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Anodal Transcranial Direct Current Stimulation Improves Impaired Cerebrovascular Reactivity in Traumatized Mouse Brain

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Abstract

Cerebrovascular reactivity (CVR) is a compensatory mechanism where blood vessels dilate in response to a vasodilatory stimulus, and is a biomarker of vascular reserve and microvascular health. Impaired CVR indicates microvascular hemodynamic dysfunction, which is implicated in traumatic brain injury (TBI) and associated with long-term neurological deficiency. Recently we have shown that anodal transcranial direct current stimulation (tDCS) caused prolonged dilatation of cerebral arterioles that increased brain microvascular flow and tissue oxygenation in traumatized mouse brain and was associated with neurologic improvement. Here we evaluate the effects of tDCS on impaired CVR and microvascular cerebral blood flow (mCBF) regulation after TBI. TBI was induced in mice by controlled cortical impact (CCI). Cortical microvascular tone, mCBF, and tissue oxygen supply (by nicotinamide adenine dinucleotide, NADH) were measured by two-photon laser scanning microscopy before and after anodal tDCS (0.1 mA/15 min). CVR and mCBF regulation were evaluated by measuring changes in arteriolar diameters and NADH during hypercapnia test before and after tDCS. Transient hypercapnia was induced by 60-s increase of CO₂ concentration in the inhalation mixture to 10%. As previously, anodal tDCS dilated arterioles which increased arteriolar blood flow volume that led to an increase in capillary flow velocity and the number of functioning capillaries, thereby improving tissue oxygenation in both traumatized and sham animals. In sham mice, transient hypercapnia caused transient dilatation of cerebral arterioles with constant NADH, reflecting intact CVR and mCBF regulation. In TBI animals, arteriolar dilatation response to hypercapnia was diminished while the NADH level increased (tissue oxygen supply decreased), reflecting impaired CVR and mCBF regulation.

Anodal tDCS enhanced reactivity in parenchymal arterioles in both groups (especially in TBI mice) and restored CVR thereby prevented the reduction in tissue oxygen supply during hypercapnia. CVR has been shown to be related to nitric oxide elevation due to nitric oxide synthases activation, which can be sensitive to the electrical field induced by tDCS.

Keywords

Traumatic brain injury (TBI); Transcranial direct current stimulation (tDCS); Cerebrovascular reactivity (CVR); Microvascular dysfunction; Hypercapnia test

1 Introduction

Cerebrovascular reactivity (CVR), the compensatory mechanism where blood vessels dilate in response to a vasodilatory stimulus, is an essential element of cerebrovascular regulation and a biomarker of cerebrovascular reserve and microvascular health [1]. Past researches have demonstrated that CVR is impaired following traumatic brain injury (TBI) which indicates underlying microvascular dysfunction wherein the metabolically active regions of the brain will not be supplied adequately to meet demands. This impairment increases susceptibility to secondary injury following TBI and exacerbates long-term neurological deficiency [2, 3]. Since neurocentric approaches for the treatment of TBI have failed, the NIH suggested that the neurovascular unit and a larger microsystem of vessels and associated glial cells should be considered as the functional target for new therapeutic approaches [3]. Transcranial direct current stimulation (tDCS) that potentially modulates all polarizable brain cell types is a promising electroceutical therapy suggested for TBI [4]. However, due to a lack of pre-clinical studies, the mechanisms of tDCS are poorly understood. Recently we have shown that anodal transcranial direct current stimulation (tDCS) caused prolonged dilatation of cerebral arterioles that increased brain microvascular flow and tissue oxygen supply in traumatized mouse brain and was associated with neurologic improvement. Since CVR was also suggested as a therapeutic target for TBI [5], we evaluate here the effects of the anodal tDCS on impaired CVR and microvascular cerebral blood flow (mCBF) regulation in the traumatized mouse brain.

2 Methods

2.1 Experimental Design

Protocol #200247 was approved by the Institutional Animal Care and Use Committee of the University of New Mexico and the studies were conducted according to the NIH Guide for the Care and Use of Laboratory Animals. Two groups of 10 mice each were used in the study: TBI and Sham. TBI was induced by a Benchmark Controlled Cortical Stereotaxic Impactor (Leica Biosystems, Germany) through craniotomy (5 mm diam, centred at 3.5 mm posterior and left-lateral to bregma) using a 3-mm flat-tip impounder deployed at a velocity of 5 m/sec and depth of 2.0 mm from the cortical surface, as in our previous study [6]. Sham controls were subjected to craniotomy only. Anodal tDCS (0.1 mA/15 min) was done using an Animal DCS Stimulator 2100 (Soterix Medical, USA). The anode was placed around the craniotomy and the counter electrode on the thorax. Microvascular cerebral blood flow

(mCBF) concurrently with NADH were measured *in vivo* by two-photon laser scanning microscopy (2PLSM) before and after stimulation. For imaging, mice were initially anesthetized with 2% isoflurane in 70% N₂O and 30% O₂. Later on, to preserve vasoreactivity, isoflurane was gradually reduced over a 10-min period to a range of 0.5–1%.

2.2 Two-Photon Laser Scanning Microscopy

Fluorescent serum (i.v. tetramethylrhodamine isothiocyanate (TAMRA) dextran, 150 kDa in physiological saline, 5% wt/vol) was visualized using an Olympus BX 51WI upright microscope and water-immersion LUMPlan FL/IR 20X/0.50 W objective of PrairieView Ultima multiphoton system. Excitation was provided by a PrairieView Ultima multiphoton microscopy laser scan unit powered by a Millennia Prime 10 W diode laser source pumping a Tsunami Ti: Sapphire laser (Spectra-Physics, Mountain View, CA, USA) tuned to 750 nm center wavelength. Band-pass-filtered epifluorescence (570–600 nm for TAMRA and 425–475 nm for NADH) was collected by photomultiplier tubes of the Prairie View Ultima system. Images (512 × 512 pixels, 0.15 μ m/pixel in the x- and y-axes) were acquired using Prairie View software as described previously [6]. Brain tissue oxygen supply was assessed by measurement of NADH autofluorescence. In offline analyses using NIH ImageJ software, the three-dimensional anatomy of the vasculature in areas of interest was reconstructed from two-dimensional (planar) scans of the fluorescence intensity obtained at successive focal depths in the cortex (XYZ stack).

2.3 Cerebrovascular Reactivity Test

Changes in arteriolar diameters and NADH autofluorescence were measured during hypercapnia test before and after tDCS (Fig. 1). Transient hypercapnia was induced by a 60-s increase in CO₂ concentration to 10% in the inhalation mixture through the face mask. Each trial consisted of 3 min of baseline data acquisition, 1 min of hypercapnia challenge, and 3 min during the post-hypercapnic surveillance period [7].

2.4 Statistical Analyses

Statistical analyses were done using GraphPad Prism software 6.0 (La Jolla, CA, USA) by Student's t-test or Kolmogorov-Smirnov test where appropriate. Differences between groups were determined using two-way analysis of variance (ANOVA) for multiple comparisons and post-hoc testing using the Mann-Whitney U-test.

3 Results

As in our previous study, controlled cortical impact caused a decrease in arteriolar diameters leading to a reduction in cerebral blood flow volume, capillary red blood cell flow velocity and tissue oxygen supply in the peri-contusion areas of cerebral cortex. Anodal tDCS dilated arterioles leading to an increase in blood flow volume, which increased blood flow velocity in capillaries. The improved perfusion in capillaries after tDCS caused a prolonged increase in tissue oxygen supply, as reflected by NADH autofluorescence decrease in TBI and Sham mouse brain.

Baseline hypercapnia test showed that, in sham mice, cerebral arterioles dilated during inhalation of 10% CO₂ (by $44.2 \pm 4.6\%$) and constricted to the pre-hypercapnia diameter after CO₂ termination (Fig. 2a–b) reflecting intact CVR. In TBI mice, however, cerebral arterioles dilatation response to CO₂ inhalation was slower, reaching a plateau almost at the end of 1-min hypercapnia, was more gradual and had an amplitude twice as low as that in sham animals (Fig. 2a–b, $28.7 \pm 5.1\%$). The decline in dilatation after CO₂ termination was twice as slow and was more gradual than in sham animals (Fig. 2a), both reflecting impaired CVR. Anodal tDCS enhanced the reactivity of cortical arterioles: their response to hypercapnia was faster and the dilatation was stronger in both the sham ($57.6 \pm 5.6\%$, $p < 0.05$) and TBI ($39.6 \pm 4.9\%$, $p < 0.01$) groups (Fig. 2a–b).

Simultaneous NADH autofluorescence imaging did not show any significant changes in tissue oxygen supply during hypercapnia in Sham animals as a result of arteriolar dilatation and increased RBC traffic, reflecting intact mCBF regulation (Fig. 2 c–d). In TBI animals, NADH autofluorescence during hypercapnia increased by $5.3 \pm 0.5\%$, reflecting decreased tissue oxygen supply due to impaired CVR (Fig. 2 c–d). Anodal tDCS in TBI mice abolished the reduction in tissue oxygen supply during hypercapnia by improving CVR (Fig. 2 c–d, $2.2 \pm 0.4\%$, $p < 0.05$).

4 Discussion

Injury of microvasculature, blood flow, and blood flow regulation is a common complication in TBI, as reported in experimental TBI and in patients [2, 3, 8]. Our data also show that after TBI, the dilatation response of cerebral arterioles to hypercapnia was reduced, reflecting impaired CVR and an inability of the traumatized brain to adequately adjust blood flow and oxygen delivery according to metabolic demand. The decrease in tissue oxygen supply, reflected by NADH increase during hypercapnia, confirms a mismatch between cerebral blood flow and neuronal metabolism that may further damage the brain. Since CO₂ reactivity is dependent on the well-known vasodilator, nitric oxide (NO), generated by constitutive endothelial NOS (eNOS), our results suggest that NO concentration is low due to eNOS dysfunction, which has been shown in TBI [9, 10]. Anodal tDCS not only dilates cerebral arterioles thereby increasing cerebral microvascular flow and tissue oxygenation in mouse brain, as we shown in previous study [6], but also improves CVR and mCBF regulation. Because both dilatation of cerebral arterioles and CVR depend on the NO, it is highly-likely that anodal tDCS activates functional eNOS in normal brain and restores functionality of dysfunctional eNOS in the traumatized brain.

To our knowledge, this study is the first describing the influence of tDCS on CVR in cerebral parenchymal arterioles. Previous studies on human subjects reported decrease in vasomotor reactivity (VMR), the related to CVR index, of middle cerebral artery (MCA) after 15-minute 1-mA anodal tDCS [11, 12]. Vernieri et al. calculated VMR from measured blood flow velocity by Transcranial Doppler before and during hypercapnia, induced by the inhalation of a mixture of 7% CO₂/air [11]. Giorli et al. assessed VMR by Transcranial Color-Coded Sonography calling through Breath Holding Index and Heart Rate Variability before and during 30-second breath hold [12]. Both suggested that anodal tDCS can activate the sympathetic system and that increased after anodal tDCS sympathetic activity could

reduce the potential capability of further dilating vessels after a hypercapnia [11, 12]. This discrepancy with our results is only superficial, as it is known, that reactivity and reserve in parenchymal arterioles and middle cerebral arteries, as well as mechanisms involved, are sufficiently different [13]. Middle cerebral arteries are indeed regulated mainly by the adrenergic sympathetic system, while parenchymal arterioles and microvessels are innervated from within the brain tissue and tightly involved in neurovascular coupling [14]. Moreover, in our study, the maximal CVR and mCBF regulation increase were observed and traumatized brain with substantially impaired CVR and regulation, while Vernieri et al. and Giorli et al. studied healthy volunteers with intact VMR [11, 12].

5 Conclusion

Anodal tDCS restores cerebrovascular reactivity of parenchymal arterioles and regulation of mCBF in traumatized mouse brain that could contribute to neurologic improvement.

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Fig. 1. Scheme of the experimental design.
At 3 weeks after traumatic brain injury (TBI), anesthetized mouse underwent two-photon laser scanning microscopy before and after anodal transcranial direct current stimulation (tDCS). Hypercapnia test was performed at baseline and after tDCS

Fig. 2. Anodal tDCS improves cerebrovascular reactivity.

(a) Change in the diameter of the arterioles during hypercapnia test, and (b) Cerebrovascular reactivity is better after tDCS; (c) NADH autofluorescence dynamics during the hypercapnia test, and (d) NADH autofluorescence before and after tDCS; $n = 10$, * $p < 0.05$. mean $\pm$ SEM