Effects of sevoflurane and desflurane in CA1 after incomplete cerebral ischemia in rats

Ali F. Erdem, MD, Mehmet Cesur, MD, Haci A. Alici, MD, Fazli Erdogan, MD, Nazim Dogan, MD, Husnu Kursad, MD, Mustafa S. Yuksek, MD.

ABSTRACT

Objectives: We compared the postischemic cerebral protective effects of sevoflurane and desflurane in rats with incomplete cerebral ischemia.

Methods: This study was performed in Ataturk University Medical Faculty in Erzurum, Turkey in 2003. All rats were anesthetized with 5% isoflurane, intubated and mechanically ventilated, then given 2% isoflurane in 70% nitrous oxide and 30% O_2. The femoral artery was cannulated. Five minutes before ischemia, and at the end of ischemia, arterial blood was taken for plasma glucose, hematocrit and blood gas analysis. Hypotension was induced by hemorrhage, and then both common carotid arteries were clamped for 10 minutes. In the control group, the arteries were then unclamped and the rats were extubated. In the other 2 groups, isoflurane was discontinued after carotid artery unclamping, and either 2% sevoflurane or 6% desflurane in 70% nitrous oxide and 30% O_2 was given for 30 minutes, after which the rats were extubated. Five days later, they were sacrificed, and histological scores in CA1 were graded on a scale 0-3.

Results: Histopathological outcome in sevoflurane and desflurane group was not different, but there were differences between sevoflurane and control ($p<0.05$), and desflurane and control ($p<0.01$).

Conclusion: These data indicate that sevoflurane and desflurane have cerebral protective effects when given after ischemia.

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The effects of volatile anesthetic agents on neurological outcome and infarct size have been studied in animals. Some studies show that sevoflurane, halothane and isoflurane are cerebrally protective,[1,2] but others show no effect on neurological function or histopathology.[3] Volatile anesthetics reduce cerebral metabolic rate and rectify the imbalance between oxygen demand and supply.[4] They may inhibit neurotransmitter release (namely, catecholamines, glutamate) resulting in the decrease of Ca^{2+} and Na^{+} influx into postsynaptic neurons.[5,6] This inhibition reduces intracellular catabolic processes such as activation of proteases, lipid peroxidation and nitric oxide formation. In concentrations, which achieve EEG burst suppression, desflurane, isoflurane and sevoflurane reduce neuronal function.[7] Thus, desflurane and sevoflurane are potentially suitable anesthetics for neurosurgical patients.

Postischemic sevoflurane and desflurane administration was not investigated. The aim of the present study is to investigate histopathological effects of sevoflurane and desflurane administered after ischemia in rats subjected to incomplete cerebral ischemia. During ischemia period we did not give sevoflurane and desflurane.

Methods. Thirty male Sprague Dawley rats weighing 250-300 g fasted 12-16 hours before the

From the Department of Anesthesiology and Reanimation (Erdem, Cesur, Alici, Dogan, Kursad, Yuksek) and the Department of Pathology (Erdogan), Ataturk University, Medical Faculty, Erzurum, *Turkey.*

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Address correspondence and reprint request to: Dr. Ali F. Erdem, Department of Anesthesiology and Reanimation, Faculty of Medicine, Ataturk University, Erzurum, *Turkey*. Tel. +90 (442) 3166301/2298. Fax. +90 (442) 3166340. E-mail: alifuat@atauni.edu.tr

experiment. Access to water was provided. They were allocated randomly to one of the 3 groups: control (group C), sevoflurane (group S) and desflurane (group D). All animals were anesthetized in a bell jar with 5% isoflurane (Forane, Abbott, Istanbul, Turkey). The trachea was intubated and the lungs ventilated mechanically at a respiratory rate of 60 breaths per min^{-1} with a tidal volume of 12 ml per kg^{-1}. Anesthesia was then maintained with 2% isoflurane, 30% oxygen and 70% nitrous oxide. A catheter was inserted into the right femoral artery for continuous arterial pressure measurement and blood sampling. Esophageal temperature was automatically controlled at 38^{o}C during surgical preparation. Both carotid arteries were exposed and carefully separated from the vagus nerve and the cervical sympathetic chain. A loose silk suture was placed around each artery. Plasma glucose concentration, hematocrit, arterial blood gases, mean arterial pressure (MAP) and heart rate (HR) were measured 5 minutes before ischemia and at the 10th minute of ischemia. Thirty units of heparin were administered intravenously. Five minutes later, cerebral ischemia was produced by combining bilateral carotid artery occlusion and hemorrhagic hypotension. A mean arterial pressure of 35-40 mm Hg was maintained by withdrawing blood from the right femoral artery. The carotid arteries were clamped 5 minutes after the beginning of hemorrhagic hypotension. After 10 minutes of ischemia, both carotid arteries were unclamped and the withdrawn blood was reinfused over 5 minutes. Protamine (0.3 mg) and $NaHCO_3$ (0.25 mEq), were administered to reverse the heparinization and metabolic acidosis. In group C, the wounds were sutured at the end of ischemia and the anesthetic was discontinued. Mechanical ventilation was discontinued on resumption of spontaneous ventilatory effort, and the animals were extubated and transferred to heated, humidified incubators. For all rats overall duration between exposure to isoflurane during anesthesia induction, surgical preparation and the completion of ischemia was approximately 60 minutes. Animals in group S received 2% sevoflurane (Sevorane, Abbott, Istanbul, Turkey), 30% oxygen and 70% nitrous oxide for 30 minutes after reperfusion of the carotid arteries. Those in group D received 6% desflurane (Suprane, Eczacibasi, Istanbul, Turkey) in the same oxygen/ nitrous oxide mixture for 30 minutes after reperfusion of carotid arteries. In the sevoflurane and desflurane groups, the wounds were sutured and the anesthetic was discontinued after 30 minutes of anesthetic administration. Mechanical ventilation was discontinued on resumption of spontaneous ventilatory effort and the animals' was extubated and transferred to heated, humidified incubators. Twelve hours after the ischemic period all, animals had resumed intake of water and food. Five days after the ischemia, the rats were anesthetized with 150 mg/kg intraperitoneal pentobarbital and then decapitated. The brains were removed and frozen in 10% phosphate-buffered formalin for 48 hours. They were dehydrated in graded concentrations of ethanol and butanol and then embedded in paraffin. Sectional intervals were adapted to obtain specific standard levels of hippocampus for quantification of injury. Coronal brain sections were cut at a thickness of 4 mm and stained with hematoxylin and eosin.

The CA1 sector of hippocampus was evaluated at a magnification of 400× by a pathologist blinded to the randomization. Ischemic neurons were identified by cytoplasmic eosinophilia with loss of Nissl substance, and by the presence of pyknotic homogeneous nuclei. Damage was graded on a scale of 0-3, where 0 = no ischemic neurons; 1 = <10% ischemic neurons; 2 = 10-50% ischemic neurons; and 3 = >50% ischemic neurons.[8]

Table 1 - Physiologic data.

Parameter	Group C	Group S	Group D
Weight (g)	280±10	282±12	284±11
MAP (mm Hg)			
preischemia	101±8	102±6	99±7
postischemia	37. 4±1.9	37. 2±2	37.5±2.1
HR (beats/min)			
preischemia	350±25	330±29	353±24
postischemia	346±25	334±25	351±30
Temperature (^{0}C)			
preischemia	37. 9±0.1	37. 9±0.1	38±0.1
postischemia	37. 9±0.1	37. 9±0.1	37.9±0.1
Hematocrit (%)			
preischemia	42±2	43±2	44±2
postischemia	41±1	40±1	42±1
Plasma glucose (mg/dl)			
preischemia	151±21	150±20	143±22
postischemia	155±26	162±27	163±26
PaO_2 (mm Hg)			
preischemia	160±24	146±23	154±23
postischemia	159±17	131±18	158±22
$PaCO_2$ (mm Hg)			
preischemia	36.6±2.2	36.8±2.2	37±1.7
postischemia	38±1.7	37±2.3	37±20
Ph			
preischemia	7.37±0.03	7.37±0.03	7.38±0.04
postischemia	7.36±0.03	7.38±0.02	7.38±0.02

All data are represented as mean ± SD.
MAP - mean arterial blood pressure, HR - heart rate
There were no significant differences in the physiologic variables among the groups.

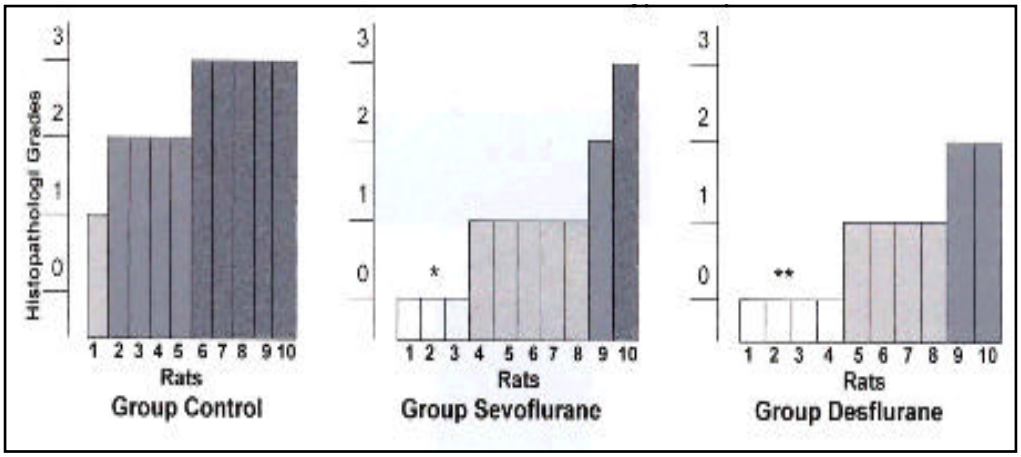

Figure 1 - Histopathologic grades for CA1 section of hippocampus: grade 0 - no damage, grade 1 - <10% of neurons damaged, grade 2 - 10-50% of neurons damaged, grade 3 - >50% of neurons damaged. Between group C and group S - $p<0.05$. Between group C and group D - $p<0.01$.

Statistical analysis was performed with SPSS for Windows (version 10). Cerebral ischemia scores between the groups were analyzed using chi-square test on a 4 × 2 table. The t-test for independent variables was used to compare the weights of the rats. Physiologic variables were analyzed by ANOVA (2-way analysis of variance). Least Square Difference was use to get *p* value. A *p* value less than 0.05 was considered statistically significant.

Results. **Table 1** shows the preischemic body weight and MAP, HR, esophageal temperature, hematocrit, plasma glucose, PaO_2, $PaCO_2$ and arterial pH 5 minutes before ischemia and at 10th minute of ischemia. There were no differences in weight or other variables among the groups ($p>0.05$). Two rats in group C and one in group D died due to unsuccessful tracheal intubation; they were replaced in order to maintain group numbers. Histopathological grades for CA1 section are represented in **Figure 1**. Signs of ischemia were observed in all animals in group C, but 3 in group S and 4 in group D showed no evidence of ischemia. Grade 3 cerebral ischemia was observed in 5 animals in group C, one in group S, and none in group D. There were no significant differences in histopathologic grades between groups S and D, but there were significant differences between groups C and S ($p< 0.005$) and groups C and D ($p<0.001$).

Discussion. Our results demonstrate that administration of sevoflurane and desflurane after ischemia offer similar degrees of cerebral protection to the CA1 area in a rat model of incomplete cerebral ischemia, when investigated 5 days after ischemia.

Different areas of the brain differ in their vulnerability to ischemia. Hippocampal neurons are very sensitive to ischemia, the most vulnerable neurons being in area CA1 and the hilus, whereas most of the CA3 pyramidal neurons and the granular cells of the dentate gyrus are resistant to ischemic damage.[9,10] Dorsal CA1 cells are more vulnerable to ischemia than ventral ones.[11] Thus, we investigated only cells from the dorsal CA1 hippocampal area. Strict control of physiological variables is important for the validity of the study, as even minor changes in blood pressure, body temperature, blood gases or glucose levels affect cell survival.[12] Our protocol avoided differences in physiological data among the groups.

We have compared our histopathological results for sevoflurane and desflurane to those of the control group, anesthetized with isoflurane. In some studies, in which focal or incomplete forebrain ischemia was induced in rats and primates, isoflurane anesthesia did not improve neurological or histopathological outcome, compared to halothane or barbiturates.[8,13-15] However, other studies show that both isoflurane and sevoflurane reduce neurological deficit, infarct size and stroke-related mortality when compared with lightly anesthetized or awake controls.[1,16-18] Thus, the protective potential of isoflurane may not be obvious when compared to halothane or barbiturate anesthesia, and its cerebral protective properties may only become evident when compared to awake or nitrous oxide-fentanyl anesthetized controls. Thus, the demonstration of neuronal and histopathological protection depends to some extent on the treatment of the control group. In our study, all rats in all groups received isoflurane until the end of bilateral carotid artery occlusion.

The development of a new cerebral ischemia model, by blocking blood flow into the middle cerebral artery with an intraluminal suture[19] has allowed comparison of the effects of anesthetic agents with the awake state. This model is a less invasive approach than temporal craniotomy. Awake rats maintain homodynamic and ventilatory stability during such ischemic events. However, the disadvantages of this technique are the difficulty of precise placement and the risk of vascular puncture. Warner et al[3] studied the effects of isoflurane on neuronal necrosis following near-complete forebrain ischemia in rats. The animals were maintained normocapnic and normothermic while subjected to a near-complete forebrain ischemic insult induced by systemic hypotension (MAP= 50± mm Hg) and bilateral carotid artery occlusion. Prior to the onset of ischemia, treated animals received isoflurane 3-4% in a 30:70 mixture of O_2 and N_2O, administered to maintain a steady state electroencephalogram burst suppression interval greater than 30s. The histopathological injury was assessed on the caudate nucleus, cerebral cortex and hippocampus. Pretreatment with isoflurane had no beneficial effect on delayed neuronal necrosis following near complete forebrain ischemia. Due to these results,[3,8,13-15,20] we gave isoflurane to all animals until unclamping of the carotid artery; we believe that this should not greatly affect our results.

There are many potential mechanisms, which volatile anesthetics can exert neuroprotective effects. The effects of excitatory amino acids in ischemic damage are well known. Efflux of glutamate aspartate or glutamate is an early event leading to neuronal death. Drugs blocking the postsynaptic actions of excitatory amino acids at N-methyl-D-aspartate (NMDA) or alpha-amino-3-hydroxy-5-methyl-4-propionic (AMPA) receptors are neuroprotective in ischemia.[21] Preventing the efflux of neurotransmitter is another approach. Volatile anesthetic agents may inhibit neurotransmitters such as glutamate and catecholamine, by decreasing Ca^{2+} and Na^{+} influx into the postsynaptic neurons.[5,6] Toner et al[22] showed that sevoflurane, at clinically relevant concentrations, can reduce the efflux of neurotoxic transmitters, and thus may be neuroprotective. Sevoflurane also blocks nicotinic receptors.[23] Warner et al[16] performed filament occlusion of the middle cerebral artery when investigating the cerebral effects of sevoflurane and halothane. Four days after ischemia, they examined neurological function and cerebral infarct volumes. Neurological function was better in rats receiving volatile agents than in the awake group. Cortical and subcortical infarct volumes were also smaller; leading the authors to conclude that sevoflurane is neuroprotective. Engelhard et al[24] reported that compared to rats anesthetized with fentanyl-nitrous oxide, isoflurane and desflurane improved neurological outcome after incomplete cerebral ischemia. Studied animals were divided to 4 groups; fentanyl-nitrous oxide, 1.0 MAC isoflurane, 1.0 MAC desflurane and 1.5 MAC desflurane. Improved neurological outcome was independent of the concentration of volatile anesthetic and neuronal activity. Outcome was, however, related to plasma catecholamine concentrations, which were suppressed by isoflurane and desflurane, but not by fentanyl-nitrous oxide. Tsai et al[25] reported that 1.0, 1.25 and 1.5 MAC desflurane reduced infarct volumes following cerebral artery ischemia and reperfusion injury.

Effects of isoflurane on long term outcome from severe forebrain ischemia in rats were studied.[26] In this study, it was found that there was both behavioral and histologic difference between the isoflurane and fentanyl-nitrous oxide groups at 5 days, but no difference for either outcome assay at 3 weeks or 3 months, so the authors concluded that protective advantage of isoflurane versus fentanyl-nitrous oxide was transient. In another study, it was reported that isoflurane only delayed the development of cerebral infarction caused by focal ischemia but did not prevent it, did not provide sustain neuroprotection.[27] In our study, we did not investigate long-term outcome of sevoflurane and desflurane administration after ischemia.

In conclusion, the use of sevoflurane and desflurane after ischemia were found to reduce histopathological injury and ischemic neurons when the injury was evaluated on early recovery period. Further investigation is required to determine the long-term outcome effects of sevoflurane and desflurane given after ischemia. This protective effect may be transient, so longer recovery periods should be studied.

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