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Research Report

Exposure of isoflurane-treated cells to hyperoxia decreases cell viability and activates the mitochondrial apoptotic pathway

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ABSTRACT

Isoflurane has either neuroprotective or neurotoxic effects. High-dose oxygen is frequently used throughout the perioperative period. We hypothesized that hyperoxia will affect cell viability of rat pheochromocytoma (PC12) cells that were exposed to isoflurane and reactive oxygen species (ROS) may be involved. PC12 cells were exposed to 1.2% or 2.4% isoflurane for 6 or 24 h respectively, and cell viability was evaluated. To investigate the effects of hyperoxia, PC12 cells were treated with 21%, 50%, or 95% oxygen and 2.4% isoflurane for 6 h, and cell viability, TUNEL staining, ROS production, and expression of B-cell lymphoma 2 (BCL-2), BCL2-associated X protein (BAX), caspase-3 and beta-site APP cleaving enzyme (BACE) were measured. ROS involvement was evaluated using the ROS scavenger 2-mercaptopropiopylglycine (MPG). The viability of cells exposed to 2.4% isoflurane was lower than that of cells exposed to 1.2% isoflurane. Prolonged exposure (6 h vs. 24 h) to 2.4% isoflurane resulted in a profound reduction in cell viability. Treatment with 95% (but not 50%) oxygen enhanced the decrease in cell viability induced by 2.4% isoflurane alone. Levels of ROS, Bax, caspase-3 and BACE were increased, whereas expression of Bcl-2 was decreased, in cells treated with 95% oxygen plus 2.4% isoflurane compared with the control and 2.4% isoflurane plus air groups. MPG attenuated the effects of oxygen and isoflurane. In conclusion, isoflurane affects cell viability in a dose- and time-dependent manner. This effect is augmented by hyperoxia and may involve ROS, the mitochondrial apoptotic signaling pathway, and β-amyloid protein.

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1. Introduction

Isoflurane is a volatile anesthetic that is commonly used in general anesthesia during surgery. Isoflurane has long been considered safe, and even neuroprotective, in cell cultures and animal models (Lee et al., 2008; Sakai et al., 2007). However, recent studies have suggested that isoflurane can induce apoptosis and might even lead to the accumulation of β-amyloid protein (Aβ), the hallmark feature of Alzheimer's disease (Fodale et al., 2010; Xie et al., 2006, 2007, 2008). Furthermore, it has been suggested that whether isoflurane is neuroprotective or neurotoxic depends on the concentration and exposure duration (Pan et al., 2011; Wei at al., 2005; 2007).

Apoptosis refers to programmed cell death that can be triggered by environment- and/or development-associated signals. The pathway by which isoflurane induces apoptosis has not yet been elucidated, but there are several hypothetical mechanisms. The first is the intrinsic mitochondrial apoptotic pathway, which is regulated by the B-cell lymphoma 2 (Bcl-2) family proteins, including the anti-apoptotic factor Bcl-2 and the pro-apoptotic factor Bcl-2-associated X protein (Bax), and involves cytochrome c release from the mitochondria into the cytosol (Zhang et al., 2010). The released cytochrome c then activates caspase-9, which consequently induces caspase-3 activation, leading to apoptosis. Second, previous studies have suggested that isoflurane may induce apoptosis by elevating cytosolic calcium levels (Wei and Anesthesia, 2009). Finally, accumulation of reactive oxygen species (ROS) has been suggested to be associated with mitochondrial damage, thereby leading to apoptosis (Szeto, 2008; Zhang et al., 2010). Apoptosis increases the level of β-site amyloid precursor protein (APP)-cleaving enzyme (BACE), which facilitates APP processing and increases the generation of Aβ (Zhen et al., 2009).

The carrier gas in anesthesia is composed of nitrous oxide/oxygen, medical air/oxygen, or pure oxygen. Although some studies have suggested that isoflurane together with nitrous oxide or hypoxia induces apoptosis and stimulates the accumulation of Aβ in neuronal cells, few reports have investigated the effects of isoflurane with pure oxygen (Pan et al., 2011; Zhen et al., 2009).

Oxygen is an important element of life and there is evidence that oxygen plays a beneficial role in the treatment of traumatic brain injury, stroke, and Alzheimer's disease (Kumaria and Tolias, 2009; Yis et al., 2008). However, a high concentration of oxygen may cause central nervous system (CNS) toxicity (Arendash et al., 2009).

We hypothesized that hyperoxia will affect cell viability of rat pheochromocytoma (PC12) cells that were exposed to isoflurane and reactive oxygen species (ROS), the mitochondrial apoptotic signaling pathway, and β-amyloid protein may be involved. To prove this hypothesis, we sought to determine the effects of hyperoxia on the viability of rat pheochromocytoma (PC12) cells exposed to isoflurane.

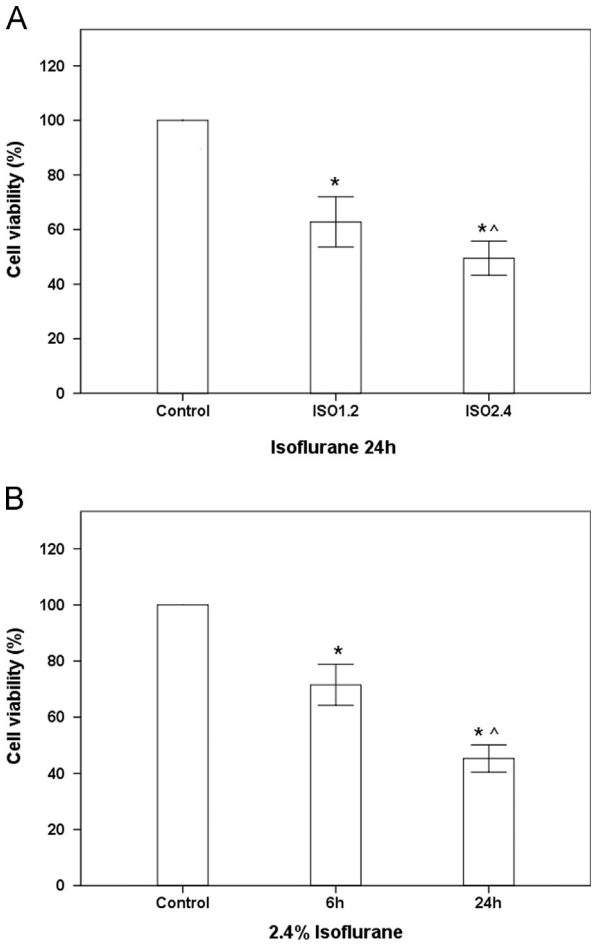

Fig. 1 – Isoflurane exposure caused a concentration- (A) and exposure time- (B) dependent decrease in cell viability. (A) Exposure of cells to 1.2% isoflurane in air for 24 h significantly decreased cell viability, and exposure to 2.4% isoflurane further decreased viability. (B) Exposure to 2.4% of isoflurane for 6 h decreased cell viability significantly, and an even further decrease was measured at 24 h. Cell viability was measured using the water-soluble tetrazolium salt (WST-1) assay. Bars represent mean (SD) and are expressed as a percent of the control ($n=10–15$). $^{*}P<0.05$ compared to control. $^{\#}P<0.05$ compared to 1.2% isoflurane or 2.4% isoflurane exposure for 6 h. ISO 1.2=1.2% isoflurane, ISO 2.4=2.4% isoflurane.

2. Results

2.1. *Exposure of PC12 cells to isoflurane resulted in a concentration- and exposure time-dependent decrease in cell viability*

Exposure of cells to 1.2% isoflurane with air for 24 h significantly decreased cell viability, and an even greater decrease in viability was observed for cells exposed to 2.4% isoflurane for 24 h (Fig. 1A). In addition, cell viability decreased with increasing exposure time (Fig. 1B).