Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 21
Sofia ● 2015

Serum IgG Antibodies to GM1, GM3 and GD1a Gangliosides in Patients with Relapsing Remitting Multiple Sclerosis under Treatment with Interferon, Copaxone and Laquinimod – Preliminary Data

V. Kolyovska, D. Maslarov[*], I. Dokova[**], S. Todorov, I. Iliev, S. Engibarov[***], R. Eneva[***]

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia 1113 Acad. G. Bonchev Str., Bl. 25
[*] Medical University of Sofia, Neurology Clinic, First MHAT – Sofia
[**] Multiprofile hospital for active treatment in neurology and psychiatry "St. Naum", Accredited university hospital, Sofia
[***] The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia 1113, Acad. G. Bonchev Str., Bl. 26

Multiple sclerosis (MS) is an inflammatory disease in which the myelin sheaths around the axons of brain and spinal cord's neurons are damaged by autoantibodies, resulting in demyelination and scarring. Our previous studies have shown that the amount of ganglioside in the brain may serve as a hallmark for the disease. Serum IgG anti-GM1 antibodies are associated as potential biomarkers for the diagnosis of demyelination. Serum IgG anti-GM3 antibodies may be accepted as biomarker for BBB integrity. Autoantibodies against gangliosides GD1a are associated with acute motor axonal neuropathy and acute motor-sensory axonal neuropathy. Interferons (INFs) and Glatiramer acetate (Copaxone) are immunomodulators. Laquinimod is a new experimental immunomodulator investigated as an oral treatment for MS.

Key words: multiple sclerosis treatment, serum IgG anti-ganglioside antibodies, Interferon, Copaxone, Laquinimod.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system (CNS), traditionally considered to be an autoimmune, demyelinating, inflammatory and neurodegenerative disease. The initial MS therapeutic strategies were directed at immune modulation and inflammation control. Disease-modifying drugs (DMDs) require long-term, regular injection or monthly parenteral infusions, which may impact the patient's compliance. The aim is to prevent demyelination and to reduce axonal loss. During the different MS phases patients use various drugs: in attacks – Corticosteroids; immunomodulators -Interferon beta-1a, Interferon beta-1b (INFs) and Glatiramer ace-

tate (Copaxone). Five oral therapies are in Phase III clinical trial development or have recently been approved for the treatment of relapsing-remitting MS. One of them is Laquinimod (ABR-215062), (ALLEGRO and BRAVO clinical studies). For evaluating the level of gangliosides in the brain, the titer of antibodies against them may be used, as they are produced due to long excess release of CNS, in case of damage of the integrity of the blood brain barrier (BBB).

Interferons (IFNs, scarce and expensive until 1980**)** are proteins made and released by host cells in response to the presence of pathogens such as viruses, bacteria, parasites or tumor cells. IFNs also activate immune cells, such as natural killer cells and macrophages; they increase host defenses by up-regulating antigen presentation by virtue of increasing the expression of major histocompatibility complex (MHC) antigens. They allow the communication between cells to trigger the protective defenses of the immune system that eradicate pathogens or tumors.

Glatiramer acetate (licensed in 1996) is an immunomodulator drug currently used to treat multiple sclerosis. It is a random polymer of four amino acids found in myelin basic protein, namely glutamic acid, lysine, alanine, and tyrosine, and may work as a decoy for the immune system. Glatiramer acetate is approved by the Food and Drug Administration (FDA) for reducing the frequency of relapses, but not for reducing the progression of disability. Glatiramer acetate has also been shown to limit the formation of new MS-related lesions in the CNS and to reduce brain atrophy.

Laquinimod is an experimental immunomodulator developed by Active Biotech and Teva. It is being investigated as an oral treatment for multiple sclerosis. Laquinimod has a high level of oral bioavailability, a small distribution volume, and a low rate of total clearance. Laquinimod is an orally administered quinoline-3-carboxamide small-molecule derivative of the parent compound, the immunomodulator linomide. In preclinical studies, evidence has accumulated suggesting that laquinimod may exhibit immunomodulatory and potentially neuroprotective properties [8]. The molar mass of laquinimod is 356.803 g/mol. As a small molecule, Laquinimod diffuses freely across the BBB without any known active transport by extra- or intracellular receptor [2]. The last studies provide evidence of the broad impact that laquinimod has on brain tissue damage [3, 9].

Materials and Methods

The MS patient's sera were provided from Multiprofile hospital for active treatment in neurology and psychiatry "St. Naum"– Sofia and from Neurology Clinic, First MHAT –Sofia. After informed consent seven patients from each group were tested. The titers of serum IgG anti-GM1, anti-GM3 and anti-GD1a antibodies were estimated by the enzyme-linked immunosorbent assay (ELISA). The results were accounted on the ELISA reader TECAN TM, Sunrise, Austria. We conclude that high serum titers IgG anti-GM3 antibodies correspond with a presence of BBB integrity damage [12]. The neurodegeneration may be presented with numerical values of the serum IgG titers of anti-GD1a antibodies but high titers IgG anti-GM1 antibodies correspond with a presence of demyelination [1, 4, 7, 10, 11].

ELISA Protocol

We made slight modifications of the Mizutamari method [5]. Briefly, 1000 ng of GD1a (or GM1 or GM3) ganglioside in 100 ml of methanol were pipetted into microtitre plate wells. After air drying, the wells were blocked with BSA-PBS (1% bovine serum

albumin in phosphate-buffered saline) for 1 h. After sixfold washing with PBS, 100 μl of sera, diluted 1:20 to 1:5000 in BSA-PBS, were added to each well and incubated overnight. After that the plates were washed sixfold thoroughly with PBS. Binding was detected following a 2 h incubation period with BSA-PBS diluted (1/3200) peroxidase-conjugated goat anti-human IgG antibodies and with BSA-PBS diluted (1/4800) peroxidase conjugated goat anti-human IgM antibodies. All the incubation steps were performed at 4 °C. After sixfold washing with PBS, color development was achieved in a substrate solution containing 15 mM O-phenilendiamine and 0.015% H_2O_2 in 0.1 M sodium acetate buffer, pH 5.0 at room temperature. The reaction was stopped after 30 min by addition of 50 μl of 1N H_2SO_4 and the optical density (OD) was measured and read spectrometrically at 490 nm in a ELISA reader (TECAN, Sunrise TM, Austria). Non specific antibody binding (OD value in a well not containing GD1a) was subtracted for each measurement. Adult patients were considered strongly positive only if the mean OD of their sera exceeded $x \pm 2$ SD (standard deviation) of the healthy controls. Determinations were carried out in duplicate [6, 10].

Results and Conclusions

The aim of the treatment is to prevent demyelination and to reduce axonal loss. Interferons are reliable and accessible drug, with good perspective for the course of the disease. All investigated medicines are shown for long-term treatment and should not be interrupted. The levels of the serum IgG anti-GD1a antibodies titers in two groups Interferons and Glatiramer acetate patients are different, but showing Interferons and Glatiramer acetate demonstrate to an equal extent, partial influence on demyelination, BBB integrity and neurodegeneration the preliminary results of this study at this stage demonstrates significantly elevated serum IgG anti-GM1 titers in Laquinimod treated patients. This implicates the presence of demyelination. On the other hand, the low titers of other antibodies demonstrate that in the Laquinimod treated patients we have full BBB integrity and indications for lack of neuronal damage. It can be concluded that Laquinimod has a neuroprotective action, although the treatment does not lead to remyelination, hence combined therapy is proposed to affect all the damages. The serum IgG titers of anti-GM1, anti-GM3 and anti-GD1a antibodies demonstrates all investigated medicines are not 100% effective in all patients. Unfortunately in some patients relapses can occur, despite the continuous treatment with a suitable medicine. The presented results are preliminaries, further researches with bigger number of patients need to be conducted to confirm the findings.

References

1. **Brettschneider J, A. Petzold, A. Junker, H. Tumani.** Axonal damage markers in the cerebrospinal fluid of patients with clinically isolated syndrome improve predicting conversion to definite multiple sclerosis. – Mult. Scler., **12**(2), 2006, 143-148.
2. **Brück, W., C. Wegner.** Insight into the mechanism of laquinimod action. – J. Neurol. Sci., **306**, 2011, 173-179.
3. **Hughes, S.** Laquinimod in MS: Latest safety data 'reassuring'. The latest data from long-term follow-up of patients receiving laquinimod. – BOSTON, Nerventra, Teva Pharmaceutical Industries Ltd/Active Biotech, 2014
4. **Kolyovska, V., D. Deleva.** Serum IgG and IgM antibodies to GD1a ganglioside in adults – preliminary data. – Acta morphol. et anthropol., **19**, 2012, 114-117.

5. **Mizutamari, R., H. Wiegandt, G. Nores.** Characterization of antiganglioside antibodies present in normal human plasma. – J. Neuroimmunol., **50**(2), 1994, 215-220.
6. **Ravindranath, M., S. Muthugounder.** Human antiganglioside autoantobodies: validation of ELISA. – Ann. N. Y. Acad. Sci., **1050**, 2005, 229-242.
7. **Susuki, K., N. Yuki, D. P. Schafer, K. Hirata, G. Zhang, K. Funakoshi, M. Rasband.** Dysfunction of nodes of Ranvier: a mechanism for anti-ganglioside antibody-mediated neuropathies. – Exp. Neurol., **233**(1), 2012, 534-542.
8. **Thöne, J., R. Gold.** Review of laquinimod and its therapeutic potential in multiple sclerosis. **–** Expert. Opin. Pharmacother, **14**(18), 2013, 2545-2552.
9. **Tur, C.** Oral laquinimod for multiple sclerosis: beyond the anti-inflammatory effect. **–** J. Neurol. Neurosurg. Psychiatry, **85**(8), 2014, 833.
10. **Uncini, A.** A common mechanism and a new categorization for anti-ganglioside antibody-mediated neuropathies. – Exp. Neurol., **235**(2), 2012, 513-516.
11. **Zaprianova, E., K. Majtenyi, D. Deleva, O. Mikova, A. Filchev, B. Sultanov, V. Kolyovska, E. Sultanov, L. Christova, X. Kmetska and D. Georgiev.** Serum IgG and IgM ganglioside GM1 antibodies in patients with multiple sclerosis. – Clin. Neurosci., **57,** 2004, 94-99.
12. **Zaprianova, E., D. Deleva, B. Sultanov, V. Kolyovska.** Serum ganglioside GM3 changes in patients with early multiple sclerosis. – Acta morphol. et anthropol., **15,** 2010, 16-18.