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SCIENTIFIC ARTICLE

Isoflurane provides neuroprotection in neonatal hypoxic ischemic brain injury by suppressing apoptosis

De-An Zhao*, **Ling-Yun Bi**, **Qian Huang**, **Fang-Min Zhang**, **Zi-Ming Han**

Department of Pediatrics, The First Affiliated Hospital of Xinxiang Medical University, Weihui, China

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KEYWORDS
Isoflurane;
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Abstract
Background and objectives: Isoflurane is halogenated volatile ether used for inhalational anesthesia. It is widely used in clinics as an inhalational anesthetic. Neonatal hypoxic ischemia injury ensues in the immature brain that results in delayed cell death via excitotoxicity and oxidative stress. Isoflurane has shown neuroprotective properties that make a beneficial basis of using isoflurane in both cell culture and animal models, including various models of brain injury. We aimed to determine the neuroprotective effect of isoflurane on hypoxic brain injury and elucidated the underlying mechanism.
Methods: A hippocampal slice, in artificial cerebrospinal fluid with glucose and oxygen deprivation, was used as an in vitro model for brain hypoxia. The orthodromic population spike and hypoxic injury potential were recorded in the CA1 and CA3 regions. Amino acid neurotransmitters concentration in perfusion solution of hippocampal slices was measured.
Results: Isoflurane treatment caused delayed elimination of population spike and improved the recovery of population spike; decreased frequency of hypoxic injury potential, postponed the onset of hypoxic injury potential and increased the duration of hypoxic injury potential. Isoflurane treatment also decreased the hypoxia-induced release of amino acid neurotransmitters such as aspartate, glutamate and glycine induced by hypoxia, but the levels of γ-aminobutyric acid were elevated. Morphological studies showed that isoflurane treatment attenuated edema of pyramid neurons in the CA1 region. It also reduced apoptosis as evident by lowered expression of caspase-3 and PARP genes.
Conclusions: Isoflurane showed a neuro-protective effect on hippocampal neuron injury induced by hypoxia through suppression of apoptosis.

* Corresponding author.
E-mail: zhaodean17@gmail.com (D.-A. Zhao).

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PALAVRAS-CHAVE
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Isoflurano fornece neuroproteção em lesão cerebral hipóxico-isquêmica neonatal por inibição da apoptose

Resumo
Justificativa e objetivos: Isoflurano é um éter volátil halogenado usado para anestesia por via inalatória. É amplamente utilizado na clínica como um anestésico para inalação. A lesão hipóxico-isquêmica neonatal ocorre no cérebro imaturo e resulta em morte celular tardia via excitotoxicidade e estresse oxidativo. Isoflurano mostrou possuir propriedades neuroprotetoras que formam uma base benéfica para o seu uso tanto em cultura de células quanto em modelos animais, incluindo vários modelos de lesão cerebral. Nosso objetivo foi determinar o efeito neuroprotetor de isoflurano em hipóxia cerebral e elucidar o mecanismo subjacente.
Métodos: Fatias de hipocampo, em fluido cerebrospinal artificial (CSFA) com glicose e privação de oxigênio, foram usadas como um modelo *in vitro* de hipóxia cerebral. O pico de população ortodrômica (PPO) e o potencial de lesão hipóxica (PLH) foram registrados nas regiões CA1 e CA3. A concentração de neurotransmissores de aminoácidos na solução de perfusão das fatias de hipocampo foi medida.
Resultados: O tratamento com isoflurano retardou a eliminação do PPO e melhorou a recuperação do PPO; diminuiu a frequência do PLH, retardou o início do PLH e aumentou a duração do PLH. O tratamento com isoflurano também diminuiu a liberação de neurotransmissores de aminoácidos induzida pela hipóxia, como aspartato, glutamato e glicina, mas os níveis de ácido γ-aminobutírico (GABA) estavam elevados. Estudos morfológicos mostram que o tratamento de edema com isoflurano atenuou o edema de neurônios piramidais na região CA1. Também reduziu a apoptose, como mostrado pela expressão reduzida da caspase-3 e genes PARP.
Conclusões: Isoflurano mostrou um efeito neuroprotetor na lesão neuronal no hipocampo induzida por hipóxia através da supressão de apoptose.

Introduction

Hypoxic brain injuries cause several pathologic conditions, which may also occur during neuro- and cardiac-surgeries and anesthesia. The mechanism underlying such hypoxic brain injury is still unclear. How to protect the brain from hypoxic injury and how to treat hypoxic brain injury remains clinically challenging. Hypothermia and pre-ischemia treatments have been shown protective effect on the brain[1,2] yet difficult to implement clinically, while pharmacological treatment clinically more practical. During the recent three decades, the neuroprotective effect of anesthetic drugs has drawn high attention from clinicians.

Isoflurane [2-chloro-2-[difluoromethoxy)-1; 1.1-trifluoroethane, CHF2-O-CHCl-CF3] is a halogenated ether used for inhalational anesthesia. Together with enflurane and halothane, it replaced the flammable ethers used in the pioneer days of surgery. Its name comes from being a structural isomer of enflurane, hence they have the same empirical formula. It is a racemic mixture of (R) and (S) optical isomers. Its use in human medicine is now starting to decline, being replaced with sevoflurane, desflurane, and the intravenous anesthetic propofol. Isoflurane is still frequently used for veterinary anesthesia. Propofol could reduce arterial blood flow in brain, intracranial pressure, and metabolism maintaining blood supply and oxygen ratio. It improved the oxygen supply during hypoxia suggesting protective effects of propofol against hypoxic brain damage.[3–5] Studies suggest that propofol plays a role in central nervous system (CNS) protection through the modulation of Ca^{2+}, oxygen free radicals, γ-aminobutyric acid (GABA) receptor and N-methyl-D-aspartate (NMDA) receptor.[6–9] Yet some data suggest that propofol had no brain protective effect after cardiac surgery and even worsened brain hypoxia[10] and that hypothermia is neuroprotective rather than propofol.[1] The volatile anesthetics all differ in potency, adverse effects, and cost, and are used extensively during surgery in human neonates and during neonatal animal research. Isoflurane was reviewed to have neuroprotective functions in studies with neonatal hypoxic ischemic brain injury.[11] Isoflurane has been studied in animal models of various diseases, such as lipopolysaccharide (LPS)-induced acute inflammation of the lung,[12] acute lung injury,[13] glucose-induced oxidative stress,[14] renal ischemia/reperfusion injury,[15] and cardiac injury.[16] Isoflurane was shown to provide protection from injury and improve various negative functional outcomes in these models.

Neonatal hypoxia ischemia is a major cause of mortality and neurological deficits such as cerebral palsy, mental retardation, and epilepsy in the perinatal period.[17] Several pathophysiological factors have been implicated in the hypoxia ischemia, including inflammatory mediators, excitotoxicity, and oxidative stress.[18] The use of isoflurane as

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