Neuroanesthesia and Intensive Care

Five percent, 7.5% or 10% hypertonic saline prevents delayed neuronal death in gerbils

[Une solution saline à 5 %, 7,5 % ou 10 % empêche la mort neuronale différée chez des gerbilles]

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Purpose: To clarify the appropriate concentration and dose of hypertonic saline solution (HSS) for preventing delayed neuronal death in the hippocampal CA1 subfield after transient forebrain ischemia in gerbils.

Methods: Thirty gerbils were randomly assigned to five groups: physiological saline solution (PSS) group, ischemia/reperfusion treated with PSS 2 $mL \cdot kg^{-1}$; 5% HSS group, treated with 5% HSS 2 $mL \cdot kg^{-1}$; 7.5% HSS group, treated with 7.5% HSS 2 $mL \cdot kg^{-1}$; 10% HSS group, treated with 10% HSS 2 $mL \cdot kg^{-1}$; 20% HSS group, treated with 20% HSS 2 $mL \cdot kg^{-1}$. Transient forebrain ischemia was induced by occluding the bilateral common carotid arteries for four minutes. Five days later, histopathological changes in the hippocampal area were examined, and the degenerative ratio of the pyramidal cells were measured according to the following formula: (number of degenerative pyramidal cells/total number of pyramidal cells per 1 mm of hippocampal CA1 subfield) $\times$ 100.

Results: In PSS and 20% groups, neuronal cell damage was observed five days after ischemia. In the other three groups, these changes were not observed. The degenerative ratios of pyramidal cells were as follows; PSS group: 91.6 ± 5.6%, 5% HSS group: 7.2 ± 1.6%, 7.5% group: 8.3 ± 1.4%, 10% HSS group: 6.2 ± 1.1%, 20% HSS group: 85.8 ± 8.7% ($P < 0.05$; PSS and 20% HSS *vs* three other groups).

Conclusion: This study demonstrates that 5, 7.5 or 10% HSS 2 $mL \cdot kg^{-1}$ may prevent delayed neuronal death in the hippocampal CA1 subfield after cerebral ischemia/reperfusion in gerbils.

Objectif : *Déterminer la concentration et la dose appropriées de solution saline hypertonique (SSH) nécessaire pour empêcher la mort neuronale différée dans le sous-champ hippocampique CA1 à la suite d'une ischémie transitoire du cerveau antérieur chez des gerbilles.*

Méthode : *Trente gerbilles ont été réparties au hasard en cinq groupes : SPS recevant une solution physiologique salée (SPS), l'ischémie/reperfusion a été traitée avec 2 $mL \cdot kg^{-1}$ de SPS ; SSH à 5 %, traité avec 2 $mL \cdot kg^{-1}$ de SSH à 5 % ; SSH à 7,5 % traité avec 2 $mL \cdot kg^{-1}$ de SSH à 7,5 % ; SSH à 10 % traité avec 2 $mL \cdot kg^{-1}$ de SSH à 10 5 % et enfin, le groupe SSH à 20 % recevant 2 $mL \cdot kg^{-1}$ de SSH à 20 %. L'ischémie transitoire du cerveau antérieur a été induite par l'occlusion des artères carotides communes bilatérales pendant quatre minutes. Cinq jours plus tard, les modifications histopathologiques de l'aire hippocampique ont été examinées et le taux de cellules pyramidales dégénératives a été mesuré selon la formule suivante : (le nombre de cellules pyramidales dégénératives/le nombre total de cellules pyramidales par mm de sous-champ hippocampique CA1) $\times$ 100.*

Résultats : *Des altérations des cellules neuronales ont été observées cinq jours après l'ischémie dans les groupes SPS et SSH à 20 % mais non dans les trois autres groupes. Les ratios de cellules pyramidales dégénératives ont été dans le groupe SPS : 91,6 ± 5,6 % ; dans le groupe SSH à 5 % : 7,2 ± 1,6 % ; dans le groupe SSH à 7,5 % : 8,3 ± 1,4 % ; dans le groupe SSH à 10 % : 6,2 ± 1,1 % et dans le groupe SSH à 20 % : 85,8 ± 8,7 % ($P < 0,05$; les groupes SPS et SSH à 20 % vs les trois autres groupes).*

Conclusion : *Cette étude démontre que 2 $mL \cdot kg^{-1}$ de SSH à 5, 7,5 ou 10 % peuvent empêcher la mort neuronale différée dans le sous-champ hippocampique CA1 à la suite d'une ischémie/reperfusion chez des gerbilles.*

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TRANSIENT forebrain ischemia induces delayed neuronal death in the hippocampal CA1 subfield of the cerebral limbic system several days after a short period of ischemia/reperfusion in gerbils.[1] A number of studies have investigated methods of cerebral protection to decrease hippocampal delayed neuronal death after transient forebrain ischemia.[2–6] Our previous study demonstrated that *iv* injection of 10% hypertonic saline solution (HSS) 2 $mL \cdot kg^{-1}$ prevented hippocampal delayed neuronal death in gerbils.[7] HSS has an osmotic effect on the brain because of its high tonicity and ability to effectively remain outside the blood-brain barrier.[8] HSS has recently been used for the treatment of secondary ischemic injury after cerebrospinal trauma and brain injury after hemorrhagic shock.[9–11] HSS reduces increased intracranial pressure, increased cerebral perfusion pressure and brain edema due to improvement of peripheral circulation. Since microvascular disturbance is responsible for delayed neuronal death after ischemia/reperfusion,[12] HSS may alleviate neuronal damage. However, the appropriate concentration and dose of HSS for prevention of delayed neuronal death remains unclear. An excessive dose of sodium ions may be cytotoxic for the central nervous system.

The purpose of this study was to clarify the appropriate concentration and dose of HSS to protect against delayed neuronal death after a short period of ischemia/reperfusion in gerbils.

Methods

This study was conducted according to the animal experimentation guidelines of Dokkyo University School of Medicine, which adheres to the National Institutes of Health Animal Experimental Guidelines.

Thirty Mongolian gerbils weighing between 65–90 g were anesthetized with 50% nitrous oxide in oxygen with halothane (end-tidal concentration: 1.0–1.5%) through a rubber mask. The animals were placed in the supine position and the common carotid arteries were exposed bilaterally. The arteries were occluded with miniature aneurysmal clips for four minutes. During this period we verified the absence of blood supply from the vertebral arteries with a microscope. Four minutes after occluding both carotid arteries, the clips were released and we visually confirmed spontaneous circulatory reperfusion with the microscope. The temperature of the tympanic membrane was monitored (Mon-a-therm Model 6510; Mallinckrodt, CA, USA) and maintained with a heating blanket at 37 ± 0.2°C during the experiment. Then, 2 $mL \cdot kg^{-1}$ of physiological saline solution (PSS; 154 $mEq \cdot L^{-1}$), 5% HSS (855 $mEq \cdot L^{-1}$), 7.5% HSS (1283 $mEq \cdot L^{-1}$), 10% HSS (1711 $mEq \cdot L^{-1}$) or 20% HSS (3422 $mEq \cdot L^{-1}$) were injected via the tail vein. Six gerbils were used in each of the five groups. The animals were kept and fed in a cage at a room temperature of 26°C.

Five days later, laparotomy and thoracotomy were performed on all gerbils under halothane anesthesia. Fixation was achieved by irrigation with a fixative solution (1:2:7 ratio of 4% formaldehyde: phosphate buffer solution: distilled water) via the heart. The bodies were stored in a refrigerator at 4°C for two hours. A craniotomy was performed and the cerebrum was extracted and embedded in paraffin which was sliced into 5 μm sections in the coronal direction and stained with hematoxylin and eosin. Histopathological changes in the hippocampal CA1 subfield were examined using a light microscope (Olympus BH-2, Olympus, Tokyo, Japan). Morphological changes were detected in the pyramidal cells and structural changes in the cell layers of the CA1 subfield of the hippocampus. In order to compare the ratios of degenerative pyramidal cells between the five groups, the total number and the number of degenerative pyramidal cells were counted over a uniform 1-mm length of the central area of the hippocampal CA1 subfield. The degenerative ratios were determined according to the following formula: Degenerative ratio (%) = (number of degenerative pyramidal cells/total number of pyramidal cells per 1-mm of hippocampal CA1 subfield) × 100.

Signs of degenerative processes such as atrophy of the pyramidal cells, deep staining of their cell bodies, vacuolation, and disappearance of the radial striated zone were taken to indicate cell degeneration, and cells in which these signs were absent were regarded as normal neurons.[1] Evaluation of the cell damage was performed by a single pathologist blinded to the experimental conditions.

Data are presented as mean ± SD. Kruskall-Wallis one-way analysis of variance was used for the statistical comparisons between each group. Statistical significance was considered to be $P < 0.05$.

Results

Histopathological findings

In the PSS group (Figure a), almost all the pyramidal cells were atrophic, and they exhibited nuclear pycnosis, deep staining of the cell cytoplasm, vacuolation, and structural disruption of the radial striated zone due to degenerative necrosis. In the 5, 7.5 and 10% groups, the normal hippocampal pyramidal cells were well maintained, and no structural disruption of the radial striated zone was detected (Figure b–d). In the 20% HSS group, however, the pyramidal cells of hip-

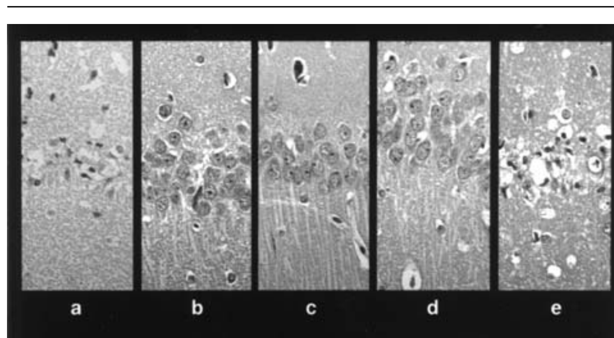

FIGURE a–e, The histological appearance of hippocampal CA1 subfield from each group. a, Physiological saline- treated group. Pyramidal cells are degenerated and the radial striated zone is disrupted. Pyramidal cell changes are present: cell shrinkage, nuclear pycnosis, dark cytoplasmic colouration and vacuolation (400 ×). b–d, 5%, 7.5% and 10% hypertonic saline-treated group. The pyramidal cell and radial striated zone stained with hematoxylin and eosin are normal (200 ×); e, 20% hypertonic saline-treated group. Pyramidal cells are degenerated and the radial striated zone is disrupted. Pyramidal cell changes are present: cell shrinkage and dark cytoplasmic colouration (200 ×).

pocampal CA1 subfield showed degenerative cell damage and structural disruption of the radial striated zone similar to the PSS group (Figure e).

Degenerative ratios

The degenerative ratios of the pyramidal cells in the PSS, 5% HSS, 7.5% HSS, 10% HSS and 20% HSS groups were 91.6 ± 5.6%, 7.2 ± 1.6%, 8.3 ± 1.4%, 6.2 ± 1.1% and 85.8 ± 8.7%, respectively. The degenerative ratios in the PSS and 20% HSS groups were significantly higher than those in the other three groups.

Discussion

The hippocampal CA1 subfield is the centre of memory and the pyramidal cells of the hippocampal CA1 are known for their selective vulnerability, similar to the Purkinje's cells and the third and fifth layers of the neocortex. We examined the histopathological changes in the hippocampal CA1 subfield of the Mongolian gerbil which has been used as a model for cerebral ischemia and infarction. Since the gerbil lacks an interconnection between the carotid and vertebrobasilar circulation, cerebral ischemia is easily produced by occlusion of the common carotid arteries. The neuronal damage in the hippocampal CA1 subfield of the gerbil is similar to that observed in humans.[13,14] The pyramidal cells of the hippocampal CA1 subfield are gradually damaged to the point of delayed neuronal death after a short period of ischemia/reperfusion in gerbils,[1] and they become degenerative on the third and fourth day. Therefore, we examined the protective effect of HSS on neuronal damage on the fifth day after cerebral ischemia/reperfusion. In this study, 5, 7.5 and 10% HSS 2 $mL \cdot kg^{-1}$ prevented delayed neuronal death after a short period of ischemia/reperfusion.

Following transient ischemia of the brain, extracellular sodium ions enter the neuronal cells and accumulate there, as depletion of intracellular adenosine triphosphate (ATP) inactivates Na-K ATPase and increases the pH-regulatory Na-H exchange. This intracellular accumulation of sodium ions leads to the extracellular increase of glutamate as an excitatory neurotransmitter and accumulation of intracellular calcium ions, which are associated with delayed neuronal death. Intravenous injection of HSS may compensate for decreased extracellular sodium ions after ischemia/reperfusion and correct the imbalance between extracellular and intracellular concentrations of sodium ions caused by hypoxia. Ho *et al.*[15] reported that hypertonic perfusion minimized intracellular sodium uptake and reduced accumulation of intracellular calcium in the hypoxic rabbit myocardium. Similarly, we speculate that less than 20% HSS 2 $mL \cdot kg^{-1}$ may minimize sodium uptake in the neuronal cells, prevent extracellular increase of glutamate and reduce the accumulation of intracellular calcium in the hypoxic hippocampus.

Excessive administration of sodium ions causes acute swelling of neuronal cells and neuronal death.[16] Following 90 min of hypernatremia, the sodium, potassium, and chloride ions enter the neuronal net tissues, especially the dendrite and soma. This alteration of electrolytes and osmotic pressure produces swelling and necrosis of neuronal cells. Lien *et al.*[17] reported that severe chronic hypernatremia (serum sodium 180 $mEq \cdot L^{-1}$, seven days) induced an intracellular increase of idiogenic osmoles, which included myoinocitol, betaine, taurine, glutamine and glutamate, to reduce the transmembrane osmolarity gradient in the brain. However, the overload of sodium ions due to acute hypernatremia may not induce an intracellular increase of idiogenic osmoles. In the present study, we could not find histopathological differences between PSS and 20% HSS 2 $mL \cdot kg^{-1}$ using a light microscope although transient ischemia and excessive administration of HSS might increase intracellular sodium ions, followed by neuronal damage. Therefore, excessive elevation of extracellular sodium after *iv* injection of higher doses of HSS may induce neuronal damage. Additional studies are needed to examine the differences between PSS and 20% HSS 2 $mL \cdot kg^{-1}$ using an electron microscope.

In addition to the prevention of neuronal damage after a short period of cerebral ischemia/reperfusion, small doses of HSS are effective for cardiopulmonary resuscitation from hemorrhagic shock in animals.[18–20] Mean arterial pressure, cardiac output, mean pulmonary arterial pressure, central venous pressure and heart rate are increased from minutes to hours after *iv* injection of HSS, and total peripheral vascular resistance and pulmonary vascular resistance decrease because of improvement of microcirculation.[21]

Petito *et al.*[13] reported a clinical case of hippocampal delayed neuronal death after cardiopulmonary arrest. The *iv* administration of an appropriate dose of HSS may not only improve hemodynamic changes but, possibly, also prevent neuronal damage such as postresuscitation encephalopathy in shock patients.

In conclusion, we have shown that administration of 2 $mL \cdot kg^{-1}$ of 5%, 7.5% and 10% HSS prevents delayed neuronal death in the hippocampal CA1 subfield after a short period of cerebral ischemia/reperfusion in gerbils.

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