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Serum antiganglioside IgG and IgM antibodies to GD1a in rat models of acute and prolonged lithium intoxication

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In the past years it is considered of critical importance to establish the significance of serum IgG and IgM anti-GD1a antibodies as potential biomarkers for neuronal damage in different neuropathies and neurodegenerative disorders. Although lithium salts are known to cause substantial neurodegeneration, the serum levels of anti-GD1a antibodies have not been studied in this type of intoxication yet. In this study, serum levels of IgG and IgM anti-GD1a antibodies were determined in rat models of acute and prolonged intoxication with LiCl using the enzyme-linked immunosorbent assay (ELISA) method. In both types of intoxication, serum antiganglioside IgG and IgM anti-GD1a antibodies titers were not elevated significantly to show that the blood-brain barrier in rats following Li treatment is not damaged. The results point out that IgG and IgM anti-GD1a antibodies cannot serve as serum markers for Li intoxication. Obviously, the acute or prolonged Li toxicity studies in rats cannot be used as models of progressive neuropathies.

Key words: serum IgG and IgM anti-GD1a antibodies, ELISA, acute lithium intoxication, prolonged lithium intoxication, rat

Introduction

Lithium is extensively used in psychiatric practice for the prevention and treatment of manic-depressive disorders. However, neurotoxicity of lithium salts within therapeutic doses has been reported in patients manifested by transient or persistent neurological deficits. Although those conditions are mostly transient and reversible, there is growing evidence that lithium can induce long lasting neurological sequelae [2, 4, 7, 9]. Side effects of Li generally correlated with the patient's serum level and often involve the central nervous system (CNS). Severe neurologic sequelae may occur in patients who take overdoses [8].

Gangliosides are a family of acidic glycosphingolipids highly concentrated in the nervous system, where they represent about 10% of the total lipid content. The ganglioside spectra of normal blood plasma are remarkably stable, but show pronounced changes in pathological conditions. GD1a is one of the major central nervous system neuronal ganglioside fractions. In our previous studies, a considerable increase of serum GD1a ganglioside was determined in both human cases and animal models of multiple sclerosis (MS – neurodegenerative multifactorial disorder with an autoimmune component). Autoantobodies against gangliosides GM1 or GD1a are associated with acute motor axonal neuropathy and acute motor-sensory axonal neuropathy. That is why over the past few years it is of critical importance to establish the clinical significance of serum IgG and IgM anti-GD1a antibodies as potential biomarkers for the diagnosis, classification, disease activity and prediction of clinical courses in antiganglioside antibody-mediated or other types of neurodegenerative disorders [5].

Although lithium salts are known to cause substantial neurodegeneration, no immunological studies about the possible involvement of serum IgG and IgM anti-GD1a antibodies in rats under the models of Li intoxication have been performed thus far.

The aim of the present study is to follow up the changes in serum IgG and IgM anti-GD1a antibodies in rat models of both acute and prolonged lithium chloride intoxication. The results are expected to elucidate the possible predictive value of those antibodies for lithium salts intoxication, as well as the extent of blood-brain barrier damage caused by Li-salts.

Materials and Methods

Mature Wistar rats (four-month-old) were subjected to acute lithium intoxication by a single dose of lithium chloride (250 mg/kg body weight, 0.2 ml dosing volume in saline, i.p.). Treated animals were sacrificed 24 hours following the administration under light anesthesia [9]. Healthy aged rats (eighteen-month old) were injected with the same volume of saline and used as controls.

Seven-month-old adult Wistar rats were subjected to a prolonged Li intoxication by receiving four administrations of lithium chloride with a quarter of the acute dose (250 mg/kg body weight) in the course of eight days (0.2 ml dosing volume in saline, i.p.) [4]. Animals were sacrificed under light anesthesia 24 hours after the last Li administration.

Three series of sera were obtained from the rats under the above experiments of acute and prolonged lithium intoxication. Isolation of serum antigangliosides antibodies was performed by the enzyme-linked immunosorbent assay (ELISA) method of Mizutamari [6] with slight modifications, as described before [1, 5]. Four independent analysis and quantification at various dilutions were conducted for each group and for control (no-Li) rats. The optical density (OD) was measured and read spectrometrically at 490 nm in ELISA reader Tekan Sunrise. The antigangliosides antibodies in the rat sera with Li-acute and Li-chronic intoxication, as well as healthy aged controls were calculated. The Student test was used to determine statistical differences between the groups using $p<0.05$ as the level of confidence.

Results and Discussion

Different studies show a significant increase of serum GD1a ganglioside in both human cases and animal models of multiple sclerosis [1, 5] as well as in other neuropathies [6]. The increase of titers of serum IgG and IgM anti-GD1a antibodies is usually con-

sidered as an indicator for neurodegeneration and blood-brain barrier damage. On the other hand, both acute and prolonged intoxications with lithium salts are shown to cause major pathomorphological changes in many regions of rat brain [4, 9] detected by the method of silver-copper impregnation for neurodegeneration [3]. Li is known to cross the blood-brain barrier leading to impairment of neuronal processes and neuronal death. However, it is not known yet whether the blood-brain barrier could be destructed as a result of the harmful action of Li on brain.

In our experiment, no statistically significant changes in the titers of serum IgG and IgM anti-GD1a antibodies were found (Fig. 1, Fig. 2). Optical density of the sera taken from Li-intoxicated animals did not exceed x ± 2 SD of the healthy controls to show a lack of abnormal antiganglioside antibodies values in both experimental and control animals' sera.

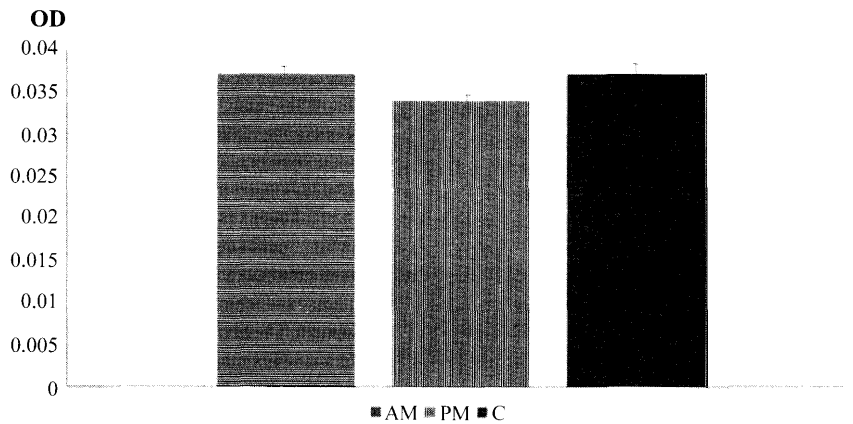

Fig. 1. Optical density (OD) of the titer of serum antiganglioside IgG antibodies to GD1a in rats subjected to acute model (AM) and prolonged model (PM) Li intoxication in comparison to control rats (C)

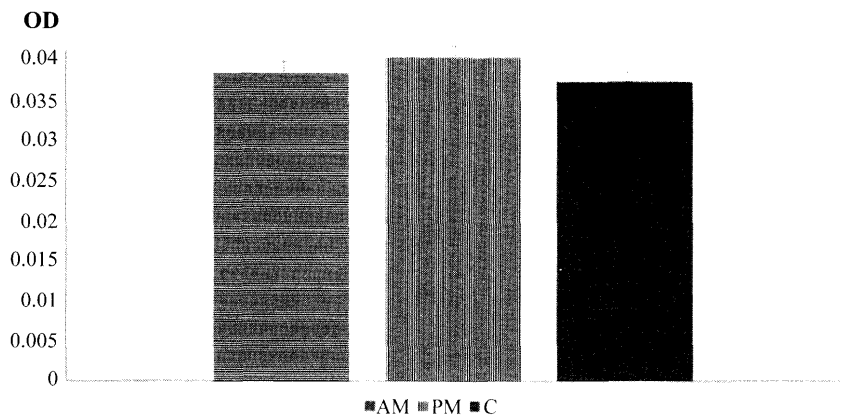

Fig. 2. Optical density (OD) of the titer of serum antiganglioside IgM antibodies to GD1a in rats subjected to acute model (AM) and prolonged model (PM) Li intoxication in comparison to control rats (C)

Legend: OD – optical density; AM – acute model; PM – prolonged model; C – control rats

In view of these results, it seems logical to conclude that lithium induced toxicity does not damage the blood-brain barrier. On the other hand, the lack of increased serum GD1a antiganglioside IgG and IgM antibodies titers suggests that rat models of acute or prolonged lithium intoxication cannot be used for the studies of highly advanced neuropathies, since the blood-brain barrier is not compromised.

References

1. Acarin, N., J. Rio, A. Fernandez, M. Tintore, I. Duran, I. Galan, X. Montalban. Different antiganglioside antibody pattern between relapsing-remitting and progressive multiple sclerosis. – Acta Neurol. Scand., **93** (2-3), 1996, 99-103.
2. Cerqueira, A., M. Reis, F. Novis, J. Bezerra, G. Magalhães, M. Rozenthal, A. Nardi. Cerebellar degeneration secondary to acute lithium carbonate intoxication. – Arq. Neuropsiquiatr., **66**, 2008, 578-580.
3. De Olmos, J., W. Ingram. An improved cupric-silver method for impregnation of axonal and terminal degeneration. – Brain Res., **33**, 1971, 523-529.
4. Dimitrova, M., E. Petrova, S. Dimitrova, Y. Gluhcheva, V. Kolyovska, D. Deleva, D. Kadiysky. Morphological changes in the rat brain provoked by prolonged lithium intoxication. – Acta morphol. et anthropol., 2012, **18,** 18-23.
5. Kolyovska, V., D. Deleva. Serum IgG and IgM antibodies to GD1a ganglioside in adults – preliminary data. – Acta morphol. et anthropol., **19**, 2012, 114-117.
6. Mitzutamari, R., L. Kremer, E. Basile, G. Nores. Anti-GM1 ganglioside IgM-antibodies present in human plasma: affinity and biological activity changes in a patient with neuropathy. – J. Neurosci Res., **51** (2), 1998, 237-242.
7. Niethammer, M., B. Ford. Permanent lithium-induced cerebellar toxicity: Three Cases and review of literature. – Mov. Disord., **22**, 2007, 570-573.
8. Ormandzhieva, V., E. Petrova, D. Kadiysky. Lithium: Specifics, toxicity and effects on the blood-brain and blood-cerebrospinal fluid barriers. – Medical data, **4** (1), 2012, 37-42.
9. Petrova, E., M. Dimitrova, S. Dimitrova, Y. Gluhcheva, V. Kolyovska, D. Deleva, D. Kadiysky. Comparison of the effect of acute LiCl intoxication on rat and mouse brain. – Acta morphol. et anthropol., **19**, 2012, 179-182.