UDC 616.441-002: 616.831: 616.892 Вестник СПбГУ. Медицина. 2019. Т. 14. Вып. 4

Reactions of mice brain to intracerebroventricular injection of thyroid peroxidase antibodies*

N. S. Novikova[1,2], K. Z. Derevtsova[1,2], A. S. Diatlova[1,2], E. A. Korneva[1,2], T. V. Fedotkina[1,3], E. V. Efimova[1], P. A. Sobolevskaia[1], L. P. Churilov[1,4]

[1] St. Petersburg State University,
7–9, Universitetskaya nab., St. Petersburg, 199034, Russian Federation

[2] Institute of Experimental Medicine,
12, ul. Akademica Pavlova, St. Petersburg, 197376, Russian Federation

[3] Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences,
44, pr. Thoreza, 194223, St. Petersburg, 194223, Russian Federation

[4] St. Petersburg Research Institute of Phthisiopulmonology, Health Ministry of Russia,
2–4, Ligovskiy pr., St. Petersburg, 191036, Russian Federation

For citation: Novikova N. S., Derevtsova K. Z., Diatlova A. S., Korneva E. A., Fedotkina T. V., Efimova E. V., Sobolevskaia P. A., Churilov L. P. Reactions of mice brain to intracerebroventricular injection of thyroid peroxidase antibodies. *Vestnik of Saint Petersburg University. Medicine*, 2019, vol. 14, issue 4, pp. 299–301. https://doi.org/10.21638/spbu11.2019.410

Hashimoto's encephalopathy is a rare disease of a presumable autoimmune aetiology. It has been shown that patients with this disease have high levels of anti-thyroperoxidase autoantibodies both in blood and cerebrospinal fluid. Specific astrocyte binding of anti-thyroperoxidase autoantibodies suggests a role of these autoantibodies in the Hashimoto's encephalopathy pathogenesis. In this study the binding of anti-thyroperoxidase with various regions of mice brain after intracerebroventricular stereotaxic injection was assessed. The results revealed no specific immunopositive labels in various brain structures of the mice which were subjected to the intracerebroventricular stereotaxic anti-thyroperoxidase injection. Direct application of human polyclonal IgG with high anti-thyroperoxidase titer on brain sections of intact mice demonstrated the same results. Apparently, absence of immunopositive staining in brain structures in the experiment can be explained by single administration of polyclonal IgG which may not contain enough of antibodies with this specificity. Further investigation of anti-thyroperoxidase binding with brain tissue in conditions of repeated intracerebroventricular administration is required.

Keywords: Hashimoto's encephalopathy, Hashimoto's thyroiditis, intracerebroventricular stereotaxic injection, neuroimmune interactions.

Introduction

Hashimoto's encephalopathy (HE) is a rare disease (2.1 cases/100,000 population) with nonspecific symptoms, associated with elevated levels of anti-thyroperoxidase (anti-TPO) and/or anti-thyroglobulin (anti-TG) autoantibodies. It predominantly affects adults starting at middle age, and females (female-to-male ratio of 5:1). Hashimoto's encepha-

* The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists. Part of the work was performed using the equipment of the science park of St. Petersburg State University.

lopathy has a presumable autoimmune aetiology. Its clinical presentation, as well as its predominance in females and its coexistence with other autoimmune diseases such as systemic lupus erythematosus, myasthenia gravis, and other disorders in up to 30 % of the cases, makes it possible to place this entity within the group of immunopathologic diseases. Due to its autoimmune nature and susceptibility to glucocorticoid treatment, recently some authors have renamed it as "steroid-reactive encephalopathy associated with autoimmune thyroiditis — SREAT" [1].

It has been shown that patients with Hashimoto's encephalopathy have high levels of anti-TPO aAb both in blood and some anti-TPO activity in cerebrospinal fluid. In immunofluorescence assays on monkey brain cerebellum sections, both Hashimoto's encephalopathy patients' sera and anti-TPO monoclonal antibodies were able to bind cerebellar cells expressing glial fibrillary acid protein. Normal human astrocytes from primary cultures also reacted with anti-TPO mAb. Specific astrocyte binding of anti-TPO aAb suggests a role of these aAb in the Hashimoto's encephalopathy pathogenesis [2].

The aim of the present study was to assess the process of polyclonal IgG enriched with anti-TPO fraction binding with various regions of mice brain, including the cerebellum, hypothalamus, thalamus, hippocampus and cerebral cortex after intracerebroventricular (ICV) anti-TPO stereotaxic injection.

Material and methods

Affinity purified human polyclonal IgG with high activity against TPO from patients with Hashimoto's encephalopathy were ICV passively transferred (using special stereotaxic apparatus) to 8 mice BalbC line (the 1st group). Control mice (n = 8, the 2nd group) were ICV injected with PBS. Also 4 mice were intact (the 3rd group). Over 24 hours after injection 2 mice from the 1st group were exposed for subsequent transcardial perfusion and fixation in 4 % paraformaldehyde (PFA). After 7 days upon injection remaining 6 mice from the 1st group, as well as mice from the 1st and 2nd groups, also were exposed for subsequent transcardial perfusion and fixation. After perfusion and fixation brain samples were embedded in paraffin. The 5 μ sections were made from paraffin-embedded samples using a microtome. Sections were placed on Poly-L-lysine glasses for subsequent immunohistochemical and immunofluorescence study. After that, brain sections were exposed to deparaffinization, dehydratation and non-specific binding blocking. Further investigation was divided in 2 parts. Brain sections from the 1st and 2nd groups of mice were incubated with secondary antibody to human IgG conjugated with HRP (BioLegend, USA) during 18h in 4 °C. Brain sections from the 3rd group of mice firstly incubated in vitro directly with human IgG with high activity to TPO from patients with Hashimoto's encephalopathy, and then incubated with secondary antibody to human IgG conjugated with HRP. After that, staining was visualized with DAB or Alexa Fluor 488. Staining was investigated by light microscope (Leica DM 2500) microscope and confocal laser scanning microscope (Leica TCS SP5) at x40, x60, x100 magnifications.

Results and discussion

On the brain sections of the 1st and 2nd groups of mice there were no specific immunopositive labels in the cerebellum, hypothalamus, thalamus, hippocampus and cerebral cortex after ICV anti- TPO injection. These results were obtained by light microscopy as

well as confocal laser scanning microscopy that allows identifying the immunopositive label more precisely. Direct application of human anti-TPO on brain sections of intact mice also demonstrated the absence of specific binding of anti-TPO with various brain structures.

It has been described that the anti-P-ribosomal protein antibody (anti-P), one of the systemic lupus erythematosus autoantibodies, binds to specific areas in the normal mouse brain tissue including the limbic and olfactory areas in conditions of repeated ICV administration. ICV injection of anti-P induces both depression-like behavior and impaired olfactory function in mice. [3]. Current research was directed to analogous investigation of anti-TPO binding with brain tissue. Apparently absence of immunopositive staining in brain structures in the experiment can be explained by single administration of IgG which may not contain enough of IgG with this specificity.

Conclusion

Further investigation of anti-TPO binding with brain tissue in conditions of repeated ICV administration is required. Single ICV administration of anti-TPO does not exert to binding with various brain structures including the cerebellum, hypothalamus, thalamus, hippocampus and cerebral cortex.

References

1. Pinedo-Torres I., Paz-Ibarra J. L. Current knowledge on Hashimoto's encephalopathy: a literature review. *Medwave,* 2018, vol. 18, no. 06, e7298.
2. Blanchin S., Coffin C., Viader F., Ruf J., Carayon P., Potier F., Reznik Y. Anti-thyroperoxidase antibodies from patients with Hashimoto's encephalopathy bind to cerebellar astrocytes. *Journal of neuroimmunology*, 2007, no. 192 (1–2), pp. 13–20.
3. Katzav A., Ben-Ziv T., Chapman J., Blank M., Reichlin M., Shoenfeld Y. Anti-P ribosomal antibodies induce defect in smell capability in a model of CNS-SLE (depression). *Journal of autoimmunity*, 2008, no. 31 (4), pp. 393–398.

Received: February 2, 2020
Accepted: June 3, 2020

Authors' information:

Nataliya S. Novikova — PhD (Biology); novikiem@gmail.com
Kristina Z. Derevtsova — PhD (Biology), Senior Researcher; kristina-shainidze@yandex.ru
Anastasia S. Diatlova — Research Engineer; nasya-nastasya@yandex.ru
Elena A. Korneva — Academician of the Russian Academy of Sciences, MD, D. Sci. (Medicine), Professor; korneva_helen@mail.ru
Tamara V. Fedotkina — PhD (Biology), Associate Professor, Senior Researcher; t.v.fedotkina@gmail.com
Evgeniya V. Efimova — PhD (Biology), Senior Researcher; e.v.efimova@mail.ru
Polina A. Sobolevskaia — Researcher; dr.polinasobolevskaia@bk.ru
Leonid P. Churilov — MD, PhD (Medicine), Associate Professor; elpach@mail.ru