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Final Report

Clinical Trial

Double-Blind Study of Bio-normalizer Effects on Oxygen Radical Production and Carbohydrate Binding with Circulating Blood Leukocytes in Patients with Insulin-Independent Diabetes Mellitus

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Introduction

Earlier, we have performed pilot clinical trial, in which the clinical efficacy, free radical- and immuno-modulating activity of Bio-normalizer (BN) in the treatment of insulin-dependent diabetes mellitus patients have been studied. It was found that BN administration improved significantly clinical conditions in more than 80% patients, the markers of diabetes mellitus being decreased in patients' blood and urine. The daily requirement of insulin decreased in average by 20-25%. We have shown for the first time that insulin injections suppressed the generation of reactive oxygen species by circulating leukocytes that may be regarded as a causative reason for a high risk of bacterial and fungal infections in diabetic patients. Along with beneficial clinical effects, BN therapy enhanced significantly (up to the normal value) the level of superoxide production by circulating monocytes and neutrophils as well as activity of nitric oxide synthase. On the grounds of these results, we concluded that surprisingly enough BN may act not merely as an antioxidant but appear to be a stimulator of enzymatic systems producing the physiologically essential oxygen radicals such as nitric oxide and superoxide. NO is synthesized in the reaction of L-arginine oxidation by a calcium activated NADPH-dependent NO-oxidase, which is blocked by L-arginine analogues N-monomethyl-L-arginine and N-iminoethyl-L-ornithine [1]. This enzyme was found in endothelial and neuronal cells, and in macrophages, neutrophils and monocytes after their "priming" with bacterial or yeast polysaccharides [1, 2]. It has been reported also that nitric oxide and superoxide radicals are produced simultaneously by cells isolated from an inflammatory exudate [3]. Those two free radicals interact with a rate constant of 6.7×10^9 M^{-1} s^{-1} to form peroxynitrite anion, which is known as a strong oxidant with bactericidal activity [4]. This decomposes spontaneously producing hydroxyl-like radicals, which could initiate lipid peroxidation [5]. Several studies have suggested that

elevated glucose levels in both diabetic patients and experimental animals can generate oxygen radicals and cause membrane and lipoprotein lipid peroxidation [6, 7, 8]. Although the role of NO in inflammation is not quite clear yet, it was suggested that it can modulate the edema formation, increase chemotaxis of cells, and inactivate an excess of superoxide derived from inflamed neutrophils [3]. Interestingly enough, that inhibition of NO-synthase by N-monomethyl-L-arginine led to abolishion of the normally potent bactericidal activity of macrophages [9].

NO causes relaxation of smooth muscle by activation of soluble guanylate cyclase that leads in turn to increased cyclic 3',5'-guanosine monophosphate (GMP) level [10].

The idea that oxidative stress contributes to diabetic complications such as vascular and renal dysfunctions is rather attractive because imbalance of consrictor (superoxide) and relaxing (NO) factors could result from variations in oxidant status. Josefsen et al., [11] have shown that the circulating monocytes in newly diagnosed type I diabetes patients are activated in terms of chemotaxis and superoxide production. At the same time they failed to find any changes in the monocyte functions 6 months after the primary diagnosis of IDDM. Hiramatsu and Arimori [12] have clearly shown that significantly elevated rate of superoxide generation in the diabetic hypertriglyceridemic monocytes was a reflection of a hypertriglyceridemic condition and did not correlate with the diabetic symptoms. If hyperglycemia itself is the cause of the diabetic complications, then it seems to be reasonable to suggest that elevated glucose level is toxic and that increased plasma and tissue glucose is a main source of the oxidative stress apparent in diabetes. Thus, glucose-dependent oxidative stress was found to be critical for determining cell damage in cultures [13]. Besides, oxygen free radicals enhance release of vasoconstrictor prostanoids or/and act as a direct vasoconsrictor factor. They can also inactivate NO, leading to excessive vasoconstriction. However, the exact role and

mechanism of an excessive oxygen radical production in diabetes is yet to be determined. A clearer understanding of these processes may provide a more rational approach to the pharmacological combating of the diabetes-caused complications. Diabetes mellitus is known to be associated with extensive disease of both large and small blood vessels [14].Because of that, diabetic patients often develop cardiomyopathy characterized mainly by left ventricular contractile dysfunction and congestive heart failure [15]. Diabetic microangiopathy includes diabetic retinopathy, diabetic glomerulosclerosis, and diffuse cappilary disease. Actually, no real progress has been made in prevention or treatment of progressive angiopathy in diabetes. Vascular disease accounts for the majority of the clinical complications of diabetes mellitus. Changes in local control of vascular tone such as unbalanced production of relaxing and contracting factors by the endothelial or/and the blood circulating cells may lead to the initiation and maintenance of abnormal vascular reactivity which is characteristic feature in diabetic patients. The endothelial and circulating cells participate in the local regulation of vascular smooth muscle tone by generating vascular relaxing factors such as nitric oxide (NO) and leukotriene E_4, D_4, and others, as well as vascular contracting factors including thromboxane A_2 and superoxide anion [16].

To our knowledge little is known about redox state of diabetic patients treated by various sugar-decreasing drugs. A few last studies [17, 18] have shown that free radical-damaging processes are probably of essential importance for this type of diabetes due to the overproduction of oxygen radicals by monocytes and granulocytes. So far, we became curious to find out the *in vivo* features of oxidative stress such as oxygen radical overproduction by monocytes, which might be induced by elevated level of glucose itself but insulin injections. A main goal of the present clinical laboratory study was to obtain further evidence, if any, on the BN regulatory activity regarding

oxygen radical production by circulating monocytes of patients with insulin-independent diabetes mellitus of both types.

The success of our previous trial made it possible to organize a new pilot study of BN administration for the insulin-independent diabetes mellitus patients. Since the effects of BN on clinical efficacy and immuno-modulating activity of diabetes patients have already been studied thoroughly in the first trial, we paid special attention to its effects on superoxide and nitric oxide formation and the expression and affinity of glucose- and galactose-binding receptors on the surface of circulating monocytes. Nonetheless, in accord with our previous observations, we registered a significant improvement of clinical conditions of most patients participated in the trial.

Patients and Study Design

Randomized double-blind study was carried out at Institute of Diabetes of Russian Academy of Medical Science. 24 adult patients suffered from insulin-independent diabetes mellitus (IIDM, Type I and type II) were randomised into two groups. Experimental group contained 15 patients and control group - 9 patients. 9 adult healthy people were in the reference group. The experimental group patients were treated with BN (2 sachets a day, daily) for 3 weeks. The control group patients were given 2 sachets containing plain sugar powder. The following parameters were measured in the beginning and after cessation of clinical trial: luminol-dependent chemiluminescence of blood monocytes, NO-synthase activity and NO production by blood monocytes, number and affinity of the glucose and galactose receptors on the monocyte membrane. Eligibility criteria for the patients participating in the trial were similar to those in the previous trial with insulin-dependent diabetes patients (See Final Report on "The study of Bio-Normalizer (BN) clinical efficacy and immunomodulating activity in the treatment of insulin-dependent diabetes mellitus").

Materials and Methods

Practically all the reagents including chemiluminescence probes luminol and lucigenin, activators of the cell oxygen radical-generating enzymes 12-O-phorbol-13-myristate acetate (PMA) and zymosan, inhibitor of NO-synthase N-monomethyl-L-arginine, enzymes CuZnSOD, MnSOD, and catalase, adrenaline, Monoprep, Ficoll-Hepaque, tris-HCl buffer, polyacrylamide gel (PAAG), and Hank's balanced salt solution were purchased from Sigma Co, St. Louis. Monoclonal antibodies to the lymphocyte and monocyte subpopulations were from Serva, Germany. Glucose- or galactose-containing PAAG conjugates were synthesized and kindly gifted by Prof. Bovin (Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia).

Blood drawing. Venous blood (10 ml) was obtained by venipuncture using heparinized vacutainers (Becton-Dickenson, Rutherford, USA) in the morning before any food. Freshly drawn blood was used for the further cell isolation.

White blood cells (WBC) and mononuclear cell isolation. White blood cells (WBC) were isolated from 10 ml blood sample, which was layered onto an equal volume of the mixture containing dextran sulfate (6% in potassium phosphate buffer) and metrizoate (32%) in ratio 5:2 v/v. To sediment erythrocytes, samples were incubated at 37^{o} C for 30 min. Then the WBC-rich supernatant was placed on the top of Monoprep® solution and centrifuged at 150 x g for 40 min, and a thin layer between Monoprep solution and plasma, containing mononuclear leukocytes (monocytes and lymphocytes) was carefully collected with a Pasteur pippette and washed twice in a cold Hank's balanced salt solution (HBSS). Finally, mononuclear cells were resuspended in a minimal essential medium (MEM) supplemented with 5% fetal calf serum [19].

Production of reactive oxygen species (ROS) by monocytes. The release of ROS from monocytes was measured by the chemiluminescence (CL) method[19] on a LKB 1251 chemiluminometer (Sweden) at 37^{o} C under continuous mixing. A mononuclear

cell suspension (10^5 cells) was added into a polystyrene cuvette containing 1 ml preheated HBSS with 50 μM luminol (LDCL) and incubated for 5-6 min. The intensity of spontaneous CL was recorded continuously. After that, 1mg/ml of zymosan particles opsonized by human serum proteins were added to cells, and the amplitude of the CL response to the activator was measured as a difference between the maximal intensities of the activated and spontaneous CL. The results were expressed as mV per 10^6 monocytes [19].

Activity of NO-synthase was determined using L-arginine monomethyl ester (L-NAME) as a specific inhibitor of the enzyme activity. Briefly, LDCL of monocytes was measured in the absence and in the presence of 50 mM of L-NAME. The inhibition of LDCL expressed in per cent (%) corresponds to the nitric oxide synthase activity.

NO production. Nitric oxide generation by monocytes was measured spectrophotometrically using reaction of the phenol nitration. Monocytes ($2x10^6$ cells/ml) were suspended in the 50mM potassium phosphate buffer in the presence of phenol red (5 mM) and FeEDTA (2.5 mM) and stimulated with PMA (100 ng/ml). After incubation at 37°C for 2 hours, reaction was stopped by centrifugation at 300 x g for 10 min. Supernatant was collected, and its pH was adjusted to pH 6.2-6.5 by 1M HCl, and then to pH 10.0-10.6 by 3M NaOH. Absorbance at 420 nm was measured at each pH value. The amount of NO_2-phenol formed was calculated using the pH-dependent difference in absorbances ($\Delta\varepsilon = 18300\ M^{-1}\ cm^{-1}$).

A number and affinity of carbohydrate receptors were determined using polyacrylamide-glucose (PAAG-glu) and polyacrylamide-galactose (PAAG-gal) conjugates loaded with fluorescein. PAAG conjugates were obtained as described in [20] and contained the polyacrylamide molecules with the polymerization equal 100. The amide groups were partly substituted by glycosyle-$OCH_2CH_2CH_2$, where glycosyl was the residue of glucose or galactose. The content of carbohydrates in the PAAG conjugates was equal 30 mol. %. The expression of carbohydrate receptors on the surface of mononuclear leukocytes was determined by flow cytometry using a FACScan (Becton Dickinson, USA). Isolated cells were fixed in the 1%

paraformaldehyde solution and then used for the flow cytometry measurements. Five thousand cells from each sample were scanned and the results were analysed automatically with a PC and the Facscan Software. To discriminate a non-specific spontaneous fluorescence in cells, the internal control was performed during each measurement. The control cell samples were processed as the experimental ones but in the absence of the fluorescein-loaded conjugates. The mean values of the fluorescence intensity and the number of fluorescent cells were determined. The results were expressed in the arbitrary units and per cent, respectively [20].

Statistical analysis

Dynamics of laboratory parameters before and after the BN administration in both experimental and control groups were analysed uisng of the non-parametric Wilcockson criteria. The results for the experimental and control groups were expressed as mean ± S.E.M. Statistically significant difference was assumed at the 5% level.

Results and Discussion

Type II diabetes, noninsulin dependent diabetes mellitus, or maturity-onset diabetes, is on the rise, affecting more than 100 million people worldwide. In The United States alone disease incidence has more than doubled in the past 20 years, and 500,000 new cases are expected every year. India reports 30 million patients [21]. Currently, the term "type II diabetes" (IIDM) means that there is no indications of autoimmune disease. While, "type I diabetes" indicates the presence of autoimmune markers such as islet cell or glutamic acid decarboxylase antibodies. Meantime, type II diabetes is defined as a syndrome capable of being initiated by a number of causes. A condition known as "insulin resistance" is thought to be the initiating disorder. Normally, pancreatic beta cells secrete insulin enough to maintain circulating blood glucose levels. On one hand, insulin inhibits glucose production in liver and on the other hand, it stimulates glucose

binding to circulating leukocytes, which deliver glucose to sceletal muscles. In insulin resistance, the muscles become unavailable to uptake glucose, some is reversed into fat, and the rest continues to circulate in the blood. The circulating blood glucose levels reach those of type I diabetes. At this point, the body faces a physiological paradox: both insulin and glucose levels are high, and patients have already exhibibted the micro- and macrovascular disorders, which cause severe complications such as heart attacks, angina, strokes, and amputations. The researchers and physicians have been searching for years now for new preparations relevant to diminish the "insulin resistance" syndrome and ameliorate to certain extent the vascular complications in both type I and type II diabetes mellitus.

What makes cells insensitive to insulin remains unclear. However, we suggested that impaired balance of oxygen radicals produced by circulating leukocytes may easily cause oxidation of proteins on the cellular surface that turns out to change the sugar-binding receptor pattern. In this study we showed that monocytes of IIDM patients produced an abnormal amount of reactive oxygen species that was revealed by the chemiluminescence approach. Indeed, the monocytes of some patients a two-fold increased level of spontaneous LDCL as compare to normal one (Table 1). Moreover, the intensity of zymosan-activated LDCL in diabetic patients was about 5 times greater than that of healthy donors. A short-term BN administration lowered both spontaneous and activated LDCL practically to the normal levels (Table 1). At the same time, there were patients with dramatically decreased levels of spontaneous and activated LDCL. After taking BN, the monocytes of those patients produced practically normal amount of oxygen free radicals (Table 2). (In monocytes of the placebo group patients the initially increased spontaneous LDCL decreased, though rather high zymosan-activated LDCL further increased after the clinical trial cessation, Table 3). The double effect of

BN could be explained in terms of (1) its well-established free radical scavenging activity and (2) its potency to stimulate cellular enzymes either producing or utilizing the oxygen free radicals. Thus, it has been shown in a number of studies that lipopolysaccharides of bacterial and yeast origin possessed the "priming effect" towards NADPH-oxidase as well as they can induce manganese SOD and NO-synthase *via* the production of interleukin-1 and TNF-α [22, 23, 24]. BN, enriched with the yeast polysaccharides may be regarded as an inducer of some initially depressed enzymatic systems. However, we found that NO production by circulating monocytes was significantly higher in the IIDM patients comparing with healthy donors (Table 4). In this case, BN administration suppressed NO generation by cells. In the placebo group there were not any changes in the levels of NO produced by cells upon stimulation (Table 5). As nitric oxide is able to interact with superoxide radical forming peroxinitrate, which is thought to be the most powerful endogenous toxicant [3], it could be assumed that beneficial effects of BN on the IIDM patients is a consequence of its superoxide- and nitric oxide-scavenging activity.

Completely new data were obtained studying the binding of PAAG conjugates with the monocyte membrane. It was found that a number of cells bound to the PAAG-glucose and PAAG-galactose conjugates was lower for diabetic patients in comparison with donors (Tables 6 and 8). The course of BN therapy improved situation for both saccharides (glucose and galactose). Unfortunately, we failed to find any abnormalities in binding of both conjugates with monocytes of the placebo group patients in the beginning of trial (Tables 7 and 9). Therefore, it is very hard to make a definite conclusion on the difference found in the experimental and control groups. We could only assume that BN is capable increasing the impaired carbohydrate binding to monocyte membrane that allows to diminish glucose levels in blood plasma.

Conclusions.

1. BN administration improved significantly patients' clinical conditions and their quality of life. This conclusion is based on the patients' and physicians' observations.

2. BN administration resulted in normalization of oxygen radical production by blood monocytes: it inhibited initially enhanced oxygen radical generation in patients with IIDM of both types. At the same time, BN administration induced an elevation of initially suppressed oxygen radical generation by monocytes from patients with IIDM. This finding can be considered as an universal molecular mechanism by which BN improves the clinical conditions in patients with diabetes mellitus.

3. BN administration inhibited the increased nitric oxide production by blood monocytes in IIDM patients. As a consequence, BN administration normalized the vessel contraction/relaxation and permeability.

4. BN administration increased the carbohydrate binding with monocytes that in turn improves the glucose utilization and its transportation to tissues.

5. These results provide a theoretical basis for BN implementation in diabetic patients with vascular and infectious complications.

6. As we know from the previous studies that BN supplementation significantly lowers blood lipid peroxidation products, it could be beneficial in reducing risk of cardiovascular disease in diabetic patients.

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Table 1. Effect of BN on the intensity of spontaneous and zymosan-activated luminol-dependent chemiluminescence (LDCL) by blood monocytes (Experimental subgroup of patients with enhanced LDCL level)

Patient	Type of diabetes	Intensity of spontaneous LDCL (mV/10^6 cells)		Intensity of zymosan-activated LDCL (mV/10^6 cells)	
		before	after	before	after
Ka-ova	II	1160	188	16910	1630
Va-tzov	II	750	95	13560	162
Mel-ko	II	1030	201	23670	1919
Ko-in	I	1060	1540	15320	35660
So-ka	II	670	760	10470	2900
Tem-ov	I	790	630	17120	3740
Rav-ich	II	860	1160	15590	6260
Af-in	II	1400	440	23200	11000
Ef-ov	I	1440	520	54300	960
m ± SD		1018 ± 222	615 ± 362	21127 ± 8398	7137 ± 1969
Normal values		525 ± 215		4216 ± 2461	

Table 2. Effect of BN on the intensity of spontaneous and zymosan-activated LDCL by blood monocytes. (Experimental subgroup with diminished LDCL level)

Patient	Type of diabetes	Intensity of spontaneous LDCL (mV/10^6 cells)		Intensity of zymosan-activated LDCL (mV/10^6 cells)	
		before	after	before	after
Kr-ova	I	178	220	3855	4161
Ta-an	II	220	1100	3300	8780
Sa-va	II	30	360	810	1420
Ko-yaev	II	88	360	810	1420
Kar-eva*	II	40	700	1180	4540
Obr-ova	II	30	420	394	2400
m ± SD		98 ± 68	527 ± 249	1725 ± 1235	3787 ± 2040
Normal values		525 ± 215		4216 ± 2461	

* - Insulin-dependent patient

Table 3. Effect of placebo on LDCL in blood monocytes.

Patient	Type of diabetes	Spontaneous LDCL ($mV/10^6$ cells)		Zymosan-activated LDCL ($mV/10^6$ cells)	
		before	after	before	after
Pro-na	I	900	510	8500	26990
Ibra-ova	II	760	320	7300	8100
Ali-va	II	1190	n.a	3070	n.a
Sil-aya	II	1020	780	7810	2920
Vet-aya	II	2620	380	34680	1480
Shu-ov	II	360	1000	3040	4400
Per-chay	II	1150	360	3750	760
Alyu-va	II	1540	1200	7590	4050
m ± SD		1192.5±452	650±487.9	8045±643.4	15520±16221
Normal values		525 ± 215		4216 ± 2461	

Table 4. Effect of BN administration on nitric oxide production by blood monocytes. (Experimental group)

Patient	Type of diabetes	NO-synthase inhibition (%)		NO production ($pmol/10^6$ cells/min)	
		before	after	before	after
Kru-ova	I	60.4	88.2	n.a.	n.a.
Kas-va	II	38.9	44.2	n.a.	n.a.
Vas-tzov	II	41.3	40.1	n.a.	n.a.
Mel-ko	II	36.2	48.2	n.a.	n.a.
Kos-in	I	43.5	40.6	45.1	8.6
Sor-ka	II	54.8	43.4	43.9	6.6
Tem-ov	I	47.1	58.6	29.5	53.3
Rav-vich	II	36.4	33.9	27.0	34.6
Ef-ov	I	48.2	69.8	15.0	17.3
Ta-an	II	44.8	46.9	28.0	13.6
Sav-va	II	39.3	47.9	28.9	20.3
Kon-ev	II	43.5	45.8	36.7	8.2
Kar-eva	II*	44.9	69.7	27.3	7.7
Af-in	II	43.8	51.6	15.4	9.7
Obr-ova	II	39.6	52.5	28.9	18.8
m ± SD		43.0 ± 1.3	50.7 ± 2.7	29.6 ± 6.7	18 ± 10
Normal values		51.5 ± 0.6		14.1 ± 4.4	

Table 5. Nitric oxide production in the control (placebo) group.

Patient	Type of diabetes	NO-synthase inhibition (%)		NO production (pmol/10^6 cells/min)	
		before	after	before	after
Kru-ova	I	60.4	88.2	n.a.	n.a.
Kas-va	II	38.9	44.2	n.a.	n.a.
Vas-zov	II	41.3	40.1	n.a.	n.a.
Mel-ko	II	36.2	48.2	n.a.	n.a.
Kos-in	I	43.5	40.6	45.1	8.6
Sor-ka	II	54.8	43.4	43.9	6.6
Tem-ov	I	47.1	58.6	29.5	53.3
Rav-ich	II	36.4	33.9	27.0	34.6
Ef-ov	I	48.2	69.8	15.0	17.3
Ta-an	II	44.8	46.9	28.0	13.6
Sav-va	II	39.3	47.9	28.9	20.3
Kon-ev	II	43.5	45.8	36.7	8.2
Ḱar-eva	II*	44.9	69.7	27.3	7.7
Af-nin	II	43.8	51.6	15.4	9.7
Obr-va	II	39.6	52.5	28.9	18.8
m ± SD		43.0 ± 1.3	50.7 ± 2.7	29.6 ± 6.7	18 ± 10
Normal values		51.5 ± 0.6		14.1 ± 4.4	

Table 6. Effect of BN administration on binding of the polyacrylamide-glucose conjugates with monocyte membrane. (Experimental group)

Patient	Type of diabetes	Intensity of fluorescence (arb. units)		Number of fluorescent cells (%)	
		before	after	before	after
Kos-in	I	n.a.	337	3.5	10.4
Sor-ka	II	n.a.	371	2.9	2.1
Tem-ov	I	n.a.	363	2.5	2.5
Rav-vich	II	n.a.	442	2.0	2.2
Ef-ov	I	312	374	3.9	3.4
Ta-an	II	315	384	4.4	3.3
Sav-va	II	n.a.	n.a.	n.a.	n.a.
Kon-ev	II	361	374	1.3	5.1
Kar-eva	II*	351	363	1.2	4.3
Af-in	II	321	n.a.	4.8	7.0
Obr-ova	II	367	347	1.1	5.7
m ± SD		343 ± 7	373 ± 10	2.8 ± 1.1	4.6 ± 1.9
Normal values		341 ± 30		3.6 ± 1.1	

Table 7. Binding of fluorescein-labeled polyacrylamide-glucose conjugates to the monocyte membranes (the placebo group).

Patient	Type of diabetes	Intensity of fluorescence (arb. units)		Number of fluorescein-labeled cells (%)	
		before	after	before	after
Pro-na	I	391	396	4.6	2.4
Ibra-va	II	369	315	6.8	3.1
Al-va	II	323	n.a.	1.0	n.a.
Sil-aya	II	352	n.a.	1.6	n.a.
Vetr-aya	II	329	386	5.3	2.2
Shu-ov	II	336	374	3.0	0.7
Per-chay	II	306	263	1.1	3.3
Sel-va	II	338	349	2.9	1.4
Alush-va	II	380	274	2.6	4.7
m ± SD		347 ± 23	337 ± 45	3.2 ± 1.6	2.5 ± 1.0
Normal values		341 ± 30		3.6 ± 1.1	

Table 8. Effect of BN administration on binding of fluorescein-labeled polyacrylamide-galactose conjugates to the monocyte membranes. (Experimental group)

Patient	Type of diabetes	Intensity of cell fluorescence (arb. units)		Number of fluorescein-labeled cells (%)	
		before	after	before	after
Kos-in	I	363	492	10.2	14.7
Sor-ka	II	413	427	7.2	7.4
Tem-ov	I	385	489	6.8	10.8
Rav-ich	II	412	460	5.6	9.6
Ef-ov	I	367	370	5.2	15.5
Ta-an	II	417	476	4.3	15.8
Sav-va	II	405	443	3.6	6.7
Kon-ev	II	471	555	3.9	14.3
Kar-eva	II*	409	471	4.2	15.0
Af-nin	II	453	494	5.2	12.1
Obr-ova	II	406	485	3.1	12.4
m ± SD		409.2±21.8	469.3 ± 32.2	5.4 ± 1.5	12.2 ± 2.6
Normal values		478 ± 27		10.0 ± 3.2	

409.2±2

Table 9. Binding of fluorescein-labeled polyacrylamide-galactose conjugates to the monocyte membranes (the placebo group).

Patient	Type of diabetes	Intensity of fluorescence (arb. units)		Number of fluorescein-labeled cells (%)	
		before	after	before	after
Pro-na	I	440	393	13.5	9.7
Ibra-va	II	490	433	18.5	12.2
Al-va	II	479	n.a.	8.6	n.a.
Sil-aya	II	368	502	11.1	19.7
Vetr-aya	II	495	466	18.8	6.1
Shu-ov	II	422	451	9.8	8.0
Per-chay	II	495	406	6.1	11.0
Sel-va	II	385	448	9.4	9.4
Alush-va	II	361	425	9.9	14.0
m ± SD		437 ± 47	440 ± 26	11.7 ± 3.4	11.3 ± 3.0
Normal values		478 ± 27		10.0 ± 3.2	