Protective Effect of Isoflurane and Sevoflurane on Ischemic Neurons and Expression of Bcl-2 and ICE Genes in Rat Brain

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Objective To study the protective effect of volatile anesthetics, isoflurane and sevoflurane, on ischemic neurons after cerebral ischemia-reperfusion in rats and its possible molecular mechanism. Methods Rat cerebral ischemia-reperfusion model was developed by occlusion of the middle cerebral artery (MCA) and bilateral common carotid arteries (CCAs) 1 h after reperfusion. Using flow cytometry (FCM) and Northern blot hybridization, we calculated the number of apoptotic bodies and detected the expression of bcl-2 mRNA and interleukin-1β converting enzyme (ICE) mRNA. Results The apoptotic bodies in hippocampus analyzed by FCM peaked at appeared 24 h after reperfusion, and decreased about 54% and 40%, respectively, after treatment with isoflurane and sevoflurane, as compared with ischemic group. There was no significant difference in the expression of bcl-2 mRNA and ICE mRNA between the inhaled anesthetic groups and ischemic group in hippocampus 24 h after MCA/CCAs occlusion. Conclusion Isoflurane and sevoflurane partially inhibit apoptosis but have no significant effect on the expression of bcl-2 and ICE genes.

Key words: Isoflurane; Sevoflurane; Apoptosis; bcl-2; ICE

INTRODUCTION

Apoptosis, a type of programmed cell death, plays an important role in the embryogenesis, normal development and maintenance of many adult tissues. It is strictly regulated by the expression or activation of several genes and proteins. The proto-oncogene bcl-2 is one of such genes that were first cloned in a B cell lymphoma line. Bcl-2 is a member of a family of related genes encoding proteins that either promote (e.g., bax, bcl-xS) or suppress (e.g., bcl-2, bcl-xL) programmed cell death. Bcl-2 is expressed in cells that survive fetal development, and inhibits programmed cell death in many in vitro systems. In the nervous system, bcl-2 protects against various stimuli that induce apoptotic neuronal death.

Sevoflurane and isoflurane are volatile anesthetics suitable for both induction and maintenance of anesthesia[1]. Clinically, they produce a pattern of rapid recovery with little excitation on emergence[2]. Their capability of increasing cerebral blood flow[3] and preserve cerebral autoregulation[1] makes them an attractive agent for the preservation of neuronal function. There is some evidence that sevoflurane and isoflurane are neuroprotective agents[4]. However, the protection afforded by volatile anesthetics in later neuronal death, i.e. apoptosis, caused by local ischemia has not been investigated. In this study, the influence of isoflurane and sevoflurane on the apoptosis of ischemic neurons and the possible molecular mechanism were investigated.

MATERIALS AND METHODS

Materials

Adult male Wistar rats weighing 320-350 g were purchased from Animal Center, Chinese Academy of Medical Sciences. The materials used were as follows: isoflurane (Abbott Laboratories), sevoflurane (Maruishi Pharmaceutical Co, Osaka, Japan), propidium iodide (Sigma-Aldrich Co, USA), DNAase-free RNAase and RNA isolation kit (Promega Co., USA), nylon membrane (Hybond-N, Amersham International, UK), oligodeoxynucleotide probe to bcl-2 and ICE (Gibco Co., USA), α-32P-dATP (DuPont Co., USA), terminal deoxynucleotidyl-transferase (TdT) (Life Technologies, Gaithersburg, MD), operating microscope (×16, ×10) (Shanghai Medical Instruments Co. Ltd., China), microchip
Statistical analyses were performed by Student’s two sample t-test using SPSS 10.0 software. P<0.05 was considered statistically significant.
RESULTS

FCM Measurement

The number of apoptotic bodies reached the maximum 24 h post-reperfusion; the majority of cells were at G1/G2 of the cycle. As the time of reperfusion prolonged, the residual dead cells gradually increased and became the main content. It indicated that cells mainly died via necrosis thereafter. In groups 3 and 4, the number of apoptotic bodies decreased about 54% and 40%, respectively, compared with group 1 (*P < 0.01) (Fig.1).

Northern Blot Hybridization Analysis

The total RNA of bcl-2 mRNA in group 1 was shown at a low level. Compared with sham-ischemia group, its expression after 1 h ischemia increased to 1.73 folds at 8 h, 1.91 folds at 16 h, and 2.10 folds at 24 h post-reperfusion, respectively (Fig. 2). Compared with group 2, the bcl-2 mRNA expressions in groups 3 and 4 were 1.32 folds and 1.30 folds higher, respectively. There was no difference between anesthetic-inhaled groups and ischemic group. The ICE mRNA expression after 1 h ischemia increased 1.23 folds at 8 h, 1.35 folds at 16 h, and 1.47 folds at 24 h post-reperfusion, respectively, compared with sham-ischemia group (Fig. 2). The ICE mRNA expressions at 24 h post-reperfusion after 1 h ischemia in groups 3 and 4 were 0.87 fold and 0.91 fold higher than that in group 2. There was no difference between anesthetic-inhaled groups and ischemic group.

TUNEL Staining

There were almost no TUNEL-positive cells in the hippocampus in sham-operated group. Clear TUNEL-positive cells were detected in the CA1 field in the other groups. As noted in Fig. 3, the number of TUNEL-positive cells significantly decreased in anesthetic-inhaled groups compared with that in ischemic group.

DISSCUSSION

The present data show that incomplete cerebral ischemia could enhance the expressions of bcl-2 mRNA and ICE mRNA in rat hippocampus at 16 h and 24 h after 1 h cerebral ischemia and reperfusion, which could not be suppressed by volatile anesthetics, isoflurane and sevoflurane. The peak of apoptotic bodies in the FCM analysis appeared 24 h after reperfusion, and the peak of apoptosis treated with isoflurane or sevoflurane was lowered by about 54% and 40%, respectively, compared with ischemic group. These data indicate that the neuroprotective effects of volatile anesthetics, isoflurane and sevoflurane, may be related to the reduction of apoptosis neuron. Sevoflurane has been reported to be an effective neuroprotective agent in cerebral ischemia[8-9] in our study; it has been confirmed that the apoptosis of neurons could be inhibited by isoflurane and sevoflurane after ischemia-reperfusion. However,
compared with ischemic group, there was no difference between anesthetic-inhaled groups. Bcl-2 plays a key role in preventing the entry of cytochrome C into cytoplasm. It also plays a role in stabilizing mitochondria and maintaining its membrane potential, thus preventing the generation of free radicals. Bcl-2 also regulates calcium flux into the mitochondria. Thus, the expression of bcl-2 in neurons induced by ischemia might be an important event that ensures neuronal survival\(^\text{10-11}\). This indicates that sevoflurane and isoflurane protect ischemic neurons not by inducing overexpression of bcl-2, but by some other mechanisms.

ICE shares homology with the gene ced-3, one of the death genes in the nematode Caenorhabditis elegans, and is the first identified member of the caspase family which is believed to execute apoptosis\(^\text{12}\). ICE and related members of the caspase family are involved in apoptosis; several recent studies support the involvement of ICE itself (caspase 1) in ischemic brain damage\(^\text{13-14}\). In this study, the expression of ICE mRNA did not change significantly after reperfusion and ischemia, suggesting that there is no relationship between the expression of ICE and the protection of sevoflurane and isoflurane against ischemic neurons.

REFERENCES


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