The Effect of Transcranial Direct Current Stimulation on Cognitive Function and Biochemical Change of Rats with Alzheimer's Desease

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Purpose: The objective of this study was to offer clinical primary data that it' s aims to examine effects of transcranial direct current stimulation (tDCS) on cognitive function and biochemical change of rat with alzheimer' s disease(AD) induced by injecting scopolamine.

Methods: Subjects were instructed cognitive dysfunction model, rat of Sprague-Dawley system was injected with scopolamine and each experimental group was classified into three; group Ⅰ (n=16) is non-treatment groups; group Ⅱ (n=16) is applied with the tacrine; group Ⅲ (n=16) is applied with the tDCS. The ziggurat task test was conducted to observe behavioral changes and cognitive function ability and 7, 14, 21, 28 days after the model. Acetylcholine Esterase (Ach E) activity was examined for biochemical assessment of which the results are followed.

Results: Participants showed as to behavioral change, tacrine application group was the most significantly responded, following tDCS application group. As to biochemical change, same as above, tacrine application group was the most significantly responded, following tDCS application group.

Conclusion: From these results, confirm that tDCS application to rat with alzheimer' s disease leads to positive effects on behavioral, cognitive function changes, and biochemical changes, lasting for certain period of time. This study, in particular, tDCS, which can change excitability of brain cells non-invasively, could provide basic data that is useful as a new treatment way for the patients with cognitive dysfunction.

Key Words: Acetylcholine Esterase, Alzheimer's disease,, Cognition

I. Introduction

Alzheimer' s disease (AD) is a neurodegenerative brain disease that insidiously robs patients of their cognition, function, independence, and identity and results in morbidity and eventual mortality.[1] The cognition dysfunction can be cause of problem in doing activities of daily living, makes it more difficult to do various activities than before the damage of cognitive function appeared.[2] It has been investigated that Loss of synapse which is room between neurons and Tau which made by over-phosphorylation in NFTs (neurofibrillary tangles) have high correlation with severeness of AD and until now, there have been many research that these factors are main cause of AD.[3] Nitsc and Paulus reported that motor evoked potential was significantly increased after stimulating primary motor area of usual person using 1mA positive direct current during 5minutes.[4] However, safety in the case of clinically applying tDCS to patients was not conducted yet and also, research about therapy intervention using it was not done yet. Once, as a kind of neurotransmitters, Acetylcholine; Acetylcholine which is involved in signal transduction of brain that is biochemical change in our body used in checking

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rehabilitation of cognitive ability developed, it becomes possible to observe change appearance of Acetylcholine; Acetylcholine synthesized through activity of enzyme choline acetyltransferase.[5] It corresponds to work of acetylcholine, which leads to increase of acetylcholinesterase activity; Acetylcholine E so that acetylcholine; Ach is disassembled rapidly.[5] Due to this work, concentration of acetylcholine; Ach decrease. As small quantity of this decrease can cause damage of memory, it is known as neurotransmitter which can be used as barometer to check damage on cognitive ability.[6] Neuron transmission system that takes pivotal tole in memory and learning become more aggravated, when acetylcholine,[7] neurotransmitters 's content decreases and acetylcholine E which decomposes it increases.[8] It can be assumed acetylcholine content' s decrease as well as increase of acetylcholine E activity consequently have negative effects on various cognitive ability. To investigate the effects on cognitive ability, in the experiment, Ach content and Ach E activity were observed by biochemical evaluation. Among those, various traditional cognitive rehabilitation therapy is difficult to be implemented on the purpose of training cognition damaged patients due to therapist' s subjective intervention and shortage of diversity. Although computerization in cognition rehabilitation treatment has recently been introduced, it may cause difficulty in moving actually owing to image training separated from real life.[9] This study is intended to provide basic study foundation, observing improvement on function center nerve system and mechanism of cognition ability improvement through verifying safety of tDCS.

II. Methods

1. Animals

In this study, 48 rats Sprague-Dawleys based paper randomly were assigned to perform experiments. All animal experimental protocols were performed in accordance with the guide lines of the Dongshin University Animal Care and Use Committee. Temperature of raising room was set at 22 ± 1℃ and humidity was maintained at 55 ± 10%. Light and shade periodicity was 12 hours in order to keep the raising room in regular condition. Hard food was used as forage, food and water are freely provided. Each experimental group was classified into three groups; group Ⅰ(n=16) is non-treatment groups; group Ⅱ (n=16) is the tacrine group; group Ⅲ (n=16) is the tDCS application group.

2. Experimental methods

Evaluation of theory in behavior reaction (ZTT) according to recovery of cognition function was conducted right after the induction and continuously conducted 4 times more after each 7, 14, 21, 28 days later. Biochemical inspection of was also conducted on the last day, victimizing 8 rats in each group. Scopolamine induced memory loss by inhibiting the cholinergic nerve signaling in non-specific antagonist which acts as a muscarinic acetylcholine receptor. In general, signaling of cholinergic nervous system, significantly reduced in the brains of patients with dementia, and is closely related to the degree of memory damage. A method of inhibiting acetylcholine decomposition enzyme is utilized development of medication to increase the cognitive and memory. To induce the cognition dysfunction on normal rats, scopolamine (Sigma, S1875, USA) 1mg/kg which was lysed and diluted in physiological saline at 0.9% had been injected in abdominal cavity during 30days (1time, a day). In addition, to compare it with experiment group that induced by scopolamine, tacrine (Sigma, A3773, St. Lous, MO. USA) 10 mg/kg that do detoxication and was lysed and diluted in physiological saline at 0.9% was injected in abdominal cavity during 4 weeks (1time, a day) after injecting scopolamine to experiment as well as comparison group. According to Kim' s method,10 tDCS applied in this experiment used direct current machine (Cyber Medic Co. Jeonju, Korea) which can control even 0.1mA. To let bipolar electrode be on transcranial region of cerebral cortex, pad 1 ㎝ wide and long. Also to fix the movement, plastic cup made to fit in experimental animal' s head was put on and fixed. Negative electrode was applied to body in a purpose of keeping shunting effect. Gel was used to minimize resistance between skin and electrode after shaving. Considering the strength point that beard of white rats are contracted, the strength of applying electric current was set at 0.1㎃ and applying time was 20minutes in one time. It had been done 2 time in a day, 5 time in a week during 4weeks at same time everyday(Figure 1).

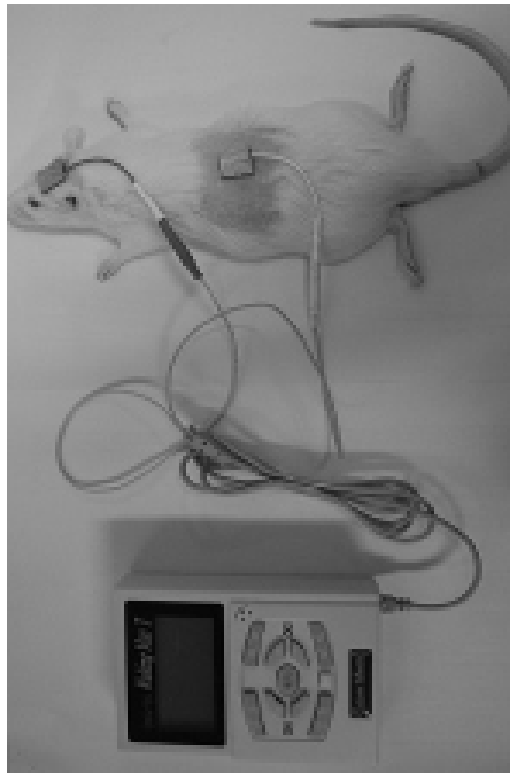

Figure1. Application of tDCS.

3. The ziggurat task test

To evaluate cognitive function in terms of theory of behavioral reaction, Ziggurat task test was conducted before and each 7, 14, 21 days later after the induction. Preceding training (first and second stage) was conducted during a week, 2 times in a day, before the induction of cognitive impairments. When it is acknowledged as the induction of cognitive impairments, used as a tool for behavior evaluation after preceding (second stage) had been conducted during 10 days. During 5 minutes, If rats find a block of pyramid where pallet was placed, catch pallet and once eat it up, it's was acknowledged as a correct choice and time to bring food to mouth was measured. In the case that rats climb other pyramids that haven't pallet or drop it is regarded as a error choice. The experiment was evaluated 4 times in a day.[11]

4. Measurement of ach E activity

As Ellman's method wrote, measurement of Ach E activity was conducted by checking optical density using acetylcholine iodide as a substrate.[12] Manufacturing of acethylcholine breakdown enzyme was done through process which extracts cerebrum cortex and using Teflon homogenizer (Eyela, Tokyo, Japan) and goes through homogenization by 10times homogenization buffer (12.5 mM sodium phospate buffer pH 7.0, 400 mM NaCl) and lastly use supernatant as a zymogen after centrifugation. 200 ㎍/㎖ was diluted to become 0.1 M phosphate buffer (pH 8.0) and the mixture of 1.5 ㎖ target material, 2.6 ㎖ buffer solution, 20 ㎕ 75 mM acetylthiocholine iodide solution, 100 ㎕ Ellmans solution (10mM DTNB, 15 mM sodium bicarbonate) was exposed at 25℃. In addition, 400 ㎕ eczyme source was added and at 410 nm, the optical density was measured after 5minutes (OPTIZEN 2120UV, Mecasys Co. Ltd., Korea). At that time, instead of adding acetyl cholinesterase sample as a comparison group, reaction solution including saline was used. Additionally, it was confirmed that unusual reaction did not happen without adding. acetylthiocholine iodide.

5. Data analysis

The static analysis of this study was done using SPSS 18.0 ver. for window and values of each result were presented as average and standard deviation. Behavioral measured value comparison among groups at each measurement time was done through one-way analysis of variance. Post hoc is conducted by Tukey's multiple range test. To check changes among each groups according to time period, paired t-test was conducted. Significance level was set at α=0.05.

III. Results

1. The time to choose by ziggurat task learning

In the differences among groups according to time interval for measurement, since the 7th day, there has been significant difference in decrease of result value among each group at every measuring time. the 7th day after the result of post hoc test, there have been more significant differences in experiment group Ⅱ than group Ⅰ ($p<0.001$). In the 14th day, there was the most significant decrease in experiment group Ⅱ as a result of post hoc test ($p<0.001$) and group Ⅲ also showed significant decrease ($p<0.01$). In addition, in the 21 and 28th day every experiment group showed same significant

Table 1. Take-time of correct choice on the ziggurat task in each group (sec)

Groups	Day				
	pre	7 days*	14 days**	21 days***	28 days***
Ⅰ	253.44 ± 18.91	246.88 ± 19.04	238.75 ± 20.68	225.88 ± 28.76	213.25 ± 30.9 †
Ⅱ	249.75 ± 12.55	223.75 ± 14.56 ††††	195 ± 22.65 ††††	178.44 ± 34.66 ††††	165.38 ± 26.17 ††††
Ⅲ	248.63 ± 19.39	232.88 ± 20.27	220.81 ± 20.23 †† †	202.56 ± 22.35 †† †	186.06 ± 18.15 †† †

All values showed mean±SD, Test by one way-ANOVA(*p<0.05, **p<0.01, ***p<0.001) and post hoc were compared with group Ⅰ (†††p<0.001) and tested by paired T-test († † † p<0.001),
Group Ⅰ :Scopolamine induce+nontreatment
Group Ⅱ :Scopolamine induce+Tacrine
Group Ⅲ :Scopolamine induce+tDCS

Table 2. The number of error choice on ziggurat test in each group (ea)

Groups	Day				
	pre	7 days*	14 days**	21 days***	28 days***
Ⅰ	12.13 ± 2.22	11.50 ± 1.97	10.94 ± 1.61	10.13 ± 1.5	9.25 ± 1.57 †
Ⅱ	11.06 ± 1.55	9.06 ± 1.46 †††††††	8.19 ± 1.20 †††††††	7.75 ± 1.5 †††††††	6.00 ± 1.41 †††††††
Ⅲ	10.56 ± 1.45	10.00 ± 1.53	9.13 ± 1.00 †† †	8.13 ± 0.77 †† †	7.50 ± 1.41 †† †

All values showed mean±SD, Test by one way-ANOVA(*p<0.05, **p<0.01, ***p<0.001) and post hoc were compared with group Ⅰ (†††p<0.001) and tested by paired T-test († † † p<0.001),
Group Ⅰ :Scopolamine induce+nontreatment
Group Ⅱ :Scopolamine induce+Tacrine
Group Ⅲ :Scopolamine induce+tDCS

Table 2. The number of error choice on ziggurat test in each group (ea)

Groups	Acetylcholinesterase activity	
	14 days***	28 days***
Ⅰ	284.51 ± 9.01	120.71 ± 8.77
Ⅱ	243.81 ± 8.3†††††††	106.92 ± 6.36†††††††
Ⅲ	265.25 ± 9.71†††††††	109.68 ± 5.96†††††††

All values showed mean±SD, Test by one way-ANOVA(***p<0.001) and post hoc were compared with group Ⅰ (†††p<0.001) and tested by paired T-test († † † p<0.001),
Group Ⅰ :Scopolamine induce+nontreatment
Group Ⅱ :Scopolamine induce+Tacrine
Group Ⅲ :Scopolamine induce+tDCS

decrease that was same with that in the 14th day. Paired-t test was conducted to check variation of result value among each group according to time interval for measurement. Experiment group I didn't show significant until the 21th day but did in the 28th day ($p<0.05$). Group Ⅱ has shown significant decrease ($p<0.05$) since the 7th day and same result appeared in the 14, 21 and 28th day. The group Ⅲ has shown significant decline ($p<0.05$) since 14th day (Table 1).

2. The error selecting number by ziggurat task learning

In differences among groups according to time interval for measurement, there have been significant differences in decrease of result value among each group at every measuring time. the 7th day after the result of post hoc test, there have been more significant differences in experiment group Ⅱ than group I ($p<0.001$). In the 14th day, there was the most significant decrease in experiment group Ⅱ as a result of post hoc test ($p<0.001$). In addition, in the 21 and 28th day every experiment group showed same significant decrease that was same with that in the 14th day. Paired-t test was conducted to check variation of result value among each groups according to time interval for measurement. Group Ⅱ has shown

significant decrease ($p<0.05$) since the 7th day and same result appeared in the 14, 21 and 28th day. the group Ⅲ has shown significant decline ($p<0.05$) since 14th day (Table 2).

3. Measurement of ach E activity

In differences among groups according to time interval for measurement of Ach E activity, there were significant differences in both 14th and 28th day and experiment group Ⅱ, Ⅲ all showed more significant differences ($p<0.001$) rather than group. Especially, experiment group Ⅱ showed most significant increase ($p<0.001$). Also, there was significant difference in the 14th day and as a result of post hoc test experiment group Ⅱ, Ⅲ all showed more significant increase than group Ⅰ ($p<0.01$). Especially, experiment group Ⅱ showed most significant increase ($p<0.001$), and so did group Ⅲ ($p<0.05$). There is also significant difference in the 28th day and as a result of post hoc test Ⅱ, Ⅲ all showed more significant increase than group Ⅰ ($p<0.01$). Especially, experiment group Ⅱ showed most significant increase ($p<0.001$) and so did group Ⅲ ($p<0.05$) (Table 3).

IV. Discussion

The exact treatment for AD is not known, but so far, various studies have been conducted. In which the two types of brain stimulation current stimulation of the motor cortex activity can be controlled.[13] Boggio reported the effect of tDCS can be varied according to polarity of electrode.[14,16] Cathodal current stimulation minimize excitement of cerebrum and anode current have a function to increase excitement of cerebrum.[5,15] Nitsche and Paulus reported that cathodal tDCS is the technology that cures damage on center nerve system including excitement of increased neuron.[4] In terms of time to choose and error selecting number by Ziggurat Task learning, group Ⅱ rather significantly decreased than goup Ⅰ in the 7th day, and in the 14th day, group Ⅱ, Ⅲ rather significantly decreased in sequence than group Ⅰ, but in the 28th day, group Ⅱ, Ⅲ showed significant differences in sequence compared with group Ⅰ. Figure of the experiment group Ⅱ which was injected Tacrine was most well maintained, being compared with Ziggurat test and the following was the group Ⅲ in terms of maintaining figures, which is considered that the number of error and gluttony for food was maintained at certain level owing to rehabilitation of motor ability and simple characteristic of maze, even after the death. In the open field and Gluttony which are evaluating method of theory in behavior reaction related with cognition, group Ⅰ of white rat with AD showed impairment with no change in time and number of error during measuring period, but in the other groups the time to get food and number of error was minimized. This result correspond with preceding thesis which consider this result as improvement of cognition.[17] When cognition dysfunction is severe after occur of Alzheimer's disease, Ach E restrainer such as donepezil, tacrine, galantamine is clinically used to maintain cognitive function and memory, keeping Ach from decomposing rapidly.[6] Although these Ach E restraining drugs have low possibility of side effects, common side effects such as nausea, anorexia, vomiting, diarrhea are accompanied and period of remedial effect is also very short. Accordingly, it is necessarily needed to conduct more research about tDCS and electrical stimulation as a medical technology that offset side effect of Ach E restraint drugs and control long tolerance of it.[18,19] Furthermore, Intervention with tDCS is considered to have the most priceless value in aspect of treatment. Taking all above into account, it is considered that tDCS promote neuron activity of white rats induced by AD and through this, it activates neurotransmitter of hippocampus and cerebral cortex. Also it is thought that tDCS would have more beneficial effect on maintaining cognitive ability, being applied to patient who have Alzheimer's disease. In conclusion, it is confirmed that the apply of tDCS after induction of cognitive impairment by AD have positive effects on change in theory of behavior reaction, rehabilitation of cognitive ability and biochemical change. The effect of it is maintained during certain period. Therefore, this study is a non-invasive method of treatment failure in patients with a new way to offer the possibility of using tDCS.

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