



Clinical Example of Effective use of Biocompatible Magnetite (Fe_3O_4) Nanoparticles in the Complex Treatment of Multiple Sclerosis

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Abstract

Multiple Sclerosis (MS) is a multifactorial disease characterized by a variety of pathogenic mechanisms and pathways. Successful treatment of multiple sclerosis requires a fundamentally new approach in solving a number of problems related to the pathogenesis of the disease. Currently, nanotechnology opens a new stage of innovative medicine. Previously accumulated clinical experience in the use of biocompatible magnetite (Fe_3O_4) nanoparticles, the study of their mechanisms of action on biological environments and systems of the living organism creates the preconditions for their effective use in the complex therapy of MS. The primary purpose of the study was to slow down the progression of MS, improve the neurological status and general condition of the patient, reduce the dynamics of demyelinating foci in the brain by including biocompatible Micromage-B magnetite nanoparticles in the complex therapy. Following the application of Micromage-B nano-particles in the treatment of MS, objective improvement of the neurological state, reduction of stiff-ness and rapid fatigue of the lower extremities were observed. Improved gait and coordination, de-creased trembling of the hand, depression and signs of concentration disorders gone, appetite re-stored, speech improved. During the entire period of use of Micromage-B, positive dynamic normalization of the neurological condition was observed. After six months, the total score fell from 210 to 45. The EDSS Disability Scale score has decreased from 6.0 to 5.0. Examination of the brain by MRI with contrast enhancement for the first time showed a decrease in the number of new foci of demyelinating in the brain. The procedure of restoration of the central nervous system activity in MS is due not only to the immunosuppressive properties of magnetite nanoparticles, but probably also to the activation of remyelination mechanisms and differentiation of oligodendrocytes due to enzymatic methylation. Considering the above, the use of biocompatible nanodevices in the complex treatment of MS is a promising innovative area and claims to be further considered for the purpose of subsequent inclusion of nanotechnology in the protocols for MS treatment.

Keywords: Multiple Sclerosis; Treatment; Nanodevice; Micromage-B; Neurological Status Assessment; Remyelination

Abbreviations: MS: Multiple Sclerosis; CNS: Central Nervous System.

Introduction

Multiple sclerosis (MS) is a serious neurological problem due to its widespread occurrence, chronic course, frequent disability of patients and propensity to affect young people. The average age of onset is 30 years. The main hypothesis of immunopathogenesis of MS is that immunological tolerance is violated and autoreactive cells, sensitized to antigens of nervous tissue, actively penetrate through the blood-brain barrier into the brain tissue. B-lymphocytes recognize myelin and order T cells to launch an immune attack [1-7]. T- and B-cells secrete chemicals that attract other immune cells that cause inflammation [8,9]. Plasma cells produce and release antibodies that attack myelin and enlist the help of other immune cells [10,11]. T- and B-cells create a permanent residence in the central nervous system (CNS) and continue the attack [12,13]. There are two hypotheses of MS pathogenesis: the outside-in hypothesis, based on the penetration into the brain tissue of immunocompetent cells activated in the periphery, and inside-out, when the primary is damage to the nervous tissue, the expression of damage receptors - from the family DAMPs (Danger-associated Molecular Patterns), resulting in activation of immunity and failure of tolerance to myelin antigens [14]. Antibodies, which are actively produced by plasma cells, destroy the protective covering of nerve cells. Inflammation develops in the damaged area. After some time the tissue is scarred, which leads to a disturbance of conduction. As a result of such processes, impulses from the brain do not reach the limbs and organs. Because of this a person loses the ability to control his body [15]. The most common initial clinical symptoms are weakness and impaired sensation in one or more limbs, decreased vision, impaired urination and cerebellar ataxia.

Currently, immunomodulatory and immunosuppressive drugs that modify the course of MS form the basis of pathogenetic treatment. Their mechanism of action is related to: 1) selective or 2) complete immunosuppression, 3) prevention of migration of activated cells from lymph nodes or into brain tissue, or 4) a combination of immunoregulatory, anti-inflammatory, antioxidant, and possibly neurotrophic actions. Possible directions in the development of new treatments for MS include selective local immunocorrection, remyelination and neuroprotection, enhancement of neuroplasticity and relocalization of function, evaluation of the effectiveness and safety of cell therapy, individual selection of therapy based on prediction of pathological process variants and possible response to treatment based on the results of molecular and cellular biology of MS [16-22].

The development of inflammation and demyelination in MS is caused by an impaired immune response, imbalance of T regulatory and T effector cells, activation of B-cell immunity and microglia. All drugs affecting the course of MS either deplete T- or B-cells or alter the signaling pathways associated with the formation of an immune response [23].

Particular attention has recently been paid to the role of B-cells in the chronic inflammatory process in the CNS, as evidenced by their role in both autoantibody formation and in the presentation of antigens and constant activation of T-cells in the brain parenchyma. Anti-B-cell therapy (Rituximab, Ocrelizumab, Ofatumumumab) has proven effective in both remitting and progressive MS. Stimulation of remyelination in MS is associated with the development of new preparations of monoclonal antibodies: anti-Lingo, human immunoglobulin M (IgM). These drugs promote remyelination and differentiation of oligodendrocytes and their precursors [24].

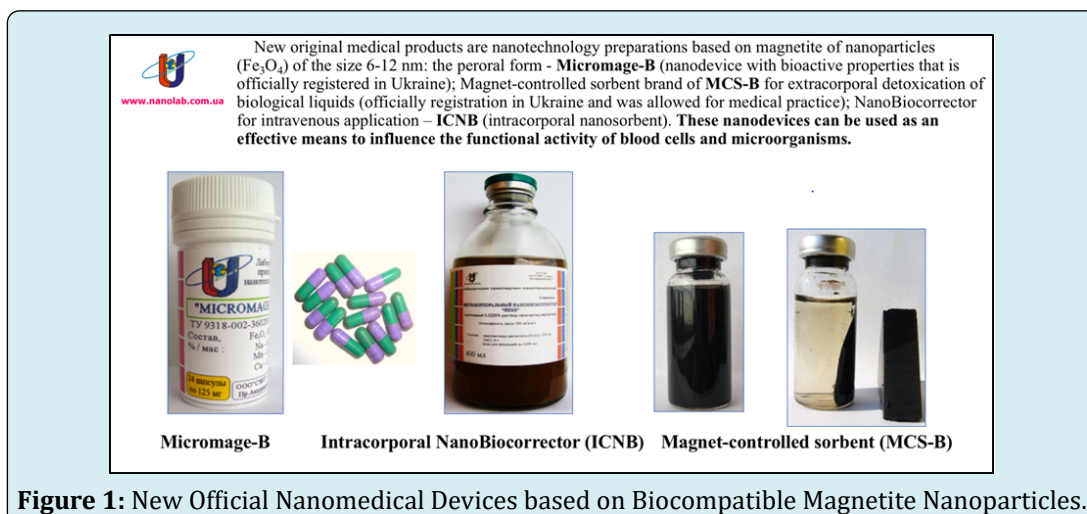


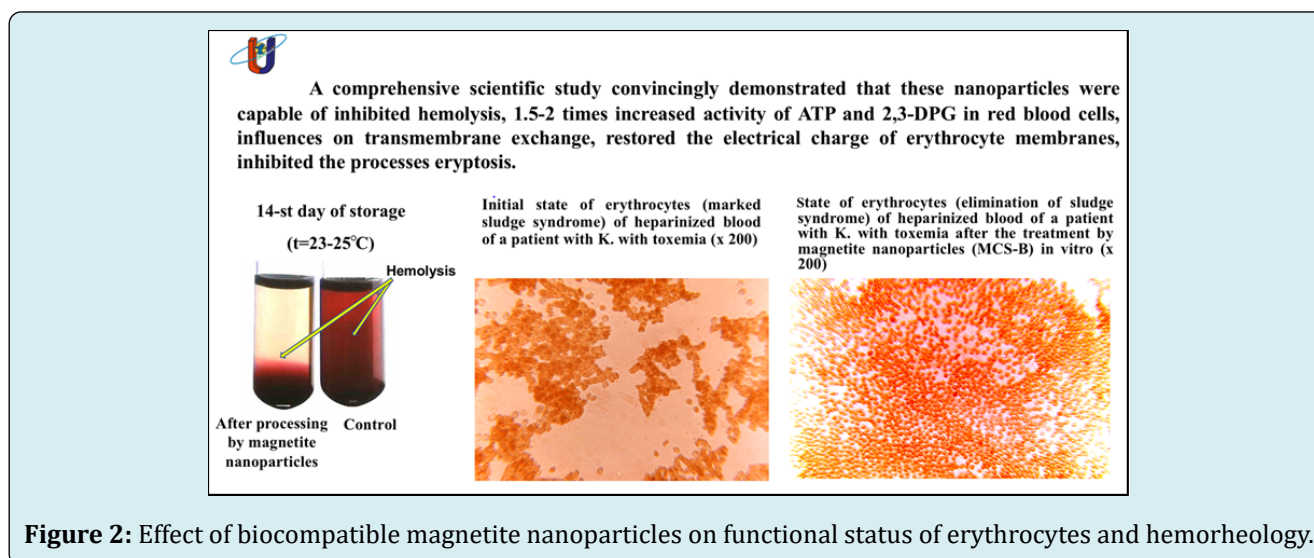
Figure 1: New Official Nanomedical Devices based on Biocompatible Magnetite Nanoparticles.

One of the key mechanisms of axonal degeneration is mitochondrial dysfunction. Some drugs (Dimethylfumarate, Idebenone, Biotin) can be considered promising in this direction [25]. The use of drugs affecting the redistribution of ion channels in the demyelinated axon (Lamotrigine, Amiloride, Fampridine) can help to reduce the energy deficit in the axon and neuron [26].

Recently, biocompatible nanotechnological preparations have been increasingly used in medicine. Since 1998, Ukrainian clinics have been officially using nanodevices developed by Applied Nanotechnology Laboratory of Belousov: Micromage-B, MCS-B and ICNB brands (Figure 1) [27].

These nanodevices are based on biocompatible magnetite nanoparticles. The peculiarity of physical and

chemical characteristics provides a condition for a wide range of their application in order to influence the quantitative and qualitative compositions of the fluid environment of the body, metabolic and biochemical processes, the energy balance of cells. Selective sorption activity against surface proteins of cell membranes, circulating immune complexes, lymphocytotoxic antibodies, complement system, the effect of increasing phagocytic activity and leukocyte phagocytosis completion index [28] allow the effective use of these nanodevices for immunocorrection. In addition, the nanopreparations presented have an effect on glycolysis processes, the activity of cell membrane ion channels, normalize erythrocyte function, improve microcirculation (Figure 2), reduce platelet aggregation index [29-31], activate the system of antiradical enzymes, and inhibit lipid peroxidation processes [32,33].



The results of these studies are a prerequisite for the development of innovative techniques for the effective and safe use of biocompatible magnetite nanoparticles in the treatment of severe autoimmune diseases, including multiple sclerosis. The main goal of the study is to slow down the progression of MS, improve the neurological status and general condition of the patient, and reduce the dynamics of the spread of demyelinating foci in the brain.

Materials and Methods

Patient K was diagnosed with multiple sclerosis, secondary progressive type of course, cerebrospinal form, clinical aggravation stage, pronounced spastic tetraparesis with accent in lower extremities with walking impairment, pronounced urinary-ataxic syndrome, sphincter and sensory disorders. MRI - signs of multifocal diffuse brain lesions (more than 30) of demyelinating nature. Active phase. Diffuse atrophic process in the cerebral cortex. The mean number of

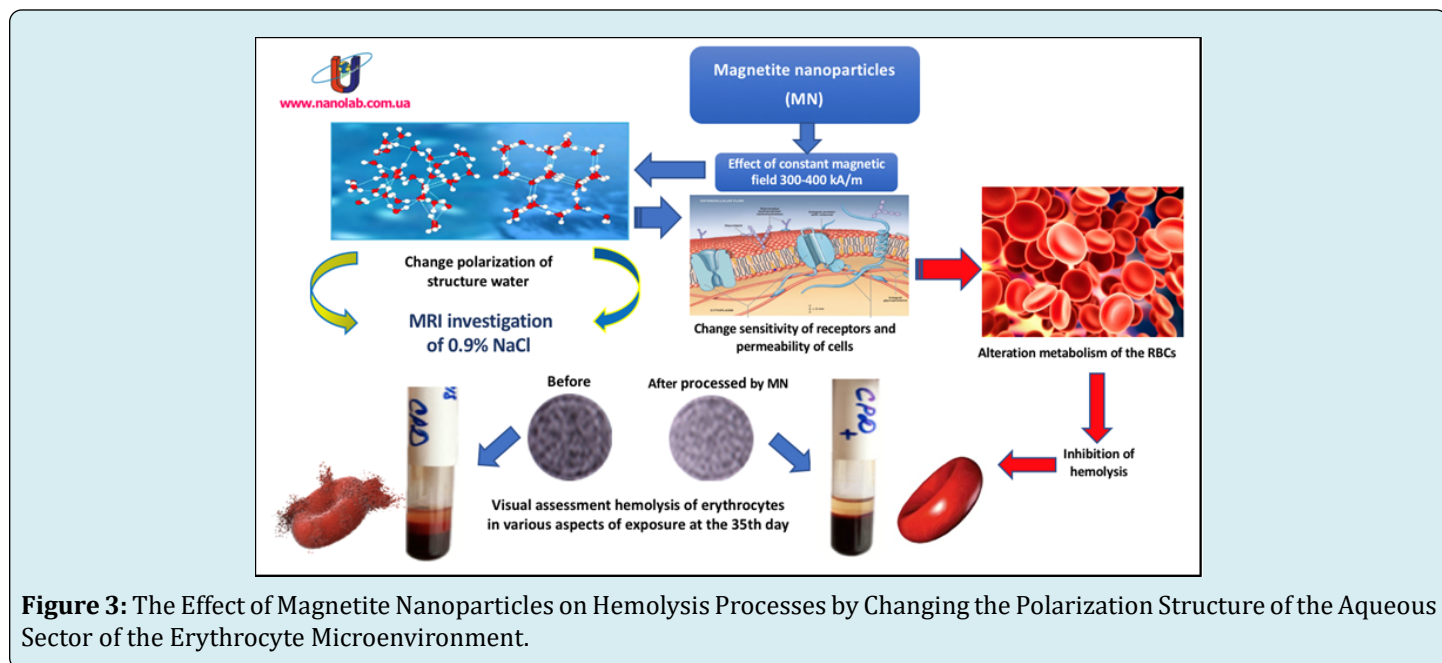
relapses during the year before study inclusion was 1.0. The EDSS disability score was 6.0. The duration of the disease since the onset of the first symptoms was 24 years. For 14 years, the patient was regularly treated with vascular and metabolic drugs in combination with hormonal therapy. In the 6th year, against the background of active treatment, the disease transformed into a secondary-progressive type of course. The therapy was supplemented with the immunosuppressive drug Teriflunomide.

However, despite all treatment, the patient's general condition progressively worsened. The neurological status has not stabilized. MRI examination showed a progressive increase in the number of new foci of demyelination in the brain over the last 4 years.

Considering the above, the complex therapy was supplemented with the prescription of Micromage-B [34]. Micromage-B is an oral form of nanodevice officially

registered by the Ministry of Health of Ukraine. Micromage-B is a powder form of magnetite (Fe_3O_4) nanoparticles, which is designed for the prevention and treatment of various diseases, as well as to increase the body's resistance to adverse environmental factors. Micromage-B is a device of nanotechnology. The size of magnetite nanoparticles is from 6 to 12 nm. The therapeutic effect of the nanopreparation is based on the mechanism of sorption and action of a constant magnetic field on cellular and subcellular structures that causes by magnetite of nanoparticles.

The area of the sorption surface of nanoparticles is from 800 to 1200 m^2/g , and the value of the induced magnetic field is 300-400 kA/m . The application point of Micromage-B is the microenvironment of the cell aqueous spaces, surface membrane proteins. Magnetite nanoparticles by selective sorption change the quantitative and qualitative composition of cell surface proteins, and the permanent magnetic field induced by magnetite nanoparticles changes the mobility and orientation of hydrogen protons in the water sectors of the cell microenvironment (Figure 3).



The latter contributes to the activation of hydrolysis of the phosphate residue ATP. In general, the transmembrane exchange and metabolism of the cell are modified, and its susceptibility is altered.

Nanopreparation causes increase of adaptive mechanisms and potential of cell organelles, accelerates of the reparative processes at the level of membranes and macromolecules. Micromage-B significantly increases the level of synthetic intracellular reactions and compensatory possibilities of the organelles of the cells of the renal glomerulus, the epithelial cells of the proximal and distal sections of the nephron, that is structurally expressed in increasing bioenergetic support of synthetic intracellular processes, increasing the reparative and adaptive capabilities of nephrons. By increasing the activity of redox phosphorylation processes, providing energy requirements of synthetic intracellular reactions proceeding at the level of membranes and macromolecules in liver cells, Micromage-B acts as a direct activator of reparative intracellular processes in hepatocytes, enhances glycogenogenesis. This fact allows to use it effectively as a

powerful hepatoprotector in the treatment of acute and chronic liver diseases.

Micromage-B can act as an active stimulator of erythropoiesis, thereby quickly restoring the level of hemoglobin in the blood. Micromage-B stimulates the synthesis of pulmonary surfactant, which provides stretchability and elasticity of lung tissue. Micromage-B provides the stability of the lungs to negative influences of factors of external and internal environment by means increasing the activity of alveolar macrophages. Clinically and laboratory: Micromage-B improves microcirculation and rheological properties of blood by stabilizing the bioelectric charge of erythrocyte membranes. It has a pronounced immunomodulatory effect. Micromage-B has a selective bacteriostatic effect in relation to the pathogenic microflora, without adversely affecting the normoflora. Delays the growth and reproduction of various kinds of fungi. Stimulates the growth and activity of lactic acid bacteria in the intestine. In 2-3 times increases the effectiveness of antibacterial and antifungal agents. These facts allow the effective use

of Micromage-B for the treatment of dysbacteriosis and candidiasis. Nanoparticles of Micromage-B adsorbed toxic substances and circulating immune complexes. It's fact that greatly increases the effectiveness of treatment of various allergic diseases, autoimmune processes (for example, rheumatoid arthritis, acute and chronic polyarthritis, eczema etc.), acute poisoning. Improving the renal blood flow by Micromage-B has a moderate diuretic effect. Having in its nature magnetic moment the magnetite nanoparticles brakes down and promotes the dissolution of stones in the kidneys and bile ducts of the liver, which are subsequently eliminated through the excretory systems of the organism in the form of magnetically sensitive crystals forms. Micromage-B actively contributes to the normalization level of lipids and proteins in blood. Affects the factors of atherogenesis, preventing the development of atherosclerotic processes. Active decline the level of release into the blood of an excessive number of mediators of the aggressive state is result of actions permanent magnetic field of magnetite nanoparticles of the Micromage-B into the macrophage cells. In turn, this causes pronounced anti-inflammatory and moderate analgesic effects. Micromage-B modulates activity of the enzyme link of antioxidant system, sorbs products of the lipid peroxidation, corrects balance between antiradical and proradical products. Under the influence of Micromage-B, the monocytes actively synthesized a tumor necrosis factor (cachexin), which has cytotoxic and cytostatic effects on tumor cells. When topical application (in the form of powders, ointments or aqueous solutions of colloids) the Micromage-B promotes rapid wound healing of mucous membranes and skin, change of wet tissue necrosis to dry. Micromage-B does not cause disturbances in the functional activity of organs and body systems, it is non-toxic [35,36].

Dosing regimen Micromage-B: the first month - 500 mg daily, the second month - 500 mg every other day, subsequent

months - 500 mg once every three days. The choice of the scheme and dosage of Micromage-B had an individual approach. The time and speed of the appearance of signs of improvement in the patient's general state and recovery of neurological status were taken into account.

The dynamics of changes in the neurological status were studied using a modified Multiple Sclerosis Patient Assessment Scale [37,38]. This scale is based both on the severity of motor disorders and on their combination with other signs of damage to the nervous system. For quantitative assessment of disability, Kurtzke's online EDSS calculator was used [39]. The manifestations of foci of cerebral demyelination were studied using contrast-enhanced MRI.

The patient's general condition and neurological status were evaluated every 7 days for 6 months. According to the plan, a contrast-enhanced MRI of the brain was performed once a year. In this case, the timing of the MRI coincided with the 5th month of use of Micromage-B.

Results

One week after the use of Micromage-B, there was a noticeable improvement in the patient's neurological status. The patient noted a significant decrease in stiffness and rapid fatigue in the lower extremities.

Objectively, the gait and coordination improved, the hand tremor decreased, depression and concentration disorders completely disappeared, appetite restored, and speech improved. Positive dynamics of normalization of the neurological status was constantly observed during the whole period of Micromage-B application. Evaluation of the neurological status of a patient with multiple sclerosis before and after 6 months of Micromage-B is presented in (Table 1).

1. Movements (pyramidal system)				
Input data		Six months after application Micromage-B		Clinical manifestations
Arm	Leg	Arm	Leg	Norma
0	0	0	0	Absence of loss symptoms, revival of tendon reflexes, enlargement of reflexogenic zones, clonus, presence of pathological signs, anisoreflexia, (absence of paresis)
5	10	5*	10*	Raises a limb independently, full volume of active movements, signs of pyramidal lesion, overcomes not only the gravity of his own limb, but also an additional obstacle of moderate strength, positive Barre-Rusetsky's symptom
10	20	10	20	Raises a limb independently, the volume of active movements is full, cannot hold a limb in a given position for a long time, and cannot overcome an additional obstacle.

15	40	15	40	Can pull a limb off the plane, the amount of active movement is limited, cannot hold a limb in a given position.
20*	60*	20	60	Cannot detach a limb from the plane, active movements in the joints of the fingers, ankle and wrist, elbow, knee joints are possible only on the plane.
40	80	40	80	Complete absence of movement (paralysis)
50	100	50	100	
2. Sensitivity				
Before	After		(a) superficial sensitivity	
0	0*		Norma	
5	0		Paresthesias, burning sensation, numbness, coldness of a limb (no objective disorders)	
10*	10		Hyperesthesia or hypoesthesia	
15	15		Anesthesia phenomena	
b) deep sensitivity				
0*	0*		Norma	
10	10		Disorder of joint and muscle feeling in small joints	
20	20		Disorder of joint and muscle feeling up to the level of the middle joints (wrist, ankle)	
40	40		Disorder of joint and muscle feeling up to the level of large joints (elbow, shoulder, knee, hip)	
3. Coordination				
0	0		Norma	
10	10*		Unsteadiness, swaying in the sensitized Romberg's test while standing on one leg, mild intensional trembling (in the complicated test), slight ataxia in the heel-knee test, deviation when walking with eyes closed.	
40*	10		Unsteadiness in simple Romberg's pose, atactic gait with open eyes and legs spread wide apart, "drunkenness," moderately pronounced intensional tremor and ataxia in the heel-knee test.	
100	100		Because of ataxia, the patient cannot move without assistance, sharp hypotonia of muscles, intensional shaking of the head, trunk, coarse - upper extremities, coarse ataxia during heel-knee test, trembling of upper extremities when trying to perform purposeful movement, chanted speech.	
4. Psycho-emotional sphere				
0	0*		Norma	
10	10		Mild impairment of the intellect in combination with euphoria, rapid change of mood, neurasthenic syndrome.	
20*	0		Euphoria, depression, decreased criticism of one's condition, decreased memory.	
100	100		Severe mental disorder, complete intellectual disintegration, Korsak's syndrome, etc.	
5. Nystagmus				
0	0*		Norma	
5	5		Nystagmus is detected only in the extreme leads (degree 1)	
10*	10		Nystagmus when looking straight ahead (degree 2)	

15	15	Sharp beating nystagmus, nystagmus in both directions, even toward the slow component (3rd degree)
6. State of the sphincters		
0	0*	Norma
10*	10	Impulsive urges, inability to hold urine for a long time, difficulty urinating
20	20	Urinary incontinence, urinary retention, intermittent urination disorders, persistent constipation
7. Sexual function		
0	0*	Norma
5*	5	Decreased sexual activity in men (intermittent impotence), sexual coldness in women
10	10	Total impotence, menstrual disorders in women
8. The ocular fundus		
0	0	Norma
5	5	Disturbance of vascular pattern, narrowing of arteries, dilation of veins, changes on fluorescence ophthalmoscopy
10*	10*	Partial optic atrophy (bitemporal pallor), optic neuritis
15	15	Complete atrophy of the optic nerve
9. Visual acuity		
0	0	Norma (vision within 1.0 or myopia)
5	5	Occasional blurring of vision, rapid fatigue when reading and performing work without impaired visual acuity
10*	10*	Visual acuity from 0.9 to 0.7
15	15	Visual acuity from 0.6 to 0.4
20	20	Visual acuity from 0.3 to 0.1
25	25	Visual acuity 0.1 and below
100	100	Blindness in one or both eyes
10. Oculomotor nerves		
0	0*	Norma (absence of subjective and objective symptoms)
5*	0	Concealed insufficiency, without visible dysfunction of one of the oculomotor nerves, inter-nuclear ophthalmoplegia syndrome
10	10	Mild visible impairment, visible insufficiency of one or more nerves, diplopia, ptosis, anisocoria
15	15	Convergent or divergent strabismus
20	20	Complete ophthalmoplegia (in one or both eyes)
11. The trigeminal nerve		
0*	0*	Norma (отсутствие объективной и субъективной симптоматики)
5	5	Subjective sensations in the form of pain, numbness, sense of "creeping chills", oppression in the face.
10	10	Objective signs of lesions, hypoesthesia, loss or decrease in the corneal reflex.
20	20	Severe anomalies with loss of trigeminal nervous functions, with or without neuralgic disorders.
12. The facial nerve		

0	0*	Norma
5*	0	Moderate weakness of facial muscles (eye closes completely, but cannot actively close it), asymmetry of frontal and nasolabial folds
10	10	Moderate weakness of mimic muscles (lagophthalmus, positive Bell's symptom, facial asymmetry in grinning), with preservation, to some extent, of mimic movements
20	20	Complete paralysis of facial muscles
13. Bulbar group of cranial nerves (nerves 9,10,11,12)		
0	0*	Norma
5*	5	Mildly pronounced bulbar phenomena (gagging when taking liquid food, change in speech, without gross organic symptoms of prolapse)
10	10	Severe dysphagia, dysarthria, decreased soft palate and posterior pharyngeal wall reflexes
100	100	Complete bulbar paralysis
14. Auditory nerve		
0*	0*	Norma
5	5	Conversational speech at a distance of 4 to 6 m, whispered speech at a distance of 1 to 3 m.
10	10	Speech - from 2 to 4 m, whisper - from 0.5 to 1 m.
15	15	Spoken speech 2 m or less, whispered speech 0 to 0.5 m
20	20	Complete deafness in one or both ears
Total:	Total:	
210*	45*	

Note: *estimated scores of the neurological status before and after using the Micromage-B.

Table 1: Neurological Status Assessment Scale for Patient K. with Multiple Sclerosis.

The data presented in Table 1 demonstrate positive dynamic normalization of neurological status after 6 months of Micromage-B use. At the initial stage the total number of points was 210. After 6 months of using Micromage-B - 45. Thus, the total number of points dropped by 165. The maximum positive effect was observed in the evaluation of the pyramidal system and coordination. The EDSS disability scale score decreased from 6.0 to 5.0.

Contrast-enhanced MRI examination of the brain after 4 months of use of Micromage-B showed for the first time a decrease in the number of new foci of demyelination in the brain.

The positive dynamics of the neurological status correlated with the results of the MRI examination of the brain.

Analyzing the data obtained, special attention should be paid to the fact that against the background of the predicted positive clinical effect caused by immunosorption, the use of magnetite nanoparticles (Micromage-B) actively contributed

to the restoration of neurological status. In this case, it can be caused by the processes of remyelination and differentiation of oligodendrocytes. Oligodendrocytes are a type of neuroglia cells that cover the myelin sheath of neurons in the CNS. The molecular mechanisms underlying cell differentiation and specialization are perhaps the most confusing area of cell biology and developmental biology. The development and maturation of many cell types is still poorly understood.

However, it is now known that one of the mechanisms of oligodendrocyte maturation is the process of enzymatic methylation - the addition of a methyl group (-CH₃) to the nitrogen atom N6 in the nitrogenous base of adenosine, known as m6A-methylation. Such a seemingly insignificant change can have enormous consequences for further stages of protein biosynthesis. The role of m6A-methylation has been shown in many processes related to oligodendrocyte maturation [40]. The universal donor of methyl groups in the body is s-adenosine-methionine, which is formed as a result of the interaction of the amino acid methionine with the ATP molecule.

Considering that due to the activation of glycolysis Micromag-B significantly increases the amount of macroergic compound (ATP) [41,42], as well as the formation of the reduced form of the coenzyme nicotinamide-adeninucleotide phosphate (NADPH₂) with the transition of oxidized form of glutathione to reduced [43], conditions for starting enzymatic methylation processes are created. The latter most likely complements the mechanism of action of magnetite nanoparticles (Micromage-B). Eventually, it ensures differentiation of oligodendrocytes and the remyelination process. It is also necessary to take into account the fact that these magnetite nanoparticles polarize the aqueous sector of the cellular microenvironment [44]. As a result, hydrolysis of the phosphate residue of ATP is activated, a quantum of energy is reset, and ADP is formed.

Taking into account the positive dynamics of the neurological status, it was decided to continue the use of the nanopreparation Micromage-B according to the specified dosage. The therapy was supplemented with a complex of rehabilitation exercises, aimed at the earliest restoration of physical, cognitive and psychosocial functions of a patient with MS.

Conclusion

The results of the study expanded the scope of clinical effectiveness of biocompatible magnetic nanoparticles in the treatment of serious autoimmune diseases [45-48]. The use of Micromage-B nanopreparation in the treatment of MS had a marked positive clinical effect. The positive dynamics of normalizing the neurological state were observed throughout the period of application of Micromage-B.

After 6 months of processing, the overall score dropped from 210 to 45. It was found that the greatest positive effect was observed in the assessment of the pyramidal system and coordination. The EDSS Disability Scale score has decreased from 6.0 to 5.0. Contrast-enhanced MRI examination of the brain for the first time registered a decrease in the number of new foci of demyelination in the brain by the 4th month of Micromage-B administration. Normalized neurological condition correlated with positive brain MRI results. The process of restoration of the central nervous system activity in MS is due not just to the immunosuppressive properties of magnetite nanoparticles, but probably also to the activation of remyelination mechanisms and oligodendrocyte differentiation due to enzymatic methylation. The design and method of using biocompatible magnetite nanoparticles to improve the efficiency of MS treatment require further improvements and studies. The use of biocompatible nanodevices in the complex treatment of MS is a promising innovation and the near future claims to include magnetite nanoparticles in the protocols of complex MS treatment [49-

51].

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