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Propofol prevents delayed neuronal death following transient fore-brain ischemia in gerbils

Purpose: This study was conducted to ascertain whether propofol may protect against delayed neuronal death in the hippocampal CA1 subfield in gerbils.

Methods: Thirty-five gerbils were randomly assigned to five groups: Group I, the control group, a sham operation treated with physiological saline solution (PSS); Group II, ischemia/reperfusion treated with PSS; Group III, ischemia/reperfusion treated with 50 mg·kg⁻¹ propofol; Group IV, ischemia/reperfusion treated with 100 mg·kg⁻¹ propofol; Group V, ischemia/reperfusion treated with 150 mg·kg⁻¹ propofol. Transient forebrain ischemia was induced by occluding the bilateral common carotid arteries for four minutes under N₂O/O₂/halothane anesthesia after administration of propofol or PSS. Five days later, histopathological changes in the hippocampal CA1 subfield were examined using a light microscope and 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) × 100.

Results: In group II, the pyramidal cells were atrophic and pycnotic; vacuolation and structural disruption of the radial striated zone was observed. In the other four groups, these changes were not observed. The degenerative ratios of pyramidal cells were as follows; group I: 5.9 ± 1.9%, group II: 94.6 ± 2.5% (*P* < 0.01), group III: 10.7 ± 1.7%, group IV: 9.7 ± 1.8%, group V: 9.2 ± 1.9%.

Conclusion: This study suggests that propofol may prevent delayed neuronal death in the hippocampal CA1 subfield after cerebral ischemia/reperfusion in gerbils.

Objectif : La présente étude a été menée pour vérifier si le propofol peut protéger contre la mort neuronale retardée du sous-champ CA1 hippocampique chez des gerbilles.

Méthode : Trente-cinq gerbilles ont été réparties au hasard en cinq groupes : le groupe I, témoin, subit une opération fictive traitée avec une solution physiologique salée (SPS); les autres gerbilles subissent une ischémie/reperfusion traitée dans le groupe II avec une SPS; dans le groupe III, avec 50 mg·kg⁻¹ de propofol; dans le groupe IV, avec 100 mg·kg⁻¹ de propofol et dans le groupe V, avec 150 mg·kg⁻¹ de propofol. L'ischémie transitoire du cerveau antérieur a été induite par l'occlusion bilatérale des artères carotides communes pendant quatre minutes sous anesthésie au N₂O/O₂/halothane après l'administration de propofol ou de SPS. Cinq jours plus tard, on a vérifié les changements histopathologiques survenus dans le sous-champ CA1 hippocampique à l'aide d'un microscope optique et le taux de dégénérescence des cellules pyramidales a été mesuré comme suit : (nombre de cellules pyramidales dégénératives/nombre total de cellules pyramidales par mm de sous-champ CA1 hippocampique) × 100.

Résultats : Dans le groupe II, on a noté des cellules pyramidales atrophiques et pycnotiques; on a aussi observé la vacuolisation et la rupture structurale de la zone radiale striée. Dans les quatre autres groupes, ces changements n'ont pas été notés. Les taux de cellules pyramidales dégénératives étaient pour le groupe I : 5,9 ± 1,9 %, le groupe II : 94,6 ± 2,5 % (*P* < 0,01), le groupe III : 10,7 ± 1,7 %, le groupe IV : 9,7 ± 1,8 %, le groupe V : 9,2 ± 1,9 %.

Conclusion : Cette étude suggère que le propofol peut prévenir la mort neuronale tardive dans le sous-champ CA1 hippocampique à la suite d'une ischémie/reperfusion chez les gerbilles.

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Accepted for publication March 13, 1999

PROPOFOL, 2,6-diisopropylphenol, has cerebral vascular and metabolic effects similar to barbiturates.¹ It is used for maintenance of neurosurgical anesthesia because it reduces the cerebral metabolic rate,²⁻⁴ cerebral blood flow,^{5,6} and intracranial pressure.^{1,7} However, consensus has not been reached as to a protective effect of propofol on cerebral ischemia. Both positive⁸ and negative⁹⁻¹² results have been reported with different experimental materials and different methods of ischemia. Most negative results were obtained from extended cerebral ischemia. In this study, we examined the cerebral protective effect of propofol on 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 adhere to the National Institute of Health Animal Experimental Guidelines.

Experimental protocol

Thirty-five Mongolian gerbils weighing between 75 - 95 g were divided into the following five groups.

Group I: 15 ml·kg⁻¹ physiological saline solution (PSS) were administered intraperitoneally 30 min before a sham operation (n=7).

Group II: 15 ml·kg⁻¹ PSS were administered intraperitoneally 30 min before ischemia/reperfusion (n=7).

Group III: 50 mg·kg⁻¹ propofol with 10 ml·kg⁻¹ PSS were administered intraperitoneally 30 min before ischemia/reperfusion (n=7).

Group IV: 100 mg·kg⁻¹ propofol with 5 ml·kg⁻¹ PSS were administered intraperitoneally 30 min before ischemia/reperfusion (n=7).

Group V: 150 mg·kg⁻¹ propofol were administered intraperitoneally 30 min before ischemia/reperfusion (n=7).

The sedation level was recorded five minutes after administration of PSS or propofol using the grade of sedation reported by Lowson *et al.*¹³ (Table I).

Then, a rubber bag was placed over the head of each gerbil and nitrous oxide 50% in oxygen with halothane (end-tidal concentration: 1.0-1.5%) was administered throughout the procedure. The animals were placed in the supine position and the common carotid arteries were exposed bilaterally. In the control group, no further procedures were performed. In groups II through V, the common carotid arteries were occluded with miniature aneurysmal clips for four minutes. During this period we verified the

TABLE I Grades of sedation

grade 1: Mongolian gerbils active and grooming with normal exploratory activity. No apparent sedation.
grade 2: Decreased coordination with dragging of hind quarters, still active.
grade 3: Staggering with inability to walk along the edge of a tray. Easier to handle.
grade 4: Severe loss of coordination, with tendency to slither on the stomach rather than walk.
grade 5: Mongolian gerbils unable to walk and slithering.
grade 6: Lying immobile, righting reflex lost. Response to tail pinch reduced but present in gerbil.
grade 7: Righting reflex lost. Response to tail pinch was also markedly reduced or lost in gerbil.

TABLE II Cell degenerative ratio

Group		Degenerative ratio (mean ± SD)
I	Sham operation, saline-treated	5.9 ± 1.9%
II	Ischemia-reperfusion, saline-treated	94.6 ± 2.5%*
III	Ischemia-reperfusion, propofol (50 mg·kg ⁻¹)-treated	10.7 ± 1.7%
IV	Ischemia-reperfusion, propofol (100 mg·kg ⁻¹)-treated	9.7 ± 1.8%
V	Ischemia-reperfusion, propofol (150 mg·kg ⁻¹)-treated	9.2 ± 1.9%

* $P < 0.01$ vs other groups I, III, IV, V. Values are mean ± SD.

Degenerative ratio (%) = number of degenerative pyramidal cells/(total number of pyramidal cells per 1 mm hippocampal CA1 subfield) x 100.

absence of blood supply from the vertebral arteries with a microscope. Four minutes after occluding the bilateral 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) and maintained with a heating blanket at $37 \pm 0.2^\circ\text{C}$ during the experiment.

Respiration of the animals, by visual inspection, was temporarily shallow during the procedure but this improved after reperfusion. The animals were kept and fed in the 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 hema-

FIGURE 1 The grade of sedation was recorded five minutes after administration of PSS or propofol. Each closed circle represents a gerbil. All gerbils that received propofol were apparently sedated. Incremental doses of propofol produced mild sedation to deep anesthesia.

toxylin and eosin. Histopathological changes in the hippocampal CA1 subfield were examined using a light microscope (Olympus BH-2, Olympus, 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 among the five groups, the total number and the degenerative number of 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) $\times$ 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.^{14,15}

Statistical analysis

Data are presented as mean $\pm$ SD. Kruskal-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

Sedation

The grades of sedation for each group are shown in Figure 1. In groups I and II, no gerbil showed any

sedation after administration of PSS. All gerbils which received propofol were sedated. Incremental doses of propofol produced mild sedation to deep anesthesia.

Histopathological findings

The histological appearances from each group are shown in Figure 2. In group I, normal pyramidal cells were well preserved and no disruption of the radial striated zone was detected. In group II, almost all the pyramidal cells were atrophic and pycnotic and exhibited deep staining of the cell cytoplasm, vacuolation, as well as structural disruption of the radial striated zone due to degenerative necrosis. In groups III, IV and V, the pyramidal cells of the hippocampal CA1 subfield were maintained as morphologically normal structures and no structural disruption was observed in the radial striated zones.

Degenerative ratios

The degenerative ratio in group I was $5.9 \pm 1.9\%$ and was the lowest among all groups. In group II, it was $94.6 \pm 2.5\%$ ($P < 0.01$ vs group I). The degenerative ratios in groups III, IV and V were lower than those in group II ($P < 0.01$) (Table II). The administration of propofol before ischemia/reperfusion produced an inhibitory action on delayed neuronal death. In this study the cerebral protective effects of propofol were not dose-dependent.

Discussion

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.¹⁶ The hippocampal CA1 of the Mongolian gerbil (*Meriones unguiculatus*) has, in particular, been used as a model for cerebral ischemia and infarction.¹⁵ Since the gerbil lacks an interconnection between the carotid and vertebro-basilar circulation, cerebral ischemia is easily produced by occlusion of the common carotid arteries. The neuronal damage in hippocampal CA1 of the gerbil was similar to that observed in humans^{17,18} and other experimental animals.¹⁹ Kirino¹⁵ showed that the pyramidal cells were gradually damaged to the point of delayed neuronal death after a short period of ischemia/reperfusion in gerbils. There was no histopathological change in the pyramidal cells on the first or second day after cerebral ischemia/reperfusion, and they became degenerative on the third or fourth day. In the recent studies, the mechanisms of the delayed neuronal death after transient ischemia have been considered as apoptosis or programmed cell death.²⁰⁻²³ However, the delayed neuronal death has been still unclear and a consensus has not been reached. In this study, we examined the protective effects

A

D

B

E

C

FIGURE 2 (a) - (e)

The histological appearances of hippocampal CA1 from each group. (a) Group I; the normal structure of the CA1 pyramidal cells is well preserved. (b) Group II; changes of the CA1 pyramidal cells are present: cell shrinkage, nuclear pycnosis, dark cytoplasmic coloration and vacuolation. In (c-e) Groups III- V, the normal structure of the CA1 pyramidal cells is preserved.

of propofol on delayed neuronal death after a short period of ischemia/reperfusion in gerbils. Pre-administration of propofol during N_2O/O_2 /halothane anesthesia attenuated histopathological changes on the fifth day after the ischemia/reperfusion. Since N_2O/O_2 /halothane anesthesia without propofol does not prevent delayed neuronal death, propofol may have a cerebral protective effects on delayed neuronal death after a short period of

ischemia/reperfusion.

Delayed neuronal death may be due to an abnormal release of glutamate as an excitatory neurotransmitter,^{24,25} an increase of intracellular Ca^{2+} ,^{26,27} followed by cellular death. In addition, since N-methyl-D-aspartate (NMDA) receptors, a type of glutamate receptor, are concentrated in the hippocampal CA1,²⁸ greater glutamate excitotoxicity affects delayed neuronal death. Hans

*et al.*²⁹ showed that propofol attenuated the neurotoxicity of NMDA receptor-mediated glutamate using cultured hippocampal neurons. They also reported that propofol acted as a noncompetitive antagonist of NMDA receptors because of the presence of a phenyl-cycle, which was also contained by MK801 and ketamine, noncompetitive antagonists of the NMDA receptors. Propofol also prevents the neurotoxic vacuole reaction induced by MK801.³⁰ Orser *et al.*³¹ found that propofol inhibited the NMDA subtype of glutamate receptors, possibly through an allosteric modulation of channel gating rather than by blocking the open channel. Bianchi *et al.*³² showed that propofol slightly inhibited the release of glutamate and glutamate dependent Ca^{2+} entry because it depressed the voltage-gated calcium channels using rat synaptosomes. However, Zhu *et al.*³⁰ demonstrated that propofol enhanced NMDA-induced neuronal damage using the recovery of the population spike amplitude as an indicator of neuronal viability. Amorim *et al.*³³ reported that propofol attenuated the increase of intracellular Ca^{2+} but does not depress the release of glutamate using hippocampal slices.¹¹ Although the mechanism of cerebral protective effects of propofol *vs* cerebral ischemia is not well understood, it may be due to the inhibition of increase of intracellular Ca^{2+} , which is provoked by glutamate.^{29,32}

Different methods have been used to determine the amount of brain damage after transient ischemia.^{14,34-36} These include a volume estimate of the infarcted zone using measurements of infarcted surface area³⁴ and stereological methods.³⁵ Another is the count of degenerative neurons.^{14,36} In the present study, we used the latter method because it was easy to estimate the neuronal damage of a local area such as pyramidal cells in the hippocampal CA1.

We administered 50, 100 and 150 mg·kg⁻¹ propofol intraperitoneally 30 min before inducing ischemia. Each dose of propofol protected the pyramidal cells of hippocampal CA1 from degeneration, followed by delayed neuronal death. Lowson *et al.*¹³ showed that 50, 100 and 150 mg·kg⁻¹ propofol administered intraperitoneally, caused sedation, light and deep anesthesia, respectively. We also confirmed this in the present study.

We conclude that the administration of propofol may prevent delayed neuronal death after a short period of cerebral ischemia/reperfusion, and that small doses of propofol may depress delayed neuronal death.

Acknowledgments

We thank H. Sakurai and F.S. Davidson for their assistance in editing the English text of this paper.

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