

Effect of Short Period Simultaneous Stimulation of Transcranial Direct Current Stimulation on Occupational Therapy to Brain-Derived Neurotrophic Factor Serum in Stroke Patients

Adrian Utomo, Sri Mardjiati Mei Wulan*, I Lukitra Wardhani

Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Universitas Airlangga-Dr. Soetomo Academic General Hospital, Surabaya 60131, Indonesia

ABSTRACT

Transcranial direct current stimulation (tDCS) is a non-invasive modality stimulating brain-derived neurotrophic factor (BDNF) secretion as one of important factors of neuroplasticity in recovery after stroke. This study aimed to determine the effect of tDCS stimulation on BDNF serum in subacute ischemic stroke patients. The subjects were divided into 2 groups. Control group had occupational therapy for 5 days consecutively, while intervention group had occupational therapy and tDCS stimulation simultaneously for 5 days consecutively. Value of BDNF serum was evaluated before and after the treatment. Among twenty-two stroke patients included in this study, the mean age were 54.73 ± 6.25 years and 55.00 ± 10.64 years, and the mean BDNF serum were 1147.7 ± 533.9 pg/ml and 1101.8 ± 680.7 pg/ml in control and intervention group, respectively. There were no significant differences of BDNF serum on control group ($p = 0.536$) and intervention group ($p = 0.594$). There was no significant difference of changes BDNF serum in control group and intervention group ($p = 0.749$). There was no benefit application of tDCS stimulation on occupational therapy in subacute ischemic stroke patients.

Keywords: Transcranial direct current stimulation, neuroplasticity, subacute ischemic stroke, occupational therapy, BDNF

Correspondence:

Sri Mardjiati Mei Wulan, MD, PhD

Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Universitas Airlangga-Dr. Soetomo Academic General Hospital, Surabaya 60131, Indonesia

Mayjen Prof. Moestopo Number 6-8, Airlangga, Gubeng, Surabaya 60131, Indonesia

Tel: +628165430480; +6285640612459, Fax: +6231-503-8838

Email: meiwulan21@gmail.com

INTRODUCTION

Stroke is the most common neurological disorder and top killers among the non-infectious diseases in the world (1,2). Stroke is a cerebrovascular condition that states the localized or global brain injury with symptoms lasting 24 hours or longer or progressing to death, with no apparent trigger other than the vascular origin, is rapidly increasing. Stroke is the third leading cause of death in the world after heart disease and cancer. Hypertension and dyslipidemia are some of the major risk factors for stroke causing blood supply to stop flowing to the brain (3,4). Stroke does not only affect in elderly, but also young people (5). Patients who have stroke, especially in transient ischemic attack (TIA) or ischemic stroke (IS), have higher risk of recurrence (6). The American Heart Association estimated that there are 795,000 strokes annually with 610,000 new cases and 180,000 recurrent cases. Stroke causes the highest level of disability compared to any other disease. National Stroke Association stated that 10% stroke survivors recovered completely without any disability, 25% recovered with mild disability, and 40% suffered moderate to severe disability requiring long term care (7,8). Stroke incidence rate according to 2013 Indonesian Basic Health Research showed an increase number, from 8.3 per 1000 people in 2007 to 12.1 per 1000 people in 2013 (9).

Stroke causes damage or dead of many neurons due to ionic imbalance in the membrane and oxygen deprivation. This condition leads to a loss function of the brain, depending on the stroke area. The stroke's area does not show enhanced activity. The area surrounding the stroke, peri-infarct zone,

shows excitatory or inhibitory activity in which activity stimulates neuroplasticity. Recovery of brain function after stroke depends on neuroplasticity activation. Neuroplasticity is an ability of neurons or brain structures to adapt and adjust the nervous system at functional and structural levels when exposed to new experiences. Many exercises and modalities have been developed to stimulate neuroplasticity. In this last decade, non-invasive modality have been developed to modulate cortex which was named transcranial direct current stimulation (tDCS). Transcranial direct current stimulation delivers weak electrical current, sub-threshold stimulation, through scalp and bone directly applied to a targeted brain area to modulate the excitability of the brain and will induce neuroplasticity. Previous research showed that tDCS has an effect on nitrosodimethylamine/N-methyl D-aspartate (NMDA) receptor efficacy and on brain derived neurotrophic factor (BDNF), which are both important factors of neuroplasticity (10).

The mechanisms of action are, however, poorly understood. A hypothesis stated that a sub-threshold stimulation from anodal tDCS will enhance excitability of the targeted brain area, altering the chance of neuron to fire and resulting in more activity. A sub-threshold stimulation will cause depolarization on neuron's membrane and fragile the NMDA receptor density, and intracellular Ca^{2+} concentration will increase and stimulate activity dependent BDNF-secretion that will induce neuroplasticity (11). BDNF, one member of the family of Nerve Growth Factor (NGF), is a neurotrophin that has functions on neuron survival, axonal growth, proliferation, differentiation, myelination, degeneration recovery,

regeneration, and neuroplasticity (12,13). Through utilizing a high-capacity saturable active transportation network, BDNF could cross the blood-brain barrier. Many researches showed strong correlation between BDNF serum and cortical and hippocampal BDNF expression (14,15).

Many researches have investigated the effect of tDCS to BDNF serum level in animals with stroke, especially rat, but rare in human (16). Many studies have been done, and no serious side effects have occurred. Slight itching under the electrode, fatigue, nausea, and headache have been reported in a minority of cases in a series of more than 550 subjects. Retinal phosphenes can be perceived at the start and end of stimulation especially with frontopolar electrode position (17). This study was performed to evaluate whether there was any differences in BDNF serum levels, as a neuroplasticity factor, after 5 days tDCS stimulation.

MATERIALS AND METHODS

An experimental study enrolled in subacute ischemic stroke patients with a randomized control group design. The subjects of this study were 22 subacute ischemic stroke patients from Rehabilitation Medicine Outpatient Clinic, Dr. Soetomo Academic General Hospital, Surabaya, Indonesia appropriate the inclusion criteria. Inclusion criteria were subacute ischemic stroke, hemiparesis, could understand instruction, manual muscle test 0-4, no severe cardiac disease, good static and dynamic sitting balance, agree to be the subject and follow the protocol by signing the informed consent. Exclusion criteria were uncontrolled hypertension, visuospatial disturbance, hemineglect, aphasia, apraxia, using metal implant, pace maker or hearing aid device and presence of head skin lesion. Drop out criteria of the subject were discontinuing the programs once and unwilling to continue the programs.

Subjects was randomized to determine whether the subject include the control or intervention group. Subjects were divided into 2 groups. The first group had occupational therapy and tDCS simultaneously with current 2 mA (intervention group) for 5 days consecutively, and the second group had only occupational therapy (control group) for 5 days consecutively. BDNF serum was measured before and

after the treatment between 7-9 a.m. A continuous current electric stimulator (Caputron Activa Dose II, Gilroy, USA) attached to a pair using sponge electrodes in saline-soaked sponge electrodes (5 cm² x 5 cm²) was used to induce transcranial direct current stimulation (2 mA, 20 min). The active anodal electrode was placed on premotor cortex area (PMC). Premotor cortex area was defined as being 2.5 cm anterior to M1 motor area. In relation to the international framework 10-20 electroencephalogram (EEG), M1 is the main region for motor cortex (C3 or C4). The anode was placed on the affected hemisphere and the reference electrode on the supraorbital region in the contralateral hemisphere (18).

BDNF in ELISA sandwiches was found under guidance of the manufacturer (Human BDNF ELISA Kit, Elabscience Biotechnology, USA). The detection range was 31.25-2000 pg/ml. Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS version 20.0). The characteristics baseline were compared using Fisher's exact test and independent sample t-test. We evaluated the differences of BDNF serum level before and after the treatment of both groups, if the data were normal distributed we use paired sample t test but if the data were distributed abnormally we use Wilcoxon test. We also compared between-group differences (delta) of BDNF serum levels using independent sample t test. The differences were considered statically significant at $p < 0.05$. All study subject had signed the informed consent form and this study had ethical clearance from the ethical committee of Dr. Soetomo Academic General Hospital, Surabaya.

RESULTS

All 22 subjects completed the sessions and study protocol. None of the subjects reported any adverse effects during or after the electrical stimulation. Table 1 presents the sociodemographic characteristic of subject. The mean age were 54.73±6.25 years and 55.00±10.64 years, and the mean BDNF serum were 1147.7±533.9 pg/ml and 1101.8±680.7 pg/ml in control and intervention group, respectively. The homogeneity test of subjects' characteristics of age, sex, and BDNF serum level before treatment between control and intervention group found no significant differences; thus they did not influence the result of the study.

Table 1: Sociodemographic characteristic of subjects at baseline

Characteristics	Control group	Intervention group	p value
Age (year)			
Mean±SD	54.73±6.25	55.00±10.64	0.942*
Sex			0.659**
Male (N)	8	6	
Female (N)	3	5	
BDNF serum (Pre) (pg/ml)			
Mean±SD	1147.7±533.9	1101.8±680.7	0.862*

*Independent sample t test; ** Fisher's Exact test.

Table 2 shows there are no significant differences of BDNF serum level before and after treatment, either in control group ($p = 0.536$) nor intervention group ($p = 0.594$). The delta

BDNF serum levels of control groups was -44.1±539.06 pg/ml and intervention group -127.4±659.27 pg/ml ($p = 0.749$).

Table 2: BDNF serum levels of control and intervention group before and after treatment

	Control	<i>p</i>	Intervention	<i>p</i> value
BDNF (pg/ml)	Mean±SD	0.536*	Mean±SD	0.594**
Before	1147.7±533.9		1101.8±598.5	
After	1020.2±473.2		1057.7±680.7	

* Paired sample t test, ** Wilcoxon test; Sig: *p* <0.05

DISCUSSION

The present study showed application of tDCS stimulation on occupational therapy to subacute ischemic stroke patients for 5 days consecutively did not make any differences in value of BDNF serum. There were no significant differences on BDNF serum after the treatment in both groups was reported in this study. There was no cellular and molecular adaptation yet due to short treatment. Another study reported no increment on BDNF serum after 30 minutes exercise using static cycle, while Ferreira said no increment on BDNF serum either after 30 minutes treadmill exercise on rats. There is an increase of BDNF serum after having adaptation with aerobic exercise, moderate intensity for 5 weeks using static cycle (19,20). These studies indicated that exercise in a long time with specific intensity would increase BDNF serum. A previous study was conducted in 7 stroke patients with chronic aphasia using real bihemispheric tDCS intensity 2 mA for 20 minutes with speech therapy for 1.5 hours, 5 times/week for 2 weeks. The BDNF serum level was evaluated three times: before treatment, soon after complete treatment, and one week after the last treatment. There were no differences in BDNF serum value on each evaluation time (21).

Recovery of oxygen perfusion and nutrition in brain is not followed by the increase of BDNF serum. The intimate contact between blood vessels, neurons and glia is a unique feature of brain circulation. In a network named the “neurovascular unit”, neurons, glia, and vascular cells are related in case of their structures and functions. Stimulation of tDCS increases brain perfusion to enhance brain function, as shown in several animal and human studies. The application of tDCS may improve the treatment of diseases associated with vascular dysfunction. An in vitro study about the effect of electric stimulation to endothelial cells showed the increase of vascular endothelial growth factor (VEGF) production that may modulate angiogenesis and the increase of nitric oxide (NO) that may increase brain perfusion (22).

Inhibition of BDNF secretion is due to some drugs. Some drugs may influence the effectivity of tDCS stimulation. Flunarizine, a calcium channel blocker, and Carbamazepine, a sodium channel blocker, may reduce the effect of anodal tDCS stimulation. Dextromethorphan, an NMDA receptor antagonist, and Lorazepam, a GABA_A receptor agonist may influence the modulatory effect on the intra-stimulation response. Propranolol, a β receptor antagonist, may shorten the after effect of anodal tDCS stimulation (17).

Subjects has expression of BDNF Val66Met polymorphism. Subject with BDNF Val66Met polymorphism influence the secretion of BDNF activity dependent that will disturb the motor skill acquisition and sensitivity to tDCS stimulation (17). A 5-day tDCS stimulation study to a subject with BDNF Val66Met polymorphism showed lower motor skill acquisition than in healthy volunteers (23). A retrospective study showed that in comparison with Val66Val carrier, Val66Met BDNF polymorphism carriers increased the tDCS-induced plasticity, while other study showed the contrary findings which found no differences between these groups for cathodic tDCS-induced plasticity (21).

BDNF in blood serum may not reflect BDNF levels in brain. Many studies said that BDNF serum level reflects the BDNF level in brain. A study showed that the BDNF levels in serum and rat's front cortex were positively correlated. Intravenous BDNF is distributed in a broad capacity via a saturable transport system through blood-brain barrier (BBB) of healthy mice. The positive correlation between whole blood BDNF and hippocampus in rats and between the plasma BDNF and the hippocampus in pigs was reported by a study. However, several findings indicate that BDNF serum could not be related to a level of BDNF in the brain. One study has shown that after stroke in rodents, BDNF plasma was not increased, indicating that circulating BDNF levels may not reflect BDNF levels in the brain in the model of this rodent stroke. The serum BDNF serum level, therefore, became a significant goal for the evaluation, predicting and treatment assessment of different central nervous system diseases (15).

This study has several limitations. First, no BDNF polymorphism data are accessible in this analysis. For the use assessment of BDNF polymorphism and BDNF serum, the correlation between BDNF polymorphism and BDNF serum level and functional results over time are important in the prediction of stroke recovery. Second, we do not have complete data about drugs consumed by the patients at the time besides their routine drugs. We already know few drugs will influence the effectivity of tDCS stimulation. Third, the subjects were only on subacute ischemic patients; thus it is difficult to generate the results to all stroke patients.

CONCLUSION

Application of tDCS stimulation on occupational therapy to subacute ischemic stroke patients for 5 days consecutively did not make any differences in value of BDNF serum.

REFERENCES

1. Willey. Acute Ischemic Stroke The Neuro ICU Book. Lee IK, editor. New Yor: Mc Graw Hill Medical; 2012. 91–122 p.
2. Rachmawati M, Sugianto P, Wardhani RIL. LDL Level in Ischaemic Stroke Patients at Dr. Soetomo General Hospital Surabaya. *Biomol Heal Sci J*. 2019;2(1):41–3.
3. Arina CA, Amir D, Siregar Y, Sembiring RJ. Correlation between homocysteine and dyslipidemia in ischaemic stroke patients with and without hypertension. In: *IOP Conference Series Earth and Environmental Science*. Institute of Physics Publishing; 2018.
4. Marbun JT, Seniman, Andayani U. Classification of stroke disease using convolutional neural network. In *Department Information Technology, Universitas Sumatera Utara, Indonesia: Institute of Physics Publishing*; 2018.
5. Puspitasari V, Wahid S, Aliah A, Suhadi B, Kaelan C, As'ad S, et al. Serum vascular endothelial growth factor as a predictor of clinical outcomes in anterior circulation ischemic stroke. *Med J Indones*. 2015;24(2):109–14.

6. Akbar M, Misbach J, Susatia F, Rasyid A, Alfa AY, Syamsudin T, et al. Clinical features of transient ischemic attack or ischemic stroke patients at high recurrence risk in Indonesia. *Neurol Asia*. 2018;23(2):107–13.
7. Zorowits R, Baerga E, Cuccurullo S. Stroke. In: Cuccurullo SJ, editor. *Physical Medicine and Rehabilitation Board Review*. 2nd ed. New York: Demos medical; 2015. p. 1–27.
8. Jauch EC, Saver JL, Adams HP, Bruno A, Connors JJ, Demaerschalk BM, et al. Guidelines for the early management of patients with ischemic stroke. A scientific statement from the Stroke Council of the American Stroke Association. *Stroke*. 2013;44(2):870–947.
9. Indonesia Ministry of Health. *Riset Kesehatan Dasar (RISKESDAS) tahun 2013*. Jakarta; 2013.
10. Busser Y, Gerrits L, Steenbakkers K, Waarlo S. *Uncovering the mechanisms of transcranial direct current stimulation-induced neuroplasticity after stroke*. Radboud University; 2016.
11. Fritsch B, Reis J, Martinowich K, Schambra HM, Ji Y, Cohen LG, et al. Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. *Neuron* [Internet]. 2010 Apr 29;66(2):198–204. Available from: <https://pubmed.ncbi.nlm.nih.gov/20434997>
12. Hidayati A, Joewono HT, Widjiati W. Increased Brain Derived Neurothropic Factor in the cerebrum and cerebellum of *Rattus norvegicus* newborn with exposure to Mozart's music in default sequence compared with the reversed sequence and without exposure during gestation. *Maj Obstet Ginekol*. 2018;26(2):67–73.
13. Utami N, Effendy E, Amin MM. BDNF (brain-derived neurotrophic factor) serum levels in schizophrenic patients with cognitive deficits. In: *IOP Conference Series Earth and Environmental Science*. Institute of Physics Publishing; 2018.
14. Semkova I, Krieglstein J. Neuroprotection mediated via neurotrophic factors and induction of neurotrophic factors. *Brain Res Brain Res Rev*. 1999 Aug;30(2):176–88.
15. Luo W, Liu T, Li S, Wen H, Zhou F, Zafonte R, et al. The Serum BDNF Level Offers Minimum Predictive Value for Motor Function Recovery After Stroke. *Transl Stroke Res*. 2019 Aug;10(4):342–51.
16. Podda MV, Cocco S, Mastrodonato A, Fusco S, Leone L, Barbati SA, et al. Anodal transcranial direct current stimulation boosts synaptic plasticity and memory in mice via epigenetic regulation of *Bdnf* expression. *Sci Rep*. 2016 Feb;6:22180.
17. Stagg CJ, Nitsche MA. Physiological basis of transcranial direct current stimulation. *Neurosci a Rev J bringing Neurobiol Neurol psychiatry*. 2011 Feb;17(1):37–53.
18. Andrade SM, Batista LM, Nogueira LLRF, Oliveira EA de, de Carvalho AGC, Lima SS, et al. Constraint-induced movement therapy combined with transcranial direct current stimulation over premotor cortex improves motor function in severe stroke: a pilot randomized controlled trial. *Rehabil Res Pract*. 2017;2017.
19. Gold SM, Schulz K-H, Hartmann S, Mladek M, Lang UE, Hellweg R, et al. Basal serum levels and reactivity of nerve growth factor and brain-derived neurotrophic factor to standardized acute exercise in multiple sclerosis and controls. *J Neuroimmunol*. 2003;138(1–2):99–105.
20. Zoladz JA, Pilc A, Majerczak J, Grandys M, Zapart-Bukowska J, Duda K. Endurance training increases plasma brain-derived neurotrophic factor concentration in young healthy men. *J Physiol Pharmacol*. 2008;59(Suppl 7):119–32.
21. Marangolo P, Fiori V, Gelfo F, Shofany J, Razzano C, Caltagirone C, et al. Bihemispheric tDCS enhances language recovery but does not alter BDNF levels in chronic aphasic patients. *Restor Neurol Neurosci*. 2014;32(2):367–79.
22. Pulgar VM. Direct electric stimulation to increase cerebrovascular function. *Front Syst Neurosci*. 2015;9:54.
23. Pelletier SJ, Cicchetti F. Cellular and molecular mechanisms of action of transcranial direct current stimulation: evidence from in vitro and in vivo models. *Int J Neuropsychopharmacol*. 2014 Oct 31;18(2):pyu047.