPN) is one of the most common, serious complications of diabetes mellitus (DM) and is considered one of the most important medical-social problems of modern neurology [4]. It is known that if there is DPN in patients with type 2 diabetes mellitus, it continues to progress despite adequate glycemic control and exclusion of risk factors [3]. Pathogenesis of DPN is multifactorial, is not completely found out, it continues to be learnt. Currently, the model of diabetic neuropathy is a multifactorial and multistage process involving a cascade of pathogenetic mechanisms trigger mechanism of which is chronic hyperglycemia that activates polyol pathway of glucose utilization, which leads to the accumulation of sorbitol, fructose in the nervous tissue, and hexosamine pathway of its utilization with the accumulation of intermediate products of glucose metabolism, the formation of a large number of terminal products of excessive glycosylation of proteins and destruction of mitochondrial DNA with one of the most active free radicals – superoxide. This oxidative stress, the cause of which in DM is the formation of free radicals excess on the background of the weakness of their own antioxidant system, responsible for the development of disorders of glucose metabolism. Excess of superoxide even without initiation of alternative ways of glucose utilization may be the only factor of dramatic inhibition of endothelial enzymes and may lead to violations of endothelium-dependent responses and functions structures of nerve cells. There is a theory that points to the role of a genetic defect that is in the high sensitivity of peripheral tissues to hyperglycemia, the factors of natural aging are also significant. According to various studies about the factors that contribute to the development of DPN, there are also patient's age, duration of DM, the level of glycosylated hemoglobin (HbA_{1c}) [1, 5, 6, 10, 12]. In addition, recently there is an emphasis on the role of immune factor in the progression of the peripheral nervous system damage [4, 9]. It is believed that due to metabolic disorders, including diabetes, there are phenomena of axonal lesion of peripheral nerves [7, 10]. But most authors indicate the mixed type of nerve lesions, which are the phenomena of not only axonopathy but also myelinopathy [6, 8, 10]. Demyelination may be a secondary process that is not specific and is observed in the progression of axonal

nerve damage of any genesis, and as it is known, immune disorders may also contribute to demyelination of peripheral nerve fibers [1, 2]. It is known that phenomena of myelinopathy are characterized by very active recovery compared with axonal lesion [1, 9]. Currently, there are few active substances that affect the physiological regeneration of peripheral nerves. These active ingredients can include pyrimidine nucleotides (uridine monophosphate and cytidine monophosphate). These substances play an important role in the synthesis of phospholipids and glycolipids of neuron membranes, affecting the synthesis of myelin membranes and accelerate the regeneration of nerve fibers [11, 12].

In our opinion, DPN therapeutic strategy in this case would provide a comprehensive correction of damaged nerves using the means of neurotrophic part of medicine of pyrimidine nucleotides.

The aim of the study was to assess the potential impact of immune homeostasis imbalance in the pathogenesis of myelin damage in patients with diabetic polyneuropathy and to observe the pyrimidine nucleotides' medication efficiency in process of nerves' myelination.

Materials and Methods

We observed 120 patients with type DM type 2 complicated by DPN. The average age was 49.18±1.86 years. Glycated hemoglobin level was 9.26±0.64%, indicating the decompensation of DM. The study included patients with symptomatic stage of DPN: IIA, IIB and III stage according to classification of P.J. Dyck et P.K. Tomas (1999) [5]. IIA stage of DPN was diagnosed in 52 (37.7%) patients, 24 (46.2%) of them are women, 28 (53.8%) – men; IIB – 28 (20.3%) patients: 11 (39.3%) women, 17 (60.7%) men; III stage of DPN was found in 40 (29.0%) patients: 18 (45%) women, 22 (55%) men. For comparison of the received results there were taken 20 practically healthy individuals (PHI) that was the control group. Patients were performed the general clinical examination to exclude other causes of peripheral nerve lesions and clinical neurological examination. Electroneuromyographic (ENMG) study of peroneal and tibial nerves was performed on computer dual channel electroneuromyographic "Neyro-EMG-Mikro" ("Neyrosoft", RF) three times on both legs with the averaging parameters. To assess the functional status of axons the amplitude of the potential maximum motor response was studied – M-response (mV) to stimulation of nerve in distal point, to identify the functional state of myelin – residual latency period (RL) (m/s) and conduction velocity of arousal (CVA) (m/s) nerves. Immune status was determined by the following characteristics: immunocompetent cells: CD8+ – T-suppressors, CD3+ – T-mature lymphocytes, CD4+ – T-helper cells, CD16+ – NK-natural killer cells, CD19+ – B-cells, –were determined by immunofluorescence set of monoclonal and polyclonal antibodies of company "Sorbent" (c. Podolsk, OKPO 13180653); levels of immunoglobulins IgM, IgG by method of radial immunodiffusion in gel (RID) according to Mancini (FUP "NPO" Mikrogen", RF). Immunoregulatory index was calculated (IRI): CD4+/CD8+.

In order to study the effectiveness of the proposed treatment, the examined patients were divided into two clinical groups: I group (n=36) – patients who were used basic therapy; II group (n=30) – in combination with basic therapy patients were taking the drug, which contains a set of pyrimidine nucleotides: cytidine-5-monophosphate disodium salt – 5 mg, uridine-5- trisodium triphosphate, uridine-5- disodium diphosphate, uridine- 5-disodium mono phosphate – 1.33 mg 1 capsule orally 3 times daily for 30 days. The inclusion of patient into the group of patients receiving a particular treatment was performed by the method of "sequence numbers" using a table of random numbers. Examination was performed at admission, discharge and 3 months after the treatment.

Statistical analysis of the received results was performed using statistical methods of analysis with STATISTICA (StatSoft, Inc.) and MS Excel. There were used parametric and nonparametric methods: arithmetic mean value was calculated (M), standard error of the mean (m), the significance level (p) of verification of statistical hypotheses. Correlation analysis was performed using Spearman correlation coefficient.

Results and Discussions

The study found that in patients with stage IIA of DPN the likely decrease in the amplitude of M-response was noted in the study of peroneal and tibial nerves compared with the data of practically healthy persons (PHI) to 41.1% ($p<0.05$) and 41 28% ($p<0.05$), respectively. Along there were observed significantly altered indicators of markers of the functional state of myelin. Thus, the rate of residual latency period (RL) was extended as to the normative data to 92% ($p<0.05$) and 81.2% ($p<0.05$), and the conduction velocity of arousal (CVA) by the nerves decreased by 15.4% ($p<0.05$) and 16.5% ($p<0.05$), respectively. The received data show that damage of motor fibers of nerves in patients with type 2 diabetes complicated by IIA stage of DPN, was characterized by mixed character: along with the damage of axon there were signs of demyelination in not only the most distal nerve fibers, as was evidenced by elongation of RL but also in proximal areas, as was indicated by slowdown of CVA. Moreover, signs of myelin damage in this cohort of patients were observed with the same intensity on both motor nerves of the lower extremities.

All figures of ENMG testing of the nerves of the lower extremities in patients with type 2 DM complicated with DPN stage IIB, differed from the data of the control group ($p<0.05$), and parameters of RL and CVA in the study of peroneal nerves varied significantly compared to patients with stage IIA polyneuropathy ($p<0.05$), along with a small change in the amplitude of the M-response ($p>0.05$). In addition, the rate of CVA by peroneal nerves was significantly lower in comparison to that in III stage of DPN ($p<0.05$). These pronounced changes in the functional state of myelin of peroneal nerves in this cohort of patients actually reflect the strength of extensors of the foot and toes and determine the degree of paresis of innervated by them muscles.

In patients with type 2 diabetes complicated by DPN of III stage, a marked progression of peripheral nerves: all indicators of ENMG study were significantly worsened compared to data of patients with DPN stage IIA, and some – with the results of tests of nerves in the stage IIB. Compared to the parameters of PHI amplitude of M-response in III stage of DPN decreased by 23.2% ($p<0.05$) and 28.4% ($p<0.05$), RL elongated more than twice ($p<0.001$), and CVA decelerated to 73.6% ($p<0.05$) and 74.8% ($p<0.0$), respectively, indicating a mixed type of lesion of peripheral nerves of the legs in type 2 DM with prevalence of axonopathy (Table 1).

Table 1: Electron euromyo graphic indices of the function of peroneal and tibial nerve in patients with type 2 diabetes mellitus on stage of polyneuropathy (M±m)

ENMG indices	PHI, n=20	ENMG mean of indices in patients with DPN		
		IIA stage, n=52	IIБ stage, n=28	III stage, n=40
Peroneal nerves				
Amplitude of M-response, мV	6.08±0.45	3.58±0.11 *	2.08±0.21 *	1.41±0.08 */**/***
Residual latency period, мs	1.93±0.07	3.72±0.12 *	4.39±0.11 */**	4.58±0.13 */**
CVA movable fibers of the nerve, м/s	48.38±0.74	40.95±0.31 *	33.78±0.39 */**	35.62±0.34 */**/***
Tibial nerves				
Amplitude of M-response, мV	6.76±0.38	3.97±0.13 *	2.52±0.23 *	1.92±0.13 */**/***
Residual latency period, мs	1.97±0.04	3.57±0.06 *	3.89±0.05 *	4.27±0.05 */**
CVA movable fibers of the nerve, м/s	48.70±0.55	40.67±0.32 *	39.07±0.39 *	36.44±0.32 */**/***

Notes: 1. * – the difference is reliable concerning practically healthy individuals ($p<0.05$);
2. ** – the difference is reliable concerning patients with DPN stage IIA ($p<0.05$);
3. *** – the difference is reliable concerning patients with DPN stage IIB ($p<0.05$).

Thus, assessing the dynamics of changes in the parameters of ENMG according to the degree of severity of DPN in patients with type 2 diabetes, it should be noted that ENMG peculiarity in IIA and III stages were signs of mixed lesions of motor trunks of the nerves with dominated axonopathy, and in patients with IIB stage in parallel with the deepening of phenomena of axonal lesions, heavy damage of myelin, more pronounced in the peroneal nerves, was observed.

When analyzing the results of immunological study, the breach in the system of cellular and humoral immunity compared to the PHI, was determined. Significant difference of the contents of T-helper cells (CD4+) in all studied patients compared with the control group was absent ($p>0.05$). At the same time the reliable reduction of T-suppressors (CD8+) in stage IIB and III of DPN compared with PHI ($p<0.05$) and patients with stage IIA of DPN ($p<0.05$) with probable intergroup difference in IIB ($p<0.05$) and in the third stage ($p<0.05$), was determined. This may lead to an increase in functional activity of B-cells that will be manifested in increasing the synthesis of Ig, with the progression of peripheral nerve dysfunction in patients with type 2 DM. In determining CD16+ – NK-natural killer cells, a reduction in the content of this subpopulation of lymphocytes compared with PHI was found: up to 12.21±0.47% in IIA ($p<0.05$), 11.78±0.67% in IIB ($p<0.05$) and 10.03±0.38% in stage III of DPN ($p<0.05$); the difference was reliable compared with parameters of patients with different stages of DPN ($p<0.05$). High contents of CD19+ – B-lymphocytes was established in patients with stage IIA up to 16.02±0.17% ($p<0.05$), with stage IIB up to 18.11±0.83% ($p<0.05$) and with stage III up to 22.16±0.55% ($p<0.05$), indicating the active production of antibodies. Changes of helper-suppressor ratio are reflected in the significant increase of immunoregulatory index ($p<0.05$) as a measure of activity of the process that took place with the progression of DPN.

Table 2: The analysis of parameters of cellular and humoral immunity in patients with type 2 DM complicated by DPN, %

Parameters	PHI, n=20	Stages of DPN		
		IIA, n=52	IIB, n=28	III, n=40
CD3+	59.16±0.42	51.18±1.36*	50.86±0.38*	49.15±1.08*/**
CD4+	40.10±1.13	41.76±1.02	43.47±1.16	44.81±1.23*
CD8+	27.82±0.52	28.33±0.93	22.12±1.18*/**	21.08±0.46 */**
CD16+	16.54±0.77	12.21±0.83*	10.08±0.47*	10.03±0.38*/**
CD20+	10.34±0.36	16.02±0.17*	18.11±0.83*/**	22.16±0.55*/**
IRI	1.44±0.13	1.47±0.11	1.89±0.08*/**	1.92±0.13*/**
Ig M, g/l	1.47±0.35	1.49±0.95	1.38±0.23	1.51±0.98
Ig G, g/l	10.23±0.58	19.61±1.04*	22.58±1.06*/**	25.67±0.56*/**/***

Note: 1. * – possible compared to PHI ($p<0.05$);
2. ** –difference is probable compared with the parameter of patients with stage IIA of DPN ($p<0.05$);
3. *** – the difference is possible compared with the parameter of patients with stage IIB of DPN ($p<0.05$).

Compared to controls it was observed a significant increase of IgG contents up to 20.82±1.06 g/l in stage IIA ($p<0.05$), 23.81±1.12 g/l in IIB ($p<0.05$) and 25.75±0.66 g/l in III stage of DPN ($p<0.05$) and tendency to the increase of IgM up to 1.55±083 g/l in IIA ($p>0.05$) and 1.82±0.64 g/l in III stage of DPN ($p>0.05$).

As it was detected that patients with type 2 DM are characterized by deviation of all parts of the immune system with the progression of the degree of destruction of peripheral nerves; to determine the influence of the immune status of that cohort of patients on the degree of nerve fibers damage we have performed a correlation analysis between data of ENMG – indicators of functional status of myelin and indicators of the immune system.

Correlation between markers of functional status of myelin of CVA and RL and some indicators of the immune system was determined: between CVA and CD19+ (r= - 0,72, p=0,003), CD8+ (r=0,65, p=0,04), IRI (r= - 0,73, p=0,016); between RL and CD19+ (r= 0,75, p=0,001), CD8+ (r - =0,84, p=0,002), IRI (r=0,87, p=0,001). The most sensitive were characteristics of ENMG of tibial nerves, in most cases – RL, that is, indicator of functional status of myelin of the most distal nerve segments. This fact shows that in patients with type 2 DM complicated by DPN, myelinopathy accompanying axonal lesions of peripheral nerves, is also the result of immune dysfunction in these patients.

Thus, in patients with type 2 DM complicated by symptomatic stage of DPN besides secondary myelinopathy that is usually observed with progression of axonopathy, there is also a destruction of myelin due to the immune disorders. The determined immune-pathological and electroneuromyographic shifts can be used for the development of pathogenic therapy.

As a result of performed complex treatment with the use of medicine of pyrimidine nucleotides most significant was its effect on the dynamics of ENMG study, although not all indicators of the studied nerves varied uniformly and equally (Table 3). Thus, in the study of peroneal and tibial nerves against the background of both the basic and the proposed treatment there was a tendency to the increase of indicator of M-response amplitude, characterizing the dynamics of the functional state of axon, retained in 3 months after the treatment ($p>0.05$). Shortening of RL in the study of peroneal and tibial nerves was also observed in patients of both studied groups in the course of performed therapy, and in II group there was reliable improvement of this indicator compared to the data of the patients of I group and before the treatment ($p<0.05$), which was detained in the late period, i.e. in 3 months ($p<0.05$).

Table 3: Electron euromyo graphic parameters of the function of peroneal and tibial nerves in patients with type 2 diabetes mellitus in the dynamics of treatment (M±m)

ENMG indicators	Groups of observation	
	I group, n=36	II group, n=30
	1	2
	Peroneal nerve	
Amplitude of M-response, мV		
before treatment	2.48±0.43	2.51±0.46
in 3 months	2.87±0.52	3.36±0.42
Residual latency period, мs		
before treatment	4.22±0.11	4.18±0.16
in 3 months	3.86±0.18	2.86±0.15*/**
CVA, м/s		
before treatment	37.43±0.87	36.88±0.78
in 3 months	39.84±0.75*	44.03±0.82*/**
Tibial nerve		
Amplitude of M-response, мV		
before treatment	2.52±0.36	2.54±0.33
in 3 months	2.71±0.42	3.46±0.48
Residual latency period, мs		
before treatment	4.18±0.12	4.06±0.13
in 3 months	3.82±0.16	3.01±0.11*/**
CVA, м/s		
before treatment	36.71±0.58	37.12±0.65
in 3 months	40.02±0.68*	43.88±0.72*/**

Notes: 1. * – the difference is possible concerning indicators before the treatment ($p<0.05$);
2. ** – the difference is possible concerning indicators of I group (basic treatment) ($p<0.05$);

As the table shows, the most revealing were the results of the study of the dynamics in the analysis of CVA data by motor fibers of the studied nerves in patients of group II ($p<0.05$). This demonstrates the significant effect of the medicine, and its ability to restore the functional state of myelin and to stimulate maximally the potential for reinnervation.

Probable improvement of indicators of cellular and humoral immunity against the background of the proposed treatment as a result of the performed study was not noted. This fact encourages further search of pharmacological tools that could also block the abnormal autoimmune mechanisms of pathogenesis of polyneuropathy in patients with type 2 diabetes mellitus.

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