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RESEARCH

Therapeutic effects of transcranial direct current stimulation on loss of motor function caused by experimental mild traumatic brain injury

Transkraniyal doğru akım stimülasyonunun deneysel hafif travmatik beyin hasarının neden olduğu motor fonksiyon kaybı üzerine terapötik etkileri

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Abstract

Purpose: Traumatic brain injury (TBI) is a serious illness that causes behavioral disorders such as locomotor activity, learning, and memory. This study aims to investigate the behavioral effects of transcranial Direct Current Stimulation (tDCS) treatment on locomotor activity in an experimental traumatic brain injury model and to investigate the levels of IL-1β and IL-18 in the motor cortex tissue.

Materials and Methods: 30 male 3-month-old Wistar albino rats were used. The TBI model was established using the Marmarou method. 2 hours after TBI, sham and TBI+tDCS groups were treated with 0.5 mA 30 minutes anodal tDCS treatment for 2 days. Locomotor activity was evaluated in open field test. IL-1β and IL-18 levels in motor cortex tissue were measured by the ELISA method..

Results: Compared to the sham group, locomotor activity results showed significant decreases in the TBI group while the TBI+tDCS group showed significant increases compared to the TBI group. There were significant increases in IL-1β and IL-18 values in the motor cortex of the animals in the TBI group compared to the sham group, while there was a significant decrease in the TBI+tDCS group compared to the TBI group.

Conclusion: tDCS treatment was shown to have therapeutic effects on neuroinflammation against traumatic brain injury.

Keywords:. Motor function, therapeutic treatment, traumatic brain injury, transcranial direct current stimulation

Öz

Amaç: Travmatik beyin hasarı (TBH) lokomotor aktivite, öğrenme ve hafıza gibi davranışsal bozukluklara neden olan ciddi bir hastalıktır. Bu çalışmanın amacı, deneysel travmatik beyin hasarı modelinde transkraniyal Doğru Akım Stimülasyonu (tDAS) tedavisinin lokomotor aktivite üzerindeki davranışsal etkilerini araştırmak ve motor korteks dokusunda IL-1β ve IL-18 düzeylerini incelemektir.

Gereç ve Yöntem: 30 adet 3 aylık erkek Wistar albino sıçanlar kullanıldı. TBH modeli Marmarou yöntemi kullanılarak oluşturuldu. Sham ve TBH+tDAS gruplarına TBH'nın 2. saatinde başlanıp 2 gün boyunca günde 0.5 mA 30 dakika anodal tDAS tedavisi uygulandı. Lokomotor aktivite açık alan testinde değerlendirildi. Motor korteks dokusunda IL-1β ve IL-18 düzeyleri ELİSA yöntemi ile ölçüldü.

Bulgular: Lokomotor aktivite sonuçları sham grubuna kıyasla TBH grubunda anlamlı düzeyde azalma görülürken), TBH+tDAS grubunda TBH grubuna kıyasla anlamlı artma görüldü. TBH grubunun motor korteks IL-1β ve IL-18 değerlerinde sham grubuna kıyasla anlamlı artma tespit edilirken, TBH+tDAS grubunda TBH grubuna kıyasla anlamlı bir azalam tespit edildi.

Sonuç: Çalışmamızda, tDAS tedavisinin travmatik beyin hasarına karşı nöroinflamasyon üzerinde terapötik etkiye sahip olduğu gösterildi.

Anahtar kelimeler: Motor fonksiyon, terapötik tedavi, travmatik beyin hasarı, transkraniyal doğru akım stimülasyonu

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INTRODUCTION

Traumatic brain injury (TBI) is defined as damage caused by direct and indirect forces applied to the brain. TBI is the formation of temporary or permanent neurological dysfunction in the central nervous system in the face of a blow from outside the body. TBI is a major cause of disability worldwide. Since the brain is the most vulnerable and complex organ of the body, traumatic brain injuries affect the life of the person in many ways. It leads to physical, cognitive, and behavioral losses. It also causes psychological and social problems. TBI is one of the major causes of death and chronic disability in individuals under the age of 35[1]. The incidence of severe and moderate head injuries is 14 per 100,000 and 15 per 100,000 population, respectively. The incidence of mild head trauma was reported to be 131 per 100,000 population. Mortality rates are 0% for mild head injuries, 7-10% for moderate head injuries, and 30% for severe head injuries. Twenty percent of people with traumatic brain injury are hospitalized. Approximately 200,000 people die or become disabled as a result of these traumas annually. Despite being such a common and serious problem, there are problems in diagnosis and prognosis prediction[2]. Clinically, TBI may result in changes in cognitive characteristics such as memory loss, perception difficulties, distraction, and logical thinking, as well as physical problems such as partial or complete paralysis, balance disorders, swallowing, and speech disorders. Unless traumatic brain injury is treated in the early period, the mortality rate is quite high[2,3].

Primary brain injury occurs first in the central nervous system as a result of trauma. Primary brain injury consists of scalp injury, skull fracture, contusion, brain laceration, diffuse axonal damage, and intracranial hemorrhage. However, only primary damage is not responsible for the damage caused by head trauma. Secondary brain injury also occurs hours or days after the primary brain injury due to many complex physiopathological events. In patients with TBI, secondary damage is said to have a poor prognosis. Secondary damage occurs hours or days later and includes calcium-dependent cell damage, neurotransmitter release, reactive oxygen species formation, gene activation, mitochondrial dysfunction, and inflammatory response[4]. Brain trauma increases the extracellular concentration of the excitatory neurotransmitter glutamate[5]. Disruption of presynaptic membrane-bound ion pumps and calcium-mediated exocytosis cause glutamate release from neurons as a result of depolarisation[2,6]. This excess neurotransmission is thought to cause a toxic increase in intracellular calcium concentration. Especially in the last two decades, the increase in knowledge about the physiopathology of head traumas, the application of appropriate treatment methods by solving the physiology of secondary neuronal damage after traumatic brain injury, and high-level developments in patient care techniques in intensive care have reduced mortality rates and significantly improved prognosis. When the pathophysiology of traumatic brain injury is analyzed, it is seen that apoptosis, neuroinflammation, and cell death processes that occur after free radical production plays a major role in the formation of the damage[4,7,8].

The effects of many pharmacological agents such as anti-inflammatory drugs, excitotoxicity blocking drugs, antiapoptotic, free radical scavengers, calcium channel blockers, growth factors, steroids, and statins have been tested on different animal models to limit the biochemical damage and cell death after TBI. In animal experiments, statins, which are 3-hydroxy-3-methylglutaryl-coenzyme reductase inhibitors, have been reported to reduce glial activation and inflammatory response, which are the causes of cerebral edema and secondary neuronal damage in closed head trauma[9]. Treatment modalities such as deep brain stimulation especially improve motor and non-motor dysfunctions[10]. However, the high risk and cost associated with invasive neurosurgical procedures remain an important problem to be solved. On the other hand, transcranial direct current stimulation (tDCS) is a relatively easy and safe method to modulate cortical polarisation by applying low-intensity current (1.0-2.0 mA) to the scalp. Anodal stimulation in tDCS increases cortical excitability, while cathodal stimulation decreases it[11]. Recent studies show that tDCS in combination with rehabilitation has long-term effects on the improvement of symptoms in various neurological disorders[12]. Especially in recent years, tDCS applications have been used to reduce pain in diseases such as traumatic spinal cord injury, central pain, in psychiatric diseases, neurological diseases, and stroke rehabilitation research; and studies investigating the effects of cognitive functions such as learning, memory and decision-making in healthy people[13-16].

Studies have shown that tDCS treatment reduces especially motor and cognitive function losses after TBI[17-19]. TBI initiates a cascade of inflammatory

processes that include the release of proinflammatory mediators that lead to neuronal damage. It is known that brain Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6) levels increase significantly 4 hours after TBI[20]. However, although there are antioxidant studies against inflammatory damage after TBI, there are no studies investigating the neuroinflammatory effects of tDCS treatment. The effect of tDCS treatment, which is known to have neurotherapeutic effects, on motor function after mild traumatic brain injury and its relationship with neuroinflammation has not been fully elucidated. Although it is known that traumatic brain injury causes motor dysfunction and increased cytokine neuroinflammation, the effects of tDCS on motor function through cytokine regulation should be investigated. In light of this information, our study aims to investigate the effects of tDCS on motor function and neuroinflammation after TBI.

MATERIALS AND METHODS

Animals and experimental design

In this study, 3-month-old male Wistar Albina rats weighing 250-300 g were used. All animal use and experimental protocols were approved and implemented by the Animal Care and Ethics Committee of Erciyes University (Erciyes University, 02.02.2022/Approval no: 22/022). The mild traumatic brain injury (mTBI) model was created using the Marmarou method[21]. Locomotor activity was evaluated in the open field test (OF). After the behavioral experiments, the subjects were sacrificed, the brain was removed and IL-1β and IL-18 levels in the motor cortex tissues were evaluated by the ELISA method.

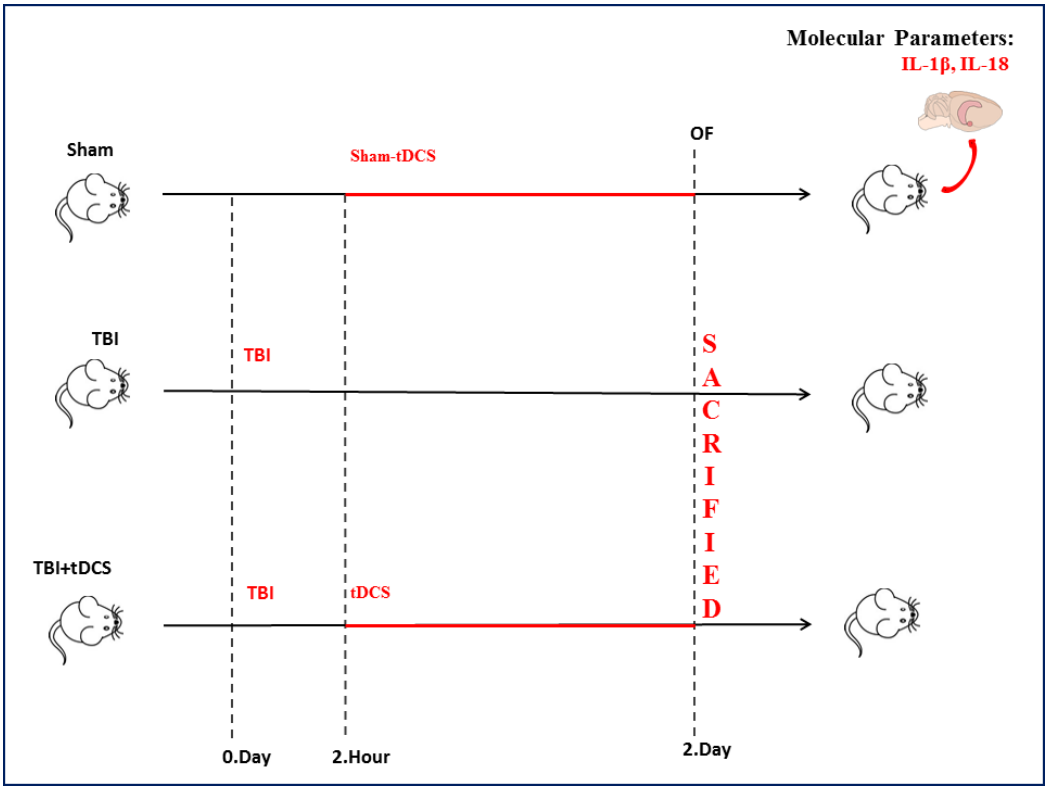

Figure 1. Experimental design

Marmarou weight drop model

Marmarou's weight reduction method, which is the most preferred among all weight reduction models, was used[21]. Animals were anesthetized with 5% isoflurane. The skull was placed on a stainless steel disc (10 mm diameter, 3 mm depth) between the lambda and bregma without surgical operation using a polyacrylamide adhesive. A model of chronic traumatic encephalopathy of moderate severity was created by dropping a 450g brass weight from a height of 1 meter directly onto the steel disc. Previous studies have shown that this injury produces an average linear acceleration force of 110 g, typically observed in human concussive blows[22,23].

tDCS application

The Animal DCS Stimulator (model 2100) device with a maximum current intensity of ±1000 μA, a current resolution of 0.01mA, and a temporal resolution of 1 min was used for experiments. Anodal tDCS stimulation (0.5 mA, 30 min) was applied with a disc electrode placed on the heads of rats under isoflurane anesthesia. tDCS was performed on the TBI+tDCS and Sham groups for consecutive 3 days.

Behavioral tests

Open-field test (OF)

Locomotor activity was carried out in a setup with a base of 80x80 cm and a wall height of 40 cm. For rats to explore the apparatus, they were placed in the center of the field and monitored and recorded by the video camera for 5 minutes. Total distance (cm) and frequency were calculated to evaluate locomotor activity[14].

Tissue collection

Animals were killed by decapitation on day 2 after the behavioral experiments. For biochemical analysis, motor cortex tissue was dissected from the brain and stored frozen at -80 °C. Tissues were homogenized in phosphate-buffered saline (PBS, pH 7.4) centrifuged at 12,000 rpm for 20 minutes at 4 °C, and supernatants were used for biochemical analyses.

Biochemical analysis

Enzyme-Linked Immunosorbent Assay (ELISA)

The levels of IL-1β and IL-18 were quantified using enzyme-linked immunosorbent assay (ELISA) kits. Commercially available ELISA kits (R&D Systems, Minneapolis, USA) for rat IL-1β (EK710260) and IL-18 (EK710281) were performed according to the manufacturer's instructions. IL-1β and IL-18 concentrations in the samples were calculated from their corresponding absorbance values via the standard curve. Data were normalized to total tissue protein and expressed as pg·mg−1 tissue protein.

Protein measurements

Protein concentrations were measured in the motor cortex tissues at 595 nm by a modified Bradford assay using Coomassie Plus reagent with bovine serum albumin standard (Pierce Chemical Company, Rockford, IL, USA).

Statistical analysis

The SPSS software package 20.0 program was used for all analyses. The results were given as mean ± standard error of the mean (SEM). P values less than 0.05 were considered significant. The results of the behavioral experiments and biochemical analyzes were analyzed with the One-way ANOVA test after the Shapiro-Wilk test normality analysis. The Tukey test was used for post-hoc analysis.

RESULTS

The behavioral experiments of the groups were measured in terms of locomotor activity in the OF (Figure 2). Total distance and frequency in the OF were significantly decreased in the TBI group compared to the sham group ($p<0.05$) (Figure 2. A). After tDCS treatment, a significant increase in locomotor activity was observed in the TBI+tDCS group compared to the TBI group ($p<0.05$) (Figure 2.A. and Figure 2.B.). IL-1β and IL-18 levels in the motor cortex are shown in Figure 3. IL-1β and IL-18 levels of the TBI group were significantly increased compared to the sham group ($p<0.05$) (Figure 3). tDCS treatment significantly decreased all proinflammatory cytokine levels in the TBI+tDCS group ($p<0.05$ for all). IL-1β and IL-18 levels in the motor cortex are shown in Figure 3. IL-1β and IL-18 levels of the TBI group were significantly increased compared to the sham group ($p<0.05$) (Figure 3). tDCS treatment significantly decreased all proinflammatory cytokine levels in the TBI+tDCS group ($p<0.05$ for all).

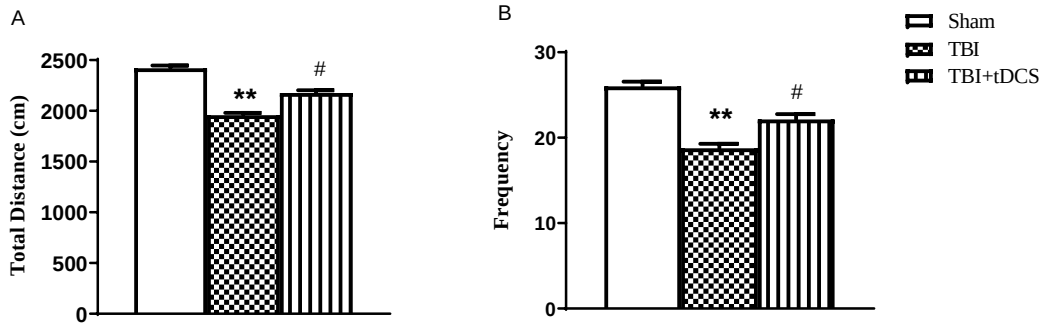

Figure 2. Behavioral results of experimental groups. A) Total distance (cm) in OF, B) Frequency in OF. (n=10, for each group;

** p<.01 shows the difference compared to the Sham group, # p<.05 shows the difference compared to the TBI group, one-way ANOVA test, followed by Tukey post hoc test). All data are presented as means ± SEM.

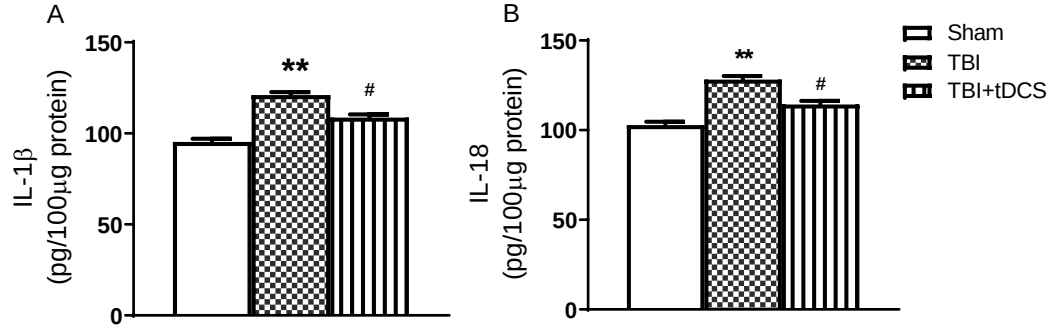

Figure 3. Neuroinflammation results in the motor cortex. A) IL-1β levels, B) IL-18 levels (n=10, for each group;
** p<.01 shows the difference compared to the Sham group, # p<.05 shows the difference compared to the TBI group, one-way ANOVA test, followed by Tukey post hoc test). All data are presented as means ± SEM.

DISCUSSION

Today, traumatic brain injury that occurs after head trauma still maintains its place as an important health problem despite groundbreaking developments in medicine. After trauma, primary brain damage occurs in the central nervous system first[1]. However, secondary damage rather than primary damage is responsible for the damage caused by head trauma. In patients with TBI, secondary damage has been shown to adversely affect the prognosis[1]. Mechanisms that cause secondary damage include neurotransmitter release, formation of reactive oxygen species, calcium-dependent cell damage, and inflammation[1]. TBI causes motor dysfunction. In our study, it was determined that there was motor dysfunction in the TBI group. tDCS treatment has been shown to have a therapeutic effect on loss of motor function. It has also been observed to have a therapeutic effect on neuroinflammation.

This study demonstrated that TBI causes loss of motor function and affects the level of neuroinflammation. Furthermore, anodal tDCS administered immediately after brain injury has been proven to provide therapeutic benefits for loss of motor function. Decreased motor function caused by mild TBI (mTBI) was restored by tDCS treatment.

Furthermore, tDCS treatment after mild traumatic brain injury may be thought to reduce neuroinflammation levels. In our study, it was determined that there was motor dysfunction in the TBI group. tDCS treatment has been shown to have a therapeutic effect on loss of motor function. It has also been observed to have a therapeutic effect on neuroinflammation.

In animals, decreased locomotor activity can mean loss of motor function. In this study, locomotor activity was decreased after mTBI in rats, but stimulation of anodal tDCS to rats after mTBI resulted in recovery of locomotor activity compared to rats with mild traumatic brain injury. Moreover, the locomotor activity of the anodal tDCS-treated group was similar to that of the sham group. It was observed that locomotor activity parameters, total distance, and frequency results decreased after mTBI. It was determined that anodal tDCS treatment improved the loss of motor function caused by mTBI and increased the locomotor activity values of the TBI+tDCS group compared to the TBI group and approached the sham group. These results are similar to other studies in the literature[17-19]. Kim and Han showed that 0.2 mA 30 min anodal tDCS treatment reduced the loss of motor function after TBI[18]. Yu et al. also reported that 0.2 mA 30 min anodal tDCS treatment improved the loss of motor and cognitive function caused by TBI[19]. In our study, anodal tDCS treatment (0.5 mA 30 min. for 3 days) decreased the loss of motor function caused by mTBI.

In this study, it was shown that tDCS treatment after TBI in motor cortex tissue reduced IL-1β and IL 18 neuroinflammation levels. It was determined that there was a decrease in IL-1 and IL-18 levels in the TBI+tDCS group compared to the TBI group. The results of the study suggest that tDCS treatment may reduce the neuroinflammation damage caused by TBI. Inflammation has been shown to increase after TBI. Antioxidant treatments with anti-inflammatory effects reduce NF-ϰB, IL-1 β neuroinflammation levels after TBI[24]. Sahin et al. showed that ginger treatment administered 50 mg/kg intraperitoneally immediately after TBI decreased infarct volume by decreasing NF-ϰB, IL-1 β, and GFAP and increasing neurotrophic factors BDNF, GAP, ICAM, and Nrf2 in the brain They also reported that ginger administration inhibited inflammation, NFkB and GFAP levels increased GAP-43 and Nrf2 levels and showed neuroprotection following[24]. In our study, it is seen that tDCS treatment reduces neuroinflammation levels after TBI. This study not only demonstrated the therapeutic effects of tDCS therapy on post-traumatic impaired motor function and inflammation, but also suggested that tDCS may be effective against neuronal damage caused by glutamate and calcium excitotoxicity, such as epilepsy and stroke.

This study has several limitations. Firstly, the use of only male rats and not female rats is among the limitations of the study. The effects of long-term tDCS could have been investigated, because this is important for the implementation of treatments in clinical settings. In addition, investigating the free radical levels and apoptosis pathways that lead to neuroinflammation would have made a great contribution.

This study showed that tDCS treatment had positive effects on motor function and neuroinflammation recovery after mTBI in rats. In particular, early treatment of tDCS was seen to reduce locomotor activity loss. tDCS acts as an antioxidant that reduces neural damage after mTBI. tDCS contributes to locomotor function recovery by increasing motor cortex excitability. Therefore, we think that tDCS treatment may be a treatment option for mTBI patients by reducing neuroinflammation and improving motor function.

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Ethical Approval: All animal use and experimental protocols were approved and implemented by the Animal Care and Ethics Committee of Erciyes University (Erciyes University, 02.02.2022/Approval no: 22/022).
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