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Lower doses of isoflurane treatment has no beneficial effects in a rat model of intracerebral hemorrhage

Elga Esposito*, Emiri T Mandeville and Eng H Lo*

Abstract

**Background:** Intracerebral hemorrhage is a subtype of stroke that has a poor prognosis without an adequate therapy. Recently, the use of anesthetics such as isoflurane has been shown to be protective after cerebral ischemia. However, the potential therapeutic effect of isoflurane after intracerebral hemorrhage (ICH) has not been fully explored.

**Results:** In this study, male Sprague–Dawley rats (SD) were subjected to ICH and randomized into controls and 1.2% or 1.5% isoflurane posttreatment groups. Brain water content, neurological outcomes and matrix metalloproteinase-2 and -9 (MMP2-MMP9) plasma levels were quantified at 24 hours. Isoflurane treatment did not reduce brain edema compared with controls in any of the applied isoflurane concentrations. Moreover, consistent with this lack of effect on brain edema, isoflurane posttreatment did not affect neurological outcomes in any of the tests used. Plasma MMP levels did not change.

**Conclusion:** Our data suggested that there is no neuroprotection after isoflurane posttreatment in a rat model of ICH.

**Keywords:** Anaesthesia, Neuroprotection, Brain edema, Intracerebral hemorrhage, Animal models

Background

Intracerebral hemorrhage (ICH) is a subtype of stroke with the highest mortality of all stroke subtypes. ICH is characterized by extravasation of blood into the brain parenchyma with hematoma formation and concomitant edema and secondary brain damage [1,2]. The prognosis after ICH is poor and no widely effective treatments exist. Recently, two endogenous neuroprotective mechanisms have been shown to be broadly effective after ischemic stroke: ischemic preconditioning and ischemic postconditioning [3,4]. These two strategies are thought to recruit natural adaptive responses that brain and other organs utilize to protect themselves from various insults. What is interesting is that many of the conditioning pathways are highly conserved, i.e. protection can be induced by a wide range of sublethal triggers [5,6]. Recently, isoflurane has been shown to be highly effective conditioning inducer in various animal models of cerebral injury. This may be translationally relevant since isoflurane can be easily applied in a clinical setting. In particular, isoflurane postconditioning had been widely demonstrated to be protective after the ischemia [7]. Here, we ask whether isoflurane may also have some neuroprotective effect when administrated after ICH.

**Methods**

**Rat intracerebral hemorrhage model**

All experiments were performed following protocols approved by Massachusetts General Hospital Institutional Animal Care and Use Committee in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All procedures were performed in a blinded and randomized fashion. ICH was induced by collagenase injections. Male Sprague–Dawley rats (7 rats for each group) (Charles River Laboratories, Wilmington, MA) were anesthetized with isoflurane (1.2%) in 30%/70% oxygen/nitrous oxide. A catheter in the tail artery was used for measuring blood pressure, pH, PaO₂, and PaCO₂. Using a stereotactic frame, 1 μL of saline containing 0.5 U collagenase VII-S (Sigma) was...
injected through a 25-gauge needle over 5 minutes into the right striatum (from bregma: 1.2 mm anterior, 3.5 mm lateral, 5.3 mm depth). 10 minutes after the collagenase injection, rats were randomized into isoflurane (1.2% and 1.5% isoflurane through a face mask for 1 hour) versus control groups. All endpoints were assessed in a blinded manner.

**Measurement of brain edema**
A standard wet–dry method was used for the measurement of total brain water content as an indicator of edema. At 24 hours after ICH, rats were euthanized and brains were removed immediately, without fixation. A single 4-mm section was cut centered around the hematoma, and edema was calculated as: [(wet weight – dry weight)/wet weight] × 100%.

**Gelatin zymography**
Matrix metalloproteinase-2 and −9 levels in plasma were assessed with standard gelatin zymography. EDTA blood samples were immediately centrifuged at 10000 rpm for 10 minutes to obtain supernatants. Equal amounts of prepared protein samples were loaded and separated. After electrophoretic separation, the gel was renaturated and then incubated with developing buffer at 37°C for 20 hours. After developing, the gel was stained and then destained appropriately. Proteolytic bands in the zymography were quantified via densitometry.

**Neurological tests**
The rats were assessed blindly at 24 hours using 3 tests: forelimb placement, body swing test, and a 5-point neuroscore scale. For the forelimb-placing test, animals were held close to a tabletop and scored for the ability...
to place the forelimb on the tabletop in response to whisker, visual, tactile, or proprioceptive stimulation. For the body swing test, animals were held approximately one inch from the base of their tails and elevated to an inch above a surface of a table. A swing was recorded whenever the rat moved its head out of the vertical axis to either side by more than 10° from vertical and then returned to the vertical position. Thirty total swings were counted per animal. Neuroscores were graded as 0 = no apparent deficit; 1 = slight deficit; 2 = circling; 3 = heavy circling or no movement at all; or 4 = death.

Statistic analysis
Values are expressed as means ± S.D. Statistical analysis was performed with ANOVA, followed by Newman-Keuel’s test. Neurological outcomes (forelimb placement test, swing test, neuroscore) were analyzed using the nonparametric Kruskal-Wallis test. Statistical significance was accepted at the 95% confidence level (p < 0.05).

ARRIVE (animal research: reporting in vivo experiments) guidelines
All experiments were performed following protocols approved by Massachusetts General Hospital Institutional Animal Care and Use Committee in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All studies herein, including experimental design, animal care and use, randomization, blinding, statistical powering and analyses, inclusion/exclusion criteria, outcomes etc. are explicitly defined following ARRIVE guidelines.

Results
As expected, this standard model of collagenase injections resulted in reproducible intrastriatal hematomas. There were no statistically significant differences in hematoma volumes in controls vs 1.2% or 1.5% isoflurane treatment groups. Accordingly, brain edema developed in all groups - brain water content in the ipsilateral hemisphere was significantly greater than the contralateral hemisphere (control group: ipsilateral, 81.5% ± 0.8% versus contralateral, 78.9% ± 0.4% P < 0.05; 1.2% isoflurane group: ipsilateral, 81.6% ± 0.2% versus contralateral, 78.8% ± 0.6% P < 0.05; 1.5% isoflurane group: ipsilateral, 81.3% ± 0.4% versus contralateral, 78.7% ± 0.3% P < 0.05). However, isoflurane treatment did not reduce brain edema compared with controls in any of the applied isoflurane concentrations (P = 0.653) (Figure 1).

Consistent with this lack of effect on brain edema, isoflurane posttreatment did not affect neurological outcomes in any of the tests used (Figure 2A,B,C). In the forelimb placement test, 1.2% and 1.5% isoflurane had no effect on the outcomes (control ipsilateral group, 5.43 ± 1.8; 1.2% isoflurane ipsilateral group, 6.33 ± 1.15;
Our data demonstrated that isoflurane posttreatment well-established rat model of collagenase-induced ICH. It is illustrated after ICH. May also have some neuroprotective effect when administered with 1.2 or 1.5% isoflurane, as showed during ischemic postconditioning, a sublethal ischemic episode applied before a longer harmful ischemia [9], and ischemic postconditioning, a sublethal ischemia subsequent to a prolonged harmful ischemic episode, are both able to produce remarkable neuroprotection in various models of stroke and neuronal injury. However, preconditioning or postconditioning with an ischemic stimulus, although useful for proof-of-concept and dissecting molecular mechanisms, may be difficult to apply in a clinical setting. More recently, it has been suggested that conditioning may also be stimulated with various pharmacologic approaches. Emerging studies now suggest that the widely used anesthetic isoflurane can induce postconditioning and protect heart and brain against ischemia [10]. However, the potential therapeutic effect of isoflurane has not been fully explored after intracerebral hemorrhage (ICH). Here, we ask whether isoflurane, as showed during ischemic postconditioning, may also have some neuroprotective effect when administered after ICH.

Two concentrations of isoflurane were tested in a well-established rat model of collagenase-induced ICH. Our data demonstrated that isoflurane posttreatment did not affect edema. Since neurovascular damage after ICH is closely related to MMP activation [11,12], we also assessed potential MMP biomarker levels in plasma. Once again, no effects of isoflurane were detected. Finally, consistent with the lack of morphological or biochemical effects, there were no differences in a wide battery of neurological tests at 24 hrs post-ICH. The observed lack of beneficial effects following isoflurane treatment in the present study may be due to the lower doses tested here. Blood pressure effects prevented us from assessing higher isoflurane concentrations. However, we cannot unequivocally exclude the possibility that higher doses may have had beneficial effects in this ICH model system.

In contrast to our negative results, Khatibi et al. [13] recently suggested that 1.5% isoflurane posttreatment significantly reduced peri-hematoma edema, ameliorated apoptotic cell death and rescued neurologic function in a different model of ICH. These different results could be related to a species difference (mice vs rats) or to the different methods used to produce ICH. In the previous Kathibi et al. study, ICH was induced by infusing autologous blood into the striatum of mice. In the present study, collagenase injections were used to produce cerebrovascular rupture and hemorrhage in rat brains. There are no perfect animal models of ICH. But the potential advantage of the collagenase model is that it replicates the primary vascular trauma and rupture that is present in human ICH [14]. However, a potential drawback is that compared to the blood infusion model, the collagenase approach may be associated with altered inflammatory responses [15].

Discussion

In the last few years, many advances have been obtained in term of dissecting the signaling pathways activated by endogenous neuroprotective mechanisms after an ischemic insult [8]. Interestingly, it has been recently reported that ischemic preconditioning, a sublethal ischemic episode applied before a longer harmful ischemia [9], and ischemic postconditioning, a sublethal ischemia subsequent to a prolonged harmful ischemic episode, are both able to produce remarkable neuroprotection in various models of stroke and neuronal injury. However, preconditioning or postconditioning with an ischemic stimulus, although useful for proof-of-concept and dissecting molecular mechanisms, may be difficult to apply in a clinical setting. More recently, it has been suggested that conditioning may also be stimulated with various pharmacologic approaches. Emerging studies now suggest that the widely used anesthetic isoflurane can induce postconditioning and protect heart and brain against ischemia [10]. However, the potential therapeutic effect of isoflurane has not been fully explored after intracerebral hemorrhage (ICH). Here, we ask whether isoflurane, as showed during ischemic postconditioning, may also have some neuroprotective effect when administered after ICH.

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References
1. Katsuki H: Exploring neuroprotective drug therapies for intracerebral hemorrhage. J Pharmacol Sci 2010, 114(4):366–378.
2. Lok J, Leung W, Murphy S, Butler W, Noviski N, Lo EH: Intracranial hemorrhage: mechanisms of secondary brain injury. Acta Neurochir Suppl 2011, 111:53–69.
3. Obrenovitch TP: Molecular physiology of preconditioning-induced brain tolerance to ischemia. Physiol Rev 2008, 88(1):211–247.
4. Pignataro G, Scorziello A, Di Renzo G, Matthysse M, Lo EH: Post-ischemic brain damage: effect of ischemic preconditioning and postconditioning and identification of potential candidates for stroke therapy. FEBS J 2009, 276(1):46–57.
5. Burda J, Danielova V, Heinen A, Weber NC, Hollmann MW, Schlack W, Preckel B: Postconditioning by xenon and hypothermia in the rat heart in vivo. Eur J Anaesthesiol 2010, 27(8):734–739.
6. McMurtrey RJ, Zuo Z: Isoflurane preconditioning and postconditioning in rat hippocampal neurons. Brain Res 2010, 1358:184–190.
7. Zhao H, Sapolsky RM, Steinberg GK: Interrupting reperfusion as a stroke therapy: Ischemic postconditioning reduces infarct size after focal ischemia in rats. J Cereb Blood Flow Metab 2006, 26(9):1114–1121.
8. Gidday JM: Cerebral preconditioning and ischaemic tolerance. Nat Rev Neurosci 2006, 7(6):437–448.
9. Lee JJ, Li L, Jung HH, Zuo Z: Postconditioning with isoflurane reduced ischemia-induced brain injury in rats. Anesthesiology 2008, 108(6):1055–1062.
10. Del Bigio MR, Yan HJ, Buijt R, Peeling J: Experimental intracerebral hemorrhage in rats. Magnetic resonance imaging and histopathological correlates. Stroke 1996, 27(12):2322–2329.
11. Rosenberg GA, Mun-Bryce S, Wesley M, Kornfeld M: Collagenase-induced intracerebral hemorrhage in rats. Stroke 1990, 21(5):801–807.