Effects of intravenous infusion of hydrogen-rich fluid combined with intra-cisternal infusion of magnesium sulfate in severe aneurysmal subarachnoid hemorrhage: study protocol for a randomized controlled trial

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Abstract

Background: The failures of recent studies intended to prevent cerebral vasospasm have moved the focus of research into delayed cerebral ischemia away from cerebral artery constriction towards other mechanisms. Recent accumulating evidence has suggested that early brain injury is also involved in the development of delayed cerebral ischemia, and that hydrogen can prevent early brain injury. Therefore, we have established a combination therapy of intravenous hydrogen infusion and intra-cisternal magnesium sulfate infusion for the treatment of both early brain injury and cerebral vasospasm. The present randomized controlled clinical trial is designed to investigate the effects of this novel therapeutic strategy on the occurrence of cerebral vasospasm, delayed cerebral ischemia, and clinical outcomes after high-grade subarachnoid hemorrhage.

Methods: This study is a randomized, double-blind, placebo-controlled design to be conducted in two hospitals. A total of 450 patients with high-grade subarachnoid hemorrhage will be randomized to one of three arms: (i) Mg + H2 group, (ii) Mg group, and (iii) control group. Patients who are assigned to the Mg + H2 group will receive intra-cisternal magnesium sulfate infusion (2.5 mmol/L) at 20 mL/h for 14 days and intravenous hydrogen-rich fluid infusion (200 mL) twice a day for 14 days. Patients who are assigned to the Mg group will receive intra-cisternal magnesium sulfate infusion (2.5 mmol/L) at 20 mL/h for 14 days and intravenous normal glucose-electrolyte solution (200 mL) without added hydrogen twice a day for 14 days. Patients who are assigned to the control group will receive intra-cisternal Ringer solution without magnesium sulfate at 20 mL/h for 14 days and intravenous normal glucose-electrolyte solution (200 mL) without added hydrogen twice a day for 14 days. Primary outcome measures will be occurrence of delayed cerebral ischemia and cerebral vasospasm. Secondary outcome measures will be modified Rankin scale score at 3, 6, and 12 months and biochemical markers.

Discussion: The present protocol for a randomized, placebo-controlled study of intravenous hydrogen therapy with intra-cisternal magnesium infusion is expected to establish the efficacy and safety of this therapeutic strategy.

Trial registration: UMIN-CTR: UMIN000014696

Keywords: Subarachnoid hemorrhage, Early brain injury, Vasospasm, Delayed cerebral ischemia, Hydrogen-rich fluid, Oxidative stress, Magnesium, Cerebrospinal fluid

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Background

Aneurysmal subarachnoid hemorrhage (SAH) accounts for 5% of all strokes [1]. The mortality rate is approximately 50%, and 20% to 30% of surviving patients have significant neurologic deficits [2,3]. In particular, high-grade SAH, which is classified as Hunt and Kosnik grades 4 and 5, accounts for approximately 20-40% of patients with SAH, and its prognosis is extraordinarily poor [4]. Delayed cerebral ischemia (DCI), a clinical diagnosis previously proposed by Vergouwen et al., is considered to be the most important cause of mortality and morbidity [5]. Angiographic cerebral vasospasm develops in approximately 70% of patients between 4 and 14 days after SAH, and the primary mechanism underlying DCI was widely believed to be cerebral vasospasm [1,6].

Numerous experimental and clinical studies have been conducted to prevent and/or treat cerebral vasospasm, and various prophylactic strategies against cerebral vasospasm have been advocated [7-12]. For example, magnesium sulfate has been studied as one of the most attractive therapeutic agents for decades [10-18]. Magnesium sulfate exhibits several beneficial effects such as vasodilation of vessels and attenuation of neuronal death. The mechanisms of these effects include blockage of the voltage-dependent calcium channels, inhibition of excitatory glutamate release, and interference with N-methyl-D-aspartate-glutamate receptors [19]. Several randomized controlled trials (RCTs) have been conducted to investigate the effects of intravenous magnesium sulfate administration on outcome after SAH [12-18]. However, these RCTs and meta-analyses showed that magnesium sulfate did not decrease DCI or improve the poor functional outcome after SAH [10-18].

Two main reasons for these negative results have been identified. Firstly, intravenous magnesium sulfate infusion results in only limited increases in cerebrospinal fluid (CSF) magnesium levels, whereas serious adverse events (such as bradycardia and hypotension) can occur at serum magnesium levels >2 mmol/L [20]. Therefore, these contradictory effects may be difficult to overcome for clinical use of intravenous magnesium infusion. Secondly, recent accumulating findings have suggested that early brain injury (EBI) is involved mainly in the development of DCI and causes the high mortality and morbidity observed after SAH [21,22]. EBI is the product of pathological mechanisms triggered in the brain during the first 72 hours after SAH. However, the time from the onset to initiation of intravenous magnesium administration was approximately 30–40 hours in most RCTs, whereas more time is required to achieve significant CSF magnesium levels after infusion [12,18,23]. This time latency can also be considered as one of the reasons for the negative results in RCTs.

Previously, we studied the safety and efficacy of intra-cisternal magnesium sulfate infusion and found that significant increases in CSF magnesium levels can be achieved without changes in serum levels [23-25]. However, the time latency from infusion to achievement of significant CSF magnesium level was also present with intra-cisternal infusion, and remains unresolved [23]. Theoretically, magnesium can also exert effects on EBI [21,22], but we consider that effective CSF magnesium levels are difficult to achieve during the period of EBI development, regardless of administration route. Therefore, other treatment strategies against EBI are more promising.

Increasing evidence has suggested that enhanced oxidative stress is involved in EBI as well as cerebral vasospasm following SAH [26-29]. Hydrogen can selectively reduce hydroxyl radicals and peroxynitrites, which are very strong reactive oxygen species that react indiscriminately with nucleic acids, lipids, and proteins, resulting in DNA fragmentation, lipid peroxidation, and protein inactivation [30-38]. Previous studies have shown that hydrogen has antioxidant, anti-apoptotic, anti-inflammatory, and cytoprotective properties that are beneficial to the cell [30]. Hydrogen is highly diffusible and could potentially reach subcellular compartments, such as the mitochondria and nuclei, which are the primary sites of reactive oxygen species generation and DNA damage [30,38]. In addition, hydrogen has no side effects, and we previously showed that hydrogen can be safely administered intravenously in patients with ischemic stroke [33]. Furthermore, recent experimental studies showed that hydrogen can alleviate EBI after SAH via attenuation of neuronal apoptosis [39-42].

These findings suggest that combination therapy using intravenous hydrogen infusion and intra-cisternal magnesium sulfate infusion may be effective to treat both EBI and delayed cerebral vasospasm. The present randomized controlled clinical trial is intended to investigate the effects of intravenous hydrogen therapy with intra-cisternal magnesium sulfate infusion on the occurrences of cerebral vasospasm and DCI, and clinical outcomes in patients with high-grade SAH.

Methods

Overview

This study is a randomized, double-blind, placebo-controlled trial to be conducted in two hospitals (National Defense Medical College Hospital and Mishuku Hospital) and includes three arms: (i) intravenous hydrogen-rich fluid infusion with intra-cisternal magnesium sulfate infusion (Mg + H2 group), (ii) intra-cisternal magnesium sulfate infusion only (Mg group), and (iii) placebo (control group). The protocol was approved by the ethics committee of the National Defense Medical College in May 2013 (#1126) and was registered on UMIN-CTR (UMIN000014696).
The study follows the Declaration of Helsinki and good clinical practice guidelines. Written informed consent will be obtained from each patient or family members before inclusion in the study. The procedures performed during the study are outlined in Figure 1.

Participants
All patients presenting with a diagnosis of SAH will be checked for eligibility by the treating physician. Inclusion criteria are: 20 to 80 years of age, aneurysm rupture, Hunt and Kosnik grade 4 or 5, aneurysm treated by surgical clipping within 72 hours after the onset, and written informed consent from the patient or family member. Exclusion criteria are: severe brain edema, heart dysfunction (New York Heart Association Class III or IV), renal insufficiency (calculated creatinine clearance rate of less than 30 mL/min), Fisher grade 4 with massive intracerebral hematoma, and rejection of randomization.

Randomization
Patients who fulfill the eligibility criteria will be assigned to either the control group, Mg group, or Mg + H2 group by stratified block randomization, which will be carried out using a computer system by a statistician not related to the project team to protect the double-blind design and integrity of the study. Randomization will be stratified by sex, age (20–60 years and older than 60 years), Hunt and Kosnik grade, and Fisher group. The allocation ratio will be 1:1:1. Patients, treating physicians, and investigators assessing outcomes and analyzing data will be unaware of the allocation.

Interventions
All participating patients will be treated according to state-of-the art SAH management, comparable with recent published international guidelines [43]. During aneurysm clipping, a ventricular drainage tube will be placed in the lateral ventricle, and a cisternal drainage tube placed in the basal cistern. A spinal drainage tube will be placed in the lumbar spine immediately after the clipping procedure. Fasudil hydrochloride (90 mg/day, 14 days) will be administered but not triple-H therapy. If severe cerebral vasospasm is detected by cerebral angiography at 7–10 days after surgery, fasudil hydrochloride (15 to 60 mg) will be administered via the proximal internal carotid artery [44,45].

Patients who are assigned to the Mg + H2 group or the Mg group will receive intra-cisternal magnesium sulfate infusion [23]. Figure 2 illustrates the irrigation system. Continuous infusion of 2.5 mmol/L magnesium sulfate in Ringer solution will be administered at 20 mL/h for 14 days. Irrigation will be performed through the cisternal to spinal drainage. The cisternal drainage tube and the pressure control system at 20 cm H2O will be connected by a T-connector for safe irrigation. The ventricular drainage and spinal drainage will be set at a pressure of 10 cm H2O and 5 cm H2O, respectively, which can be adjusted by the drainage volume. The respective drainage volumes will be checked hourly to avoid overdrainage or elevated intracranial pressure. CSF and serum magnesium ion concentrations will be measured daily until 14 days after surgery, but the principal investigators of the trial will remain unaware of the results. Patients assigned to the control

Figure 1 Flow diagram.
group will receive continuous infusion of only Ringer solution at 20 mL/h for 14 days.

Patients who are assigned to the Mg + H2 group will receive intravenous hydrogen-rich fluid infusion. Hydrogen-rich fluid is produced using a non-destructive hydrogen diffusion apparatus (Miz Co., Fujisawa, Japan; Patent No. 4486157, Patent Gazette of Japan 2010) as reported before [23,33]. Bags of glucose-electrolyte solution (Soldem 1, 200 mL/bag, Terumo, Tokyo, Japan) are immersed, without opening or altering the bag, in a water tank in which water is electrolyzed periodically to produce water with hydrogen concentrations of up to 1.6 ppm. The concentration of hydrogen in the bag reaches saturation, increasing to more than 1.0 ppm, because of diffusion through the wall of the bag. Additional information describing this process can be found at: http://www.e-miz.co.jp/english/technology.html#non_destructivewebsite. Intravenous hydrogen-rich fluid (200 mL) will be administered at 200 mL/h twice a day (every 12 h). Patients assigned to the control group and the Mg group will receive glucose-electrolyte solution (Soldem 1, 200 mL) without added hydrogen at 200 mL/h twice a day (every 12 h).

If severe brain edema, heart failure, or renal failure occurs, the study intervention will be stopped immediately.

CT, CT angiography, and CT perfusion
Head computed tomography (CT) will be performed on admission, and at 3, 7, 10, and 14 days after surgery. Head CT angiography will be performed on admission and at 7 days after surgery. Head CT perfusion will be performed at 7 days after surgery. All images will be reviewed by an experienced neuroradiologist to identify the occurrence of DCI and cerebral vasospasm.

Digital subtraction angiography
Cerebral digital subtraction angiography will be performed on admission and at 7–10 days after surgery. Digital subtraction angiography will be reviewed by an experienced neuroradiologist to identify the occurrence of cerebral vasospasm.

Transcranial Doppler
Transcranial Doppler imaging will be performed daily until 14 days after surgery. The mean velocity of the proximal middle cerebral artery (M1) will be recorded in both hemispheres.

Biochemical markers
Venous blood and CSF samples will be taken for assays of several biochemical markers at 1, 3, 7, and 14 days after surgery. Malondialdehyde will be assessed as an indicator of oxidative stress [33,46]. Neuron-specific enolase and S-100 calcium binding protein B will be assessed as markers of neuronal and glial injuries [21,47]. C-reactive protein will be assessed as an inflammatory marker [21,48].

Outcome measures
Primary outcome measures will be as follows.
– Occurrence of DCI: new focal neurological deficits (motor or speech deficits) that developed after SAH, decrease in Glasgow Coma Scale of ≥2 points for >6 hours, or new cerebral infarction not related to surgery, rebleeding, progressive hydrocephalus, electrolyte or metabolic disturbance, or infection [5,15].

– Occurrence of cerebral vasospasm: angiographic vasospasm which is defined as moderate-to-severe arterial narrowing on CT angiography and/or digital subtraction angiography not attributable to atherosclerosis, catheter-induced spasm, or vessel hypoplasia. Transcranial Doppler vasospasm which is defined as a mean flow velocity in the M1 of >120 cm/s [49].

Secondary outcome measures will be as follows.

– Modified Rankin scale score at 3, 6, and 12 months.
– Biochemical markers (malondialdehyde, neuron-specific enolase, S-100 calcium binding protein B, and C-reactive protein).

**Sample size**

Assuming that the incidence of DCI is 35% in the control group, 15% in the Mg group, and 5% in the Mg + H2 group, a total of 413 patients will be required (80% power and 2-sided α = 0.05). However, assuming approximately 10% loss to follow up, 450 patients will need to be recruited. Therefore, 150 patients must be randomized to each intervention arm.

**Statistical analysis**

The study design accords to the ‘intention to treat’ principle. A test for overall comparison (e.g. analysis of variance, or if the conditions for analysis of variance are not met, a non-parametric equivalence such as the Kruskal-Wallis test) will be employed for each outcome across all three interventions, and if found to be significant, pair-wise comparisons will be made. We appreciate that many pair-wise comparisons suffer from Type I (false-positive) error, so we will adjust for multiplicity of comparisons by using steps such as Bonferroni and Tukey’s procedure. The statistical procedures for pair-wise comparisons will depend on the nature of the data: for example, for dichotomous outcomes, we will use Fisher’s exact test or chi-square test as appropriate, and for continuous outcomes we will use the t-test if the observations in each arm are normally distributed; or the Mann–Whitney U-test if non-normally distributed. A value of p < 0.05 will be considered significant.

**Discussion**

The present study is intended to investigate therapeutic strategy against both EBI and cerebral vasospasm to prevent the development of DCI using combination therapy with intravenous hydrogen infusion and intracisternal magnesium sulfate infusion. Previously we used intracisternal infusion of 15 mmol/L magnesium sulfate, but 20% of patients experienced respiratory suppression [23]. Therefore, we selected 2.5 mmol/L for the concentration of magnesium sulfate solution in the present study, because our recent experimental study showed that intra-cisternal infusion of 2.5 mmol/L (5 mEq/L) magnesium sulfate could prevent cerebral vasospasm after SAH [50]. We expect the intra-cisternal infusion of 2.5 mmol/L magnesium sulfate to maintain the beneficial effects and minimize side effects. In addition, the dose regimen of hydrogen-rich fluid in the present trial was based on our previous study, in which 38 patients with acute ischemic stroke received intravenous infusion of hydrogen-rich fluid, and adverse events included diarrhea in one patient (2.6%) and heart failure in one (2.6%) [33]. Heart failure was considered to be due to volume overload, so that heart dysfunction and renal insufficiency will be excluded in the present study.

The present protocol for a randomized, placebo-controlled study of intravenous hydrogen therapy with intracisternal magnesium infusion is expected to establish the efficacy and safety of this therapeutic strategy.

**Trial status**

The study is currently ongoing.

**Abbreviations**

CSF: Cerebrospinal fluid; CT: Computed tomography; DCI: Delayed cerebral ischemia; EBI: Early brain injury; RCT: Randomized controlled trial; SAH: Subarachnoid hemorrhage.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

ST and KM contributed to the conception and design of the trial, and data acquisition, and drafted the manuscript. HA, KF, KN, ST, NO, HO and KW made contributions to the conception and design of the trial and critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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