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Research Article

Changes in Blood Brain-Derived Neurotrophic Factor (BDNF) Levels in Experimental Animals with Traumatic Brain Injury after Magnesium Sulfate Administration: An Experimental Study

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ABSTRACT

Background: Traumatic brain injury (TBI) results in notable impairments in neurological function and is associated with poor outcomes. Various processes occur at the cellular level, one of which is neuroinflammation. Brain-derived neurotrophic factor (BDNF) is a neurotrophin protein produced by the brain that circulates in plasma post-injury. It has functions such as anti-apoptosis, anti-neurotoxicity, and anti-inflammatory effects. Therapeutic approaches aimed at modulating or synergizing BDNF are anticipated to reduce inflammation and enhance outcomes in TBI patients. Magnesium sulfate administration is known for its anti-inflammatory and neuroprotective effects. **Methods:** This study employed a true experimental post-test-only group design. The subjects, male Wistar rats (*Rattus norvegicus*), were subjected to weight-drop-induced TBI and divided into three distinct groups: a control group (Group A), a TBI group without therapy (Group B), and a therapy group (Group C). Group B received TBI without magnesium sulfate administration, while Group C received TBI with magnesium sulfate administered at 250 µm/kg BW. BDNF levels in blood plasma were assessed at the conclusion of therapy utilizing ELISA. ANOVA was used to conclude the inquiry after all groups underwent a Shapiro-Wilk test. **Results:** Plasma BDNF levels were significantly lower in the TBI rat models treated with magnesium sulfate at 250 µm/kg BW within 4 hours after injury than in the untreated group ($p = 0.005$). Compared to the untreated group, the magnesium sulfate-treated group had reduced plasma BDNF levels. **Conclusions:** Administration of MgSO4 to the TBI treatment group resulted in decreased BDNF levels compared to the untreated group.
Keywords: Traumatic brain injury, Magnesium sulfate, BDNF, Neuroinflammation.

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BACKGROUND

Traumatic brain injury (TBI) is a kind of physical damage that may result in either a permanent or temporary interruption of brain function, and it can be caused by a variety of events, including a bump, blow, or collision to the head.[1] Different geographic areas have varied rates of TBI. According to the Global Burden of Disease Study 2019, Africa has the lowest rate of traumatic brain injury, whereas North America and Europe have the greatest rate.[1] Because secondary brain injuries may vary in kind, including axonal injury, ischemic brain damage, and mass lesions, treatment options for them have not always been successful. Examples of these treatments include lipid peroxidation, apoptosis, glutamate excitotoxicity, free radical injury, edema, and calcium influx.[2] Hypoxia, hypotension, increased intracranial pressure, decreased blood perfusion to the brain[3], and pyrexia are the main factors that cause secondary brain injury. Preventing secondary brain injury in cases of traumatic brain injury can enhance various outcomes.[4] TBI may activate microglia and neuroinflammation, and cause damage to axons, neurons, and glia. Therefore, modifications to the intracellular proteins found in brain tissue or other organic compounds found in certain bodily fluids that serve as an indirect indicator of the ensuing brain injury and recovery are referred to as cerebral biomarkers.[1] The central nervous system (CNS) has systemic indicators that may be used to predict immediate mortality after a severe traumatic brain injury. Additionally, in relation to the molecular pathways they represent, biomarkers depict TBI-specific disease.[5] Neuroinflammation, neuronal ischemia, or hypoxia caused by surgeries and general anesthesia can cause brain cell harm, resulting in the elevation of biochemical markers for brain damage in the bloodstream.[6]

An autocrine factor that promotes cell regeneration, differentiation, and development is brain-derived neurotrophic factor (BDNF), one of a group of neurotrophic proteins. BDNF is involved in maintaining the survival of central nervous system neuron, promoting the growth and development of new neurons, enhancing synapse formation, contributing to neurogenesis, and safeguarding neural precursor cells (NPC) and neural stem cells (NSC).[7] Moreover, synaptic plasticity and neuronal cell regeneration depend on this protein. The human chromosome 11p contains the BDNF gene, composed of one 3' exon (exon V) that generates the mature BDNF protein and four 5' exons (exons I–IV) with unique promoters. Eight different mRNAs are produced; exon IV is mostly located in the heart and lung, while transcripts with exons I–III are typically located in the brain.[8] BDNF shares approximately 50% of its amino acid sequence with neurotrophin-4/5 (NT-4/5), neurotrophin-3 (NT-3), and nerve growth factor (NGF). Every neurotrophin includes a homodimer linked noncovalently and consists of a signal peptide after the start codon

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and a proregion that has an N-linked glycosylation site.[1,2,9] BDNF is a fascinating indicator in TBI rehabilitation because of its significant influence on the autonomic nervous system through hypothalamic metabolic regulation and brainstem management of the cardiovascular system. TBI results in a prolonged stress reaction and individual variations in stress hormone levels, which can result in diverse post-injury effects. Lowered levels of serum BDNF have also been linked to death in non-injured populations.[8] It has been shown that BDNF may strengthen the connections between them after trauma, protect nerve cells, and decrease the degree of secondary brain damage.[5] TBI can lead to BDNF gene methylation, which hinders its gene transcription and secretion, resulting in limited brain neuroregeneration after TBI and worsening the patient's clinical outcomes.[8] Studies on TBI management might benefit from focusing on BDNF, a neurotrophin present in the brain, because of its role in neuronal plasticity, neurogenesis, and survival.[5]

Magnesium is utilized in various areas of medicine with minimal negative impacts and affordable pricing. Several studies have examined the benefits of magnesium for neuron protection and found positive effects following a lack of blood flow to the brain.[3] The use of magnesium sulfate (MgSO4) during surgery, which acts as a blocker for N-methyl-D-aspartate receptors, is recognized for its ability to reduce pain and the amount of anesthesia and pain medication required, even though its specific mechanism is not fully understood. Magnesium has the ability to shield neurons from ischemic harm and promote neuronal survival following traumatic brain injury by employing different mechanisms, such as preventing the release of presynaptic excitatory neurotransmitters, obstructing voltage-gated calcium channels and NMDA channels, increasing presynaptic adenosine, reducing cortical spreading depression.[10] Magnesium is recognized for its involvement in expanding blood vessels.

This compound may play roles in hemostasis and protecting the blood-brain barrier, potentially serving as a neuroprotective agent for acute stroke and brain hemorrhage.[11] Magnesium plays a critical role in transmitting nerve signals and regulating ionic balance within the body.[12] Magnesium can protect neurons from damage caused by lack of oxygen and help neurons survive after a traumatic brain injury by blocking the release of excitatory neurotransmitters, NMDA channels, and calcium channels, as well as by enhancing adenosine and suppressing cortical spreading depression.[10] Lower levels of MgSO4 are linked to neurological disorders by causing various biological and metabolic issues, which are worsened by reduced ATP generation and changed Na/K-ATPase function.[13] The BBB defends the brain from toxins and pathogens and regulates the movement of molecules between the blood and the CNS in both directions. It is also an important provider of multiple neurotrophins, one of which is BDNF, which is essential for neuronal plasticity and cognitive processes like learning and memory.[12] Numerous clinical studies have shown that MgSO4 has protective effects on the brain, particularly after inducing experimental cerebral ischemia.[3] Elevated levels of Mg also increase the expression of BDNF, a neurotrophin found throughout the central nervous system crucial for the development and maintenance of neurons and synapses.[12] This study examines the administration of magnesium sulfate on BDNF plasma levels after traumatic brain injury treatment.

MATERIALS AND METHODS

Animal Model

This study was approved by the veterinary research ethics committee at Universitas Airlangga (reference number: 2.KEH.040.03.2024). In this study, the in vivo model of TBI involved male Wistar rats (*Rattus norvegicus*) aged 2.5–3 months and weighing 150-250 grams. A veterinary assistant, who was unaware of the study's categorization strategy, assigned samples to each group before arranging them randomly in cages without labels.

The following formula establishes the quantity of samples needed for the hypothesis test:

$$E = Total\ Number\ of\ Animals\ -Total\ Number\ of\ groups$$

The following formula establishes the sample size:

$$E = (10\ x\ 3) - 3 = 27$$

Three main groups of subjects were created: Group A, which served as the control group and did not receive magnesium sulfate; Group B, which had a TBI but did not receive magnesium sulfate; and Group C, which received magnesium sulfate treatment. Group C was administered magnesium sulfate at a dose of 250 µm/kg BW 30 minutes post-TBI treatment. After 4 hours, the evaluation of BDNF by ELISA was conducted in the control group, the TBI treatment group without the administration of MgSO4, and the TBI treatment group with the administration of MgSO4.

For seven days, all the rats were provided the same food and drink, exposed to a regular cycle of light and dark every twelve hours, and housed in cages with a constant temperature of 22 ± 2°C for acclimatization. Group C received treatment 30 minutes after trauma induction and was terminated 4 hours later. Groups B and C were terminated 4 hours after the trauma started. Group A was terminated immediately after the acclimatization procedure.

The exsanguination approach was used to terminate the subjects while they were under general anesthesia. This method was selected since it lowers the possibility of extended hypoxia during the termination procedure.

Traumatic Brain Injury Induction

The rats were placed under a weight drop device after the administration of anesthesia. The brain injury treatment utilized a modified Barzo "weight drop" model that dropped a rod-shaped iron weighing 40 grams, 4 mm in diameter, from a height of 100 cm directly through a guiding tube onto the exposed bony surface of the rat brain. This caused a standardized parietal contusion.

Magnesium Sulfate Administration

In this study, 20% magnesium sulfate was taken using a 1 ml syringe with a dose of 250 µm/kg BW using normal saline dissolution. The drug was administered through a vein in the tail of the rats. This experiment was conducted in the laboratory of experimental animals of the Faculty of Veterinary Medicine, Universitas Airlangga.

Blood Sampling

Blood samples were collected from all groups by inserting a syringe directly into the heart and drawing all the blood (exsanguination) under general anesthesia. Rat blood plasma samples were obtained from rat blood that had been centrifuged (2,000-3,000 RPM for 20 minutes) at room temperature. BDNF levels in the plasma of the experimental rats were analyzed using an ELISA device (catalog number E0013Mo, Bioassay Technology Laboratory, Shanghai Korain), following the manufacturer's instructions. The ELISA microplate reader system was used to analyze the data at a wavelength of 450 nm. The unit of measurement was ng/mL.

Statistical Analysis

The collected data were processed using SPSS Statistics and Microsoft Excel. Since there were less than 50 samples, a data normalcy test using the Shapiro-Wilk test was conducted to make sure the data in

each group were distributed normally. Data were considered normally distributed if the probability was above 0.05. In the case of regularly distributed data, the ANOVA test was used for data analysis; otherwise, the Kruskal-Wallis test was applied. ANOVA was chosen because it compares three or more groups and uses interval ratio scale data and numerical data. Researchers used a significance level of 5%, meaning that p-values less than 0.05 were regarded as statistically significant.

RESULTS

This study demonstrated a decreased in BDNF levels in Group C (brain injury treatment with MgSO4 administration) compared to Group B (brain injury treatment without MgSO4 administration). This result suggests that magnesium sulfate, which is an anti-inflammatory agent, has a similar function to BDNF in terms of neuroinflammation. Based on these findings, the administration of magnesium sulfate is thought to reduce BDNF expression after BTI and can serve as an alternative therapy in brain injury management by acting as a neuroinflammatory agent.

The Shapiro-Wilk data normality test (Table 1) found that the BDNF data was normally distributed ($P > 0.05$). The ANOVA test results revealed substantial differences in BDNF levels among Groups A, B, and C ($P = 0.005$) (Table 1).

Following the discovery of significant differences in the ANOVA test findings, a post hoc test was conducted with a significance value of $P < 0.05$. The post hoc test findings revealed differences between the TBI group with MgSO4 administration and the control group ($P = 0.150$). There was also a difference between the TBI group without MgSO4 administration and the TBI group with MgSO4 administration ($P = 0.383$) (Table 2).

DISCUSSION

This research examines the impact of MgSO4 injection on BDNF level variations in the blood of rats treated for brain damage. Since it inhibits several secondary injury factors, such as the production of free radicals, lipid peroxidation, mitochondrial permeability transition pore opening, the formation of edema, calcium channels, N-methyl-D-aspartate channel activity, glutamate release, magnesium has been suggested as a highly effective pharmacological intervention in traumatic brain injury. Neurotrophins—in particular, BDNF—are crucial for cellular processes, including neuronal survival, axonal sprouting, and synaptogenesis, that occur throughout the healing process after TBI.[14] Magnesium is crucial for maintaining balance in regulating processes related to the delayed secondary phase of brain injury.[15] Hypomagnesemia, a prevalent electrolyte imbalance in medical settings, has been identified in numerous studies, especially among ICU patients, where it has been linked to higher mortality rates and longer hospital stays.[16] Hypomagnesemia in critically ill patients can be attributed to several factors, such as diabetes mellitus, malnutrition, reduced magnesium absorption caused by gastrointestinal issues, other electrolyte disturbances like hypokalemia and hypocalcemia, and the use of specific medications, including proton pump inhibitors, gentamycin, and loop diuretics.[16] Nuclei, mitochondria, and ER/SR separate intracellular Mg2+ with overall concentrations ranging from 15 to 18 mm.[14] Administering magnesium following experimental TBI enhances psychological, motor, and cognitive recovery, lessens the activity of the proapoptotic protein p53, and reduces post-injury edema formation.[17] By competing with calcium receptors or obstructing calcium channels, magnesium inhibits the flow of calcium into the brain, acting as a vasodilator.[18] The blood-brain barrier's integrity determines whether magnesium can enter the central nervous system. Such permeability is not always present in human trauma, despite the fact that significant opening of the blood-brain barrier in animal models of trauma allows magnesium access into the central nervous system for at least 24 hours.[19] In experiments, research conducted in various labs has shown that magnesium levels in the serum and brain decrease after experimental traumatic brain injury. It has been observed that outcomes improve with magnesium supplementation, whether administered before, shortly after, or hours after the injury.[20]

Within 30 minutes of treating rats for traumatic brain damage in this investigation, rats received a dosage of 250 µm/kg BW of magnesium sulfate. BDNF levels were evaluated by ELISA in the control group, the traumatic brain injury treatment group without MgSO4 administration, and the traumatic brain injury treatment group with MgSO4 administration. According to the study's findings, the treatment group receiving MgSO4 had reduced BDNF levels than the group receiving no MgSO4 at all.

Table 1. BDNF Results.

	Animal	$\bar{X} \pm$ SD (ng/mL)	P-value
Control	9	0.539 ± 0.244	
TBI models without MgSO4	9	0.990 ± 0.369	0.005
TBI models with MgSO4	9	0.794 ± 0.098	

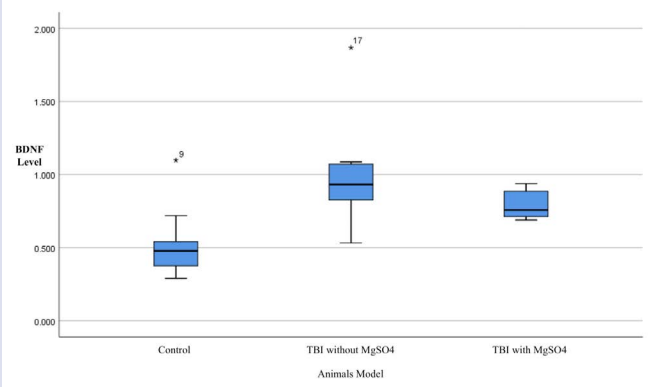

Table 2. BDNF Results Graph.

CONCLUSION

The administration of MgSO4 to animals with traumatic brain injury decreased BDNF levels compared to animals without MgSO4 administration. The drop in BDNF levels in the animal group post-MgSO4 treatment may be attributed to MgSO4 acting as an anti-inflammatory agent, with BDNF also being an endogenous anti-inflammatory generated from cellular activities following a head injury. Therefore, with the decrease in BDNF levels, there may be a cellular mechanism triggering the downregulation process due to MgSO4 interfering with the anticipated anti-inflammatory process.

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CONFLICTS OF INTEREST

The authors reported no conflicts of interest in this study.

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ETHICAL CLEARANCE

Ethical committee approval for this study was obtained from the Faculty of Veterinary Medicine at Universitas Airlangga. The approval certificate number is 2.KEH.040.03.2024.

AUTHOR CONTRIBUTION

All authors contributed to article preparation and paper revision and have collectively assumed responsibility for all aspects of this study.

DATA AVAILABILITY

The article contains all the necessary data to support the results; no supplementary source data are needed.

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