



# Sevoflurane has postconditioning as well as preconditioning properties against hepatic warm ischemia–reperfusion injury in rats

Saki Shiraishi<sup>1</sup> · Sungsam Cho<sup>2</sup> · Daiji Akiyama<sup>3</sup> · Taiga Ichinomiya<sup>1</sup> · Itsuko Shibata<sup>3</sup> · Osamu Yoshitomi<sup>3</sup> · Takuji Maekawa<sup>3</sup> · Eisuke Ozawa<sup>4</sup> · Hisamitsu Miyaaki<sup>4</sup> · Tetsuya Hara<sup>1</sup>

Received: 15 October 2018 / Accepted: 6 April 2019 / Published online: 3 May 2019  
© Japanese Society of Anesthesiologists 2019

## Abstract

**Purpose** Ischemia–reperfusion (IR) injury is inevitable after liver transplantation and liver resection with inflow occlusion. Sevoflurane has been widely used during hepatobiliary surgery and was reported to exhibit preconditioning (PreC) properties against hepatic IR injury; however, its postconditioning (PostC) properties remain unknown. This study examined whether a clinically applicable dose of sevoflurane has PostC and PreC properties against hepatic IR injury and roles of heme oxygenase-1 (HO-1).

**Methods** Warm ischemia was induced in male Wistar rats, excluding the sham group, for 1 h, followed by 3 h of reperfusion. Group C received propofol from 60 min before ischemia until the end of the experimental procedure. In the SPreC and SPostC groups, propofol was replaced by 2.5% sevoflurane for 30 min from 35 min before ischemia in the SPreC group and for 30 min from 5 min before reperfusion in the SPostC group. The SPreC+Z and SPostC+Z groups received a HO-1 inhibitor, zinc protoporphyrin (Znpp), 60 min before ischemia, and sevoflurane PreC and PostC were induced.

**Results** Serum aspartate aminotransferase, alanine aminotransferase, and lactic dehydrogenase levels, and histological damage scores in the SPreC and SPostC groups were significantly lower than those in group C. Inhibiting HO-1 with Znpp partially blocked these protective effects of sevoflurane. Sevoflurane PreC and PostC significantly increased the number of HO-1-positive Kupffer cells in comparison with group C, and Znpp prevented sevoflurane-induced HO-1 expression.

**Conclusion** PostC and PreC by sevoflurane at a clinically applicable dose have equally protective effects against hepatic IR injury by increasing HO-1 expression.

**Keywords** Sevoflurane · Preconditioning · Postconditioning · Ischemia–reperfusion injury · Hepatic · Heme oxygenase-1

## Introduction

The prevention of major hemorrhage during hepatic resection is important because the transfusion influences postoperative recovery and long-term outcomes [1, 2]. The Pringle

maneuver, which involves inflow occlusion by clamping of the portal triad, prevents blood loss during liver transection, but hepatic ischemia–reperfusion (IR) injury caused by this method affects the morbidity and mortality after operation [3]. Hepatic IR injury includes the generation of reactive oxygen species (ROS), leukocyte migration and activation, microcirculatory abnormalities, sinusoidal endothelial cell damage, activation of the coagulation cascade, Kupffer cell activation due to the release of inflammatory cytokines, and mitochondrial dysfunction [4, 5]. One of the hepatoprotective methods against IR injury is ischemic preconditioning (PreC). Ischemic PreC, which is defined as brief periods of ischemia prior to sustained ischemia, prevents hepatic IR injury [4–6]. Several studies have demonstrated that not only ischemic PreC but also ischemic postconditioning (PostC) comprising several brief cycles of ischemia and reperfusion at the onset of sustained reperfusion after ischemia,

✉ Saki Shiraishi  
sakiono0103@gmail.com

<sup>1</sup> Department of Anesthesiology, Nagasaki University Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan

<sup>2</sup> Department of Anesthesiology, National Hospital Organization Kokura Medical Center, Kitakyushu, Japan

<sup>3</sup> Department of Anesthesiology, Nagasaki University Hospital, Nagasaki, Japan

<sup>4</sup> Department of Gastroenterology, Nagasaki University Hospital, Nagasaki, Japan

can protect against hepatic IR injury [7–9]. In addition to ischemic PostC, some agents, such as adenosine A2A receptor agonists [10], recombinant erythropoietin (rhEPO) [11], and a phosphodiesterase inhibitor, milrinone [12], can be used as pharmacological inducers of PostC.

Sevoflurane, a volatile anesthetic agent, and the intravenous anesthetic propofol have been widely used during hepatobiliary surgery. Pharmacological PreC induced by sevoflurane has been reported to attenuate hepatic IR injury in randomized controlled trials among patients undergoing liver surgery with inflow occlusion and in animal models [13–15]. PostC is more likely than PreC to be feasible for clinical application because the onset of reperfusion is more predictable. Both ischemic PreC and PostC have equally protective effects in terms of alanine aminotransferase (ALT), aspartate aminotransferase (AST), the generation of ROS, the expression of inflammatory cytokines, and apoptosis [16], but it is unknown whether sevoflurane has PostC properties.

Therefore, this study compared the protective effects of pharmacological PostC and PreC induced by a clinically applicable dose of sevoflurane against hepatic IR injury in rats. Our secondary aim was to clarify whether heme oxygenase-1 (HO-1) is involved because the inhibition of HO-1 expression abolishes the protection against hepatic IR injury [4, 17], suggesting the importance of HO-1 in ischemic PreC and PostC.

### Materials and methods

All experimental procedures and protocols in this study were approved by the Institutional Animal Care and Use Committee of Nagasaki University of Medicine, Japan (no. 1402181119-3, 2014). All animals were maintained in accordance with the recommendations of the National

Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

### Surgical procedure and experimental protocol

The instrumental methods were used as described in our previous report [12], with slight modifications. Male Wistar rats weighing from 350 to 450 g were anesthetized with a 50 mg/kg intraperitoneal bolus of sodium pentobarbital. After the rats were sufficiently sedated to ensure that pedal and palpebral reflexes were absent, catheters were inserted into the right jugular vein for fluid or drug administration and the right carotid artery for measurement of arterial blood pressure. Thereafter, lactated Ringer’s solution was infused at a rate of 10 ml/kg/h until the experimental procedures. Hemodynamics were continuously monitored with a transducer (blood pressure monitor link sck-9082; Becton–Dickinson, Tokyo, Japan) and a blood pressure amplifier (AP-641G, Nihon-Kohden, Tokyo, Japan), and displayed using a polygraph system (Ohmeda BCWT00963-0, Nihon-Kohden, Tokyo, Japan). After tracheotomy, the trachea was intubated with a cannula connected to a small animal ventilator (SAR-830, CWE, PA, USA) and the lungs were ventilated with pure oxygen. Rats were placed on an electric heating pad under a warming light, and body temperature was continuously monitored with a rectal thermometer and maintained between 36 and 37 °C. The abdominal cavity was approached through a midline incision and the liver was exposed. At this point, all rats were divided into six groups (*n* = 8 each). The experimental protocol is illustrated in Fig. 1. Rats in the sham group and group C received propofol at 39 mg/kg/h and fentanyl at 30 µg/kg/h from 60 min before ischemia until the end of the experimental procedure, rats in the SPreC and SPostC groups received propofol and fentanyl at the same dose, and propofol was replaced by 2.5% sevoflurane for 30 min from 35 min before ischemia in the SPreC

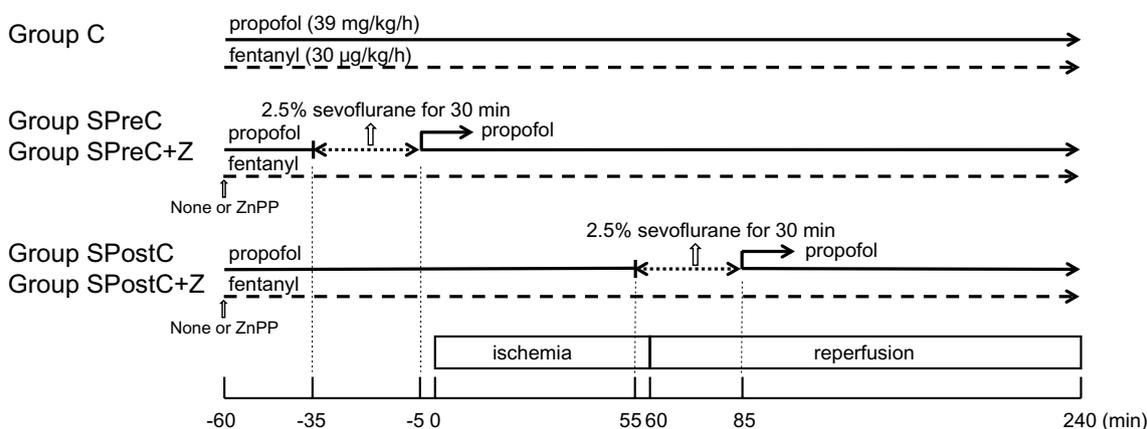


Fig. 1 A schematic illustration of the experimental protocols

group and for 30 min from 5 min before reperfusion in the SPostC group. Propofol anesthesia was reinitiated when sevoflurane administration was stopped. In the SPreC+Z and SPostC+Z groups, an HO-1 inhibitor, zinc protoporphyrin (Znpp; 25  $\mu\text{mol}/\text{kg}$ ; Sigma chemicals, Germany), was administered as an intravenous bolus 60 min before ischemia [18]. In groups C, SPreC, SPostC, SPreC+Z, and SPostC+Z, the liver was exposed, and all structures in the portal triad (hepatic artery, portal vein, and bile duct) to the median and left lateral hepatic lobes were occluded by a microvascular clip; this induced approximately 60–70% hepatic ischemia. This method of partial hepatic ischemia prevented mesenteric venous congestion by permitting portal decompression through the right and caudate lobes. The abdomen was covered with saline-humidified gauze during the ischemic period. After 1 h of ischemia, the clip was removed for hepatic reperfusion, and the abdominal cavity was closed with a 4-0 silk suture. Sham-operated animals (sham group) underwent the same surgical procedure, but hepatic vessel clips were not applied. All animals were sacrificed 3 h after reperfusion, and blood samples and liver tissues were collected for analysis.

The doses of propofol and sevoflurane were set based on previous studies. Rats were exposed to 2.5% sevoflurane corresponding to a minimum alveolar anesthetic concentration (MAC) of 1 [19], which has the same anesthetic potency of propofol at 39 mg/kg/h according to the tail-clamp technique [20]. Sevoflurane was administered via a vaporizer (SEVO-TEC3, Ohmeda, Steeton, UK). The end-tidal concentration of sevoflurane was measured using an infrared gas analyzer that was calibrated with known standards before and during experimentation. To prepare solutions of Znpp at 2 mg/ml, it was dissolved in 0.2 N NaOH and the pH was adjusted to 7.4 with 1 N HCl [21]. Phosphate buffered saline (pH 7.4) was added to increase the total volume as appropriate.

### Liver function tests

After 3 h of reperfusion, the vena cava was opened, 4 ml of blood was collected in sterile syringes and centrifuged immediately at 3000 rpm for 10 min, and then serum samples were stored at  $-80\text{ }^\circ\text{C}$  until analysis. Serum AST, ALT, and lactate dehydrogenase (LDH) levels were measured by ultraviolet spectrophotometric enzymatic assay.

### Histological examination and immunohistology of HO-1

All histopathology and immunohistology examinations were performed by a researcher blinded to the study group. Liver tissues for histological examination were excised samples from the anterior edge of the left lobe after 3 h of reperfusion. The excised liver specimens were fixed in 10% buffered

formaldehyde solution, embedded in paraffin, and stained using hematoxylin and eosin (HE). Histological analysis was performed at  $200\times$  magnification using a point-counting method for the severity of hepatic injury proposed by Suzuki et al. [22] to determine the degree of sinusoidal congestion, liver cell vacuolization, and necrosis (Table 2). Histological changes were scored from 0 to 4, and the total score range was 0–12.

To assess cell type-specific expression patterns of HO-1, slides from the same paraffin-embedded livers for HE staining were used. Briefly, antigen retrieval by microwave irradiation was carried out in a citrate buffer according to a standard protocol. Endogenous peroxidase activity was blocked by incubation in 1%  $\text{H}_2\text{O}_2$ /methanol. After subsequent treatment with skim milk, slides were incubated with the rabbit polyclonal anti-HO-1 antibody (ab13243; Abcam plc, Cambridge, UK) diluted 1:500 overnight at  $4\text{ }^\circ\text{C}$ . After washing with phosphate buffered saline, the sections were stained with Histofine Simple Stain Rat MAX-PO (MULTI) (Nichirei Biosciences Inc, Tokyo, Japan). The slides were then counterstained with hematoxylin. HO-1-positive cell counts were expressed as the number of cells in six random high-power fields (magnification  $400\times$ ) per sample.

### Statistical analysis

The *N*-query advisor program (PASS13) was used to determine an adequate *n* value using our previously published data [12]. The *n* value was calculated assuming a one-way analysis of variance (ANOVA) and gave a minimum sample size of seven animals per group. At this sample size, one-way ANOVA has  $>80\%$  power to detect significant differences in means at the 0.05 level based on previous data characterized by a variance of means of 0.05. Therefore, eight animals per group were used. The results are expressed as the mean  $\pm$  standard deviation or median (range). Differences except for serum AST, ALT, and LDH levels among experimental groups were evaluated by ANOVA followed by the Welch *t* test, or with one-way ANOVA followed by the Student–Newman–Keuls test, and  $P < 0.05$  was considered significant. Differences in serum AST, ALT, and LDH levels among experimental groups were evaluated by Bonferroni's post hoc correction, and  $P < 0.01$  was considered significant. Statistical analysis was performed using IBM SPSS Statistics version 24.0 software for Windows (IBM Japan, Tokyo, Japan).

### Results

Sixty-eight rats were used, with 48 successful experiments. Five rats were excluded due to technical difficulties or error in the experimental preparation. Circulatory collapse or

shock-induced death developed in 15 other rats before completion of the experiment; five in group C, one in SPreC, three in SPostC, three in SPreC+Z, and three in SPostC+Z. These rats were excluded from further analysis.

There were no significant differences in body weight or age among the groups. Hemodynamic variables of the mean blood pressure, heart rate, and body temperature were not significantly different among the groups at any measurement point (Table 1).

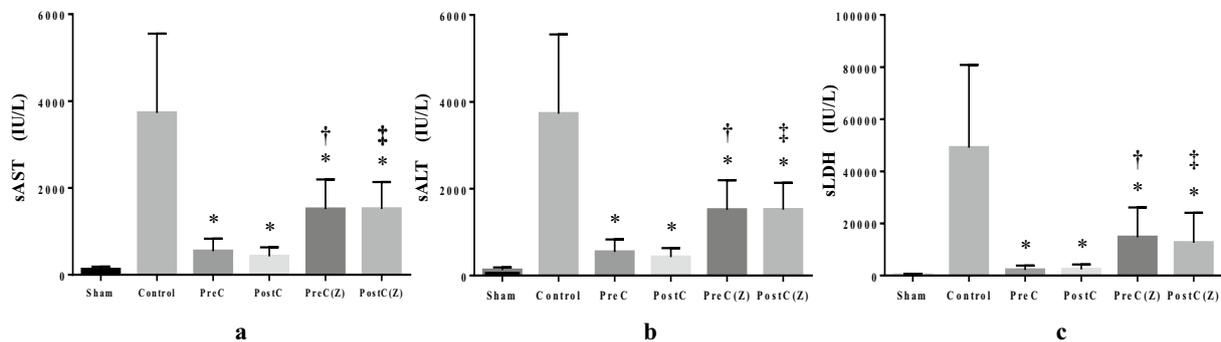
### Serum AST, ALT, and LDH levels

The serum AST, ALT, and LDH levels in group C were higher than those in the sham group. The livers of rats in the SPreC and SPostC groups had less damage, as evidenced by significantly lower serum ALT, AST, and LDH levels at 3 h after reperfusion than those in group C (Fig. 2). Sevoflurane PreC and SPostC have equally protective effects in terms of the serum ALT, AST, and LDH levels. In Znpp-treated

**Table 1** Hemodynamic variables and body temperature

	30 min before ischemia	45 min after ischemia	30 min after reperfusion	3 h after reperfusion
<b>MBP (mmHg)</b>				
Group C	117 ± 21	109 ± 28	98 ± 18	91 ± 7
SPreC group	131 ± 14	117 ± 22	105 ± 18	91 ± 16
SPostC group	125 ± 12	120 ± 21	102 ± 16	103 ± 15
SPreC+Z group	131 ± 14	121 ± 20	107 ± 16	100 ± 23
SPostC+Z group	124 ± 17	116 ± 33	108 ± 32	100 ± 29
Sham group	110 ± 16	119 ± 21	117 ± 19	112 ± 16
<b>HR (beats/min)</b>				
Group C	318 ± 19	312 ± 41	310 ± 23	304 ± 20
SPreC group	315 ± 10	304 ± 21	292 ± 17	274 ± 17
SPostC GROUP	321 ± 19	302 ± 28	280 ± 17	266 ± 12
SPreC+Z group	319 ± 9	325 ± 20	316 ± 16	305 ± 11
SPostC+Z group	322 ± 11	325 ± 15	315 ± 14	302 ± 13
Sham group	294 ± 18	315 ± 25	332 ± 18	328 ± 21
<b>BT (°C)</b>				
Group C	36.9 ± 0.5	37.0 ± 0.5	37.3 ± 0.4	36.9 ± 0.8
SPreC group	37.0 ± 0.5	36.5 ± 0.6	36.2 ± 0.3	37.1 ± 0.5
SPostC group	36.8 ± 0.7	36.6 ± 0.8	36.2 ± 0.2	36.5 ± 0.8
SPreC+Z group	36.8 ± 0.5	36.7 ± 0.8	37.2 ± 0.2	37.0 ± 0.6
SPostC+Z group	36.9 ± 0.6	36.4 ± 0.6	36.7 ± 0.4	36.7 ± 0.6
Sham group	36.8 ± 0.3	36.7 ± 0.7	36.9 ± 0.7	36.7 ± 0.7

Data are expressed as the mean ± standard deviation (n=8 each)  
 MBP mean blood pressure, HR heart rate, BT body temperature



**Fig. 2** Serum AST, ALT, and LDH levels after 3 h of reperfusion. Data are expressed as the mean ± standard deviation (n=8 in each group). \*Significantly different from group C (P<0.01). †Signifi-

cantly different from SPreC (P<0.01). ‡Significantly different from SPostC (P<0.01). AST aspartate aminotransferase, ALT alanine aminotransferase, LDH lactate dehydrogenase

groups, the sevoflurane-induced protective effects were partially inhibited. There were no significant differences between the SPreC+Z and SPostC+Z groups (Table 2).

### Histological examination and Immunohistology of HO-1

The livers of rats in group C exhibited severe IR injury such as hepatocyte vacuolization with disruption of the lobular architecture and sinusoidal congestion (Table 3; Fig. 3a). All sevoflurane-treated groups had less injury. According to Suzuki's histological classification by HE staining, the injury scores in the SPreC and SPostC groups were lower than those in group C (Table 3; Fig. 3). Compared with those in group SPreC, the injury scores were significantly higher in SPreC+Z, that score in group SPostC were significantly higher in group SPostC+Z in a similar way.

On immunohistology, HO-1 was more highly expressed in Kupffer cells in the SPreC and SPostC groups than in group C. Znpp-treated groups had significantly lower sevoflurane-induced HO-1 expression (Fig. 4). There was no significant difference between group C and sham group.

### Discussion

In the present study, AST, ALT, and LDH levels, and histological damage scores after 3 h of reperfusion in the sevoflurane PreC and PostC groups were significantly lower than those in the non-conditioning group, and there were no significant differences between the sevoflurane PreC and PostC groups. Thus, a clinically applicable dose of sevoflurane can induce both PreC and PostC, which have equally protective effects against warm hepatic IR injury in rats. Furthermore, sevoflurane PreC and PostC increased the number of HO-1-positive Kupffer cells, and Znpp-treated groups had significantly lower sevoflurane-induced HO-1 expression. In the Znpp-treated groups, the sevoflurane-induced protective effects in terms of serum ALT, AST, and LDH levels, and histological damage scores were partially inhibited. Therefore, HO-1 may play a role in sevoflurane PreC and PostC.

Although the mechanisms underlying ischemic PostC in the liver are not fully understood, some studies have reported that ischemic PreC and PostC processes have many similarities [7, 23]. Recent studies have demonstrated that some agents, such as rhEPO [11, 24] and milrinone [12, 25], can be used as pharmacological inducers of PostC and PreC. Randomized controlled trials among patients undergoing liver surgery with inflow occlusion revealed that sevoflurane PreC or PostC during propofol anesthesia can prevent postoperative liver injury, as indicated by serum ALT, AST levels, and postoperative complications [13, 26]. Zhou et al. [14] reported that PreC by a MAC of 1, 1.5, or 2 of

**Table 2** Suzuki score

Degree	Sinusoidal congestion	Liver cell vacuolization	Necrosis
0	None	None	None
1	Minimal	Minimal	Single cell necrosis
2	Mild	Mild	≤ 30%
3	Moderate	Moderate	≤ 60%
4	Severe	Severe	> 60%

**Table 3** Suzuki score for rat liver after ischemia and reperfusion

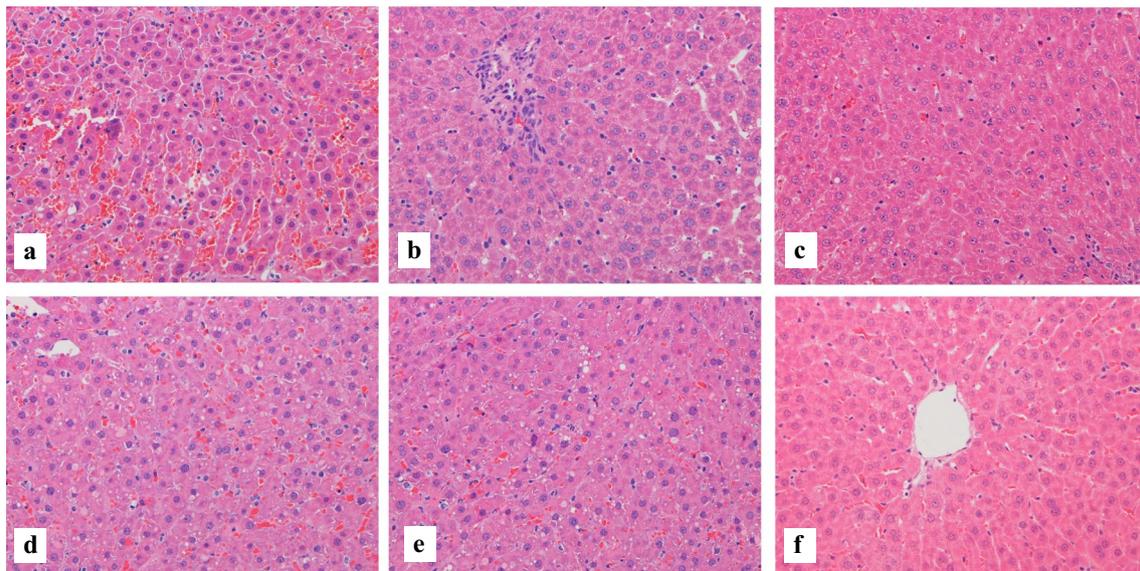
	Sinusoidal congestion	Liver cell vacuolization	Necrosis	Total
Group C	3 (2–4)	3 (1–4)	2 (1–3)	8 (6–11)
SPreC group	1 (0–2)	1 (0–2)	0 (0–1)	2 (1–4)*
SPostC group	1 (0–2)	1 (0–2)	0 (0–1)	2 (1–4)*
SPreC+Z group	2 (0–3)	1 (1–3)	0 (0–1)	4 (1–5)*,†
SPostC+Z group	2 (2–3)	2 (1–2)	0 (0–1)	4 (3–6)*,‡
Sham group	0 (0–1)	0 (0)	0 (0)	0 (0–1)

Data are expressed as the median and range ( $n=8$  each)

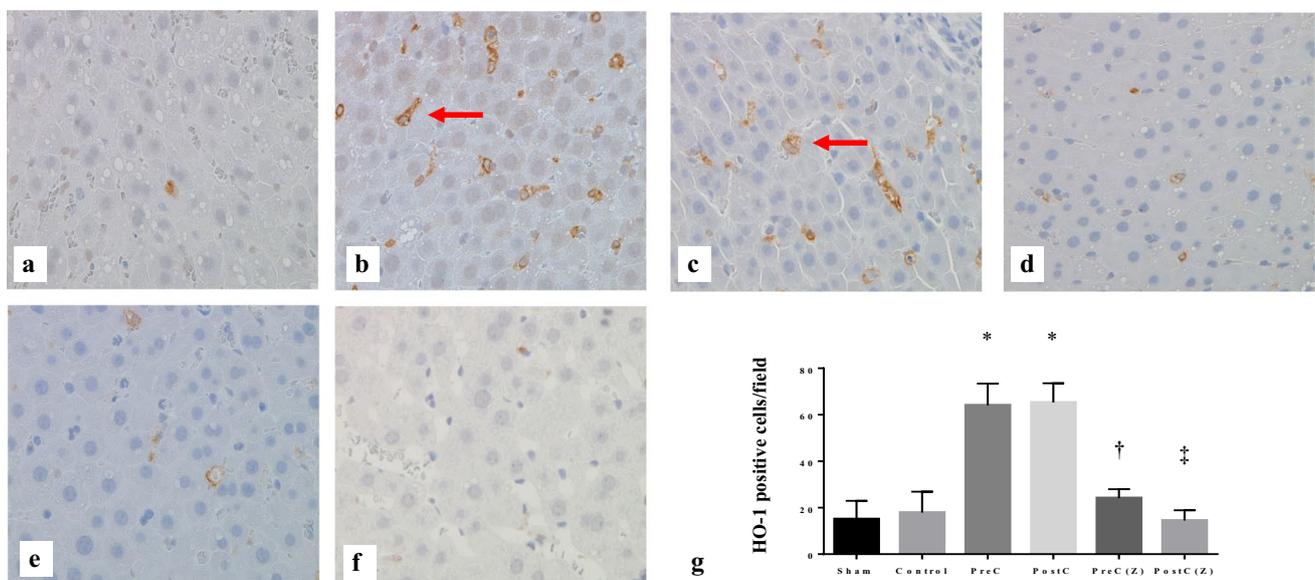
\*Significantly different from group C ( $P<0.05$ ). †Significantly different from SPreC ( $P<0.05$ ). ‡Significantly different from SPostC ( $P<0.05$ )

sevoflurane significantly attenuated IR-induced AST and ALT increases in rats, in addition to increased myeloperoxidase activity and malondialdehyde levels. Sevoflurane PostC has been found to decrease hepatocyte apoptosis by reducing ROS generation by hepatic stellate cells [27]. In this study, we found that PreC and PostC by a MAC of 1 of sevoflurane reduced AST, ALT, and LDH levels, and histological damage scores after 3 h of reperfusion as compared with the control group. Thus, a clinically applicable dose of sevoflurane has both PostC and PreC effects against warm hepatic IR injury in rats.

Several comparative studies between ischemic PreC and PostC against hepatic IR injury have been conducted. Zhang et al. [16] reported that both ischemic PreC and PostC exert equal protection against hepatic IR injury, as assessed by serum transaminase levels, superoxide dismutase activity, apoptotic index, and microscopy findings. Song et al. [28] demonstrated that ischemic PreC and PostC protocols were equally effective in reducing liver injury, as evidenced by the significant reduction of AST and ALT levels, suppression of cytokine and malondialdehyde levels, and increase in the activity of antioxidant enzymes. Consistent with these previous studies, sevoflurane-induced PreC and PostC had equally protective effects against warm hepatic IR injury in rats in our study, as evidenced by the AST, ALT, and LDH levels, and histological damage scores after 3 h of reperfusion.



**Fig. 3** Comparison of histopathology for rat hepatic ischemia–reperfusion injury. Representative photomicrographs of liver histology in group C (a), SPreC (b), and SPostC (c), SPreC+Z (d), SPostC+Z (e), and sham (f) groups



**Fig. 4** Heme oxygenase-1 expression. Immunohistology for heme oxygenase-1 in group C (a), SPreC (b), and SPostC (c), SPreC+Z (d), SPostC+Z (e) groups, and sham (f) groups. The arrow indicates heme oxygenase-1 (HO-1) expression in Kupffer cells. **g** Numbers of (HO-1)-positive cells per microscopic field were counted in 5 groups.

Data are expressed as the mean  $\pm$  standard deviation ( $n=8$  in each group). \*Significantly different from group C ( $P<0.05$ ). †Significantly different from SPreC ( $P<0.05$ ). ‡Significantly different from SPostC ( $P<0.05$ )

In contrast, it has been reported that rhEPO induces PreC and PostC reduces the increase in AST and ALT levels, but PreC has an advantage over PostC in improving hepatic IR-induced apoptosis [24].

As HO-1 is induced as a protective mechanism in response to numerous stimuli, including IR injury, targeted

induction of this stress–response enzyme may be an important therapeutic strategy to protect against inflammatory processes and oxidative tissue damage [29, 30]. HO-1 plays an essential role in ischemic PreC and PostC because the inhibition of HO-1 expression abolishes the protection against hepatic IR injury [4, 17]. Moreover, PreC with an another

volatile anesthetic isoflurane was reported to increase HO-1 expression and activity after 4 h of reperfusion, and attenuate the hepatic IR injury and inflammatory responses [18]. Moreover, administration of the HO-1 inhibitor Znpp prior to the isoflurane pretreatment significantly attenuated the isoflurane-induced increase in HO-1 protein expression and activity, and prevented PreC-induced protection against hepatic IR injury. Based on immunohistology, higher expression of HO-1 was mainly observed in Kupffer cells in the sevoflurane PreC and PostC groups, and Znpp treatment significantly reduced this HO-1 expression. However, the inhibition of HO-1 by Znpp did not completely abolish the sevoflurane-induced protective effects. Thus, the mechanism of the protective effects by sevoflurane may partially depend on HO-1.

In clinical settings, hypnotic anesthetic agents, such as sevoflurane, desflurane, isoflurane, or propofol, are combined with an analgesic agent such as fentanyl or remifentanyl. There are several reasons why propofol and fentanyl were continuously administered as background anesthesia in this study. First, anesthesia was maintained using a target-controlled infusion of propofol in a randomized controlled trial that examined sevoflurane PreC or PostC against hepatic IR injury [13, 26]. It is possible that propofol metabolism in the liver affects the hepatic function during IR. However, propofol has been reported to protect against hepatic IR injury by reducing apoptosis and the release of pro-inflammatory cytokines [31]. It has also been reported that the continuous administration of sevoflurane and propofol can protect against hepatic IR injury in rats [32]. In a retrospective study of patients undergoing liver resection with inflow occlusion, there were no significant differences in the peak serum ALT and AST levels or postoperative complications between continuous sevoflurane and propofol anesthesia [33]. Thus, the continuous administration of sevoflurane and propofol has equally protective effects against warm hepatic IR injury, and sevoflurane PreC or PostC during propofol anesthesia exerts stronger protection than continuous sevoflurane and propofol anesthesia. Second, remifentanyl-induced hepatic PreC was found to reduce hepatic IR-induced increases in ALT and AST levels, and hepatocyte apoptosis by exhausting reactive oxygen species and attenuating the inflammatory response [34]. Moreover, our preliminary study demonstrated that the continuous administration of remifentanyl at 120  $\mu\text{g}/\text{kg}/\text{h}$  combined with propofol effectively attenuates liver injury as compared with propofol and fentanyl anesthesia (data not shown). Fentanyl at 30  $\mu\text{g}/\text{kg}/\text{h}$  in this study and remifentanyl at 120  $\mu\text{g}/\text{kg}/\text{h}$  similarly reduced the isoflurane MAC by 25% [35].

Several limitations of this study should be noted. First, HO-1 plays an important role in ischemic or pharmacological PreC and PostC for the protection against hepatic IR injury [4, 17]. However, the involvement of HO-1 in the

mechanisms underlying sevoflurane PreC and PostC may be limited, and we were unable to examine the other mechanisms in this study. Morita et al. [15] found that ischemia and sevoflurane PreC attenuated the increase in ALT and AST levels after hepatic IR through the Akt-glycogen synthase-3 $\beta$ -cyclin D1 pathway using ingenuity pathway analysis. Inflammatory responses during liver IR are accompanied by the formation and vascular sequestration of platelet-neutrophil conjugates (PNCs). In addition, sevoflurane was reported to increase Adora2b transcription and expression, which inhibited IR-induced platelet and leukocyte activation, PNCs formation, cytokine release, and liver damage [36]. Beck-Schimmer et al. [27] demonstrated that the Bax/B-cell lymphoma 2 mRNA ratio in liver tissue was lower in the sevoflurane PostC group of patients in the randomized controlled trials, and sevoflurane PostC attenuated hepatocyte apoptosis by reducing ROS generation by hepatic stellate cells. Therefore, sevoflurane may protect against hepatic IR injury by reducing inflammatory responses and ROS generation. HO-1 also protects against hepatic IR injury, such as the elevation of serum transaminase levels and histological damage, by inhibiting inflammatory responses and ROS generation [29, 30]. However, no study has found other mechanisms involving HO-1 in sevoflurane PreC and PostC against hepatic IR injury, and we did not examine inflammatory responses or ROS generation in this study. Thus, the interactions among HO-1, inflammatory responses, and oxidative tissue damage remain unclear. Further studies are needed to evaluate the mechanisms involved in sevoflurane PreC and PostC. Second, we did not investigate the interaction between propofol and sevoflurane. Desflurane-induced PreC was reported to reduce myocardial infarct size, and these effects were blocked by the concomitant administration of propofol [36]. Zaugg et al. [37] found that sevoflurane PreC was protective with respect to functional recovery and  $\text{Ca}^{2+}$  overload after IR in the functional rat heart. They also noted that the concomitant administration of propofol attenuated this protection in a concentration-dependent manner. However, our results and previous reports [13, 26] suggest that a clinically applicable dose of sevoflurane has both PostC and PreC effects against warm hepatic IR injury during propofol anesthesia. As such, the concomitant administration of propofol with sevoflurane, rather than the discontinuation of propofol during sevoflurane inhalation, is what attenuated the sevoflurane PreC and PostC against hepatic IR injury. Third, we did not examine hemoglobin,  $\text{PaO}_2$ , or  $\text{PaCO}_2$  levels. As these factors may affect the results, blood gas analyses should be performed.

In conclusion, we provide new insight that the clinically applicable dose of sevoflurane can induce PreC and PostC, which have equally protective effects against warm hepatic IR injury, and the mechanism of the protective effects by sevoflurane may be partially dependent on HO-1.

**Acknowledgements** The authors are grateful to Dr. Katsuya Matsuda (Department of Tumor and Diagnostic Pathology, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, Japan) and Dr. Suntaro Sato (Clinical Research Center, Nagasaki University Hospital, Nagasaki, Japan) for their useful suggestions for histopathology and immunohistology examinations, and statistical analysis.

## Compliance with ethical standards

**Conflict of interest** The authors declare that there is no conflict of interest.

## References

- Kooby DA, Stockman J, Ben-Porat L, Gonen M, Jarnagin WR, Dematteo RP, Tuorto S, Wuest D, Blumgart LH, Fong Y. Influence of transfusions on perioperative and long-term outcome in patients following hepatic resection for colorectal metastases. *Ann Surg*. 2003;237:860–9 (**discussion 869–870**).
- de Boer MT, Molenaar IQ, Porte RJ. Impact of blood loss on outcome after liver resection. *Dig Surg*. 2007;24:259–64.
- Serracino-Ingloft F, Habib NA, Mathie RT. Hepatic ischemia–reperfusion injury. *Am J Surg*. 2001;181:160–6.
- Carini R, Albano E. Recent insights on the mechanisms of liver preconditioning. *Gastroenterology*. 2003;125:1480–91.
- Yan S, Jin LM, Liu YX, Zhou L, Xie HY, Zheng SS. Outcomes and mechanisms of ischemic preconditioning in liver transplantation. *Hepatobil Pancreat Dis Int*. 2010;9:346–54.
- Suyavaran A, Thirunavukkarasu C. Preconditioning methods in the management of hepatic ischemia reperfusion–induced injury: update of molecular and future perspectives. *Hepatol Res*. 2017;47:31–48.
- Theodoraki K, Karmanioliou I, Tympa A, Tasoulis MK, Nastos C, Vassiliou I, Arkadopoulos N, Smyrniotis V. Beyond preconditioning: postconditioning as an alternative technique in the prevention of liver ischemia–reperfusion injury. *Oxid Med Cell Longev*. 2016. <https://doi.org/10.1155/2016/8235921>.
- de Rougemont O, Lehmann K, Clavien PA. Preconditioning, organ preservation, and postconditioning to prevent ischemia–reperfusion injury to the liver. *Liver Transpl*. 2009;15:1172–82.
- Wang N, Lu JG, He XL, Li N, Qiao Q, Yin JK, Ma QJ. Effects of ischemic postconditioning on reperfusion injury in rat liver grafts after orthotopic liver transplantation. *Hepatol Res*. 2009;39:382–90.
- Dal Ponte C, Alchera E, Follenzi A, Imarisio C, Prat M, Albano E, Carini R. Pharmacological postconditioning protects against hepatic ischemia/reperfusion injury. *Liver Transpl*. 2011;17:474–82.
- Shawky HM, Younan SM, Rashed LA, Shoukry H. Effect of recombinant erythropoietin on ischemia–reperfusion induced apoptosis in rat liver. *J Physiol Biochem*. 2012;68:19–28.
- Toyoda T, Tosaka S, Tosaka R, Maekawa T, Cho S, Eguchi S, Nakashima M, Sumikawa K. Milrinone-induced postconditioning reduces hepatic ischemia–reperfusion injury in rats: the roles of phosphatidylinositol 3-kinase and nitric oxide. *J Surg Res*. 2014;186:446–51.
- Beck-Schimmer B, Breitenstein S, Urech S, De Conno E, Wittlinger M, Puhann M, Jochum W, Spahn DR, Graf R, Clavien PA. A randomized controlled trial on pharmacological preconditioning in liver surgery using a volatile anesthetic. *Ann Surg*. 2008;248:909–18.
- Zhou SP, Jiang P, Liu L, Liu H. Protective effect of sevoflurane on hepatic ischemia/reperfusion injury in the rat: a dose-response study. *Eur J Anaesthesiol*. 2013;30:612–7.
- Morita T, Ishikawa M, Sakamoto A. Identical microRNAs regulate liver protection during anaesthetic and ischemic preconditioning in rats: an animal study. *PLoS One*. 2015. <https://doi.org/10.1371/journal.pone.0125866>.
- Zhang WX, Yin W, Zhang L, Wang LH, Bao L, Tuo HF, Zhou LF, Wang CC. Preconditioning and postconditioning reduce hepatic ischemia–reperfusion injury in rats. *Hepatobil Pancreat Dis Int*. 2009;8:586–90.
- Zeng Z, Huang HF, Chen MQ, Song F, Zhang YJ. Contributions of heme oxygenase-1 in postconditioning-protected ischemia–reperfusion injury in rat liver transplantation. *Transp Proc*. 2011;43:2517–23.
- Lv X, Yang L, Tao K, Liu Y, Yang T, Chen G, Yu W, Lv H, Wu F. Isoflurane preconditioning at clinically relevant doses induce protective effects of hemo oxygenase-1 on hepatic ischemia reperfusion in rats. *BMC Gastroenterol*. 2011. <https://doi.org/10.1186/1471-230x-11-31>.
- Hirata N, Kanaya N, Kamada N, Kimura S, Namiki A. Differential effects of propofol and sevoflurane on ischemia-induced ventricular arrhythmias and phosphorylated connexin 43 protein in rats. *Anesthesiology*. 2009;110:50–7.
- Carmichael FJ, Crawford MW, Khayyam N, Saldivia V. Effect of propofol infusion on splanchnic hemodynamics and liver oxygen consumption in the rat. A dose–response study. *Anesthesiology*. 1993;79:1051–60.
- Amersi F, Buelow R, Kato H, Ke B, Coito AJ, Shen XD, Zhao D, Zaky J, Melinek J, Lassman CR, Kolls JK, Alam J, Ritter T, Volk HD, Farmer DG, Ghobrial RM, Busuttil RW, Kupiec-Weglinski JW. Upregulation of hemo oxygenase-1 protects genetically fat Zucker rat livers from ischemia/reperfusion injury. *J Clin Invest*. 1999;104:1631–9.
- Suzuki S, Toledo-Pereyra LH, Rodriguez FJ, Cejalvo D. Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. *Transplantation*. 1993;55:1265–72.
- Guo JY, Yang T, Xg Sun, Zhou NY, Li FS, Long D, Lin T, Li PY, Feng L. Ischemic postconditioning attenuates liver warm ischemia–reperfusion injury through Akt-eNOS-NO-HIF pathway. *J Biomed Sci*. 2011. <https://doi.org/10.1186/1423-0127-18-79>.
- Schmeding M, Neumann UP, Boas-Knoop S, Spinelli A, Neuhaus P. Erythropoietin reduces ischemia–reperfusion injury in the rat liver. *Eur Surg Res*. 2007;39:189–97.
- Satoh K, Kume M, Abe Y, Uchinami H, Yakubowski SV, Takahashi T, Sato T, Yamamoto Y. Implication of protein kinase A for a hepato-protective mechanism of milrinone pretreatment. *J Surg Res*. 2009;155:32–9.
- Beck-Schimmer B, Breitenstein S, Bonvini JM, Lesurtel M, Ganter M, Weber A, Puhann MA, Clavien PA. Protection of pharmacological postconditioning in liver surgery: results of a prospective randomized controlled trial. *Ann Surg*. 2012;256:837–44 (**discussion 844–845**).
- Beck-Schimmer B, Roth Z, Graggen B, Booy C, Köppel S, Spahn DR, Schlöpfer M, Schädle E. Sevoflurane protects hepatocytes from ischemic injury by reducing reactive oxygen species signaling of hepatic stellate cells: translational findings based on a clinical trial. *Anesth Analg*. 2018;127:1058–65.
- Song X, Zhang N, Xu H, Cao L, Zhang H. Combined preconditioning and postconditioning provides synergic protection against liver ischemic reperfusion injury. *Int J Biol Sci*. 2012;8:707–18.
- Farombi EO, Surh YJ. Heme oxygenase-1 as a potential therapeutic target for hepatoprotection. *J Biochem Mol Biol*. 2006;39:479–91.

30. Liu B, Qian JM. Cytoprotective role of heme oxygenase-1 in liver ischemia reperfusion injury. *Int J Clin Exp Med*. 2015;8:19867–73.
31. Wei L, Chen WY, Hu T, Tang YX, Pan BB, Jin M, Kong GY. Effect and mechanism of propofol in hepatic ischemia/reperfusion injury of rat. *Eur Rev Med Pharmacol Sci*. 2017;21:3516–22.
32. Xu Z, Yu J, Wu J, Qi F, Wang H, Wang Z, Wang Z. The effects of two anesthetics, propofol and sevoflurane, on liver ischemia/reperfusion injury. *Cell Physiol Biochem*. 2016;38:1631–42.
33. Slankamenac K, Breitenstein S, Beck-Schimmer B, Graf R, Puhan MA, Clavien PA. Does pharmacological conditioning with the volatile anaesthetic sevoflurane offer protection in liver surgery? *HPB (Oxford)*. 2012;14:854–62.
34. Yang LQ, Tao KM, Liu YT, Cheung CW, Irwin MG, Wong GT, Lv H, Song JG, Wu FX, Yu WF. Remifentanyl preconditioning reduces hepatic ischemia–reperfusion injury in rats via inducible nitric oxide synthase expression. *Anesthesiology*. 2011;114:1036–47.
35. Criado AB, Gómez e Segura IA. Reduction of isoflurane MAC by fentanyl or remifentanyl in rats. *Vet Anaesth Analg*. 2003;30:250–6.
36. Smul TM, Stumpner J, Blomeyer C, Lotz C, Redel A, Lange M, Roewer N, Kehl F. Propofol inhibits desflurane-induced preconditioning in rabbits. *J Cardiothorac Vasc Anesth*. 2011;25:276–81.
37. Zaugg M, Wang L, Zhang L, Lou PH, Lucchinetti E, Clanachan AS. Choice of anesthetic combination determines Ca<sup>2+</sup> leak after ischemia–reperfusion injury in the working rat heart. Favorable versus adverse combinations. *Anesthesiology*. 2012;116:648–57.

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.