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Research report

The additional oxygen as a carrier gas during long-duration sevoflurane exposure ameliorate the neuronal apoptosis and improve the long-term cognitive function in neonatal rats

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ABSTRACT

The effects of the oxygen concentration as a carrier gas and long duration anesthesia exposure on neuroapoptosis and cognitive impairments in the developing brain are not fully understood. This study shows that long-duration sevoflurane anesthesia with or without additional oxygen induces neuroapoptosis and long-term cognitive dysfunction in neonatal rats. Seven-day-old rats were exposed to sevoflurane anesthesia for 2, 4, and 6 h with 21% or 30% oxygen. The control group received 21% oxygen alone for 6 h. Post-anesthesia blood gas analysis resulted in hypoxia and hypercapnia. Moreover, PO_2 and base excess in the 30% oxygen group were significantly higher than the 21% oxygen group. The numbers of caspase-3-positive cells in both cortical layer 3 and the CA1 region in the hippocampus in the 6 h anesthesia exposure group with 21% oxygen were increased compared with the 6 h anesthesia exposure with 30% oxygen and control groups. Cognitive function was assessed in an additional group of rats, and the brains were stained for NeuN 6 weeks post-anesthesia. Although the Morris water maze task was acquired equally by all rats 3 weeks post-anesthesia, the escape latency was significantly longer in the 6 h sevoflurane with 21% oxygen group than the 6 h with 30% oxygen groups 6 weeks post-exposure. No difference was found with regard to freezing time among the groups in the fear conditioning test. The number of NeuN-positive cells in the CA1 region of the hippocampus in the control group was increased compared with the other groups. These findings indicate that long-duration sevoflurane exposure with 30% oxygen as a carrier gas would ameliorate neuronal apoptosis and improve long-term cognitive function in neonatal rats.

1. Introduction

Many preclinical studies have investigated the neuronal toxicity induced by general anesthesia in neonatal animals and have shown that general anesthetics induce profound neuronal apoptosis, degeneration in the developing brain and produce long-term neurocognitive deficits (Disma et al., 2016; Robert, 2010; Sanders et al., 2013; Stratmann, 2011; Walters and Paule, 2016). Moreover, there are many studies of showing the toxicity of inhalational anesthetic sevoflurane during the neonatal period (Amrock et al., 2015; Bercker et al., 2009; Briner et al., 2016; Fang et al., 2012; Istaphanous et al., 2011; Ju et al., 2016; Kodama et al., 2011; Lee et al., 2014; Liang et al., 2010; Lu et al., 2010; Ramage et al., 2013; Seubert et al., 2013; Shen et al., 2013; Wu et al., 2014; Zhang et al., 2008; Zheng et al., 2013). These reports used oxygen as a carrier gas at various concentrations, ranging from 25% to 100%. Although the use of oxygen as a carrier gas for sevoflurane anesthesia is necessary and might affect neurocognitive function in neonatal rodents, the concentration of oxygen used as a carrier gas in experimental studies investigating the toxic effects of this anesthetic in neonatal animals has not been reported. It is not clear that the carrier gas using the air only would deteriorate the neurotoxicity of sevoflurane in neonates. It can be supposed that additional oxygen would be more important than normoxic exposure of anesthesia in critical situation such as developing brain.

Multiple exposures to anesthesia lead to neuronal degeneration when compared to a single exposure (Amrock et al., 2015; Ji et al., 2016; Zhang et al., 2015). Therefore, increased exposure to anesthesia might result in worse outcomes in neonates (Disma et al., 2016; Sinner et al., 2014).

The hypotheses of this study were a long-duration sevoflurane exposure with a normoxic carrier gas would deteriorate the

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neurotoxic effects and worsen long-term cognitive function compared with the group added oxygen in developing brain. Thus, this study examined the effect of oxygen concentration and the duration of sevoflurane exposure on: (i) blood gas analyses and caspase-3 staining immediately post-sevoflurane exposure, and (ii) long term cognitive function and histological changes in later life following sevoflurane exposure.

2. Results

2.1. Blood gas analysis

The results from the blood gas analysis performed immediately after anesthesia exposure are shown in Table 1 (n = 7 in each group). Although slight acidosis, hypercapnia and hypoxemia were seen in the group due to the exposure to sevoflurane, but there were no significant differences of PCO_2 and base excess compared with the control group. According to the two-way ANOVA for sevoflurane exposed group, PO_2 and base excess in the 30% oxygen group were significantly higher than the 21% oxygen group. Namely, there was the significant interaction about O_2 concentration in both PO_2 and base excess, not but duration of exposure in the sevoflurane exposed group.

2.2. Caspase-3 staining

Fig. 1 shows typical caspase-3-positive cells and Fig. 2 shows the number of caspase-3-positive cells per 0.1722 mm^2 in the cortex and CA1 region of the hippocampus in all groups immediately after sevoflurane exposure (n = 5 in each group). The number of caspase-3-positive cells in both the cortex (26 ± 4) and hippocampus (12 ± 5) in the 6 h anesthesia exposure with 21% oxygen group, was significantly increased compared with the 6 h with 30% oxygen (15 ± 6, 6 ± 2, cortex and hippocampus, respectively) and control groups (0.3 ± 0.5, 0, cortex and hippocampus, respectively) (Fig. 2).

2.3. Morris water maze

The escape latency and swimming path length to the hidden platform in the acquisition trials during P27–29 are shown in Fig. 3. All rats acquired the task equally during this period. The escape latency was significantly longer in the 6 h sevoflurane exposure with 21% oxygen group (28 ± 17 s) than in the 4 h (11 ± 11 s) and 6 h (10 ± 9 s) exposure with 30% oxygen groups 6 weeks post-anesthesia (P47, Figs. 3 and 4(A)). There were significant differences between the groups with regard to the escape latency and the swimming times at P47, though not at P48 and P49. No significant differences were found among the groups with regard to the swimming length.

2.4. Fear conditioning test

The freezing time to tone in 6 h sevoflurane exposure with 30% oxygen group were similar with the 6 h sevoflurane group with 20% oxygen at P49, and there were no significant differences among the groups (Fig. 4(B)).

2.5. NeuN staining

Fig. 5 shows the NeuN staining in each slice at −2 mm caudal to bregma. The number of NeuN-positive cells per 0.15 mm^2 in the CA1 region of the hippocampus in the control group was significantly increased compared with that of the other groups, whereas there were no differences between 20% and 30% oxygen in the sevoflurane treated groups (n = 5 in each group) (Fig. 6). Moreover, the number of NeuN-positive cells in the cortex in 6 h sevoflurane exposure with 30% oxygen group was similar with the 20% oxygen group (Fig. 6).

2.6. Positive cell density map (PCDM) and statistical parameter mapping of positive cell density

Fig. 7(A) shows the NeuN PDCM for each group, and indicates a tendency towards an increase in the cortical PDCM. The differences among the groups are shown in more detail in Fig. 7(B). Fig. 7(B) shows that the control group had a significantly increased NeuN-positive cell density compared to the anesthesia-exposed groups and the cell density in the 30% oxygen group had a significantly increased compared with the 20% group. The differences show the regions indicated in red. Although we did not find any hippocampal differences, increased expression was observed in other brain regions in both of the 6 h anesthesia exposure groups, indicating that the numbers of normal cells in the 6 h anesthesia exposure groups were reduced compared to the control group. The several areas in red in the 2 h and 4 h sevoflurane exposure with 30% oxygen groups were found, indicating that the numbers of the normal cells in the 30% oxygen group were higher than in the 20% oxygen group in cortex and hippocampus.

3. Discussion

The present study shows that long-duration sevoflurane exposure induces an immediate increase in the number of caspase-3-positive cells. Moreover, sevoflurane exposure continues to affect long-term spatial memory retention 6 weeks post-exposure. These results were improved by the additional oxygen with sevoflurane exposure.

The results of this study with regard to the anesthesia related neural degeneration and cognitive impairments are consistent with many previous reports (Amrock et al., 2015; Bercker et al., 2009; Briner et al., 2016; Fang et al., 2012; Istaphanous et al., 2011; Ji et al., 2016; Kodama et al., 2011; Lee et al., 2014; Lei et al., 2012; Liang et al., 2010; Lu et al., 2010; Ramage et al.,

Table 1
Blood gas analysis after anesthesia exposure.

Group	Control (n = 7)	30%2h (n = 7)	21%2h (n = 7)	30%4h (n = 7)	21%4h (n = 7)	30%6h (n = 7)	21%6h (n = 7)
pH	7.391 ± 0.03	7.231 ± 0.08*	7.232 ± 0.06*	7.261 ± 0.08*	7.261 ± 0.07*	7.270 ± 0.07*	7.231 ± 0.07*
PCO_2 (mmHg)	52.7 ± 2.6	70.2 ± 10.1	67.2 ± 12.0	71.1 ± 13.9	67.3 ± 11.9	70.9 ± 11.8	67.7 ± 8.7
PO_2 (mmHg)	82.8 ± 11.1	60.3 ± 11.5*	51.2 ± 8.0*	60.4 ± 7.5*	53.0 ± 4.6*	57.4 ± 7.7*	55.0 ± 6.8*
Base excess	2.1 ± 3.2	1.9 ± 2.7	0.2 ± 3.0	1.6 ± 0.4	0.6 ± 2.1	1.8 ± 0.9	0.6 ± 2.6

PO_2 and base excess in the 30% oxygen group were significantly higher than the 21% oxygen group among the sevoflurane exposed groups. Nevertheless, there were no significant differences among the sevoflurane exposed groups due to multiple comparisons. *P < .05 vs control group. 21%2h = 2 h sevoflurane with 21% oxygen, and other groups are expressed in the same way. Data are expressed as mean ± SD.