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# Research Article
# Experimental *in vivo*-Models to Assess Protective Responses Against Neurodegenerative Disorders: A Pilot Study

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## Abstract

**Background and Objective:** Neurodegenerative diseases are associated with complex biochemical and structural alterations, including changes in intramolecular interactions that may influence disease progression or protective mechanisms. This pilot study aimed to investigate these biochemical, structural, and intramolecular changes to identify potential protective responses against neurodegenerative disorders. **Materials and Methods:** Plasma from patient with Alzheimer's disease (AD) was taken. The titers of IgG antibodies against gangliosides GM1, GM3 and from the general POOL were assessed by Enzyme-Linked Immunosorbent Assay (ELISA). The gangliosides titers were determined in mixture of patient's plasma with healthy experimental mouse brain homogenate. Histological light microscopy slides from rat experimental *in vivo*-model of neurodegenerative disorder and healthy rat were prepared. **Results:** In the patient's plasma, at dilution 1:40 the highest was the IgG anti-POOL antibodies titer, and at dilution 1:400 -the IgG anti-GM1 antibodies titer. In mixture, described above, the highest were the POOL gangliosides titers, and in the mouse brain extract-of the anti-POOL IgG anti-ganglioside antibodies. Both titers were approximately equal, but also the general POOL gangliosides titer in the mixed sample with the IgG anti-POOL antibodies titer. These data were supported by morphological differences between histological slides from brain of experimental rat *in vivo*-model and control healthy rat. Here again was confirmed antibodies production by non-lymphoid cells, tissues and organs in appropriate conditions, but also the role of gangliosides in the control of their function. **Conclusion:** The results suggested changes and processes, which become in the patient's brain, and which could lead to disease development or to prevention.

Key words: Gangliosides and anti-ganglioside antibodies, neurodegenerative disorder, morphological/histological features, experimental *in vivo*-models

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Dynamic qualitative and quantitative developmental, age-related and pathological changes in the gangliosides' structure and amounts have been suggested to correlate with changes in the neuronal function[1]. These changes have been established to affect many properties and functions of the gangliosides, including their capability to interact with other bio-molecules and thus, many metabolic pathways, in which they participate. As particularly critical about the neuronal function has been proven of ganglioside GM1[2]. As an example in this attitude has been accepted the observed age-dependent amyloid-beta (Aβ) plaques development in transgenic experimental mouse model[3]. An evidence about action through effect on the brain neuroplasticity, mediated by enhanced synaptic plasticity in the hippocampus and nigro-striatal dopaminergic pathway, has been proposed[4]. The role of gangliosides together with other glycolipids in many neurodegenerative disorders has been underlined[5]. Investigations of glycolipid metabolic alterations, accompanying the neurodegenerative disorders, have provided insight into the pathogenic mechanisms and recognition of diagnostic markers, as well as molecular structures and mechanisms, acting as therapeutic or preventive tools[6].

Cellular bio-potentials, generated by ion gradients across the membrane structures, have been established in many biological processes, in particular in the metabolite processes in the neurons and their control have[7]. In this way, the so called resting membrane potential (RMP) has been suggested as a potential against many normal biological functions and pathologies-wound healing, malignancies, etc., including by stem cell-based therapeutic strategies. As key cellular organelles in these metabolic pathways have been determined the mitochondria at the synapse zone[8]. This role of the mitochondria has been confirmed by the established declined energy supply and/or of disrupted bioenergy homeostasis in many pathologies as ischemia, stroke and Neurodegenerative disorders, but also in traumatic brain injuries[9]. Among the key functions of the neuronal mitochondria have been determined the regulation of $Ca^{2+}$ and redox signaling, which has been proven as the main phenomenon, underlining the developmental and synaptic plasticity, which importance in the prevention of cell death has been assessed[7]. Therefore, the role of the mitochondria as usable targets in the regulation and promoting the functions of the stem/progenitor cells has been proposed[10]. Increased intra-cellular $Ca^{2+}$ amounts have been proven as a result of ATP shortage[11]. On the other hand, the arising disruption in the $K^+/Na^+$ homeostasis has been established to lead to chronic membrane depolarization and thus, to neurodegeneration. The role of gangliosides as regulators of membrane-transmitted potential has been widely discussed[12]. As essential have been proven the adequate production of adenosine triphosphate (ATP) in the normal synaptic transmission and thus, in the normal neuronal function[13]. As a main metabolic regulator, which stimulates the catalytic process, providing the production of energy, but also the brain normal development and function, neuronal proliferation, migration, morphogenesis and synaptic communication, has been proven enzyme AMP-activated protein kinase (AMPK). A relationship of the ATP levels and the energy production rate with the level of NAD and thus-with $NAD^+$/NADH redox ratio has been established[14].

In this relation, the main goal of the current study was related with a better understanding of the intra- and extra-cellular inter-molecular interactions in the brain of patients with neurodegenerative disorders.

## MATERIALS AND METHODS

**Patient plasma sample:** Plasma was obtained from a patient with Alzheimer's disease (AD). The titers of IgG antibodies against gangliosides GM1 and GM3, as well as against the general pool (POOL), were determined by Enzyme-Linked Immunosorbent Assay (ELISA)[15].

The study was conducted during 2025-2026. The plasma samples were provided by the Neurological Clinic, First MHAT "St. John Krastitel", Sofia, Bulgaria. Materials related to the experiments were provided by the University Hospital for Neurology and Psychiatry "St. Naum", Sofia, Bulgaria, and the Medical University of Sofia, Bulgaria.

**Experimental animals and brain tissue collection:** The *in vivo* experiments using experimental rodents were performed at the Institute of Experimental Morphology, Pathology and Anthropology with Museum (IEMPAM), Bulgarian Academy of Sciences (BAS), Sofia, Bulgaria.

Brains were isolated from experimental healthy mouse, experimental healthy rat, and rat at the early stage of a neurodegenerative disorder induced by parasitic inoculation.

**Preparation of brain homogenates:** A total brain homogenate was prepared from the brain of a healthy mouse by treatment with a solution containing 0.25% trypsin and 0.02% EDTA, by application of method, developed by Mansuri *et al.*[16]. The prepared samples were subjected to repeated freeze-thaw cycles and subsequently centrifuged.

After removal of the supernatant, the pellet was resuspended in phosphate-buffered saline (PBS). The resulting suspensions were fixed in 96% ethanol.

**Biochemical and immunological studies:** An aliquot of the prepared total brain extract was mixed with an equal volume of plasma obtained from the tested patient. The titers of GM1, GM3, and the general ganglioside pool (POOL) were assessed using a modified ELISA, in which the prepared mixed sample served as the ganglioside mixture. The ELISA protocol was modified[15] with application of the technique, developed by Galan *et al.*[17] about quantitative assay of gangliosides in biological samples.

The titers of anti-GM1, anti-GM3, and anti-POOL IgG antibodies in the mouse brain extract were determined using a modified ELISA, in which the prepared organ homogenate served as the blood serum/plasma sample. In this case were taken in consideration the developed ELISA modifications about detection of disorders, related to microbiological, endocrine, metabolic and cardiovascular conditions[18].

**Histological studies:** Fixed histological slides were prepared from brains isolated from rats presenting an experimental model of a neurodegenerative disorder at the initial stage and from experimentally healthy control rats. The technique developed by Li *et al.*[19] was followed. The brain tissues were fixed in 10% neutral phosphate-buffered formalin and embedded in paraffin. Paraffin sections were placed on light microscopy slides and deparaffinized using successive treatments with xylene and ethanol solutions (70%, approximately 4-5 changes; 80%, 2 changes; 96%, 2 changes; and 100% absolute ethanol). The slides were subsequently treated with hematoxylin (Sigma-Aldrich), washed with PBS (Sigma-Aldrich), stained with eosin (Sigma-Aldrich), and again washed with PBS. Hematoxylin and Eosin (H&E) staining was performed. The proven qualitative and quantitative variations in staining between histological slides of organs from organisms with a disease and healthy controls, probably based on differences in dye absorbance, were taken into consideration[20]. The prepared slides were examined using a light microscope equipped with a CCD camera.

**Statistical analysis:** The obtained antibody titers were expressed as Main ± Standard Deviation (SD). Optical density (OD) was measured spectrophotometrically at 490 nm using an ELISA reader (TECAN™ Sunrise, Austria). Differences were considered statistically significant at $p<0.05$ and $p<0.01$.

## RESULTS

The titers of IgG antibodies against GM1, GM3, and the general ganglioside pool (POOL) were determined in the plasma sample from the patient with Alzheimer's disease using ELISA. At a dilution of 1:400, the plasma showed a significantly higher titer of IgG anti-GM1 antibodies compared with the titers observed at the other dilutions (Fig. 1). In opposite, the highest titer of the anti-POOL IgG antibodies was noted at dilution 1:40.

The highest titer was detected of the gangliosides from the POOL in the mixture of plasma from the patient with neurodegenerative disease with the total extract of the brain from healthy adult mouse (Fig. 2a). In the total extract of the adult healthy mouse brain was observed the highest titer of the anti-POOL antibodies (Fig. 2b). Approximately equal titers

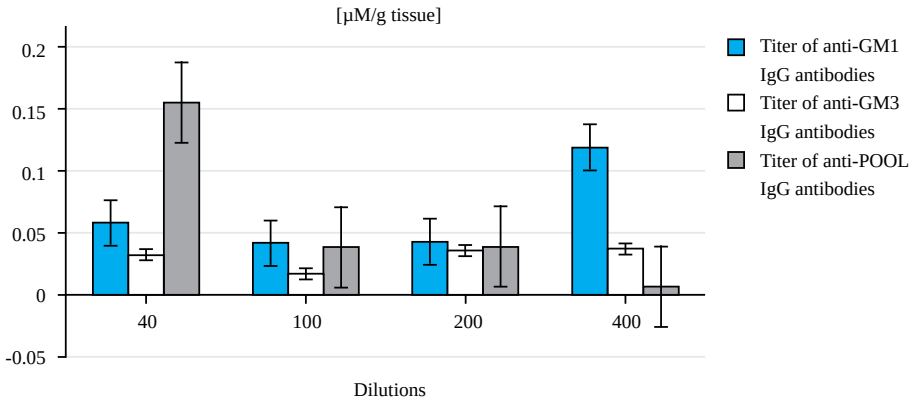



Fig. 1: Titers of the anti-GM1, anti-GM3 and anti-POOL IgG antibodies in plasma from patient with neurodegenerative disorder, at dilutions 1:40, 1:100, 1:200 and 1:400

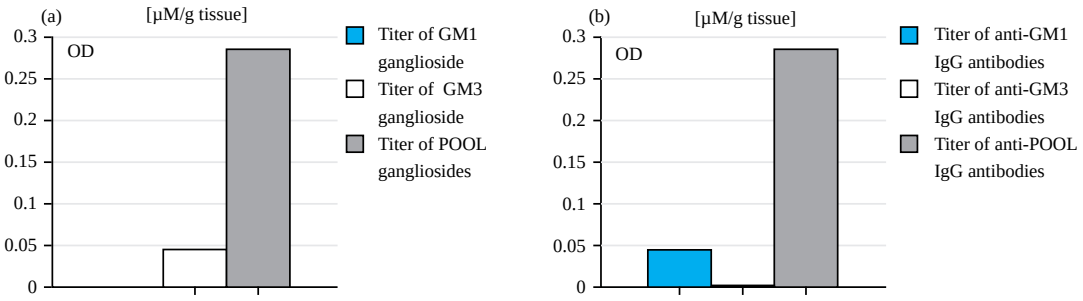



Fig. 2(a-b): (a) Titers of GM1, GM3 and POOL gangliosides in mixture of total extract from brain of healthy adult experimental mouse with plasma from the patient with neurodegenerative disease and (b) Titers of anti-GM1, anti-GM3 and anti-POOL IgG antibodies, in the total extract from the brain of healthy adult experimental mouse

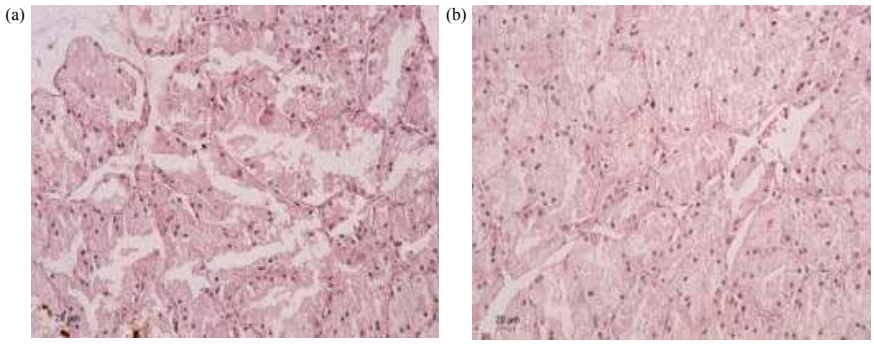



Fig. 3(a-b): Histological light-microscopy samples, (a) From brain of experimental *in vivo*-model of rat with neurodegenerative disorder at initial stage and (b) From brain of healthy control experimental adult rat

of the gangliosides from the POOL in the prepared mixture between the mouse brain extract and the patient's plasma with the titer of the IgG anti-POOL antibodies in the mouse kidneys homogenate were noted. In the mixed sample, described above, was established a low titer of ganglioside GM3 and lack of ganglioside GM1 (Fig. 2a). The titer of the POOL gangliosides in the prepared mixed sample was approximately equal (Fig. 2a) to the titer of the anti-POOL antibodies in the total extract from the brain of the healthy adult mouse (Fig. 2b). The assessed titer of anti-GM1 antibodies in the total brain extract of the healthy adult animal (Fig. 2b) was approximately equal with the titer of ganglioside GM3 in the mixed sample (Fig. 2a). These results could be explained with the protective role of the gangliosides against the pathological processes, which are available in the patient. In the histological slide of brain from the used experimental rat *in vivo*-model of initial phase of neurodegenerative disorder, injuries in the distribution of cells, irregular localization of the nuclei and of the other cellular components could be (Fig. 3a) compared to the histological slide of the brain from the healthy control rat (Fig. 3b).

## DISCUSSION

Taking in consideration the proven property of all gangliosides to transform from each to another type, in confirmation of this hypothesis was the high titer of gangliosides from the general POOL and the presence of GM3 ganglioside in the mixture of the same rodent brain with serum from patient with neurodegenerative disorder (Fig. 2a)[21]. These suggestions were in agreement with the literature data about the proven role of ganglioside GM1 and some other gangliosides as markers of neurodegeneration[22]. The contradictory data in the titers of gangliosides GM1 and GM3 in the extract from the healthy mouse brain and of the respective IgG antibodies to each one of them in the mixture between the same brain extract and plasma from the patient could be explained with the preventive role of the respective gangliosides against neurodegenerative and inflammatory events. These results also were in confirmation of the literature findings[23]. The noted co-localization of the accumulated GM3 with Aβ plaques and with *Hexa* gene expression in aged transgene mice has proposed the accumulation of GM3 as a mechanism of gangliosides' degradation[3].

The high titers of the anti-POOL IgG antibodies and the presence of such against ganglioside GM1 (Fig. 2b) in the total brain extract of healthy adult mouse. The presented result confirmed the possibility about production of antibodies/immunoglobulins by non-lymphoid types of cells, tissues and organs, and thus-about performing of immune functions by non-immune cells in appropriate conditions[24,25]. Activation of initial internal protective systems has been proven in this relation[26,27]. As underlining these events have been proven reorganization processes in the cytoplasmic organelles, including in mitochondria[28]. On this basis changes in the mitochondrial dynamics, related with mtDNA, mitochondria-located microRNAs and various proteins, have been suggested as novel targets in many brain pathologies, including due to injuries and immuno-metabolic disorders and malignancies[29]. These rearrangements have been found to lead to arising or development of a pathology process, but also to the opposite effect of its prevention[30]. In various inter-molecular interactions and cascade regulatory mechanisms, which could underline the development of multifactor diseases, particularly malignancies, psychiatric and neurodegenerative pathologies or their prevention, gangliosides have been proven to participate.

## ETHICS

The used experimental rodents were commercial, delivered from Charles River Laboratories, Quebec, Canada. In all cases were applied euthanasia methods by anesthetic, consistent with the commonly accepted norms of veterinary best practice. All ethics rights are taken in consideration according to the Declaration of Helsinki. The process of taking plasma isolation from the patient was important in the process of diagnosis, about determination of optimal therapeutic scheme about her. Informed consent to participate was obtained from all of the participants in the performed study, which is described in the current research paper. The ethical approval is approved from the Danish National Center for Ethics (Journal Number: H-23030276) and the Danish Data Protection Agency (Journal Number: BFH-2017-001). The current research paper is not under consideration elsewhere.

## CONCLUSION

The highest titer of the anti-GM1 IgG antibodies in the plasma from the patient with AD was assessed at dilution 1:400, and of the anti-POOL IgG antibodies-at dilution 1:40. In the prepared mixture of the plasma from the patient plus total extract of the brain from adult healthy control mouse, the highest titer was observed of gangliosides from the POOL, but also of the anti-POOL IgG antibodies in the extract of the healthy mouse brain, which was approximately the same as the antibodies against it in the mixture. In the same mixture, despite the noted presence of GM3 ganglioside, its titer was significantly lower than that of the gangliosides from the POOL. GM1 ganglioside was not available. In the total extract of the healthy mouse brain lack of anti-GM3 antibodies titers was assessed, and the titer of the anti-GM1 antibodies was significantly lower than of the POOL gangliosides. On the other hand, the titer of GM3 ganglioside in the prepared mixed sample was approximately equal with the titer of the anti-GM1 IgG antibodies in the mouse kidneys extract. These results were explained with the protective functions of gangliosides, available in the brain extract from the healthy adult mouse against neurodegenerative and inflammatory processes. Here again was confirmed the possibility about production of antibodies by non-lymphoid types of cells, tissues and organs in appropriate conditions, but also the importance of gangliosides in the control of their functions. The assessed biochemical features were supported by observed differences in histological samples from brains of experimental adult rat *in vivo*-models of initial phase of neurodegenerative process and of healthy control.

## SIGNIFICANCE STATEMENT

This study highlights potential interactions between anti-ganglioside IgG antibodies and brain gangliosides in Alzheimer's disease. The biochemical findings, supported by histological observations, provide insights into possible immune-related and structural changes associated with neurodegeneration and may help guide further investigations into protective mechanisms and disease progression.

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