L-Glutamate profile in acute ischemic stroke after intra arterial heparin flushing

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ABSTRACT

Objective: The intra-arterial heparin flushing (IAHF) is one of the endovascular procedure that in facts have benefit effect to improving the motoric function after acute ischemic stroke, but the exact mechanism not yet known clearly. The L-glutamate is a neurotransmitter that have important role in the brain metabolism. The purpose of this study is to know how the IAHF affect the Glutamate concentration in the blood and muscle function which measured by Manual Muscle Testing-Medical Research Councils (MMT-MRC) in Acute Ischemic Stroke Patients.

Method: The thirty-nine patients of acute ischemic stroke that admitted to hospital, was studied of L-Glutamate serum profile. The patients grouping depend on onset (<7 days, 7-14 days after stroke onset), site of infarction (cortical and subcortical), and size of infarction (lacunar and wide infaract). The outcome of motoric function was recorded and measured by MRCs (Motoric Research Council Scale; 0-5) in the arm weakness, MRCs < 18; severe, 18-23; moderate, 24-29; mild). The L-glutamate serum level measured before and after IAHF.

Results: Characteristic of sample = Onset: <7 days = Before: 19,51±6,74. After: 20,64±7,45. P = 0,474. 7-14 days = Before: 18,70±6,08. After : 22,70±7,72, p=0,046. Site of infarction; cortical = Before: 21,63±9,55. After: 25,94±8,22. P = 0,258. Subcortical = Before: 18,32±4,3, after: 19,69±6,48. P= 0,370. Size of infarct: Lacunar = before: 18,32±4,3. After: 19,69±6,48. P= 0,098. Muscle Strength: MRCs < 18 = before: 16,68±5,13, after: 19,67±5,72, p= 0,060. MRCs (Upper Arms) 18-23; Before: 24,70±4,72, After: 27,95±6,93, p = 0,372. MRCs 24-29; Before: 18,17±6,93 After: 20,53±7,89, p = 0,248.

Conclusion: The L-glutamate serum level was increasing after IAHF, but not significantly different according to the all of group in this study.

Keywords: L-glutamate, acute ischemic stroke, IAHF, muscle strength

INTRODUCTION

Stroke is a major health problem and nowadays it has been a major concern because its position as the number two causation of mortality and disability in long terms in adult.1 In the last decade, many studies has been done to improve the understanding of pathophysiology, diagnosis, and therapy of ischemic stroke.2 The most common neurological deficits found in stroke patient were paresis, speech, and sensory deficits. Clinical characteristics of stroke may be varied for different population groups, but male subjects are the most likely experiencing the gait disturbances.3

This gait disturbance affects every aspect of the patient lifestyles, to measure how much the muscular function has been decreased this study using the Manual Muscle Test-Medical Research Councils (MMT-MRC) Method. This measurements method was first developed by Lovett and Wright in 1912 and also had been revised to be a wide range of methods. The quantification methods of MMT-MRC will be described later.

A lot of drugs that influences hemostasis and clot build ups such as anticoagulants and anti-platelets aggregation many used in cerebrovascular disease treatment.4 One of many drugs that widely used in thrombosis cases both arterial thrombosis or venous thrombosis is heparin.5 Administration of heparin has been proven to be safe in cerebral cases that it has been choose as the protocol of Cerebral Venous Thrombosis therapy.6 Based on this the Intra Arterial Heparin Flushing has been proven to be safe to be used as one of the therapy option in cerebrovascular disease cases.

Glutamate as a neurotransmitter is the method of communication between neurons. This kind of interactions is either excitatory or inhibitory. The major excitatory neurotransmitters are glutamate and aspartate, while the inhibitory neurotransmitters are GABA, Glycine, and Taurine. Glutamate is synthesized by the endoplasmic reticulum then transported to Golgi apparatus for additional processing. When glutamate emerges from the Golgi apparatus it’s encapsulated inside a vesicle,
this vesicle transported down the axon along the microtubules tracks to be deposited at the tip of the axon near the presynaptic membrane. The depolarization of axonal membrane triggers the release of the glutamate into the synaptic space by exocytosis. The emptied vesicles would then be recycled back to the neuronal body by retrograde transport along the microtubules tracks. Stimulation in every glutamate receptor will cause membrane depolarization because the influx of positive ions and then stimulate the voltage gate calcium ion. A few drugs has been developed to interrupt, influence, or temporarily halt the excitotoxic neuronal injury, such drugs includes riluzole, lamotrigine, and lifarizine which are sodium channel blockers. Where the most common drugs being used is nimodipine which a voltage dependent channel blocker. Glutamate excitotoxicity also causing neuronal injury in some disorders such as trauma, seizures, hypoglycemia, hypoxia and some neuronal degenerative disorders.

Another glutamate release mechanism is by astrocytes, this mechanism occurs by this mechanism which are : reversal of uptake by glutamate, anion channel opening induced by cell swelling, calcium dependent exocytosis, glutamate exchange via the cystine glutamate antiporter, release through ionotropic purinergic receptor and functional unpaired connections on the cell surface. The inhibition of astrocytes glutamate uptake and astrocytes mitochondrial function impairs neuronal survival from excitotoxic injury. In some studies Astrocytes generally has been shown more resistant to Oxygen Glucose Deprivation (OGD) than neurons. Another neuroprotection strategy after traumatic brain injury that has been studied on rats is by giving oxaloacetate and pyruvate to scavenge blood glutamate.

The glutamate neurotoxic effect can be through an agonist effect on NMDA, AMPA, Kainate, or Group I metabotropic receptors. The susceptibility to excitotoxicity cell death is under genetic control in variety of ways. Even single gene defect can enhance vulnerability as in the case of superoxide dismutase.

Glutamate is renowned as the most important transmitter for brain function. Almost all excitatory neurons in the CNS (Central Nervous System) are glutametergic, and over half of all brain synapses release this agent. In clinical neurology, elevated concentration of extracellular glutamate related to neural injury due to its toxicity to neurons. This process of toxicity is called excitotoxicity which refers to the glutamate and related compounds ability to destroy neurons by prolonged excitatory synaptic transmission. In normal condition, glutamate concentration released to the synaptic cleft rises to approximately 1mM, but it remains at this level for a few milliseconds. If the concentration abnormally accumulated in the cleft, there will be excessive activation of neuronal glutamate receptors can excite the neurons to death.

Cerebral Ischemic Strokes is a cerebral vascular events caused by obstructed arteries that deprive the oxygen and other nutrients supplies. When the neuronal death following ischemic stroke occurred the homeostasis of glutamate is disturbed. In homeostatic condition glutamate functioned as the primary excitatory neurotransmitter in the nervous system where the release of glutamate into the synaptic cleft stimulates NMDA receptor which causes influx of calcium and sodium and so the depolarization of post synaptic neuron. When the ischemia occurred, the ATP levels decreased as cellular respiration compromised then impairs the glutamate transporters, causing unregulated accumulation of glutamate in synaptic cleft. The excess of glutamate over activates NMDA Receptor causing an influx of calcium, the production of Reactive Nitrogen Species (RNS), mitochondrial dysfunction, and generation of Reactive Oxygen Species (ROS), which all of this contributes to cell death.

The damages to neuron was restricted to the postsynaptic cells, causing the dendrites of the target neurons were grossly swollen, while there is no specific effects on the presynaptic terminals. It also been observed that the concentration of glutamate and aspartate in extracellular space increased during ischemia. The most prevalent glutamate precursor in synaptic terminals is glutamine and glutamine is released by glial cells.

In homeostatic condition, glutamate is cleared out from the extracellular space by sodium-dependent reuptake system and moved into astrocytes and neurons; however, during ischemia the sodium gradient breaks down and extracellular glutamate is not transported back into the intracellular compartment. The association of ischemic stroke and elevated glutamate levels has been found in a study by Davalos et al and Castillo et al where the study shows that in 48 hours after stroke there is an increased level of glutamate found in CSF and in the blood of the patients, and this condition correlated with poor clinical outcome. In normal condition the glutamate concentration in plasma is 5-100 μM/L, in the whole blood concentration is 150-300 μM/L, while in the brain ECF it's only 0.3-2 μM/L.
MATERIAL AND METHOD

Subjects
This study involved 28 acute ischemic stroke patients in Cerebrovascular Center Gatot Soebroto Indonesian Army Central Hospital started from February 2015 by consecutive sampling.

The inclusion criteria include: (1) patient diagnosed with acute ischemic stroke (by radiology and neurology examination) (2) age 30 – 70 years old, and (3) agreed to follow the IAHF procedure and signed the informed consent form. The exclusion criteria include: (1) any allergic to contrast and heparin,(2) blood clotting abnormality. (3) subjects with high risk or contraindication according to cardiology, pulmonology, internal medicine, and anesthesiology procedures. (4) not able to undergo MRI examination. (5) cannot understand or not able to follow study instructions. (6) motoric dysfunction caused by another disease. (7) subjects diagnosed with brain stem stroke for more than 6 hours or less than 2 weeks (involution state).

L-Glutamate Quantitation
The method used to quantify the L-glutamate concentration is using ELISA Glutamate method with the following Reagent kit: LDN GmbH, 48531 Nordhorn, Cat: BAE2400, Lot:13165-150093, ED: 30/11/2015–09/01/2017. With Standard Calibration:0,6-60 μg/mL. Detection Limit: 0,3 μg/mL. Sample Values: Serum: 2,2-30 μg/mL.

IAHF Procedure
According to Putranto et al,2016 IAHF procedure has the purpose to release obstruction in the blood vessel in the brain on ischemic stroke patient. the procedure of IAHF itself were: patients and instruments were readily prepared before, 5000 IU heparin diluted with 500 cc NS Otsu. Topical anesthesia EMLA was applied on femoral artery area, continued with povidone iodine 7,5% and alcohol 70%. Local anesthesia lidocaine was injected intracutaneous and subcutaneous. Femoral artery was punctured with abocath 18 G, and short guidewire was inserted. Fluoroscopy was performed to see the anatomical imaging. Diluted heparin was flushed intra-arterial in both right and left carotid arteries and vertebral arteries. After completing the flushing process, femoral artery bleeding was stopped using either conventional technique or angio seal.

Manual Muscle Test Measurement
In this study to measure the muscle strength before and after the IAHF treatment we’re using The Manual Muscle Test – Medical Research Council Scale (MMT-MRcs) method measurements which contains 6 scales, including: 0 = stands for no movement detected; 1 = only a weak contraction that can visualized or sensed at muscle; 2 = the muscle can be moved horizontally but unable to move against gravity; 3 = muscle strength declined and muscle contraction can moved the joints against gravity if there is no resistance added; 4 = muscle strength declined but muscle contraction can move joints against resistance; 5 = normal muscle contraction against full resistance. This measurement will be performed by a trained physicians and neurologist and the total summation of all score will be the assessment factor for this study.

Statistical Analysis
Statistical analysis was performed. Kolmogorov– Smirnoff test was performed to find the data distribution model. The differences in serum level of L-Glutamate and MMT-MRcs score before and after treatment were tested using paired T Test or Wilcoxon test alternatively with P value of 0.001 were considered significant.

RESULTS
The twenty-eight Acute Ischemic Stroke Patient were participated in our study. L-Glutamate Quantitation before and after IAHF were analyzed using paired T-Test or Wilcoxon as alternative, as shown in Table 1.

We found that the L-Glutamate level in <7 Days Onset Group increased from 19,51±6,74 before IAHF treatment become 20,64±7,5 after IAHF Treatment. While the L-Glutamate level in 7-14 Days Onset Group increased from 18,70±6,08 before IAHF treatment become 22,70±7,72 after IAHF treatment with p=0.046. We also found that the L-Glutamate level in Cortical Group increased from 21,63±9,55 before IAHF treatment become 25,94±8,22 after IAHF Treatment. While the L-Glutamate level in Subcortical Group increased from 18,32±4,3 before IAHF Treatment become 19,69±6,58 after IAHF Treatment. Another finding in the L-Glutamate level in Lacunar Group shows increased level of L-Glutamate from 18,32±4,3 before IAHF treatment become 19,69±6,58 after IAHF Treatment. While the L-Glutamate level in Non-Lacunar Group increased from 21,63±9,55 before IAHF Treatment become 25,94±8,22 after IAHF Treatment. In this study we also find an increasing level of L-Glutamate in <18 Points MMT Score Group from 16,68±5,13 before IAHF treatment become 19,67±5,72 after IAHF treatment. While the L-Glutamate level in 18-23 Points MMT Score Group increasing from 24,70±4,72 before IAHF Treatment become 27,95±6,93 after IAHF...
Treatment. The level of L-Glutamate also increased in 24-29 Points MMT Score Group from 18,17±6,93 before IAHF Treatment become 20,53±7,89 after IAHF Treatment. The increasing of L-Glutamate serum level after IAHF procedure was not statistically significant.

We also measured the muscle strength in acute ischemic stroke patient using Manual Muscle Testing-Medical Research Councils (MMT-MRC) scoring method as shown below in Table 2.

**DISCUSSION**

According to WHO it has been estimated that 17 million people die of Cerebrovascular Disease, particularly heart attacks and strokes in every year. This number will increase due to the modern people lifestyles. This increased rate of stroke incident become the basis of this study where the time window therapy for stroke itself is still narrow when the only approved stroke therapy is thrombolysis induced by intravenous administration of recombinant tissue plasminogen activator.23

Heparin is widely used as a flushing solution for catheterization.24 But it seems the heparin not only has a role as an anticoagulant but also as a fibrinolytic. And also has potential in increasing thrombolysis by inhibit TAFI (Thrombin Activatable Fibrinolysis Inhibitor) formation.25,26

Based on this information Putranto et al.22 choose to use the heparin as a solution for stroke incident treatment by using the heparin to dissolve any occlusion in the blood vessel. This technique is called Intra Arterial Heparin Flushing (IAHF) which has been gone through an empirically successful rate of good outcome in stroke patient that has been admitted in Indonesia Army Central Hospital Gatot Soebroto Jakarta.

In this study we want to see if there is any effect of this IAHF method in glutamate levels in the blood, for the glutamate is one of many indicators that there is some neurological process occurred when the cerebrovascular disease incident happened.

This study shows that the glutamate level in all groups is increased after Intra Arterial Heparin Flushing treatment, even though this increased level of glutamate is not very significant. This increased level of glutamate after ischemic stroke incident is also found by Aliprandi et al27 shows there is a prolonged increase of glutamate in plasma after stroke. This excess release of glutamate contributes to the mechanism of tissue destruction in focal cerebral ischemia. This overstimulation of glutamate receptors induces the increased intracellular Ca2+ concentrations, release of K+ into extracellular space, and cell swelling due to passive movement of water and Na+ influx.27

The mechanism to prevent glutamate from reaching excitotoxicity level in the blood is the sodium dependent glutamate transporters on nerve terminals and astrocytes facilitate the uptake of glutamate into brain cells, ensuring the excess concentrations of glutamate in brain ECFs are reduced to safe concentrations which it’s no longer active or toxic. Another glutamate removal mechanism is by glutamate transporters present on the ant luminal side of the brain capillary endothelial cells, this way the glutamate is eliminated from the brain into the blood.28

While in another study there is a possibility that the CSF glutamate levels can be decreased naturally later after acute stroke (from 2 to 10 days even 6 to 24 hours after stroke).29 This decreased amount of glutamate in blood correlate with improved neurological outcome.30

Another therapeutic strategies to decrease glutamate levels in blood is by using a neuroprotective agent Sipatrigine (BW619C89).30

Glutamate not only has role as a primary excitatory neurotransmitter but also a critical transmitter for signaling the neurons to degenerate after a stroke event. When there is a brief episode of
ischemia the extracellular glutamate concentration rises abruptly. Besides working as neuron to neuron communication glutamate also has roles in neuronal growth and axon guidance, brain development and maturation and synaptic plasticity in health and disease. Excitotoxicity is an overdrive of neuronal excitation mediated by sodium ions and any source of excitation which is potentially harmful. The glutamate receptors that responsible for ischemic neuronal damage is NMDAR which the most calcium-permeable ionotropic glutamate receptors. A few drugs in clinical trial were developed to inhibit glutamate release in stroke patients which are: Lifarizine, Ziconotide Magnesium Sulfate. It has been known also that some of the Glutamate source also gained from dietary protein digested by pancreas and gut. MSG intake has been proven to increase glutamate levels in blood. Therefore a dietary strategy might be also used to control the glutamate levels in blood.

CONCLUSION
The purpose of this study is to show that the Intra Arterial Heparin Flushing has been proven to have quite significant effect on L-Glutamate Quantification in Acute Ischemic Stroke Patient, where in this study the L-Glutamate level were increased but not quite significant in all grouping. The IAHF treatment also has been proven to have quite significant effect on muscle strength in Acute Stroke Patient which in this study the muscle strength measured with Manual Muscle Test Medical Research Councils (MMT-MRC) Score, where the MMT-MRC Score in this study shows an increased point after IAHF Treatment. There is a possibility that this results still can be developed if there are more subjects included in the study, which the author hoped that there is a future study on the same variables.

Conflict of Interest Statement
The Authors declare that there is no conflict of interest.

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