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Difference in Outcome Depending on the Timing of Therapeutic Hypothermia for Prevention of Brain Damage

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ABSTRACT

Therapeutic hypothermia, a method that prevents damage to central nerve system by lowering patient's body temperature to a certain goal temperature, is recently being appraised in neurologist communities as cases with high therapeutic outcome are being reported. Its main principle is to prevent various brain damages that may occur as necrosis of nerve cells continues by slowing body metabolism, but there is a great problem that outcome of the treatment is too uneven as it depends on various factors such as initial damage and temperature. In this study, 'time', a crucial factor directly related to brain damage, has been selected as a variable. The study confirms the effectiveness of therapeutic hypothermia while acknowledging the importance of Golden Time. After inflicting hypoxic ischemic brain damage to 7-days old rats, therapeutic hypothermia was conducted in different timings. In result of this study, rats that had some interval between the damage and treatment showed severe progression of brain damage as much as rats that did not receive the treatment, but rats that received therapeutic hypothermia immediately showed tendency to recover to a state similar to normal rats. Therefore, therapeutic hypothermia shows excellent effect in treatment of brain damage and it has been studied that reduction of time taken until start of the treatment is a crucial factor for successful recovery.
KEYWORDS: Therapeutic hypothermia, Golden time, brain damage

INTRODUCTION

1. **Proposal**

Recently, therapeutic hypothermia has been used for treatment of Lee Geonhui, the chairman of Samsung, who fell into coma and it is published by media. Therapeutic hypothermia afflicts hypothermic stimulus to prevent/slow brain damage. Generally therapeutic hypothermia is known to show more significant effect of brain protection than when patient is kept in room temperature. [1, 2] Currently therapeutic hypothermia is actively applied in clinic and is achieving evident outcomes as it shows great effect to child patients.

However, according to <Hypothermia treatment for newborns with hypoxic ischemic encephalopathy>, protocol for optimal practice of therapeutic hypothermia does not exist in a strict sense. The biggest reason is that effect of treatment heavily depends on numerous variables such as intensity of brain damage [3, 4]. While aspects of brain damage in actual patients are variable, recently developed therapeutic hypothermia does not have protocol for all situations. Such conditions reinforce the necessity of protocol that can be applied in various clinical cases.

2. Purpose and significance of the study

Existence optimized in time of action, temperature, and body area can highly raise efficiency of therapeutic hypothermia. Above all, grasping golden time is one of the most important tasks. It is because elapse of time is the most critical factor in brain damage. Therefore, we focused on timing among all the variables in practicing therapeutic hypothermia. The purpose of our study is to find which timing of the treatment is suitable for maximum effect to be achieved and if it is effective after there is a delay in treatment. We anticipate it can contribute in establishing database for optimal practice of therapeutic hypothermia. If further studies are conducted in the future, more extensive and standardized protocol can be established in near future. It has its significance in that it can allow patients to be free from the risk of permanent brain damage, receive more successful treatment, and continue their social life.

MATERIALS AND METHODS

(1) 7-days old rats as study subjects (20 rats)
(2) The rats are given anesthetic. The rats are dissected in one of anterior cervical arteries with electric knife and occluded.
(3) Rats are placed in a chamber (connected to gas tank consisted of oxygen 8%, nitrogen 92%) inside incubator for 2 to 2 and a half hours.

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(4) Rats are classified into 4 groups
A: left in incubator of 34℃(room temperature)
B: Immediate therapeutic hypothermia in 20~23℃
C: After left in 34℃, therapeutic hypothermia in 20~23℃
S: Comparison group with neither surgery nor hypothermia
(5) Rats are raised under controlled conditions for 4 weeks.
(6) M.W. test conducted in each weekend from late August
*Collection of data: Morris Water maze test (M.W. test)

METHOD OF EXPERIMENT

In Invisible test, a platform in diameter of 10cm and height, 38.5cm is placed in a tube cistern in diameter of 155cm and height, 60was used. Water of 24~26℃ is filled in the cistern so that island can be placed in 1.5 cm deep and Milk is poured to the water so that island cannot be seen. Pre-training is conducted without platform for 90s before conducting experiment.
Time of arrival to the platform was measured and Rat was given a rest of 30s after its arrival. If the rat does not reach the platform for 60s, the experimenter relocates rat to platform and gives 30s of rest. Starting position is set in each quadrant and step 1, 2, and 3 are conducted 4 times.
Above steps are repeated for 5 weeks.

In Visible test, a flag is placed on the platform without mixing milk to water and other aspects are identical to invisible test. The test is conducted once in the week after termination of invisible test.

In Probe test, platform is removed and other aspects are identical to invisible test. The test is conducted once in the week invisible test is terminated (the 5th week).

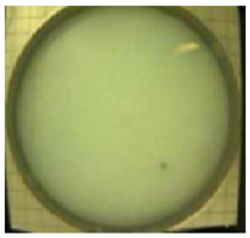


Fig.1. A platform in a tube cistern

Subject < Theoretical review on theme of the study>

A. Study subjects
--Sprague-Dawley rats that are 7 days old are used in the study.
1. Brain of rat near birth generally shows higher neuroplasticity than grown rat and relatively more excellent in altering function, chemical characteristic (characteristic of signal conduction substance), and synaptic structure of nerve cell. Therefore, when inflicting damage to central nerve system, nerve tissue in healthy part of brain substitutes for roles of the damaged parts. As synaptic connection in damaged part is replaced with interneuron reinforcement and generation and differentiation of new nerve cells, risk of serious trouble in memory, character, and cognition is significantly lowered in long-term.
Hemispherectomy is a representative case of experiment that can effectively be accomplished with neuroplasticity of children. When cause of epileptic symptoms extensively exists in one hemisphere, one hemisphere of the brain with disorder in neuron connection is removed for treatment. Then, some subjects may experience hemiplegia or visual deterioration, but the other hemisphere takes place of the role by the removed hemisphere and no serious physical or psychological shock is experienced. In one case, there was a male who received hemispherectomy at age of 5 and completed general education with higher-than-average grades, graduating from college.
As baby rats used in this study as subjects possess excellent neuroplasticity, no behavioral disorder that makes the experiment impossible to conduct is induced when brain damage is inflicted from hypoxic ischemia. They also show high recovery of neural tissue, which makes it suitable to approve effect of therapeutic hypothermia.

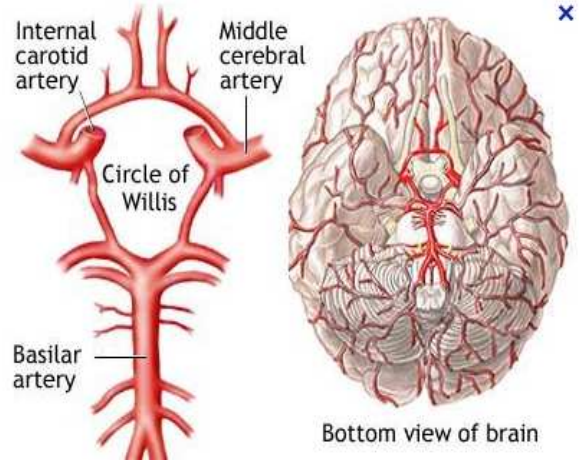
2. In neck of the subject rats, 'Willis Circle' is formed by 2 carotid arteries of both sides in the front and 2 spinal arteries in the back. In the circle, blood circulates and it supplies the blood to brain. Due to the structure with anterior and posterior arteries connected, dissection of one artery for induction of hypoxic ischemia does not pose a serious threat to survival in overall blood supply to the brain.

B. Hypoxic ischemic Brain injury

In this study, right carotid artery was cut to induce hypoxic ischemia by decreasing blood flow to brain. If blood flow to brain decreases, ATP becomes insufficient, crippling cellular respiration. Then, human compensation increases blood flow to brain and heart rate, but as hypoxic ischemia continues, the compensation process weakens, resulting in ATP insufficiency. As ATP that plays crucial role in controlling osmotic pressure in and out of cell membrane, becomes insufficient, extracellular Na+ are introduced into the cell with water, inflating the cell. As introduction continues, the cell expands continuously and starts necrosis as it contracts.

C. Therapeutic hypothermia

'Therapeutic hypothermia' is an artificial drop of body temperature to decrease tissue damage of central nerve system. Nowadays, therapeutic hypothermia as neural protection method started from Fray who first conducted therapeutic hypothermia to brain injury patients in 1940s.



Therapeutic hypothermia decreases body metabolism and slow oxygen supply and demand replenishment, but it has tissue-specific effects such as reduction of cellular excitotoxicity, anti-inflammation, prevention of ATP insufficiency and intracellular hypercalcemia to prevent cellular necrosis. Such function makes therapeutic hypothermia to be highly appraised for effective treatment of neural diseases or injuries such as stroke, subarachnoid hemorrhage, and brain trauma.

Therapeutic hypothermia can be classified into two methods depending on the method of cooling. One is topical cooling that alters external conditions and lower body temperature and the other, internal cooling that directly lowers body temperature with circulation of blood injected with cool saline. In this study, topical cooling was used for therapeutic hypothermia.

Generally, therapeutic hypothermia can be classified into mild (34.5~36.5), moderate (32~34.5℃), marked (28~32℃), profound (less than 28℃) depending on the goal temperature. In this study, profound therapeutic hypothermia with 20~23℃ was conducted to optimally observe influence of therapeutic hypothermia.

RESULTS & DISCUSSION

After inducing hypoxic state and inflicting hypoxic ischemic brain damage, timing of conducting therapeutic hypothermia was set as an independent variable and for 5 weeks after 3rd week, Morris Water Test was used to measure intensity of brain damage as dependent variable. To study spatial cognition and learning ability, Invisible Test that made rat to find destination, shape of an island, in a cistern filled with milk-mixed water was conducted weekly for 5 weeks and in the last 5th week, Probe test that released rats to the cistern after removing the destination was conducted to evaluate rat's learning on the test. Also, Visual Test that places a target on the island was conducted to study visual cognition.

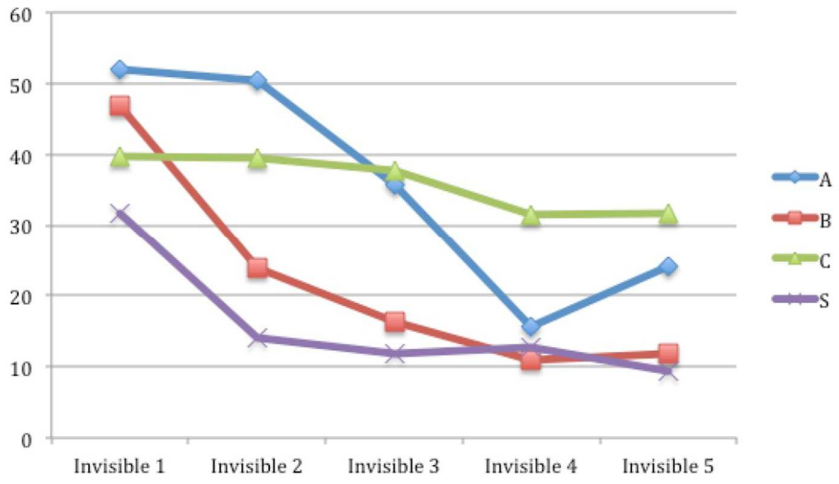
Groups in this study was 4 in total; A was group that did not receive therapeutic hypothermia; B, group with immediate therapeutic hypothermia; C, group that received therapeutic hypothermia after left in room temperature after receiving brain damage; S, a comparison group that did not have brain damage.

[Table 1] shows time taken for rats to move to destination.

[Table 1] Morris Water Test Result

	Invisible 1	Invisible 2	Invisible 3	Invisible 4	Invisible 5	Probe Test	Visible Test
A1	48.2	50.8	44	23.4	19.8	23.8	4.025
A2	77.8	45.8	27.8	20.4	23.6	47.2	3.275
A3	27.4	14.8	6.6	8.4	5.4	18.4	6.120
A4	54.8	90	64.2	10	47.2	26.6	1.141
A avg	**52.05**	**50.35**	**35.65**	**15.55**	**24.00**	**29.00**	**3.640**
B1	62	15.8	9.4	10.8	11	10	11.107
B2	35.6	45.6	18.2	8	12.6	26.2	7.721
B3	41.6	11	17.2	4.8	8	21	7.273
B4	48.6	23.2	20.2	20	16	8.8	2.915
B avg	**46.95**	**23.90**	**16.25**	**10.90**	**11.90**	**16.50**	**7.254**
C1	11.4	28.2	44.2	34.6	32.2	23.8	1.741

In Invisible Test, it takes long time for rats to reach the destination as they experience failure in reaching the destination. However, as time passes, Time of arrival decreases as rats learn visual and experiential information. However, for rats with brain injury lack in learning ability and spatial cognition, arrival time is much slower in the first trial and does not greatly decrease as the study is conducted.



[Graph 1] shows changes in Invisible Test of different groups.

[Graph 1] Change in Invisible Test Results

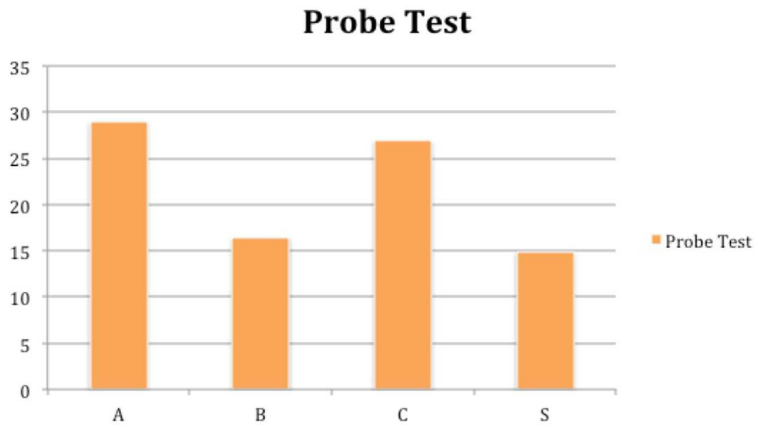
In [Graph 1], changes in Invisible Test to study spatial cognition and learning ability, one of the most prominent features is that B group, the group with immediate therapeutic hypothermia, shows similar tendency to S group, the comparison group that did not have brain damage. The results suggest that therapeutic hypothermia is effective for prevention of brain damage. However, C, the group with late therapeutic hypothermia, shows little reduction in time of arrival. It shows almost similar time to group A, the group that did not receive therapeutic hypothermia.

In this trial, the intensity of brain damage of the group with immediate therapeutic hypothermia is similar to the comparison group that did not have brain damage, but the intensity of brain damage of the group with late therapeutic hypothermia is similar time to the group that did not receive therapeutic hypothermia. So, we could conclude that therapeutic hypothermia shows great difference in effect, depending on timing of therapeutic hypothermia and its effect diminishes as time passes.

In collected 13 randomized clinical trials and 5 observational 2012 studies, concerning hypertension management in patients with Traumatic brain injury using therapeutic hypothermia. A significant reduction of Increase intracranial pressure was evident in all of the patients [5].

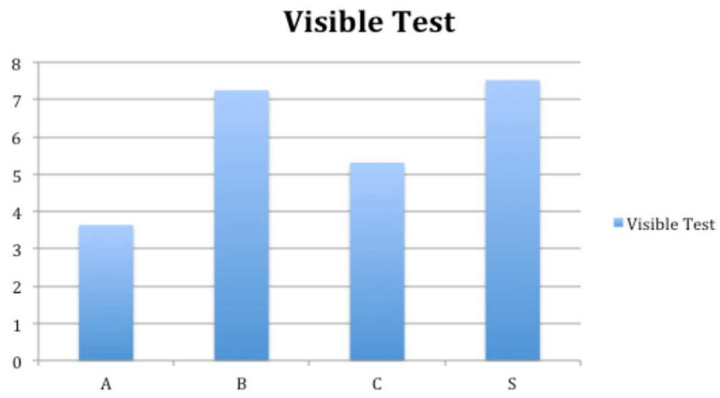
In other studies of the time-dependent effect of hypothermia for traumatic brain injury in 2006, a randomized study of 215 patients with severe traumatic brain injury, which showed that prolonged hypothermia (5 ± 1.3 days) was more effective than conventional hypothermia (2 ± 0.6 days) in reducing the number of patients with poor neurological outcomes. Both patients arms where cooling to reach 33–35°C of rectal temperature, using cooling

blankets [6].
After all Invisible Tests are complete, Probe Test was conducted after removing all destinations to study learning ability and memory. [Graph 2] shows the result of Probe Test



[Graph 2] Probe Test Results

As shown in Probe Test, group C with late therapeutic hypothermia showed similar result to group A that received no therapeutic hypothermia, while Group B with immediate therapeutic hypothermia showed similar to group D with no brain damage. Therefore, results of Probe Test confirm that therapeutic hypothermia shows difference in its effect depending on timing of the treatment.
We finally conducted Visible Test that installs a flag on island to compare intensity of brain damage. [Graph 3] shows results of Visible Test.



[Graph 3] Results of Visible Test

Visible Test shows almost no effect of therapeutic hypothermia, which is contradictive to other test results. However, as seen in S comparison group, Visible Test must be unsuitable to evaluate intensity of brain damage. Also, as seen in [Graph 1], in Visible Test, unlike other test results, results were low, similar, and sometimes extreme, thereby extreme results were not included in statistics, which posed difficulty in generalizing it statistically.

CONCLUSION

Though therapeutic hypothermia is appraised highly in not only neurologist society but also various fields of medicine as treatment for brain injury, comparative analysis on various practice methods are deficient. Therefore, we conducted treatment using therapeutic hypothermia with 4 groups of rats, grouped depending on the time taken until therapeutic hypothermia from onset of brain damage.
After inducing hypoxic ischemia and inflicting brain damage, result of therapeutic hypothermia in different

conditions were confirmed with Morris Water Maze Test. Based on the test results, group with immediate therapeutic hypothermia showed least nerve cell necrosis similar to the group with no brain damage. In contrast, group with late therapeutic hypothermia showed similar progression as group with no therapeutic hypothermia. It could be learned that Golden Time, the time taken to start therapeutic hypothermia after onset of brain damage, is very crucial and the treatment must be conducted as soon as possible after brain damage for effective therapeutic hypothermia or it would be ineffective.

From this study, we learned the importance of accurate protocol. Therapeutic hypothermia must be conducted within appropriate time under accurate protocol for patients to recover into normal condition with effective treatment.

However, the time of 'Golden Time' for therapeutic hypothermia was not found in this study. There have been studies that researched when other treatments, for example, CPR, must be conducted. But as for therapeutic hypothermia, preceding study that covers this subject does not exist and also does the study on difference of effect depending on the time passed until the treatment. Therefore, methodology of this study is to be expanded and further study on at least until when the therapeutic hypothermia should be conducted to the patient is required. From such, more effective protocol for treatment of brain injury would be established.

One shortcoming of this study is that it has some inaccuracy in its measurement. Due to technical, material, and experiential limitations, the time had to be measured with stopwatch in cell phone and though there were extreme measurements in data analysis, statistical analysis was conducted. Also, it has its limits in that M.W. Test is generally conducted every day for 5days to clearly observe effect of learning, but procuring time for the test was difficult and it had to be conducted weekly. Further study is required.

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