

Roles of cyclooxygenase-2 in sevoflurane- and olprinone-induced early phase of preconditioning and postconditioning against myocardial infarction in rat hearts

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Short title: Cyclooxygenase-2 in pharmacological pre- and postconditioning

Key words: cyclooxygenase, sevoflurane, olprinone, myocardial ischemia

Implications statement: A selective COX-2 inhibitor prevents the cardioprotective effect of sevoflurane but not olprinone in rat hearts. (16 words)

Abstract

Purpose: It is known that selective cyclooxygenase (COX)-2 inhibitors increase mortality in patients with previous myocardial infarction, and it has been suggested that COX-2 plays an important role in cardioprotection against ischemia. The present study was carried out to determine whether COX-2 is involved in the mechanisms of sevoflurane- and olprinone-induced early-phase preconditioning (E-PreC) and postconditioning (PostC) in rat hearts.

Methods: Male SD rats were anesthetized with sodium pentobarbital. After opening the chest, all rats underwent 30-min left anterior descending coronary artery occlusion followed by 2-h reperfusion, and the infarct size was measured after the reperfusion. The rats were randomly assigned to groups with pre- and post-ischemic exposure to sevoflurane and administration of olprinone with or without a selective COX-2 inhibitor, NS-398.

Results: The infarct size in the control group was $42 \pm 6\%$ of the area at risk. Infarct size was significantly reduced by pre- and post-ischemic administration of sevoflurane ($16 \pm 7\%$ and $17 \pm 6\%$, respectively), as well as by olprinone ($14 \pm 4\%$ and $15 \pm 10\%$, respectively). NS-398 prevented the protective effects of both pre and post-ischemic exposure to sevoflurane ($35 \pm 8\%$ and $42 \pm 10\%$, respectively), whereas the protective effect of both pre- and post-ischemic administration of olprinone was not influenced by NS-398 ($12 \pm 5\%$ and $19 \pm 7\%$, respectively).

Conclusions: COX-2 could be a critical mediator of sevoflurane-induced but not olprinone-induced E-PreC or PostC in rat hearts. (236 words)

Introduction

Cyclooxygenase (COX) inhibitors are universally used to produce anti-inflammatory and analgesic effects. Accordingly, selective COX-2 inhibitors have been widely used to prevent various complications such as gastric ulcer and renal dysfunction. However, it was reported that the use of selective COX-2 inhibitors at all dosages and nonselective nonsteroidal anti-inflammatory drugs (NSAIDs) at high dosages would increase the incidence of mortality in patients with previous myocardial infarction¹. COX-2 inhibitors may promote thrombosis and cardiovascular events owing to an imbalance between thromboxane A₂ and prostaglandin (PG) I₂, but this theory of balanced versus unbalanced COX inhibition is now questioned because the nonselective NSAIDs are also associated with increased cardiovascular risk. Recent studies have demonstrated that COX-2 inhibition increases the risk of development of hypertension, abnormal vascular remodeling, and inhibition of protective mechanisms against ischemia–reperfusion injury². Several studies have demonstrated that ischemic and pharmacological preconditioning (PreC) upregulate the expression and activity of COX-2 in the heart, and that this increase in COX-2 activity mediates the protective effects of late-phase PreC (L-PreC) against both myocardial stunning and myocardial infarction³.

Volatile anesthetics, sevoflurane and isoflurane, were demonstrated to produce L-PreC^{4,5}. Tanaka et al.⁴ showed that celecoxib abolished isoflurane-induced L-PreC in rabbits, whereas isoflurane did not alter COX-1 and COX-2 protein expression. It has been reported that sevoflurane and isoflurane have both early-phase PreC (E-PreC) and postconditioning (PostC) properties, and that these cardioprotective effects are mediated by protein kinase C (PKC) and mitochondrial ATP-sensitive potassium (m-K_{ATP}) channels^{6,7}. Alcindor et al.⁸ showed that COX-2 is a critical mediator of ischemic-, isoflurane-, and diazoxide-, an m-K_{ATP} channel opener, induced E-PreC in dogs. Recently, it has been reported that ischemic and intermittent bradykinin-induced

PostC require COX activation and PGI₂ release during reperfusion⁹. However, there have been no reports on the role of COX-2 in volatile anesthetic-induced PostC.

Phosphodiesterase type 3 inhibitors (PDE3-I) increase the intracellular cyclic adenosine monophosphate (cAMP) level¹⁰ and exert a positive inotropic effect. Sanada et al. reported that pretreatment with PDE3-Is has cardioprotective effects via cAMP and protein kinase A (PKA)-dependent but PKC-independent mechanisms¹¹. Our previous study showed that pretreatment with olprinone, a PDE3-I, protects the heart against myocardial infarction, and that this cardioprotective effect is mediated by the phosphatidylinositol 3-kinase pathway but not PKC or m-K_{ATP} channels¹². A recent study has also shown that cAMP/PKA-mediated preconditioning by glucose-decagon-like peptide-1 is independent of COX-2¹³. However, there have been no reports on PDE3-I-induced PostC or the roles of COX-2 in PDE3-I-induced E-PreC and PostC.

The present study was carried out to clarify whether sevoflurane and olprinone have both E-PreC and PostC properties, and, if so, whether the cardioprotective effects are mediated by the COX-2 pathway.

Materials and Methods

All experimental procedures and protocols described in this study were approved by the Institutional Animal Care and Use Committee.

Drugs

Olprinone was purchased from Eisai (Tokyo, Japan). Sevoflurane was purchased from Maruishi Pharmaceutical Company (Osaka, Japan). NS-398 was purchased from Wako Pure Chemical Industries (Osaka, Japan). Patent blue dye and 2,3,5-triphenyltetrazolium chloride (TTC) were purchased from Sigma (St. Louis, MO, USA).

General Preparation

The methods used were as described in our previous report¹². Male Sprague-Dawley rats weighing 455 ± 33 g (mean \pm SD) and aged 14 ± 1 weeks were anesthetized with sodium pentobarbital, a 50 mg/kg intraperitoneal bolus, followed by intravenous infusion at 10-20 mg/kg/h. The rats were adequately sedated to ensure that pedal and palpebral reflexes were absent throughout the experimental protocol. Catheters were inserted into the right jugular vein for fluid and drug administration, and the right carotid artery for measurement of arterial blood pressure. After tracheotomy was performed, the trachea was intubated with a cannula connected to a small animal ventilator (model SAR-830 CWE, PA, USA), and the lungs were ventilated with pure oxygen. Arterial blood PCO₂ and pH were maintained within a physiological range by adjusting the respiratory rate and tidal volume throughout the experiment. A left thoracotomy was performed in the fifth intercostal space, and the pericardium was opened. A 7-0 prolene ligature was placed around the proximal left anterior descending coronary artery (LAD) and vein in the area immediately

below the left atrial appendage. The ends of the suture were threaded through a small plastic tube to form a snare for reversible LAD occlusion. Coronary artery occlusion was produced by clamping the snare onto the epicardial surface of the heart and was confirmed by the appearance of epicardial cyanosis. Reperfusion was achieved by loosening the snare and was verified by observing an epicardial hyperemic response. Hemodynamics were continuously monitored with a transducer (blood pressure monitor link sck-9082; Becton Dickinson, Tokyo, Japan) and an AP-641G blood pressure amplifier (Nihon-Kohden, Tokyo, Japan) and shown on a polygraph system (Nihon-Kohden).

Experimental Protocol

The experimental design used in the current investigation is illustrated in Figure 1. All rats underwent 30 min of LAD occlusion followed by 2-h reperfusion. The Sprague-Dawley rats were randomly assigned to one of 10 groups (n = 7 for each).

Control group: the rats received vehicle instead of a selective COX-2 inhibitor, NS-398, and saline instead of olprinone.

Pre-S group: the rats received 2.0% end-tidal concentration of sevoflurane (corresponding to one minimal alveolar concentration (MAC) in rats)¹⁴ for 15-min starting at 30 min before LAD occlusion.

Post-S group: the rats received 1 MAC of sevoflurane for 8-min starting 3 min before reperfusion.

NS group: the rats received NS-398 (3 mg/kg) intravenously as a bolus at 60 min before the LAD occlusion.

NS+Pre-S group: the rats received NS-398 and sevoflurane as described above in NS

group and Pre-S group.

NS+Post-S group: the rats received NS-398 and sevoflurane as described above in NS group and Post-S group.

Pre-O group: the rats received olprinone (10 µg/kg) intravenously as a bolus at 30 min before the LAD occlusion.

Post-O group: the rats received olprinone (10 µg/kg) intravenously as a bolus at 5 min before reperfusion.

NS+Pre-O group: the rats received NS-398 and olprinone as described above in NS group and Pre-O group.

NS+Post-O group: the rats received NS-398 and olprinone as described above in NS group and Post-O group.

The doses of sevoflurane⁶, olprinone¹², and NS-398¹⁵ were set on the basis of previous studies. 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.

Determination of Infarct Size

Myocardial infarct size was measured as previously describe¹². Briefly, at the end of each experiment, the LAD was reoccluded, and patent blue dye was administered intravenously to stain the normal region of the left ventricle (LV), and the heart was rapidly excised. LV tissue was isolated and cut into approximately 10 cross-sectional pieces of equal thickness. The nonstained LV area at risk (AAR) was separated from surrounding blue-stained LV normal zone, and both regions were separately incubated at 37°C for 15 min in 1% TTC in 0.1 M phosphate buffer adjusted

to pH 7.4. The tissues were fixed overnight in 10% formaldehyde. AAR and blue-stained LV normal zone region were weighed for determination of AAR / LV. TTC stains living tissue a deep red color, whereas necrotic tissue is TTC-negative and appears white within the AAR slices. Each slice was scanned at 1200 dpi with a commercial scanner (CanoScan LiDE 60; Canon, Japan), and infarcted and noninfarcted areas were measured using an image analysis program. Myocardial infarct size was expressed as a percentage of the AAR.

Statistical Analysis

Statistical analysis of hemodynamic data within and between groups was performed with analysis of variance for repeated measures followed by Dunnett's test. Inter-group differences in body weight, age, LV weight, AAR weight, the ratio of AAR to LV, and the ratio of infarct size to AAR were analyzed using one-way analysis of variance followed by the Student-Newman-Keuls test. Statistical significance was defined as $P < 0.05$. All values were expressed as mean \pm SD. Statistical analyses were performed using SPSS 15.0 software (SPSS Japan, Tokyo, Japan) or GraphPad Prism 5.0 (GraphPad Software, San Diego, CA).

Results

There were no significant differences in body weight or age among the groups. Hemodynamic data for the heart rate (HR) and the mean blood pressure (MBP) are shown in Table 1. There were no significant differences either within groups or among groups in HR or MBP at any measurement point.

There were no significant differences in LV weight, AAR weight, and the ratio of AAR to total LV mass among the groups (Table 2). Myocardial infarct sizes in the study groups are shown in Table 2 and Figure 2. Pre- and post-ischemic exposure to sevoflurane and administration of olprinone reduced infarct size compared with that of the control. NS-398 alone did not affect infarct size, but prevented sevoflurane-induced E-PreC and PostC. On the other hand, NS-398 did not affect olprinone-induced E-PreC and PostC.

Discussion

The present study is, to our knowledge, the first to demonstrate that pre- and post-ischemic administration of olprinone as well as exposure to sevoflurane protects rat hearts against ischemia-reperfusion injury, and that NS-398, a selective COX-2 inhibitor, prevents sevoflurane-induced E-PreC and PostC but not olprinone-induced E-PreC or PostC.

The present results show that olprinone could have not only E-PreC but also PostC properties in rat hearts. PDEIs are used for the treatment of heart failure, since these compounds have inotropic and vasodilatory effects via an increase in intracellular cAMP level in cardiomyocytes and vascular smooth muscles, and increase myocardial contractile force with less cardiac oxygen demand compared with catecholamines. It has been reported that the administration of olprinone before ischemia could lead to a reduction in myocardial infarct size in dogs¹¹ and rats^{12,16}. Use et al.¹⁷ showed that milrinone, a PDE3-I, administered before ischemia or just after reperfusion, attenuates myocardial stunning in swine. The present results show that a bolus application of the clinical loading dose, 10 µg/kg of olprinone just prior to reperfusion, has a significant effect on reducing myocardial infarct size. The present results also show that pre- and post-ischemic exposure to sevoflurane reduces myocardial infarct size, confirming the previous report by Obal et al.⁶, which showed that pre- and post-ischemic exposure to sevoflurane reduced myocardial infarct size in rat hearts. They suggested that sevoflurane-induced PostC as well as E-PreC would be mediated via m-K_{ATP} channel, since these cardioprotective effects were inhibited by a selective m-K_{ATP} blocker, 5-hydroxydecanoic acid (5-HD).

Ischemic stimulation upregulates the activity of COX-2 in the heart, which mediates L-PreC against ischemia reperfusion injury³. Ischemic stimulation activates a complex signal transduction cascade that includes PKC and protein tyrosine kinase, leading to activation of a

transcription factor, nuclear factor-kappa B (NF- κ B), and signal transducers and activators of transcription (STAT) 1/STAT3. Binding of NF- κ B and STAT1/STAT3 to the promoters of both inducible nitric oxide synthase (iNOS) and COX-2 results in coordinated transcriptional activation of iNOS and COX-2 genes with synthesis of new iNOS and COX-2 proteins. iNOS-derived nitric oxide can produce L-PreC both via direct actions and via activation of COX-2-dependent synthesis of cardioprotective PG. However, the role of COX-2 on E-PreC and PostC was not fully understood.

The present study showed that pre- and post-ischemic exposure to sevoflurane decreased myocardial infarct size, and NS-398 blocked this protective effect. Gres et al.¹⁸ showed that a non-selective COX inhibitor, indomethacin, could prevent the ischemic E-PreC-induced reduction in myocardial infarct size in pig LAD occlusion model. Alcindor et al.⁸ showed that a selective COX-2 inhibitor, celecoxib, prevented reductions in myocardial infarct size produced by pre-ischemic repetitive brief ischemia, exposure to isoflurane, and administration of diazoxide, and that a COX-1 inhibitor, aspirin, and a COX-3 inhibitor, acetaminophen, failed to block isoflurane-induced E-PreC. They also reported that isoflurane increased myocardial 6-keto-PGF_{1 α} from baseline, and celecoxib pretreatment prevented this increase. It was reported that PostC enhanced cardioprotective effects of L-PreC through further increase in PGE₂ content but not COX-2 protein¹⁹. Penna et al.⁹ showed that ischemic PostC and administrations of bradykinin and a PGI₂ analog, iloprost, during reperfusion induced cardioprotection, and indomethacin could prevent this beneficial effect. COX-2-dependent synthesis of PGE and PGI₂ also influences the activity of m-K_{ATP} channels. Pre-ischemic administration of PGE₀ and PGE₁ decreased myocardial infarct size in anesthetized rabbits, and this beneficial effect was blocked by 5-HD and a non-selective K_{ATP} channel blocker, glibenclamide²⁰. Shinmura et al.²¹ showed that a PGI₂ analog,

carbaprostacyclin, protected cardiac myocytes from oxidative stress via m-K_{ATP} channels, and that carbaprostacyclin alone had no effect on flavoprotein fluorescence but enhanced diazoxide-induced activation of m-K_{ATP} channels. It is likely that COX-2 activity would be needed for sevoflurane-induced activation of m-K_{ATP} channels that produce E-PreC and PostC.

As shown in the present results, a selective COX-2 inhibitor, NS-398, does not affect olprinone-induced E-PreC and PostC. It is suggested that pharmacological E-PreC and PostC would be mediated via both COX-2-dependent and -independent pathways. PKC and m-K_{ATP} channels mediate ischemic and sevoflurane-induced E-PreC and PostC^{6, 22}. COX-2-dependent synthesis of PGE and PGI₂ are also known to be closely related to PKC and m-K_{ATP} channels in cardioprotective mechanisms. Pre-ischemic administration of PDE3-I induces cardioprotection through the elevation of the level of cAMP and PKA activation independent of PKC¹¹. Our previous study showed that pretreatment with olprinone protects the heart against myocardial infarction and this cardioprotective effect is independent of PKC or m-K_{ATP} channels¹². Ye et al.¹³ showed that oral sitagliptin, a dipeptidyl-peptidase-IV inhibitor, pretreatment limits myocardial infarct size via cAMP-dependent PKA activation. They also showed that sitagliptin has no effect on cytosolic phospholipase A₂ (cPLA₂) and COX-2 activity. Thus, it is likely that olprinone-induced cardioprotection is mediated by a pathway independent of PKC or m-K_{ATP} channels and that this pathway is not influenced by COX-2 activity.

Myocardial ischemia induces COX-2 expression. Abbate et al. showed that COX-2 is expressed in cardiomyocytes at the site of recent acute myocardial infarction, and its expression is associated with extremely high apoptotic rates²³. Engelbrecht et al.²⁴ showed that inhibition of cPLA₂ reduces COX-2 induction and decreases caspase-3 and poly-ADP-ribose-polymerase cleavage during simulated ischemia/reperfusion in neonatal cardiac myocytes. Chronic

pretreatment with celecoxib reduces myocardial infarct size by inhibition of apoptosis in rat²⁵. Administration of parecoxib after myocardial infarction significantly ameliorates the remodeling process through prevention of apoptosis and preservation of myocardial vascularity²⁶. Saeed et al.²⁷ reported that nimesulide, a COX-2 inhibitor, improved coronary endothelial function after myocardial infarction. Thus COX-2 inhibition may reduce apoptosis and preserve endothelial function during myocardial ischemia reperfusion. In the present study, NS-398 alone did not affect infarct size, confirming a previous report²⁸. In addition, parecoxib pretreatment does not reduce myocardial infarct size but reduces apoptosis in mice²⁹. Thus it is likely that COX-2 inhibition has only a weak property of reducing myocardial infarct size.

In conclusion, a selective COX-2 inhibitor, NS-398, prevents sevoflurane-induced E-PreC and PostC. Pre- and post-ischemic administration of olprinone exerts cardioprotective effects independent of COX-2 activity, and suggested that pharmacological E-PreC and PostC could be mediated via both COX-2-dependent and -independent pathways.

Footnotes

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Table 1. Systemic Hemodynamics

Group	Baseline	Pre15	CO20	RP20	RP60	RP120
Control						
HR (beats/min)	405 ± 24	417 ± 34	416 ± 26	416 ± 29	405 ± 22	397 ± 27
MBP (mmHg)	105 ± 11	112 ± 9	106 ± 17	99 ± 21	104 ± 8	97 ± 10
Pre-S						
HR (beats/min)	402 ± 21	375 ± 33	391 ± 6	414 ± 15	392 ± 12	389 ± 18
MBP (mmHg)	108 ± 13	98 ± 17	117 ± 8	109 ± 15	121 ± 11	120 ± 9
Post-S						
HR (beats/min)	390 ± 17	428 ± 28	411 ± 21	420 ± 27	397 ± 28	394 ± 34
MBP (mmHg)	104 ± 7	102 ± 8	112 ± 8	93 ± 29	110 ± 6	106 ± 15
NS+Pre-S						
HR (beats/min)	391 ± 17	383 ± 26	384 ± 33	414 ± 27	384 ± 32	399 ± 29
MBP (mmHg)	107 ± 11	101 ± 15	109 ± 22	101 ± 16	108 ± 15	108 ± 17
NS+Post-S						
HR (beats/min)	403 ± 8	417 ± 28	403 ± 15	421 ± 19	403 ± 26	399 ± 23
MBP (mmHg)	100 ± 6	110 ± 12	96 ± 13	96 ± 11	106 ± 13	105 ± 14
Pre-O						
HR (beats/min)	397 ± 27	422 ± 25	399 ± 39	426 ± 14	406 ± 25	395 ± 27
MBP (mmHg)	105 ± 7	93 ± 16	99 ± 5	102 ± 24	106 ± 11	106 ± 15
Post-O						
HR (beats/min)	407 ± 21	401 ± 25	409 ± 20	417 ± 28	419 ± 13	403 ± 23
MBP (mmHg)	98 ± 8	107 ± 16	96 ± 7	98 ± 17	108 ± 8	104 ± 12
NS+Pre-O						
HR (beats/min)	414 ± 21	425 ± 30	409 ± 19	430 ± 30	418 ± 21	404 ± 19
MBP (mmHg)	97 ± 6	96 ± 11	98 ± 13	102 ± 14	108 ± 9	110 ± 8
NS+Post-O						
HR (beats/min)	417 ± 10	420 ± 27	421 ± 4	420 ± 27	410 ± 12	414 ± 13
MBP (mmHg)	99 ± 11	104 ± 14	100 ± 15	102 ± 24	104 ± 13	111 ± 11
NS						
HR (beats/min)	412 ± 39	409 ± 25	422 ± 32	428 ± 18	417 ± 11	386 ± 8
MBP (mmHg)	100 ± 13	101 ± 16	92 ± 12	99 ± 21	101 ± 15	101 ± 12

Data are presented as mean ± SD and n = 7 for each.

Pre-S and Post-S = pre- and post-ischemic exposure to sevoflurane; Pre-O and Post-O = pre- and post-ischemic administration of olprinone; NS = NS-398; HR = heart rate; MBP = mean blood pressure; Pre15 = 15 min before ischemia; CO20 = 20 min after coronary occlusion; RP20, RP60, and RP120 = 20, 60, and 120 min after reperfusion.

Table 2. Left Ventricular Area at Risk and Infarct Size

Group	AAR/LV (%)	IS/AAR (%)
Control	54 ± 9	42 ± 6
Pre-S	51 ± 11	16 ± 7 *
Post-S	58 ± 10	17 ± 6 *
NS+Pre-S	52 ± 5	35 ± 8 †
NS+Post-S	48 ± 10	42 ± 10 ‡
Pre-O	53 ± 10	14 ± 4 *
Post-O	49 ± 10	15 ± 10 *
NS+Pre-O	55 ± 10	12 ± 5 *
NS+Post-O	52 ± 11	19 ± 7 *
NS	48 ± 6	35 ± 6

Data are presented as mean ± SD and n = 7 for each.

Pre-S and Post-S = pre- and post-ischemic exposure to sevoflurane; Pre-O and Post-O = pre- and post-ischemic administration of olprinone; NS = NS-398; AAR = area at risk; LV = left ventricle; IS = infarct size.

* Significantly (P < 0.05) different from control group; † significantly (P < 0.05) different between Pre-S and NS+Pre-S; ‡ significantly (P < 0.05) different between Post-S and NS+Post-S.

Legends of Illustrations

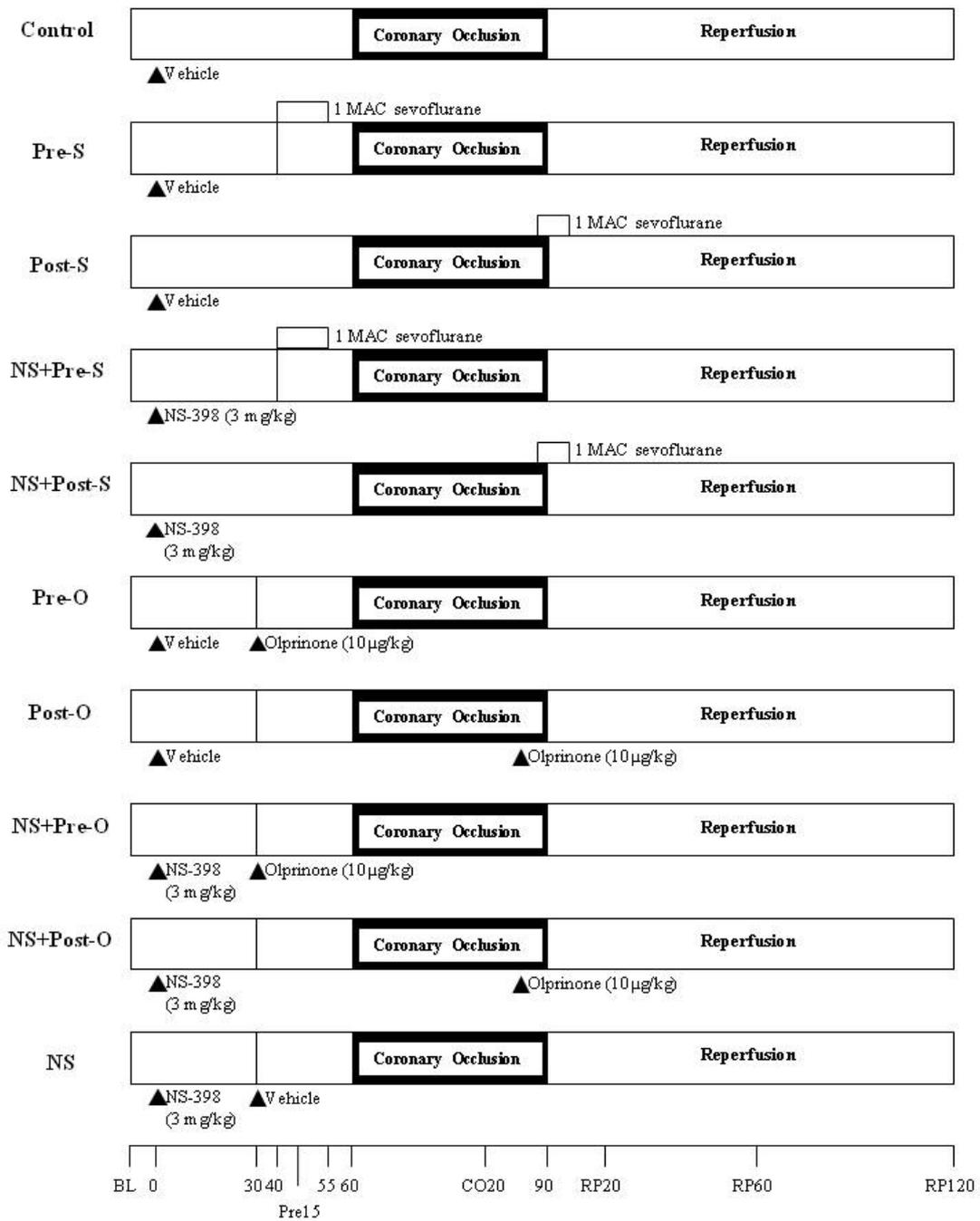


Figure 1. Schematic illustration of the experimental protocols.

Pre-S and Post-S = pre- and post-ischemic exposure to sevoflurane; MAC = minimal alveolar concentration; Pre-O and Post-O = pre- and post-ischemic administration of olprinone; NS = NS-398; HR = heart rate; MBP = mean blood pressure; Pre15 = 15 min before ischemia; CO20 = 20 min after coronary occlusion; RP20, RP60, and RP120 = 20, 60, and 120 min after reperfusion.

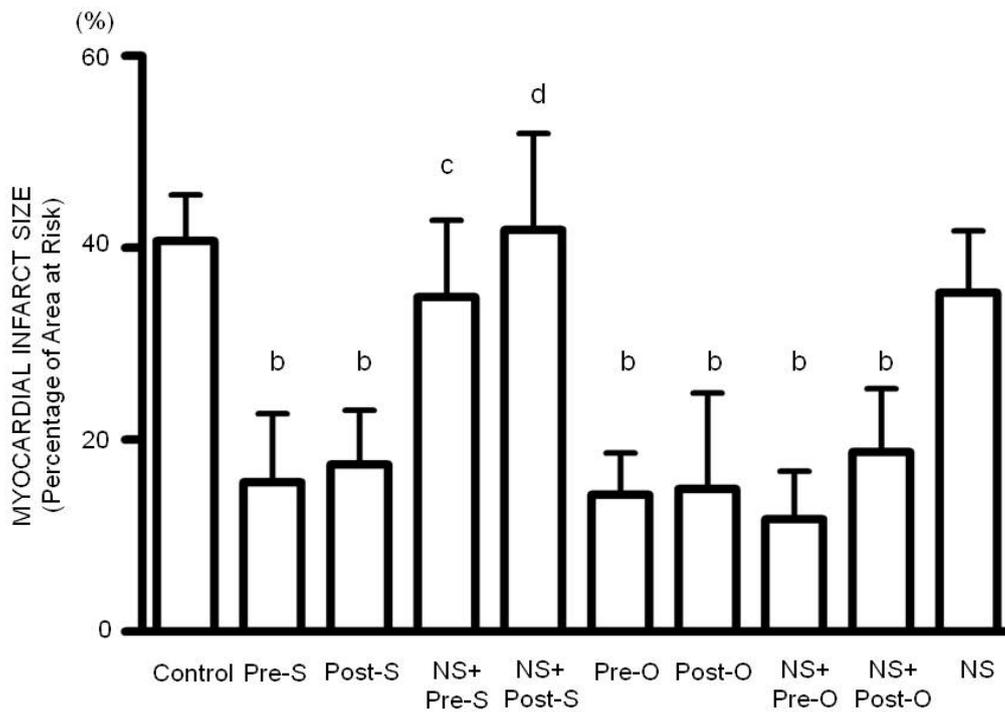


Figure 2. Myocardial infarct size expressed as a percentage of the left ventricular area at risk.

Data are presented as mean \pm SD and n = 7 for each.

Pre-S and Post-S = pre- and post-ischemic exposure to sevoflurane; Pre-O and Post-O = pre- and post-ischemic administration of olprinone; NS = NS-398.

* Significantly ($P < 0.05$) different from control group; † significantly ($P < 0.05$) different between

Pre-S and NS+Pre-S; ‡ significantly ($P < 0.05$) different between Post-S and NS+Post-S.