Isoflurane Impairs Motor Function Recovery by Increasing Neuroapoptosis and Degeneration during Spinal Ischemia-Reperfusion Injury in Rats

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INTRODUCTION. Spinal cord ischemia is one of the major concerns of postoperative paraplegia following major vascular or aortic surgery. The incidence of postoperative paraplegia during aortic surgeries is reported to be 0.5%-1.5% for coarctation repair, 10% for thoracic aneurysm repair, and up to 20% for thoracoabdominal aorta repair. Isoflurane is one of the most commonly used inhalation agents in major cardiovascular surgery and it has been shown to produce neuroprotective responses in cerebral ischemia and spinal cord ischemia. On the contrary, experimental studies indicate that isoflurane is potentially neurotoxic during ischemia-reperfusion injury (IRI) and in cognitive disorders by generation of neurotoxic peptides. Therefore, the effects of perioperative administration of volatile isoflurane on the recovery of neurological function in major vascular operations involving spinal cord ischemia remained undetermined. Using a rat model of spinal cord IRI, we analyzed the functional recovery and molecular changes in the spinal cord.

METHODS. Rats were randomly assigned to receive intramuscular injection of a cocktail anesthetic Zoletil® (50 mg/kg, Virbac) or Zoletil® (30 mg/kg) (i.e. zoletil only or zoletil+isoflurane groups, respectively) for induction of anesthesia. During the perioperative period, pure oxygen or isoflurane (1.5% v/v in oxygen) was delivered to the zoletil-treated or isoflurane-treated animals, respectively. Each ml of Zoletil contains tiletamine HCl 125 mg and zolazepam base 125 mg. Cord ischemia was induced by cross-clamping of thoracic aorta at the level of T5, and cord perfusion was resumed after 25 min. Cessation of blood flow in the lumbar cord was confirmed by absence of Evans blue dye following systemic perfusion (Figure 1). The motor function [Basso-Beattie-Bresnahan (BBB) scale (range 0-21)] was assessed independently up to 48 h after reperfusion. Spinal cords were harvested and analyzed for molecular and histological changes.

RESULTS. Six hours after release of aortic clip, the BBB locomotor rating scales were reduced in the hindlimbs of animals with spinal IRI (Figure 2), suggesting the presence of motor dysfunction due to ischemic cord syndrome. The locomotor function was gradually recovered in the rats received only Zoletil®, and was almost returned to high BBB rating scale (>16) at 48 h (Figure 2). However, the locomotor function was significantly deteriorated (<5) in the isoflurane+Zoletil® group, and most of them eventually became paralysis at 48 h after operation (Figure 2). In animals treated with isoflurane, the expression of iNOS was suppressed and HO-1 was up-regulated in the spinal cord after SCI (Figure 3). Compared with controls, the concentration of IL-6 was reduced in the spinal cord of isoflurane-treated rats with similar activity of myeloperoxidase. The protein expression of GFAP, a biomarker of neurodegenerative process, was significantly enhanced in the spinal cord of rats anesthetized with isoflurane (Figure 4). Consistent with GFAP expression, perioperative exposure to isoflurane also significantly induced the cleaved-caspase-3 and Iba-1 in the ischemic spinal cord (Figure 5). Increased numbers of apoptotic cells in the lumbar spinal cord of isoflurane treatment was observed (Figure 6). Cell markers of neuronal degeneration (GFAP, caspase-3 and Iba-1) were highly expressed in the spinal cord of rats anesthetized with isoflurane (Figure 7A). In the spinal cord subjected to IRI, more damaged motor neurons were identified in the ventral horn of grey matter of rats received isoflurane anesthesia (Figure 7B). Histological sections did not show increased infiltration of inflammatory cells in the spinal cord of isoflurane-treated animals (Figure 7B).

Figure 1. Rat model of spinal cord ischemia. Thoracic cavity was exposed at the left T5-6 intercostal space (A & B) and vascular clip was applied to the thoracic aorta to induce hypoperfusion in the lumbar spine. Complete abolish of blood flow was confirmed by absence of Evans blue dye following systemic perfusion (C).

Figure 2. The BBB scale (range 0-21) was used to assess the hind limb locomotor function after reperfusion. Compared with Zoletil (●), the BBB scales were significantly lower in the isoflurane-treated animals (○). Open boxes (□) indicate naïve treatment. *P<0.001 Zoletil vs isoflurane. n= 7-10 in each group.

Figure 3. Protein expressions of inducible nitric oxide synthase (iNOS) and heme oxygenase (HO)-1 in the tissue homogenates of spinal cord at 12-24h (A) and 48h (B) after spinal cord ischemia (SCI).. *P<0.05 Zoletil vs isoflurane. n= 5-6 in each group.

Figure 4. Expressions of glial fibrillary acidic protein (GFAP) in the spinal cord at 12h-24h (A) and 48h (B) after spinal cord ischemia (SCI).. Data are presented as mean±SD. *P<0.05 Zoletil vs isoflurane. n= 5-6 different animals.

Figure 5. Expressions of cleaved caspase-3 and Iba-1 in the spinal cord at 24h (A) and 48h (B) after spinal cord ischemia (SCI). Data are presented as mean±SD. *P<0.05 Zoletil vs isoflurane. n= 5-6 in each group.

Figure 6. Significantly higher ApoptTag®-fluorescent densities were detected in the spinal cord harvested from isoflurane-treated rats. *P< 0.001 Zoletil vs isoflurane.

Figure . (A) Representative immunofluorescence sections of spinal cord stained for GFAP (green) and astrocytes S100β (red), caspase-3 (green)/ nuclei (neuN in red) and Iba-1 (green)/neuN (red). The neurodegenerative cell markers were highly expressed in the injured cord of rats anesthetized with isoflurane. **(B)** Histological sections by HE stain (upper 3 panels) and Nissl stain (last panel). Loosen Nissl substances, pyknotic homogenous nuclei and formation of vacuolation were observed in the ventral horn of grey matter of rats received isoflurane anesthesia. n=3-4 different animals in each group.

CONCLUSION

Our study demonstrated that pretreatment with isoflurane suppressed the regional inflammatory burden in the ischemic cord that was resemble with anesthetic preconditioning response, but the locomotor function was significantly impaired in these animals. The adverse effect of isoflurane on the regeneration of spinal cord subjected to IRI was derived from the neurodegenerative process and neuronal apoptosis. This study underscores that administration of isoflurane as an anesthetic agent in patients at risk of cord ischemia could result in detrimental effect on the functional recovery of spinal cord.

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