The Effects of tDCS and Montoya Stair Task on Sensorimotor Recovery and GFAP Expression in MCAo induced Stroke Rat Model

This study is intended to examine the tDCS and Montoya stair task (MST) on sensorimotor recovery and glial scar expression in MCAo induced stroke model of rat. To achieve this goal, this study selected 80 SD rats of 8 weeks. The experiment groups were divided into four groups, and assigned 20 rats to each group. Group I was a control group; Group II was a tDCS application group after MCAo; Group III was a MST application group after MCAo; Group IV was a tDCS and MST application group after MCAo. In each group, neurological function test measurement, motor behavior test, montoya stair task test, immunohistochemical finding of GFAP expression finding were analyzed. In motor behavior test, the outcome of group I was significantly different than the other group, especially from 14 days. In montoya stair task test, the outcome of group I was significantly lower than the other group especially, group II were significantly different on 14 days and group IV was most significantly different than the other group. In immunohistochemical finding, group II, III, IV were decrease GFAP expression on depend on time stream. These results throughout the MCAo due to focal ischemic brain injury rat model four weeks tDCS and MST was applied, when the neurobehavioural, upper extremity function and ability, histopathologic data suggest that sensorimotor function recovery and a positive influence on glial scar decrease and confirmed that.

Key words: MCAo; tDCS; Montoya Stair Task; GFAP

INTRODUCTION

The motor injury were took damage of the cerebral motor cortex, premotor cortex, motor pathway (motor tract) and related fibers, cerebrum and cerebellum passage between the association pathways in the ischemic and/or hemorrhagic caused stroke(I). Especially, an experimental method that middle cerebral artery occlusion (MCAo) in the focal ischemic brain damage inducing a state of abnormal blood flow by artificially limit, and serum calcium transient decrease. Therefore, in selective areas of the MCA supplied blood cortex, cells were death cause of this action. We has attention to supplies of blood flow specific area at middle cerebral artery that the brain area is involved in limb movement and sensory motor functional recovery of upper limb, the occurred focal ischemic stroke is structural damage to the widespread connected brain that excitatory and/or inhibitory neurotransmitter deliver in each area(2, 3). This is made worse by secondary brain damage causes serious complications after a stroke.

Recently, the protocol of non invasive trans-cranial direct current stimulation (tDCS) were known that stimulate way of brain regulates the function of specific neural structures and the plasticity of the brain. Traditionally, the electrical stimulation to the brain through the nervous system in the rehabilitation process can improve the exercise training(4). The development of these brain stimulation techniques will possible to stimulate the non invasive and without pain. Also, stimulus intensity and duration, stimulation parameters such as various controlled to functional

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changes on cerebral cortex(5). Electrical stimulation from the tDCS as a way to give non invasive and excitatory transmission can check as a selective way to change the excitability of the brain has been developed.

Montoya stair task(MST) were develope the rat’s upper limb function as a way to provide a 14 pellets after the provide of visual information. It was designed to use only the upper limb for exercise of reach and grasp function(6). MST requires concentration that take the pellet directly into the mouth in rats. The dropped pellet can not be grasping and eating again. In stroke models of rat, it will be relative clinical motor skill training for task–oriented movement to a high concentration and applied task to success way is very closely relation.

The change of cells level after ischemic injured brain tissue, more attention to the protein changes in blood flow that the change of astrocytes and glial scar occurred due to the bad influence on the plasticity. The glial fibrillary acidic protein(GFAP) on the expression levels have been studied, GFAP is known to be associated between astrocytes and astrocyte specific marker protein(7), GFAP is the intermediate fiber protein of the reaction characteristic as a matter of cell fiber in all astrocyte cell of central nervous system(8, 9, 10).

Neuroglial scar can be made at the ischemic brain injury induced by MCAo. Thereby, due to damage to astrocyte expression of GFAP has been reported to have increased(11, 12). Neuroglial scar occur mainly appears to in the form of nerves on the response of the tissue damage(13). Therefore, GFAP expression increased had to mean that expansion of the neuroglial scar. It interferes with axonal regeneration in the play as a barrier to action, So, it need to therapeutic approach for the reduction of the neuroglial scar area(14, 15).

The purpose of this study were to test the effect of tDCS and MST on neural behavioral functional recovery and histological change after MCAo induced stroke model in the rat. And clinically applicable method of intervention is to evaluate the clinical effectiveness.

METHODS

Subjects

Male Sprague–Dawley rats(n=80, 230±20g) were used. Animals were housed in cage, and kept in a temperature–controlled room(22±1°C) with a 12hr cycle. Food and water were freely available. All experimental protocols were carried out according to the guidelines of the Dongshin University Animal Care and Use Committee. Care was taken to minimize numbers of animals used.

The experiment groups were divided them into four groups, and assigned 20 rats to each group. Group I was a experimental control group; Group II was a tDCS application group after MCAo; Group III was a MST application group after MCAo; Group IV was a tDCS and MST application group after MCAo.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (n=20)</td>
<td>Stroke induced + Non treatment</td>
</tr>
<tr>
<td>II (n=20)</td>
<td>Stroke induced + tDCS</td>
</tr>
<tr>
<td>III (n=20)</td>
<td>Stroke induced + MST</td>
</tr>
<tr>
<td>IV (n=20)</td>
<td>Stroke induced + tDCS + MST</td>
</tr>
</tbody>
</table>

MCAo Induced Stroke

MCAo was induced using an intraluminal filament(16). The right carotid artery was exposed and a heparinized intraluminal filament was introduced via the external carotid artery into the internal carotid artery to occlude the sources of blood flow to the MCA territory. After an MCA occlusion for 20 min, the filament was gently pulled out to allow reperfusion and the external carotid artery was permanently closed by cauteryization.

Transcranial Direct Current Stimulation(tDCS)

tDCS applied to this experiment, the Kim(17), depending on how the intensity in units of 0.1 mA adjustable DC stimulator(cybermedic Co, Jeonju, Korea) were used. The anode electrode in order to attach in the light cranial region of the cerebral cortex produces the pad at 1 cm × 1 cm sizes. For a fixation after producing plastic cup models, covered in the head and inserted and fixed and applied. The cathode electrode to prevent the shunting effect of the applied to the body. To reduce the electrical resistance, gel was used after removal of hair between skin and electrode pad. Model in plastic cup filled with a gel insert in the head was fixed. The intensity of electrification was contraction of the rat beard are visible, the intensity of the half was set to 0.1 mA. Application time is 20 minutes each, the 1 day 2 times, 5 times per week, four weeks was applied at the same time every day(Fig. 1).

Montoya Stair Task(MST)

MST was used in this experiment, including the Montoya(6), the training methods used by self–made
suit. From the 24 hours after MCAo, the 7 steps created and only use both sides of upper limb, for the prevention to use of head made acrylic panels in the middle. Providing all 14 pellet, we made eat it with upper limbs. When it dropped its pellet, we considered it fail, and reprovided it. Application time is 20 minutes each, the 1 day 2 times, 5 times per week, four weeks was applied at the same time every day(Fig. 1).

**Table 2. Neurological function assessment**

<table>
<thead>
<tr>
<th>Grade</th>
<th>State</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Rat held by tail had normal extension of both forelimbs toward the floor.</td>
</tr>
<tr>
<td>4</td>
<td>Rat with consistent flexion of forelimb on either side and adduction and internal rotation of shoulder.</td>
</tr>
<tr>
<td>3</td>
<td>Rats placed on soft plastic coated paper they could grip with forepaws.</td>
</tr>
<tr>
<td>2</td>
<td>Rats then allowed to move on floor and observed for circling behavior when pulled by tail rats circling to paretic side were grade 2.</td>
</tr>
<tr>
<td>1</td>
<td>Spontaneous circling when rat allowed to move on floor.</td>
</tr>
<tr>
<td>0</td>
<td>No spontaneous motion.</td>
</tr>
</tbody>
</table>

**Neurobehavioral Response Test**

During the experiment to evaluate the behavioral response to changes in total 4 weeks ago caused, after the induction of 1, 14, 28 days after. Each adaptive behavior score tests, 25 point behavior functional score test were assessed. How to evaluate a test by the examiner does not know what a double-blind assessment was to assess ways.

**Adaptation behavior score test**

To evaluate of spontaneous activity, symmetry of limb balance, the use of upper limb, proprioception, reaction to external stimuli on the both sides that the adaptation behavior score were examined(19). 4 point 18 of the point scoring, rating the higher the score obtained by adapting the state closer to the normal load means, Adaptive behavioral responses that appear after stroke to determine the state science assessment is a way.

**Histological Assessment**

For light microscopy, 4 weeks after the behavioral studies, the rats were deeply anesthetized with halotane/nitrous oxide and killed by transcardiac perfusion, following a brief flush of saline, with formalin-acetic acid solution(10%-2%) in distilled water at a pressure of 100–120mmHg for 12 min. The cerebrum was removed and fixed by 4% paraformaldehyde in 0.1% phosphate buffer for a week. Then the block of cerebrum was immersed in 30% sucrose-buffer solution overnight. Coronal sections at the bregma level were cut. The sections were subsequently stained with toluidine blue, and examined for histological changes.
Table 3. Adaptation behavior score test

<table>
<thead>
<tr>
<th>Function</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous activity</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Slightly affected</td>
</tr>
<tr>
<td></td>
<td>Severely affected</td>
</tr>
<tr>
<td></td>
<td>No movement</td>
</tr>
<tr>
<td>Symmetry in movement of 4 limb assessed</td>
<td>Symmetric</td>
</tr>
<tr>
<td>when rat held suspended by tail</td>
<td>Asymmetric</td>
</tr>
<tr>
<td></td>
<td>Hemiplegic</td>
</tr>
<tr>
<td>Forepaw outstretching assessed by bringing</td>
<td>Symmetric forepaws</td>
</tr>
<tr>
<td>rat to edge of table and making it walk on</td>
<td>Mild asymmetry</td>
</tr>
<tr>
<td>forelimbs while being held by tail and</td>
<td>Marked asymmetry</td>
</tr>
<tr>
<td>observing forelimb use</td>
<td>One forelimb did not move</td>
</tr>
<tr>
<td>Climbing determined by placing rat on the</td>
<td>Climbed easily, gripped tightly</td>
</tr>
<tr>
<td>wall of a wire cage and observing climbing</td>
<td>One side impaired</td>
</tr>
<tr>
<td>and strength of attachment to wall</td>
<td>Failed to climb or tended to circle instead of climbing</td>
</tr>
<tr>
<td>Body proprioception</td>
<td>Equal on both sides</td>
</tr>
<tr>
<td></td>
<td>Reacted slowly to stimulus on 1 side</td>
</tr>
<tr>
<td></td>
<td>No response on one side</td>
</tr>
<tr>
<td>Response to vibrissae touch determined by</td>
<td>Symmetric</td>
</tr>
<tr>
<td>brushing vibrissae on each side</td>
<td>Asymmetric</td>
</tr>
<tr>
<td></td>
<td>No response on 1 side</td>
</tr>
<tr>
<td>Total</td>
<td></td>
</tr>
</tbody>
</table>

Immunohistochemical Assessment

Immunohistochemistry for GFAP was carried out using the avidin–biotin–peroxidase method. Briefly, the free-floating sections were treated with 0.15% Triton–X and incubated in 1% hydrogen peroxide to block endogenous peroxidase. After pre-incubation in 5% blocking serum, the sections were incubated for 24 h at 4°C with a rabbit polyclonal anti-GFAP primary antibody(SantaCruz, USA) diluted 1:300. Sections were then incubated for 3hr with an appropriate secondary biotinylated antibody, followed by the avidin–biotin–peroxidase detection method(ABC Elite, Vector Laboratories). The positive signal was developed in a solution containing 3,3’-Diaminobenzidine(DAB) in the presence of hydrogen peroxide(0.002%). In our preliminary study, immunoreactivity specificity was tested by incubating rat brain sections with no primary antibody, in which no immunostaining was observed.

Data Analysis

Results are expressed throughout as mean±standard error of mean. The neurological function score were analysed with independent T-test and the behavioral data were analysed with a one-way ANOVA(SPSS 12.0 ver, for window). Post hoc analyses were performed using Tukey’s multiple range test, Differences were considered significant if p<.05.

RESULTS

Neurological Function Test

The neurological function test was used to assess at validation of stroke induced in each groups. Table 4 present each groups neurological function score on post time. After stroke induce, experimental group II, III, IV were significantly(p<.001) longer compared with experimental group II. There were significant differences between the Pre and Post result in each group.

Table 4. The neurological function score in each groups (score)

<table>
<thead>
<tr>
<th>Group</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>4.95±.22</td>
<td>4.90±.30</td>
<td>4.90±.30</td>
<td>4.86±.36</td>
</tr>
<tr>
<td>Post</td>
<td>1.29±.46</td>
<td>1.24±.44</td>
<td>1.29±.46</td>
<td>1.29±.46</td>
</tr>
<tr>
<td>P-value</td>
<td>.000</td>
<td>.000</td>
<td>.000</td>
<td>.000</td>
</tr>
</tbody>
</table>
Neurobehavioral Response Test

**Adaptation behavior score test**

The adaptation behavior score test was used to assess spontaneous activity, symmetry of limb balance, the use of upper limb, proprioception, reaction to external stimuli on both sides in each groups. Table 5 present each groups score. The score in the experimental groups were higher compared with group I. After 14, 28 days, the score in the experimental group IV were significantly (p<0.01) higher compared with experimental group I. There were significant differences among the four groups.

| Table 5. The changes of adaptation behavior score test score in each groups (score) |
|-----------------|-----------------|-----------------|-----------------|
|                | Pre             | 1 day           | 14 days         | 28 days         |
| I              | 17.52±0.87      | 4.86±0.79       | 8.33±0.58       | 13.38±1.07      |
| II             | 17.62±0.67      | 5.05±0.67       | 8.86±1.24       | 13.57±1.21      |
| III            | 17.67±0.66      | 5.29±0.72       | 9.86±1.28      | 13.05±1.56      |
| IV             | 17.62±0.59      | 4.90±0.77       | 10.52±1.25      | 14.71±1.68      |
| P-value        | .982            | .241            | .000            | .002            |

*: Statistically significant as compared with group I

"*: p<0.01; ""*: p<0.001

**Montoya Stair Task Test**

The Montoya stair task test was used to assess using upper extremities to reach the pellet, grasp both the behavior of complex upper extremity functional ability to perform in each groups. Table 6 present each groups score. The score in the experimental groups were higher compared with group I. After 14 days, the score in the all groups were significantly (p<0.001) higher compared with experimental group I. 28 days, the score in the experimental group IV were significantly (p<0.001) higher compared with experimental group I. There were significant differences among the four groups.

| Table 6. The changes of montoya stair task test score in each groups (score) |
|-----------------|-----------------|-----------------|-----------------|
|                | Pre             | 1 day           | 14 days         | 28 days         |
| I              | 12.57±1.2       | 1.38±0.92       | 5.77±1.45       | 8.60±1.77       |
| II             | 12.52±0.98      | 0.86±0.65       | 7.80±0.98      | 8.67±1.24       |
| III            | 12.86±0.79      | 0.67±0.66""     | 8.57±1.03""     | 10.52±1.81""    |
| IV             | 12.71±0.72      | 0.81±0.75       | 9.95±1.63""     | 11.57±1.83""    |
| P-value        | .660            | .017            | .000            | .000            |

*: Statistically significant as compared with group I

"*: p<0.05; ""*: p<0.01; """*: p<0.001

**Histological Finding**

Light microscopy of the cerebrum cortex of all groups of rats showed Toluidine blue staining in the cortical atrophy and not heavily staining was observed in pyramidal cells. The most severe morphological changes were observed in group I, and group II, III, IV has improved to those with less status was observed compared with group I. Especially, the experimental group IV increased number of pyramidal cells and axons were observed than the experimental group I (Fig. 2).

**Immuno histochemical Finding**

GFAP immunoreactivity were observed for interfere with nerve regeneration in the cerebral cortex, and sensitivity to neurological excitotoxicity used as indicators of neurotoxic. After 14 days the group I exhibited higher GFAP levels than the group II, III, IV in cerebral cortex. Especially, the experimental group IV exhibited lower GFAP levels in cerebral cortex (Fig. 3).
DISCUSSION

The tDCS anode electrode applies that increased activation of prefrontal cortex area(20). Especially, applied depending on time and intensity to normal people that were lower limb area, as well as increased activation in the upper limb area. Anode effect of direct current flowing through the anode to the cathode electrode placement, due to the flow of the current anode upper region between cathod lower region department began to have the flow of current when placed around the activity of the cells is increased(21). Moreover, anode tDCS were effective on N-methyl-D-aspartate(NMDA) receptors to excite potential and increased of cerebral cortex activity. Cathod tDCS were decreased of cerebral cortex activity(22, 23). So, in this study, after MCAo brain damage for the purpose of recovery of motor cortex at anode tDCS was applied.

Plasticity of the central nervous system rather than a fixed neurological connectivity and morphology of synapses were formed by modulating and number of synapses and morphological will be changes that experience really depends on the amount of work performed by the actual changes(24). MST is using the stairs to reach it to move with inducement of actual upper movement through the more skilled movement that able to training will affect brain plasticity.

In this study, through the evaluation of neurobehavioral response were identified recovery of cerebral injury. The results raised confidences as reducing the variation between subjects, We observed that the effect of tDCS and MST in MCAo induced ischemic stroke model of rat. In the adaptation behavior score test which designed to measure the ability to spontaneous activity, symmetry of limb balance, the use of upper limb, proprioception, reaction to external stimuli on both sides, the duration in the experimental groups were lower compared with group I and after 4 weeks, the duration in the group II, III, IV were significantly(p<.001) higher compared with group I. Especially, group IV was observed that most increased of score than group II, III on 14 days. Our results were consistent that tDCS and MST training increased functional recovery and sensorimotor recovery. In the Montoya stair task test which designed to measure the ability to upper extremities to reach the pellet, grasp both the behavior of complex upper extremity functional ability, the score in the experimental groups were lower compared with group I and after 4 weeks, the score in the group II, III, N were significantly(p<.001) higher compared with group I. Especially, group IV was observed that most increased of score than group II, III on 14 days too. These results were consistent with previous studies that dominant upper limb in the brain activation caused on a cellular level in the area of upper limb functional recovery was considered to be used. The positive effect on tDCS and training in brain damage that centralized state in performing repetitive tasks performed by experienced more efficiently as compared to baseline is thought to promote brain plasticity. In this study, upper limb dominated areas of the brain damage is when rat drops the pellet is to train them repeatedly in the final assessment is thought to increase the more success rate. Kloths study(25) in the SD rat has improved ability to more easily trained, 3 days after data was showed the best performance, Therefore, the score in the group II, III, IV were significantly(p<.001) differentment compared with group I on 14 days that application of MST training to be that can be used effectively in the initial therapeutic approach.

The structural transformations are thought to represent quantitative changes in information processing that occur in association with changes in behavior. We tried to observe the histologic change and GFAP expression of cerebrum. We observed that histological changes were in each of the degenerated axon in the brain between the connective tissue, nerves, to recover at myelin sheath.

In toluidine blue stain, experimental groups was showed swelling pyramidal cells, and clearly did not stain was observed in pyramidal cells. The most obvious morphological changes were observed in group I. The experimental group II, III, IV were observed treatment than group I. Especially, group IV was observed that decreased of degeneration of pyramidal cells and increased number of axons than group II, III on 14 days. These histologic findings were that consistent with neurobehavioural responses results and MST test result. The increase of dendrite that thought to mean the remodeling of synaptic to accept afferent information. The result suggest that it applies a variety of tDCS and the MST in the cerebral cortex thought to affect that such as the reduced the swelled pyramidal cells, number of dendrites and reduction of synaptic strength,
In immunohistochemical reaction of GFAP, the group II, III, IV observed lower GFAP expression levels in cerebral cortex. In contrast, group I observed higher GFAP expression levels than the other groups in cerebral cortex. GFAP is a protein in neuroglial scar, 8–9nm, 55kDa, are the major component of astroglial filaments in astrocytes. Motility and shape control are known to play an important role in astrocyte(26, 27). This result, Glial scar tissue formation in the central nervous system damage to the axonal growth cone is the growing frustration. Glial scar formation itself to structural barriers and the growth inhibition factor. At the central nervous system damage that occur to surrounding cells combined materials and to generate gliosis. As a result, this inhibition the nerve regeneration. The result suggest that it through the approach to physical therapy were decrease of GFAP expression and it can increase of survival rate of the neurones in the central nervous system. It seems to be able to mitigate of the various symptoms of brain injury.

As a result, tDCS and MST were thought that increasing the formation of synapses and density to promote in the cerebral cortex and involved in regeneration of synapses. These changes can be seen as a process in cellular levels of the nerve cells are connected to each other by changing the strength of synaptic connections and form a new neural network. Therefore rat’s neuro–functional behavior would have been improve and recovery. It seems that the synaptic plasticity of damaged neural network will be strengthened as well as new neural network when tDCS and MST stimuli input and neurological function and histological change.

**CONCLUSION**

This study is intended to examine the tDCS and MST on sensorimotor recovery and GFAP expression in MCAo induced rat. In each group, neurological function test measurement, motor behavior test, montoya stair task test, immunohistochemic finding of GFAP expression finding were analyzed. The following result were obtained.

1. Stroke induced confirmation was neurological function test measurement, the outcome of all groups were and significantly lower than the pre-value(p<.001).

2. In motor behavior test, the outcome of group I was significantly difference than the other group (significantly changes in other group, especially from 14 days).

3. In Montoya stair task test, the outcome of group I was significantly lower than the other group (especially, group II were significantly different on 14 days and group IV was most significantly difference than the other group).

4. In immunohistochemic finding group II, III, IV were decrease GFAP expression depend on time stream.

These results suggest that the tDCS and MST apply will be recovery and change of new neural network formation in the MCAo induced rat model.

**REFERENCES**


