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Effectiveness of Propofol Pretreatment on the Extent of Deranged Cerebral Mitochondrial Oxidative Enzyme System after Incomplete Forebrain Ischemia/Reperfusion in Rats

It has been suggested that propofol has the protective effect on cerebral ischemia-reperfusion injury. The aim of this study is to evaluate the effect of propofol pretreatment on incomplete forebrain ischemia-reperfusion injury in rats. Thirty Sprague-Dawley rats were anesthetized with isoflurane in oxygen and randomly allocated into propofol group (n=13) and saline group (n=17). In propofol group, propofol was pretreated in a step-down scheme before inducing forebrain ischemia by occlusion of both common carotid arteries and arterial hypotension. After ischemia (20 min) and reperfusion (30 min), rats were decapitated. Brain was sliced to obtain coronal slices of 4-12 mm from frontal pole, which were reacted with 2% 2,3,5-triphenyl-2*H*-tetrazolium chloride (TTC) for 10 min to differentiate the damaged tissues from normal tissues. Median (interquartile range) values of the average percent infarct area were 0.0 (8.6)% and 20.1 (41.2)% in propofol and saline groups, respectively. There was significant difference between the groups. In conclusion, propofol may have a protective effect on incomplete forebrain ischemia-reperfusion injury.

Key Words: *Brain Ischemia; Propofol; Reperfusion Injury; Tetrazolium Salts*

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INTRODUCTION

Propofol, one of intravenous anesthetics, has been widely used for many surgical procedures. Like barbiturate, propofol lowers cerebral blood flow and cerebral metabolic rate (1). Although many investigations have been performed to prove neuroprotective effect of propofol, it remains unclear until now (2-7). Mostly, outcome is improved by propofol in focal ischemia model (2, 3). However, in forebrain ischemia, neuroprotective effect of propofol is still not confirmed (7).

Forebrain ischemia is often expected in special clinical situation. We hypothesized that propofol might have protective effect on the forebrain ischemia and reperfusion injury in two-vessel occlusion ischemia model in rats. Infarct was produced by temporary occlusion and reperfusion of bilateral common carotid arteries. We evaluated the extent of brain infarct by tetrazolium salts which is known as a histochemical indicator for mitochondrial respiratory enzymes (8, 9). The purpose of this study was to evaluate the protective properties of propofol in this circumstance by observing the derangement of mitochondrial oxidative enzyme system.

MATERIALS AND METHODS

Thirty Sprague-Dawley rats (body weight 310-510 g, average 381.7 g) were randomly allocated into two groups; 17 for saline group and 13 for propofol group. Rats were not fasted, and other conditions such as cage temperature, humidity and noise were constantly controlled. Anesthesia was induced in a transparent plexiglass chamber by blowing 5% isoflurane in oxygen until the righting reflex became inapparent. Rats were intubated and anesthesia was maintained with isoflurane 1-2 vol% in oxygen. No muscular relaxants were given. Mechanical ventilation (Harvard Rodent Ventilator Model 683, Harvard Apparatus, Inc., Holliston, MA, U.S.A.) was maintained around 35-40 mmHg of arterial carbon dioxide tension. Left femoral artery was cannulated with polyethylene tubing (PE# 50, ID 0.58 mm, ED 0.965 mm, Intramedic Non-radiopaque Polyethylene Tubing, Becton Dickinson Co., Franklin Lakes, NJ, U.S.A.) for pressure monitoring, right femoral artery for blood withdrawal and reinjection, and right femoral vein for drug administration. Rectal temperature was maintained in the range of 37-38℃ by using a warm blanket (Homeother-

mic Blanket System, Harvard Apparatus, Inc.). Drugs were given as follows. In propofol group, propofol (Diprivan, Zeneca Pharmaceutical, Macclesfield Cheshire, U.K.) infusion was started at 96 mg/kg/hr for 20 min and maintained at 72 mg/kg/hr until the initiation of carotid occlusion. In saline group, the same volume of saline was infused instead of propofol.

Cerebral ischemia was produced in all rats by combination of clamping both common carotid arteries and inducing hypotension to the mean blood pressure level of 45-50 mmHg for 20 min. Intravenous labetalol and artificial blood withdrawal/reinfusion technique were used to induce hypotension. After 20 min of ischemia, both common carotid arteries were unclamped and recovery of blood flow was confirmed by observing semi-transparent vessel wall. The withdrawn blood was reinfused slowly. Reperfusion was allowed for 30 min. At the end of the reperfusion, the rats were sacrificed by decapitation and the brains were collected rapidly. Each brain was sectioned coronally along the planes 4, 6, 8, 10 and 12 mm posteriorly to the frontal pole. These sections were immersed promptly in the solution of 2% 2,3,5-triphenyl-2*H*-tetrazolium chloride (TTC) (Sigma Inc., St. Louis, MO, U.S.A.) in saline, which was incubated at 38℃ for 10 min. After incubation, the photographs of the brain surfaces in each of the five coronal sections were taken. TTC reaction represents bright red coloration, where unstained color denotes unreacted TTC, which is considered as "TTC-infarct". The photographs were analyzed to differentiate the unstained area from those of red color in the brain surfaces. The TTC-infarct areas were digitally quantified in uncalibrated square pixel units by analyzing software (SigmaScan Pro v4, Jandel Scientific, SPSS Inc., Chicago, MI, U.S.A.), and the infarcted area was determined to be an average of 4-12 mm planes.

Statistical analysis

The average percent infarcted area was compared between groups by Mann-Whitney U test. SPSS v8 (SPSS Inc.) was used for statistical analysis. Statistical decision was made within 5% range of statistical type I error. Values were expressed as median and interquartile range in brackets.

RESULTS

Median (interquartile range) value of average percent infarcted area in propofol group was 0.0 (8.6)%, which was significantly lower than 20.1 (41.2)% in saline group (p=0.001) (Fig. 1). TTC-infarct was most prominent in 6-8 mm planes. At these planes, infarct was extended to cerebral cortices (motor, somatosensory and gustatory area) and caudoputamen (Fig. 2).

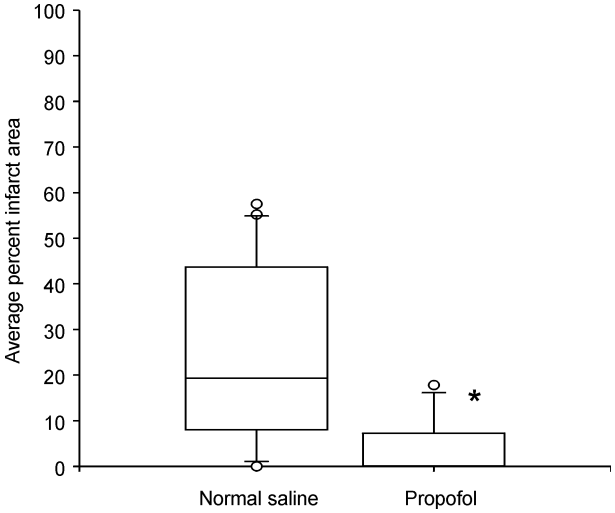

Fig. 1. Box-and-whisker plot for average percent infarct area of two groups. Median of percent mean infarct area is 0.0 (8.6)% in propofol group, which is significantly lower than 20.1 (41.2)% in normal saline group. Asterisk(*) indicates p=0.001. Boxes indicate median (inner horizontal line), the first quartile (lower margin) and the third quartile (upper margin). Whiskers indicate the 95 (upper) and the 5 percentiles (lower). Empty dots are extreme observations. Values are expressed in median (interquartile range).

DISCUSSION

Our results showed that propofol pretreatment could markedly reduce the extent of TTC-infarcted area following incomplete global cerebral ischemia and reperfusion. TTC-infarct represents cellular change of deranged mitochondrial oxidative system, which means irreversible cell death (10). Until now, neuroprotective effect of propofol has been controversial. Various results were obtained according to the ischemia model and the species of experimental animal.

Propofol reduced infarcted area (2), and improved behavior (3) in focal ischemia model in rats. However, Ridenour et al. reported that propofol did not show significant neuroprotection over halothane control (6). Also in cats, propofol did not improve neurohistopathologic outcome following incomplete cerebral ischemia (11). It does not seem to be possible to conduct a pure case-control study because most anesthetics used to anesthetize the animals will affect the neuronal injury and recovery. In fact, variable coutcomes of propofol were widely dependent on species, study model and baseline anesthetic.

Isoflurane has been reported to have relative neuro-

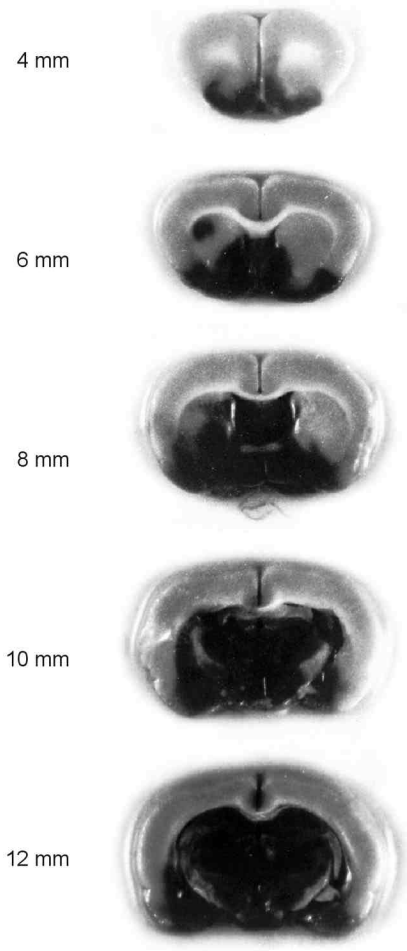

Fig. 2. Coronal sections of rat brain. Cerebral infarction is visualized by 2,3,5-triphenyl-2*H*-tetrazolium chloride. Normal tissues are stained as black (bright red color). Infarcted area is not stained, (white color) which localizes at cerebral cortices and caudoputamen. Infarction is widely distributed through 4-12 mm from frontal pole.

protective potential (12, 13). Isoflurane concentration in our study did not seem to differ between each groups as we controlled the isoflurane concentration to be 1-2 vol%. Thus, we did not consider that isoflurane distorted the outcome of this study. And protective effect of propofol over isoflurane is likely to be more important.

Several anesthesiologists use propofol during aneurysmal or carotid artery surgery whether or not believing its neuroprotective effect. However, experimental studies could not support that propofol is beneficial for the neuronal survival in forebrain ischemia model. Propofol did not improve the survival of mongolian gerbil after forebrain ischemia in study of Arcadi et al. (7). But we do not think that this is directly applicable to human because cerebral circulation is quite different in mongolian gerbil from that in human. Accordingly, it is difficult to assert that propofol has neuroprotective effects yet. However, there are several ways in which propofol may theoretically provide neuroprotection by, for example, reducing cerebral oxygen requirement (1).

Among the possible beneficial effects of propofol, Murphy et al. (4) showed and quantified the antioxidant effect of propofol in vitro and, therfore, propofol may be beneficial in reducing the damage of mitochondrial membrane during neuronal ischemia. When cells are exposed to ischemic insult, mitochondrial membranes are irreversibly fragmented and granulated gradually. Mitochondrial integrity is essential in cellular energy metabolism.

Tetrazolium reaction is one of various histochemical techniques that localize enzymes of oxidative phosphorylation (10). It has been proven useful in defining irreversible cellular death. TTC has been used to detect experimental brain ischemia, but its accuracy has not been fully established, especially in models of early brain ischemia such as the one in our study (14, 15).

Oxygen radicals are reported to take a major role in cellular injury in acute or chronic stress conditions (16). Role of oxygen radicals for tissue ischemia/reperfusion in clinical setting is well concerned. Murphy et al. (4) quantified the ability of one mole of propofol in vitro, which can scavenge two moles of oxygen radicals. Propofol resembles butyrated hydroxytoluene or vitamin E in chemical structures, which are known as antioxidants. High lipid solubility of propofol promotes its rapid accumulation to intracellular lipid layer such as mitochondrial inner layer, and clearance of oxygen radicals from cell.

We chose an infusion rate of propofol of arbitrary compensation from Werner et al. (17). In our experience, infusion rate of 120 mg/kg/hr for 10 min resulted in marked reduction of blood pressure. Infusion rate of 96 mg/kg/hr for 20 min of propofol did not result in any cardiovascular compromise and was determined to be proper for concentration for surgical procedures performed. Although the electrical silence could not be confirmed with this infusion scheme as EEG was not available, EEG silence is not likely to be an important index in this study model when considering the antioxidant effect of propofol. Recently, a report that maximal EEG suppression was not required for obtaining neuroprotection by anesthetic is encouraging (18).

The advantages of two-vessel occlusion model have been known to lie in its abrupt onset of insult and recovery (19-22); so called, "square wave" insult. As this can emulate the global cerebral ishemia resulting from acute bleeding, cardiac arrest and certain shock, it can often be highly useful. Although the procedures are relatively simple, more vigilence is required to take care of animals. In our preliminary study, several rats were sacrificed due to cardiovascular collapse during occlusion/hypotension period or reperfusion period. We accepted

mean arterial pressure of 45-50 mmHg as a target and used labetalol to minimize blood withdrawal. Use of vasodilators or artificial blood withdrawal and need of anesthesia are obligatory (23), which can confuse the ischemic outcome, and therefore act as a major disadvantage of this model. We chose the forebrain ischemia model in this experiment for one reason. It is because forebrain ischemia is often expected in clinical setting such as cardiopulmonary bypass or iatrogenic circulatory arrest during cardiac surgery. To seek a protective agent, especially propofol, as a commonly used intravenous anesthetic, would be valuable for this reason.

Conclusively, propofol pretreatment reduced the extent of TTC-infarcted area induced by global cerebral ischemia and immediate reperfusion. Although our design might simplify the mechanisms of neuronal injury in cell ischemia/reperfusion, it seems to be sufficient to obtain a conclusive picture of intrinsic neuroprotective effect of propofol.

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