Title: Sevoflurane has postconditioning as well as preconditioning properties to protect against warm hepatic ischemia-reperfusion injury in rats.

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Abstract (250 words)

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.

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, 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 Figure 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 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 μmol/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 (SEVOTEC3, 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°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 magnification using a point-counting method for 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% H_2O_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°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) 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 ± 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 hours 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 groups, the sevoflurane-induced protective effects were partially inhibited. There were no significant differences between the SPreC+Z and SPreC+Z groups.

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 and 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 and 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 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 haptic 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. 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 remifentanil. 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, remifentanil-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 remifentanil at 120 μg/kg/h combined with propofol effectively attenuates liver injury as compared with propofol and fentanyl anesthesia (data not shown). Fentanyl at 30 μg/kg/h in this study and remifentanil at 120 μg/kg/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-3β-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 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, PaO2, or PaCO2 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.

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

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Table 1. Hemodynamic variables and body temperature

	30 min before ischemia	45 min after ischemia	30 min after reperfusion	3 h after reperfusion
MBP (mmHg)				
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
HR (beats/min)				
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
BT (°C)				
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, and BT; body temperature.

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 ($P < 0.05$).

Figure legends

Figure 1. A schematic illustration of the experimental protocols

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

Figure 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.

Figure 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 ± 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 ($P < 0.05$).

Figure 1

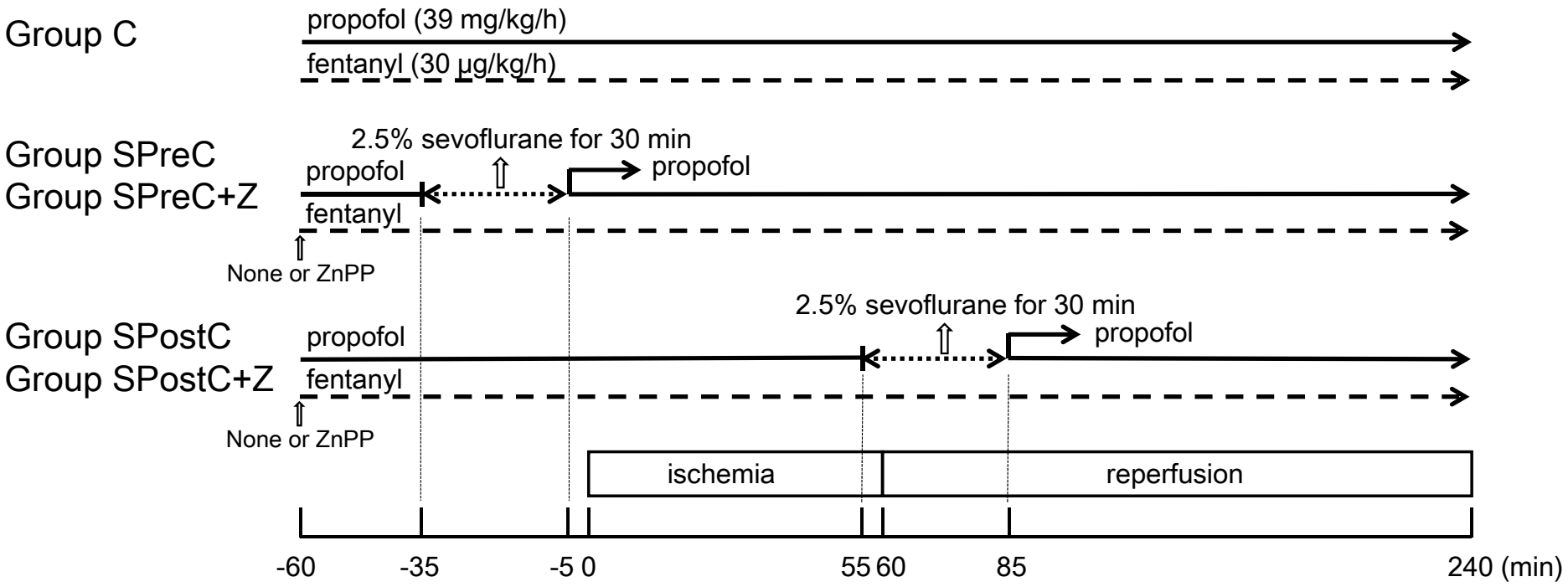

Group C
propofol (39 mg/kg/h)
fentanyl (30 μg/kg/h)
Group SPreC
Group SPreC+Z
2.5% sevoflurane for 30 min
propofol
propofol
fentanyl
None or ZnPP
Group SPostC
Group SPostC+Z
2.5% sevoflurane for 30 min
propofol
propofol
fentanyl
None or ZnPP
ischemia
reperfusion
-60
-35
-5
0
55
60
85
240 (min)

Fig 2.

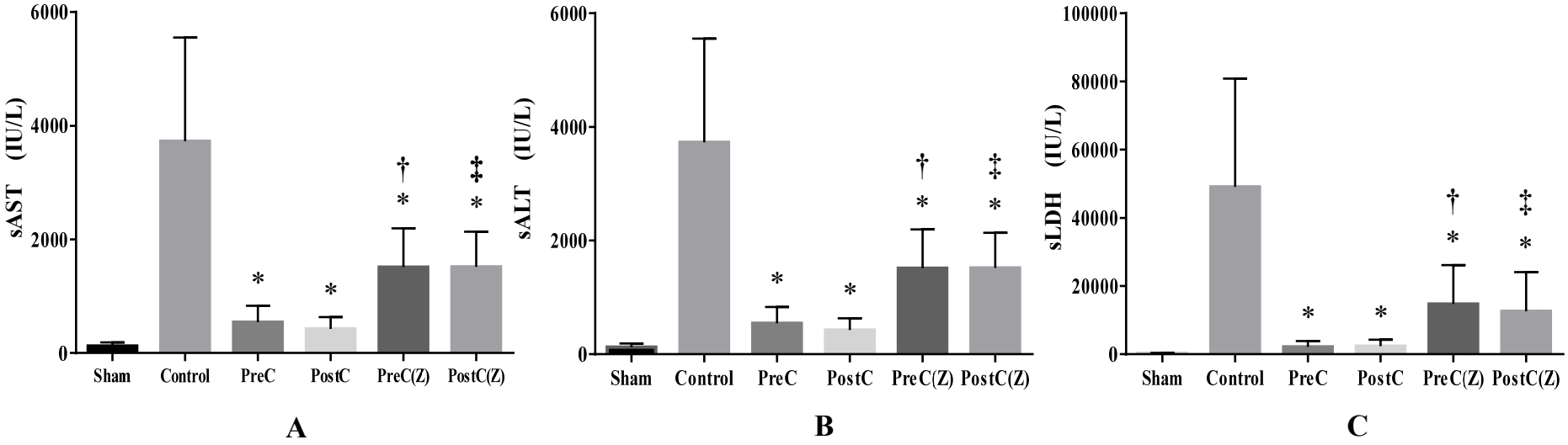

sAST (IU/L)
6000
4000
2000
0
Sham
Control
PreC
PostC
PreC(Z)
PostC(Z)
A
sALT (IU/L)
6000
4000
2000
0
Sham
Control
PreC
PostC
PreC(Z)
PostC(Z)
B
sLDH (IU/L)
100000
80000
60000
40000
20000
0
Sham
Control
PreC
PostC
PreC(Z)
PostC(Z)
C

Fig 3.

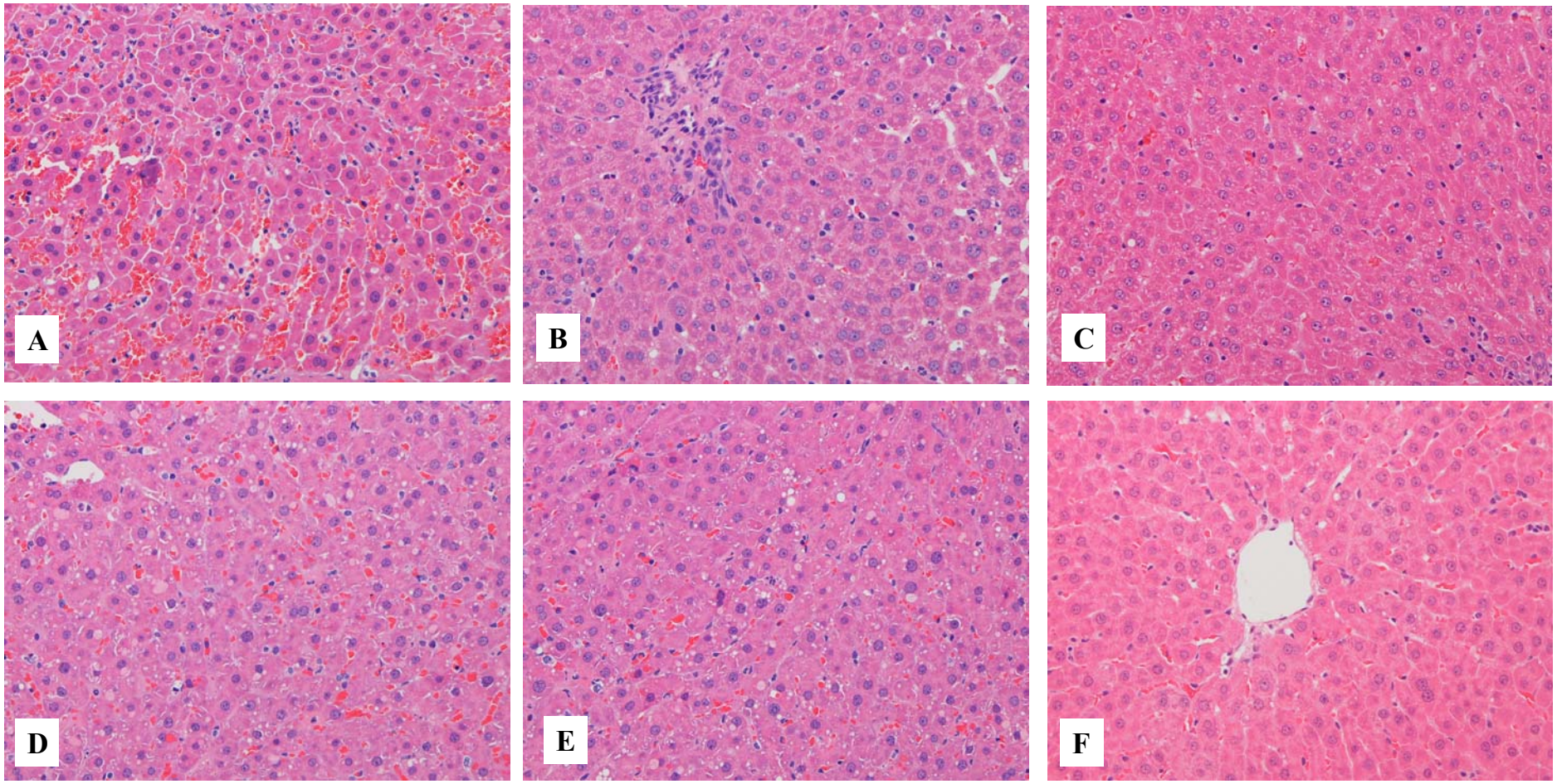
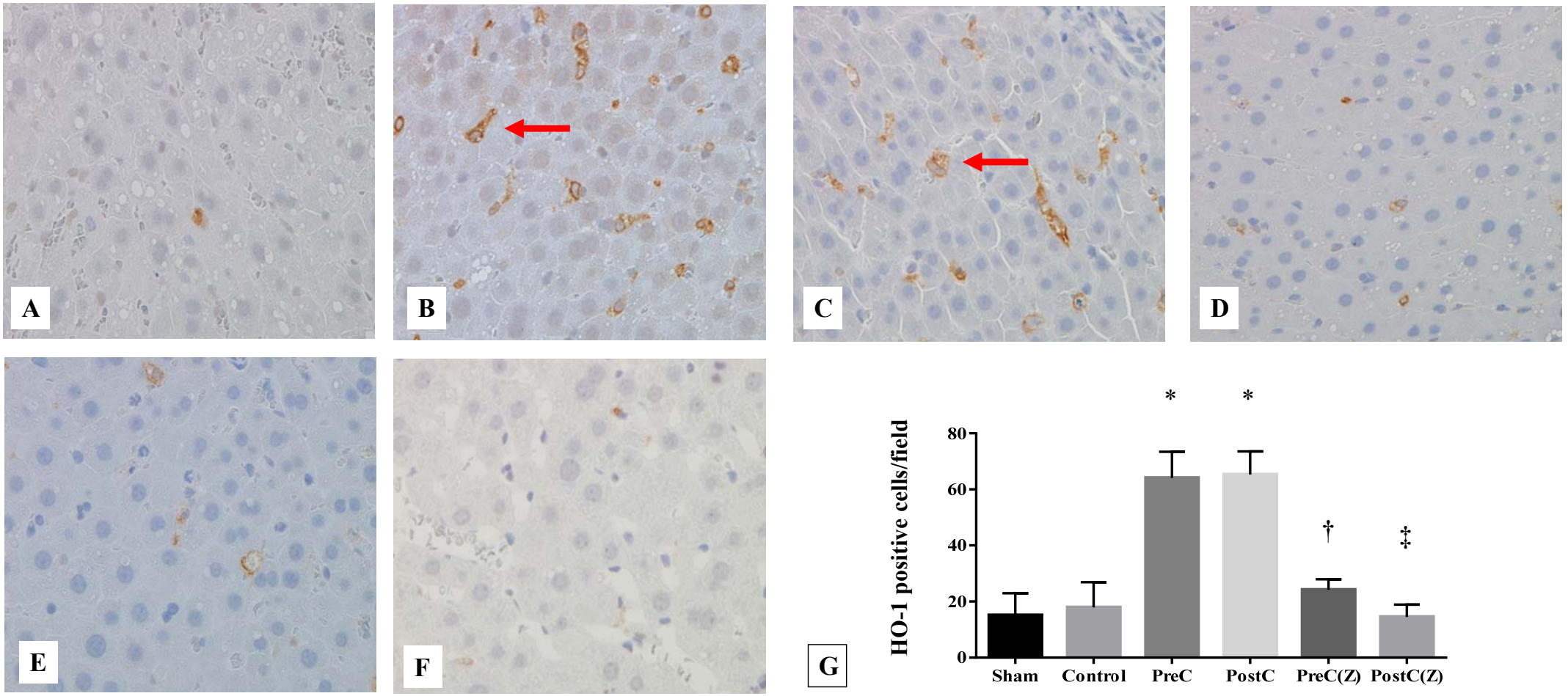


Fig 4


A
B
C
D
E
F
G
HO-1 positive cells/field
0
20
40
60
80
*
*
†
‡
Sham
Control
PreC
PostC
PreC(Z)
PostC(Z)