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Effect of Sedative Dose of Propofol on Neuronal Damage after Transient Forebrain Ischemia in Mongolian Gerbils

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This study investigated whether propofol, an intravenous, non-barbiturate anesthetic, could reduce brain damage following global forebrain ischemia. Transient global ischemia was induced in gerbils by occlusion of bilateral carotid arteries for 3 min. Propofol (50 mg/kg) was administered intraperitoneally 30 min before, immediately after, and at 1 h, 2 h, 6 h after occlusion. Thereafter, propofol was administered twice daily for three days. Treated animals were processed in parallel with ischemic animals receiving 10% intralipid as a vehicle or with sham-operated controls. In histologic findings, counts of viable neurons were made in the pyramidal cell layer of the hippocampal CA1 area 4 days after ischemia. The number of viable neurons in the pyramidal cell layer of CA1 area was similar in animals treated with a vehicle or a subanesthetic dose of propofol. In terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling (TUNEL) assay, semiquantitative analysis of dark-brown neuronal cells was made in the hippocampal CA1 area. There was no significant difference in the degree of TUNEL staining in the hippocampal CA1 area between vehicle-treated and propofol-treated animals. These results show that subanesthetic dose of propofol does not reduce delayed neuronal cell death following transient global ischemia in Mongolian gerbils.

Key Words: Propofol, Subanesthetic dose, Global ischemia, Hippocampus, Neuroprotection, Gerbil

INTRODUCTION

Transient global forebrain ischemia results in neuronal damage in vulnerable areas of the brain (Pulsinelli et al, 1982; Kirino & Sano, 1984; Schmitdt-Kastner & Freund, 1991; Ferrer et al, 1994). Neuronal damage in the CA1 area of the hippocampus in the experimental model of global forebrain ischemia is observed 2~3 days after a transient ischemic event (Kirino, 1982; Pulsinelli et al, 1982; Tortosa et al, 1994). Ischemia-induced neuronal damage involves several different mechanisms including glutamate toxicity, disturbance in calcium homeostasis, increase in free radical formation and enhanced polyamine metabolism (Choi & Rothman, 1990).

Two types of cell death, necrosis and apoptosis following cerebral ischemia, have been recognized. Necrosis is characterized by cell swelling with early loss of plasma membrane integrity, nuclear swelling, and inflammatory infiltrates and is commonly associated with an acute state of pathology (Baju et al, 1993). In contrast to the changes observed in necrosis, in apoptosis the nucleus and cytoplasm shrink and fragment with phagocytosis by neighboring cells or macrophages (Kerr et al, 1972). The DNA fragmentation in the hippocampal CA1 neurons has been considered as a main phenomenon for delayed neuronal death which has been investigated using electrophoresis, in situ DNA fragmentation, and electron microscope methods (Heron et al, 1993; Iwai et al, 1995; Nitatory et al, 1995).

Propofol (2,6-diisopropylphenol) is a new intravenous, non-barbiturate anesthetic currently used in the neuroanesthetic field. It has many advantages including the rapid onset and short duration of anesthesia with an absence of excitatory side effects (Sebel & Lowdon, 1989), and widely used for general

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anesthesia (Smith et al, 1994). Propofol decreases brain electrical activity and suppresses cerebral metabolism (Dam et al, 1990) and blood flow (Werner et al, 1990). However, its neuroprotective effect is controversial. Several studies have attempted to explain the neuroprotective actions of propofol. Propofol has been shown to improve neurologic outcome and to decrease neuronal damage after incomplete ischemia in rats (Kochs et al, 1992). Hans et al (1994) reported the protective action of propofol on neuronal toxicity induced with brief exposure to low doses of glutamate or N-methyl-D-aspartate (NMDA) in a culture model. On the other hand, propofol failed to attenuate the damage in neurons induced by kainate, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) as well as a high dose of glutamate (Hans et al, 1994). When compared with halothane, propofol does not modify neurologic outcome from temporary middle cerebral artery occlusion in rats (Ridenour et al, 1992).

Our laboratory has been shown the neuroprotective effect using sedative dose of propofol to the kainate-induced neuronal damage (Lee & Cheun, 1999). It was, therefore, investigated whether propofol can reduce delayed neuronal death in the CA1 area of the gerbil hippocampus after transient global ischemia.

METHODS

Animals and treatments

Male Mongolian gerbils (*Meriones ungiculatus*) weighing 60~80 g were used in this study. Gerbils were housed individually and maintained on a 12-/12-h light/dark cycle, with *ad libitum* access to food and water before and after surgical intervention. Seven gerbils received propofol (50 mg/kg, Zeneca, Macclesfield, Cheshire, UK) intraperitoneally 30 min before, immediately after, and at 1 h, 2 h, 6 h after occlusion followed by twice daily administrations for an additional three days. A second group of ischemic animals (n=7) received 10% intralipid (Intralipos®, Green Cross Pharmaceutical Co., Seoul, Korea) as a vehicle. Finally, sham-operated animals (n=6) were not subjected to either arterial occlusion or propofol administration.

Surgical procedures

Gerbils were anesthetized with chloral hydrate (400 mg/kg, i.p.). A midline ventral incision of 2 cm was made in the neck. Both common carotid arteries were exposed and separated carefully from the vagus nerve and occluded for 3 min with atraumatic small aneurysmal clips. When the clips were removed the incision was closed with a silk suture. Blood flow during the occlusion and reperfusion after removal of the clips was confirmed visually. The rectal temperature maintained as $37\pm0.5^{\circ}C$ with a heating pad (CMA, Stockholm, Sweden) and light for 3 h to avoid biased results by hypothermia.

Histologic evaluation

Gerbils were sacrificed 96 h after global ischemia. They were deeply anesthetized with ether and perfused transcardially with cold heparinized phosphate-buffered saline (PBS, pH 7.2) and 10% formalin in PBS. The brains were immediately removed from the skull and fixed in the same fixative solution for 24 h. The brains were then embedded in paraffin and 6-μm-thick coronal sections, which included the dorsal hippocampus, were obtained with a rotary microtome. Tissue sections were stained with hematoxylin and eosin. Blinded examiner performed histologic examiniation. Hippocampal CA1 damage was determined by counting the surviving neurons. The mean number of CA1 pyramidal neurons per millimeter for both hemispheres in a section of dorsal hippocampus was calculated for each group of gerbils.

Terminal deoxynucleotidyl Transferase-mediated dUTP Nick End-Labeling (TUNEL) staining

Histochemical staining for TUNEL was performed with a kit (Roche Molecular Biochemicals, Mannheim, Germany). Tissue sections were deparaffinized in xylene and hydrated in a sequence of ethanol washes followed by a final wash in phosphate-buffered saline (PBS). Nuclei of tissue sections were stripped of proteins by incubation with proteinase K (20 μg/ml in 10 mM Tris/HCl, at 37°C) for 15 minutes. The slices were then washed in distilled water and PBS and incubated in 0.3% hydrogen peroxide to remove endogenous peroxidases. After equilibration, each section was incubated with 50 μl of TUNEL mixture (5 μl of terminal deoxynucleotidyl transferase (TdT)

and 45 μl of fluorescence-labeled nucleotide) for 60 min at 37°C. The sections were treated with horseradish peroxidase conjugated anti-fluorescence antibodies. After a detection of double strand breaks in genomic DNA with 2,3'-diaminobenzidine tetrahydrochloride (DAB) (0.5 mg/ml in 50 mmol/L Tris-HCl buffer, pH 7.4) as a substrate for the peroxidase. The percentage of TUNEL-positive cells in the hippocampal CA1 area was determined by measuring the number of CA1 neurons possessing dark-brown staining nuclei as a proportion of the cell number in a millimeter of CA1 area. With the modification of Matsushita et al. (1996), the staining reactivities were categorized into four grades in the following: no staining (0%), small (<10%), moderate (10~50%), or large (>50 %) number of stained cells into grade 0, I, II, and III, respectively.

Statistical analysis

The data were analyzed using Student's *t*-test, and the level for statistical significance was $P<0.05$.

RESULTS

It was examined whether subanesthetic dose of propofol can affect the delayed neuronal cell death following transient global ischemia in Mongolian gerbils. Histological examination of the nervous system demonstrated a marked cell death in the pyramidal cell layer of CA1 area in gerbils treated with propofol or 10% intralipid alone, when compared with sham-operated controls (Fig. 1). CA1 pyramidal neurons showed pyknosis, eosinophilia, karyorrhexia, and chromosome condensation. Nuclear fragmentation was detected (Fig. 1B, C) when compared to sham-operated controls (Fig. 1A). No significant differences (p=0.66) were observed between gerbils treated with 10% intralipid and gerbils treated with propofol (Fig. 2).

TUNEL staining was negative in the sham-operated controls (Fig. 3A). However, the staining was strongly detected in the pyramidal cell layer of the CA1

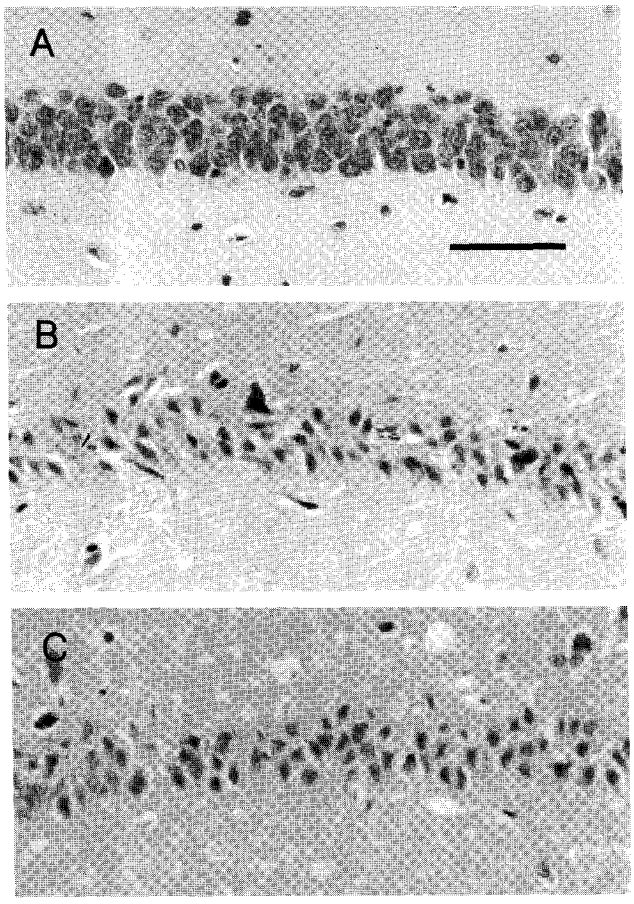

Fig. 1. Effect of propofol on the number of surviving cells in the CA1 area of hippocampus 4 days after transient global ischemia in gerbils (hematoxylin and eosin staining). CA1 area in sham-operated (A), in vehicle (10% intralipid)-treated (B), and in propofol-treated gerbils (C). Bar=100 μm.

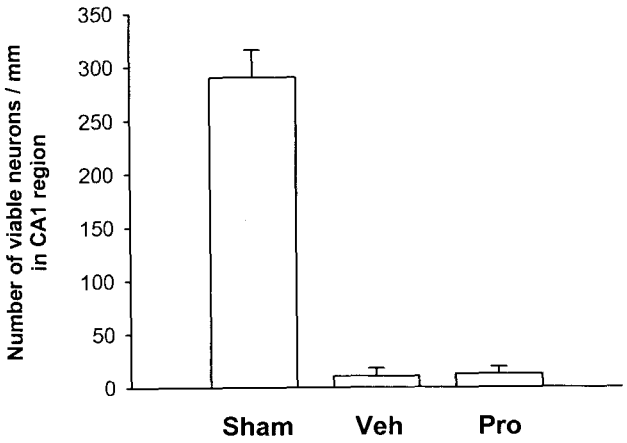

Fig. 2. Effect of propofol on the number of surviving cells in the hippocampal CA1 area 4 days after transient global ischemia in gerbils. There is no significant difference in number of surviving neurons between vehicle-treated and propofol-treated gerbils. Data are presented as the mean±S.E.M. (n=6~7 gerbils/group). Sham, sham-operated; Veh, vehicle-treated; Pro, propofol-treated gerbils.

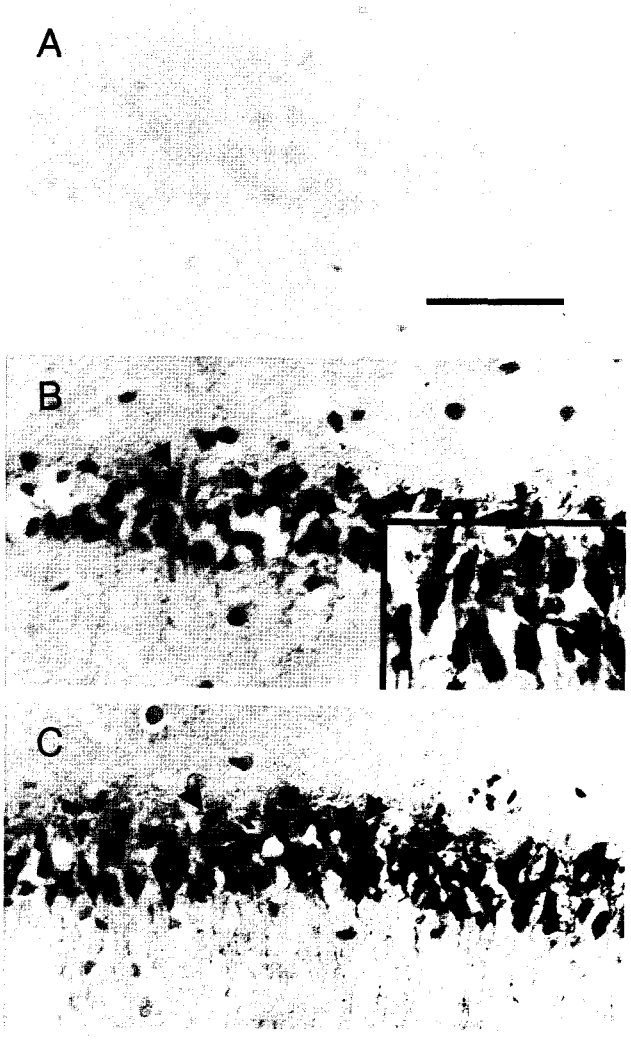

Fig. 3. Effect of propofol on the nuclear DNA fragmentation, evaluated by the TUNEL method in the hippocampal CA1 area 4 days after transient global ischemia in gerbils. The TUNEL method (A) shows no evidence of nuclear DNA fragmentation after sham-operation. A similar degree of TUNEL staining after global ischemic episode is seen in vehicle (10% intralipid)-treated. Boxed area is shown with higher magnification of TUNEL- positive cells (B), and in propofol-treated gerbils (C). Bar=100 μm. TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling.

area in the ischemic control group (10% intralipid administration) and the propofol-treated group (Fig. 3B, C). The staining was essentially found in the nucleus of neuronal cells indicating DNA fragmentation occurred exclusively in damaged CA1 neurons. However, there was no significant difference in the number of TUNEL-positive CA1 neurons between the

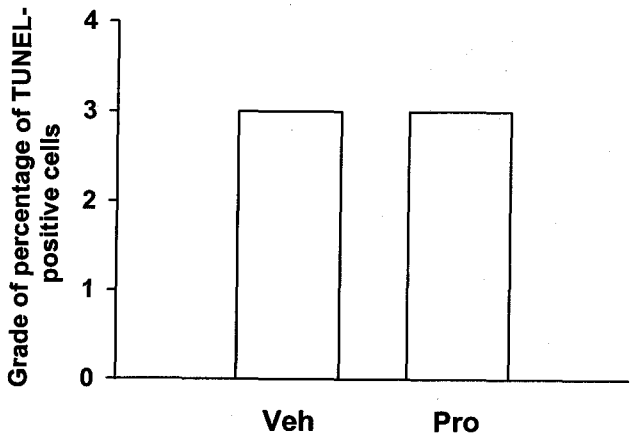

Fig. 4. Effect of propofol on the number of TUNEL stained cells in the hippocampal CA1 area 4 days after transient global ischemia in gerbil. There is no significant difference in the grade of TUNEL staining between vehicle-treated and propofol-treated gerbils. See the text for definition. Data are presented as the mean $\pm$ S.E.M. (n=4 gerbils/group). Veh: vehicle-treated, Pro: propofol-treated gerbils. TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling.

10% intralipid-treated ischemic controls and the propofol-treated animals (Fig. 4). Propofol did not show a protective effect on CA1 neurons after transient forebrain ischemia.

DISCUSSION

Our results show that propofol does not have neuroprotective properties against delayed neuronal death in a transient global ischemia model in gerbils. Administration of a subanesthetic dose of propofol did not attenuate the number of damaged neurons in the CA1 area of the gerbil hippocampus after a global cerebral ischemic event.

Several other studies showed the effects of propofol on ischemic outcome (Kochs et al, 1992; Ridenour et al, 1992; Tsai et al., 1994; Pittman et al, 1997). These produced inconsistent conclusions. Kochs et al (1992) examined the effects of propofol on ischemic outcome by using a hemispheric ischemia model. Rats anesthetized with propofol showed the attenuation of histologic injury and improved neurologic scores after a 30 min episode of unilateral carotid artery occlusion. Ridenour et al (1992) subjected propofol or halothane anesthetized rats to a 2 h interval of middle cerebral artery occlusion

followed by 96 h of recovery. There were no differences in resultant cerebral infarct volumes between the two groups, suggesting that propofol was not likely to have a prominent neuroprotective action because halothane was not viewed as a neuroprotective agent. Tsai et al (1994) studied rats anesthetized with xylaxine and ketamine, which were then given either saline or propofol intravenously. An insult of permanent middle cerebral artery occlusion combined with 60 min of bilateral common carotid artery occlusion was used in their study. Propofol administration did not affect cerebral infarct volume measured after 24 h of recovery. Pittman et al (1997) compared the effects of propofol and pentobarbital on neurologic outcome and brain infarct size following transient focal ischemia. They concluded that the outcome from ischemia was equivalent for pentobarbital and propofol. The results of the above mentioned studies indicate that propofol has complicated actions at least with respect to its effects on cerebral ischemia.

There is no previous report about the effect of consecutive treatment of propofol on delayed neuronal injury induced by global ischemia. Previous studies looked at a focal cerebral ischemia model regarding the effect of propofol only during anesthesia. Transient forebrain ischemia resulted in delayed neuronal death in the hippocampal CA1 area 48 to 72 h after injury (Kirino, 1982; Pulsinelli et al, 1982). The delay in neuronal damage provides an opportunity for therapeutic intervention. In this study, we examined the effect of propofol on the delayed hippocampal neuronal damage after transient forebrain ischemia in gerbils. To evaluate the effect of propofol on the delayed neuronal injury, unlike previous studies using a focal or hemispheric ischemic model, it may be essential to administer propofol to gerbils as a continuous infusion, or consecutive injections, because of its short action duration. So, we administered the subanesthetic dose of propofol intraperitoneally 30 min before, immediately after, and at 1 h, 2 h, 6 h after occlusion followed by twice daily for an additional three days. In addition, the reason we administered a subanesthetic dose of propofol is that the administration of propofol in anesthetic doses needs careful control of the anesthetic depth through monitoring. If clinicians try to reduce brain damage by an ischemic attack, it is important to apply any agent to patients as soon as possible.

Most sedatives can have neuroprotective properties. Propofol is primarily a hypnotic and the subanesthetic dose of propofol can induce sedation. The exact mechanism of its action has not yet been fully understood. However, evidence suggests that it acts by enhancing the function of the γ-amino-butyric acid (GABA)-activated channel just as barbiturate- and benzodiazepine-like effects (Peduto et al, 1991; Borgeat et al, 1994; Holigel et al, 1996). In addition, several reports showed the antioxidant effect of propofol (Musacchio et al, 1991; Eriksson et al, 1992; Murphy et al, 1992). Some investigators have shown that propofol attenuates NMDA-induced toxicity and inhibits NMDA receptors in cultured neuronal cells (Hans et al, 1994; Orser et al, 1995). Propofol has been shown to have an anticonvulsant or neuroprotective effect in experimental seizure models induced by pentylenetetrazol, electric shock, or kainate (Lowson et al, 1990; De Riu et al, 1992; Lee & Cheun, 1999). It was proposed that anesthetics protect neurons from injury during incomplete ischemia because of their ability to decrease metabolic demand (Newberg et al, 1983; Todd & Drummond, 1984). According to this view, we would expect the subanesthetic doses of propofol to provide a weak protection on mild ischemic injury. In the current study, administration of a subanesthetic dose of propofol failed to show the neuroprotective effect on delayed neuronal injury. Zhu et al (1997) reported that propofol does not have neuroprotective effects on glutamate excitotoxicity, whereas thiopental reduces NMDA- and AMPA-induced neurotoxicity. In their study, propofol enhanced the neuronal damage induced by NMDA.

In order to evaluate the effect of propofol on apoptosis more clearly, we used the transient global ischemic model of 3 min in gerbils similar to Matsushita et al (1996). Our approach was based on the idea that a milder ischemic insult might induce more apoptotic cell death (Bonfoco et al, 1995). Matsushita et al (1996) suggested that intranuclear DNA fragmentation is induced prior to total cell death following transient global ischemia. So, we evaluated the effect of propofol on TUNEL staining in the gerbil hippocampus after transient ischemia. Our results showed that ischemic nuclear damage could not be rescued by consecutive administrations of a subanesthetic dose of propofol. They suggest that propofol may not have the capability of inhibiting the endonuclease activity.

In conclusion, the present results suggest that

propofol does not prevent neuronal damage in the pyramidal cell layer of the hippocampal CA1 area following global forebrain ischemia in gerbils, although the drug dosage or regimen of propofol in the current study is not sufficient to determine whether propofol has neuroprotective properties. These results also point to the likelihood that, although propofol has some beneficial roles in certain forms of ischemia (Kochs et al, 1992; Pittman et al, 1997), the drug has a limited value in the clinical management of global forebrain ischemia following cardiac arrest and other causes. The current study, however, cannot rule out the possibility that the continuous administration of a maximal anesthetic dose of propofol could reduce brain damage due to transient global ischemia.

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