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Effects of Sevoflurane Exposure on Myocardial Infarction and Arrhythmia during Ischemia and Reperfusion in *in vivo* Rabbit Hearts

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Abstract

The effects of sevoflurane on myocardial ischemia and reperfusion injury have not been well studied, especially in *in vivo* model. The present study aimed to investigate the effect of timing and duration of sevoflurane exposure on the intensity of myocardial response, and ischemia/reperfusion arrhythmias.

All anesthetized open-chest rabbits underwent 30 min of left anterior descending coronary artery (LAD) occlusion followed by 3 hrs of reperfusion. In the control group (C), and sevoflurane group (S), rabbits were subjected to 30 min of LAD occlusion and 3 hrs of reperfusion under ketamine/xylazine (k/x) or sevoflurane anesthesia respectively. The ischemia-preconditioned rabbits underwent 5 min of LAD occlusion followed by 10 min of reperfusion under k/x anesthesia (C-IP), or sevoflurane (S-IP). In the sevoflurane-preconditioned group (C-SP), 30 min of sevoflurane exposure at a 1.5% end-tidal concentration was followed by 15 min of washout before 30 min of LAD occlusion and 3 hrs of reperfusion under k/x anesthesia. At the end of 3-hrs reperfusion period, area at risk was delineated by Evans blue and infarct size determined by triphenyltetrazolium chloride (TTC) staining. The rate pressure product did not alter significantly at any point among all the groups. Compared with group C, myocardial protective effect was observed in groups of S, C-IP, S-IP and C-SP. However, infarct size in sevoflurane-preconditioned group was significantly larger than those of S and S-IP groups. Also, infarct limiting effect of S-IP group was significantly intensified compared with C-IP and S. Continuous exposure of sevoflurane reduced arrhythmias during not only ischemia but reperfusion period in rabbit hearts.

These results suggested that continuous sevoflurane exposure might confer additive infarct limiting effect on ischemic preconditioning. We found that ischemic preconditioning and sevoflurane preconditioning do not have anti-arrhythmic effect, though sevoflurane exposure has anti-arrhythmic effects against ischemia and reperfusion-induced arrhythmia.

Key words; sevoflurane, ischemic preconditioning, arrhythmia

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Introduction

Repeated brief episodes of ischemia and reperfusion render the myocardium more resistant against a subsequent sustained period of ischemia and reperfusion: a phenomenon called ischemic preconditioning[1]. This phenomenon has been recognized as one of the most powerful modes of protecting the heart following ischemia. Recently, volatile anesthetics such as sevoflurane[2~4] and isoflurane[5] have been proposed as preconditioning agents. Although anesthetic pre-

conditioning against myocardial infarction by isoflurane is well characterized, the effects of sevoflurane on myocardial ischemia and reperfusion injury and anesthetic protection against arrhythmias have not been well studied, especially in *in vivo* model. Also, protection against arrhythmias by ischemic preconditioning and anesthetic preconditioning has been proposed as an additional cardioprotection, but few studies have reported those effects on ischemia/reperfusion induced arrhythmias.

The present study aimed to investigate the effect of timing and duration of sevoflurane exposure on the intensity of myocardial response, and ischemia/reperfusion arrhythmias.

Materials and methods

The present study was performed in accordance with the Guidelines of Animal Care and Use Committee of Kanagawa Dental College.

Male New Zealand White rabbits weighing 2.7–3.2 kg were allowed *ad libitum* access to standard laboratory stock diet and water. Animals were initially anesthetized with ketamine (35 mg/kg) and xylazine (5 mg/kg) given intramuscularly. Tracheotomy was performed and rabbits were intubated with an uncuffed endotracheal tube (ID 3.5 mm). The animals were ventilated with room air supplemented with additional oxygen. Inspired and expired anesthetic concentration, inspiratory O_2 percentage and end-tidal CO_2 partial pressures were continuously monitored using a multigas anesthetic monitor (Datex, Capnomac, Helsinki, Finland). The respiratory rate was frequently adjusted to maintain P_aO_2 greater than 100 mmHg, P_aCO_2 at 35–45mmHg, pH 7.35–7.45, and Base Excess between −3 and +3. After the left jugular vein was exposed and cannulated with a polyethylene catheter, 0.9% sodium chloride (0.15 ml/min) was continually administered during the experiments. The carotid artery was dissected out and fluid-filled polyethylene tube was placed in it and connected immediately to an electrocardiogram monitor (Nihon-kohden Co, Life scope 11, Tokyo, Japan) *via* pressure transducer (Nihon-kohden Co, TP-400T, Tokyo, Japan) for arterial pressure recording. An electrocardiogram was recorded throughout the experiment *via* lead II of the standard electrocardiogram. Ischemia or reperfusion-induced arrhythmias included premature ventricular contraction (PVC) and ventricular tachycardia (VT). After all the surgical procedures had been performed, a 15 min period was allowed for stabilization. Anesthesia was maintained with ketamine and xylazine solution (ketamine 35 mg/kg/hr, xylazine 5 mg/kg/hr i.m.; k/x) or sevoflurane with room air supplemented with additional pure oxygen. Left thoracotomy was performed and pericardium was opened to expose the heart. A silk thread (K-890H, Ethicon, Somerville, NJ) with taper C-1 needle was passed around the left anterior descending artery (LAD) and the end of the tie were threaded through a small vinyl tube to form a snare. The LAD was occluded by pulling the snare, which was then fixed by clamping the tube with a mosquito hemostat. Myocardial ischemia was confirmed by regional cyanosis, ST segment elevation and decreased blood pressure. Reperfusion was confirmed by reactive hyperemia over the surface after releasing the snare.

The experimental design used in the current study is illustrated in **Fig. 1**. Rabbits were randomized into five groups (n=6, respectively). All the rabbits received regional ischemia by 30 min of the LAD occlusion followed by 3 hrs of reperfusion under ketamine/xylazine (k/x) or sevoflurane anesthesia. In the control group and sevoflurane group, rabbits were subjected to 30 min of LAD occlusion and 3 hrs of reperfusion under k/x or sevoflurane anesthesia, respectively (C, S). Sevoflurane at a 1.5% end-tidal concentration was administered just from tracheal intubation to the end of reperfusion. The ischemia-preconditioned (IP) rabbits underwent 5 min LAD occlusion followed by 10 min of reperfusion before 30 min of LAD occlusion and 3 hrs of reperfusion under k/x or sevoflurane anesthesia, respectively (C-IP, S-IP). In the sevoflurane-preconditioned group (C-SP), 30 min of sevoflurane exposure at a 1.5% end-tidal concentration was followed by 15 min of washout

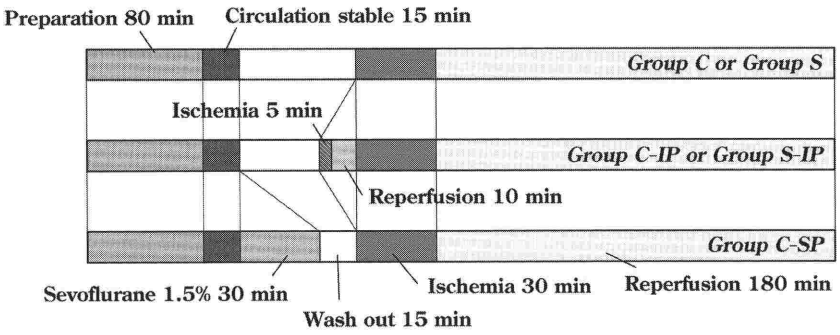

Figure 1 Schematic diagram of the protocol.

Table 1 Rate pressure product during ischemia and reperfusion

	Pre Ischemia	30 min after Ischemia	30 min after Reperfusion	60 min after Reperfusion	180 min after Reperfusion
Group C	13656.7±1379.7	13177.8±1826.5	13378.0±1979.4	12984.7±2010.6	12548.7±976.7
Group S	15193.5±2904.0	13706.8±2328.6	14285.0±3273.2	13943.0±3334.7	15333.0±4191.4
Group C-IP	14502±1570.1	14131.5±1430.9	13714.2±2389.6	14080.8±1579.9	12600.2±1736.1
Group S-IP	12709±1889.9	13477.7±2593.7	13812.3±2171.3	15797.7±3155.0	18761.3±4934.3
Group C-SP	12827±2364.1	12738.7±1874.5	12102.3±2048.9	10797.8±2348.3	10314.7±2910.0

before 30 min of LAD occlusion and 3 hrs of reperfusion under k/x anesthesia. Following completion of experimental protocol, the *in vivo* visualization of the myocardium at risk was accomplished with reocclusion of the coronary artery and injection of 10% Evans blue into the venous cannula until the eyes turned blue. The Evans blue was allowed to circulate for about 30 sec to demarcate the risk and non-risk regions. The hearts were quickly excised under deep anesthesia and frozen. The frozen hearts were then cut into six transverse slices of equal thickness. The area at risk was determined by negative staining with Evans blue. The slices were stained by incubation for 15 min in 1% triphenyl tetrazolium chloride (TTC) in isotonic pH 7.4 phosphate buffer. After staining, the sections were placed in formalin for preservation, and measurements of risk area, infarct area and left ventricle were made with computer aided morphometry. From each section, the ischemic risk area and the infarcted area were outlined and measured by planimetry. The area from each region was averaged from the slices. Infarct size was expressed both as a percentage of total left ventricle (LV) and as a percentage of the ischemic risk area.

Hemodynamic measurements included systolic, diastolic, mean arterial blood pressures and heart rate. Rate pressure product was calculated as the product of heart rate and peak arterial pressure. Baseline hemodynamic measurements were taken prior to any experimental manipulations. Subsequently, the measurements were taken at 15 min of ischemia and 15, 60, 120 and 180 min of reperfusion.

Comparisons of myocardial tissue weights and necrosis data were made by one way analysis of variance (ANOVA). Statistical comparisons of individual hemodynamic parameters between groups were made using one-way ANOVA followed by Fisher's protected least significant difference. Bartlett's test for equality of variances was used to ensure the validity of statistical comparison using the one-way ANOVA. All data are reported as group mean±SD, and were considered statistically significant at a probability value (P) less than 0.05.

Results

Rate pressure product (RPP) is shown in **Table 1**. No significant difference in the baseline levels of these parameters was observed between each group.

The RPP did not alter significantly throughout the reperfusion period at the data points in all the groups. Mean values were not significantly different among the groups at any time point for all the groups.

The ratio of areas at risk to left ventricular mass ranged from 47.4±14.7% to 65.7±14.3% with no significant difference among all the groups (**Fig. 2**). **Fig. 3** shows the infarct size expressed as percentage of area at risk in five groups. Mean infarct size was 59.2±4.8% of the risk area in Group C, and mean infarct size was decreased significantly in Group S, Group C-IP, Group S-IP and Group C-SP: 30.9±3.7%, 35.9±3.3%, 16.8±4.1%, and 45.2±3.0%, respectively ($p<0.05$ v.s. control) (**Fig. 3**). Infarct size in ischemia-preconditioned group under sevoflurane anesthesia was significant small compared with that of group S. Infarct size in group S-IP was significantly smaller compared with that of group C-IP. The mean infarct sizes in S, C-IP, and S-IP were significantly smaller than that of group C-SP ($p<0.05$ v.s. control). However, there was no significant difference in the infarct size limiting effect between ischemic preconditioning (C-IP) and sevoflurane induced preconditioning under k/x anesthesia (C-SP).

The incidence of arrhythmias during myocardial ischemia were 50% in C, 16% in S, 83% in C-IP, 0% in S-IP, 50% in C-SP (**Fig. 4**). The incidence of arrhythmias during reperfusion were 67% in C, 0% in S, 67% in C-IP, 0% in S-IP, 67% in C-SP (**Fig. 5**).

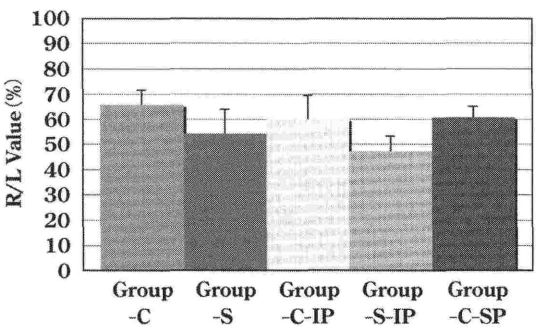

Figure 2

The figure shows R/L value (%), which means the area at risk expressed as percentage of left ventricle. Area at risk revealed no significant difference among all groups, suggesting that the changes in the infarct size observed among the groups did not depend on R/L.

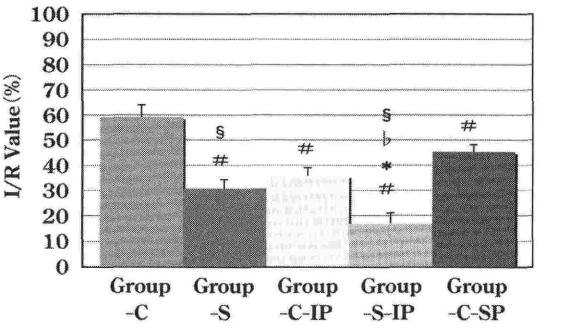

Figure 3

The figure shows I/R value (%), which means the infarct size expressed as percentage of area at risk.

Significantly different ($p<0.05$) from Group C.
* Significantly different ($p<0.05$) from Group S.
§ Significantly different ($p<0.05$) from Group C-SP.
♭ Significantly different ($p<0.05$) from Group C-IP.

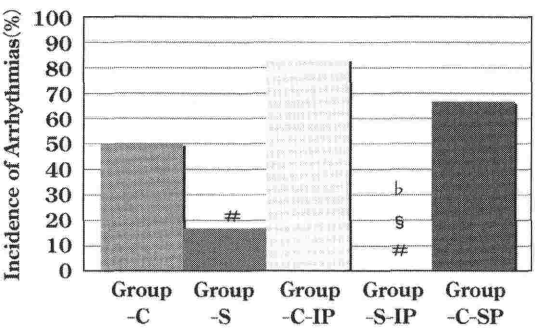

Figure 4

The figure shows incidence of arrhythmias during myocardial ischemia.

Significantly different ($p<0.05$) from Group C.
§ Significantly different ($p<0.05$) from Group C-SP.
♭ Significantly different ($p<0.05$) from Group C-IP.

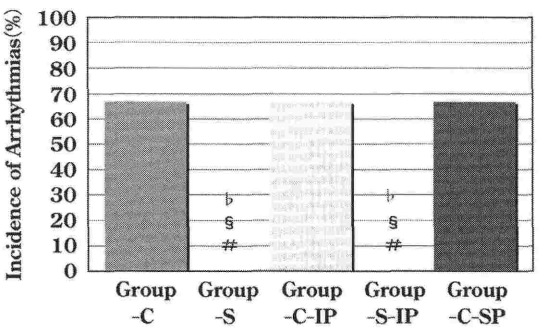

Figure 5

The figure shows incidence of arrhythmias during reperfusion.

Significantly different ($p<0.05$) from Group C.
§ Significantly different ($p<0.05$) from Group C-SP.
♭ Significantly different ($p<0.05$) from Group C-IP.

Discussion

Although the cardioprotection of sevoflurane is well established [2,6~8], there has been little information about the effects of this agent on the extent of ultimate myocardial infarction and the incidence of arrhythmias in *in vivo* ischemia/reperfusion model. The major findings of our study are that (1) the infarct size limiting effect by ischemic preconditioning was significantly augmented under sevoflurane anesthesia, and (2) continuous sevoflurane exposure reduces arrhythmias during not only ischemia but also reperfusion period, though neither ischemic preconditioning nor sevoflurane-induced preconditioning decreased the incidence of ventricular arrhythmias in *in vivo* rabbit hearts.

Infarct size is affected by some factors such as the choice of anesthetics, timing and duration of their administration, hemodynamics, and collateral blood flow of the animal used.

It has been discussed as to the possibility that the choice of anesthetics may affect hemodynamic data as well as the severity of ischemia[9,10]. However, the rate pressure product, which is one index of myocardial oxygen demand, did not significantly differ among the groups of present study. Thus, the protective effects of sevoflurane exposure on ischemia and reperfusion injury in this experimental system were probably not mediated by a decreased oxygen demand. Also, brief sevoflurane exposure before prolonged ischemic insult directly preconditions myocardium against infarction in the absence of hemodynamic effects. This suggests that the effect of sevoflurane on myocardial oxygen balance cannot contribute to improved recovery during reperfusion.

The second explanation for the infarct-limiting effect with sevoflurane exposure is that this agent might be a trigger to increase collateral blood flow to the ischemic area during coronary occlusion. However, it was determined that coronary collateral flow in rabbits is less than 2%, which is similar to human hearts [11,12]. Thus, the beneficial effects of sevoflurane exposure in our models are not explained by the increased collateral blood flow to the ischemic area.

The lower infarcts observed in our results do not appear to be attributable to factors other than the anesthetic; thus sevoflurane itself seems to possess cardioprotection. Experimental observations have extensively shown that sevoflurane markedly decreased myocardial infarct size after prolonged coronary occlusion[13~15]. The results of the current study indicated that the cardioprotective effects of sevoflurane were related to timing and duration of its administration. The cardioprotective effects of sevoflurane seem more potent when administered throughout the procedure than when used only in the preconditioning period. In clinical studies, the cardioprotective effects of sevoflurane were also most apparent when it was administered throughout the operation[16].

The ability of volatile anesthetics to reduce myocardial damage when administered before coronary artery occlusion and reperfusion has been repeatedly demonstrated in experimental animals [17~19] and humans[20].

A recent investigation[2] from our laboratory demonstrated that simultaneous administration of sevoflurane-induced preconditioning and ischemic preconditioning does not induce additional infarct limiting effect over that provided by each intervention alone. On the other hand, cardioprotective effect by ischemic preconditioning was significantly augmented by sevoflurane anesthesia. Although the exact mechanisms underlying the cardioprotective effects of sevoflurane-induced preconditioning are unknown, opening of K_{ATP} channels seems to play a pivotal role in the signal transduction cascade as well as ischemic preconditioning[8,17].

Although the potential interaction between anesthetic used and cardioprotection is still clearly unknown, protocol to induce sevoflurane-induced cardioprotection may vary greatly among researchers with regard to the anesthetic concentration, the exposure and washout time and whether applied once or in repeated cycles and *in vivo* or *in vitro* study. For example, 3.7% of sevoflurane had no significant pre-

conditioning-like effect in the same rabbit model[21]. In preliminary experiments, we have administered a variety of dose and duration of sevoflurane exposure and found that sevoflurane does not always produce more profound reduction in infarct size in concentration-dependent manner, and that optimal concentration of sevoflurane for maximum obtaining infarct size limiting effect was 1.5% and duration of sevoflurane exposure was 30 minutes following 15 minute washout. Kehl et al.[22] observed that low concentrations of isoflurane (0.25 or 0.5 MAC) were sufficient to precondition against myocardial infarction but that the efficacy of this treatment was diminished when coronary collateral blood flow was low. On the other hand, at higher concentrations of isoflurane (1.0 or 1.25 MAC), the cardioprotective effects were independent of the extent of collateral flow[16]. In the present investigation, pretreatment with 30 min of sevoflurane exposure at a 1.5% endtidal concentration followed by 15 min of washout before the prolonged ischemia succeeded in reducing infarct size in comparison with controls. Thus, we could confirm that pretreatment with sevoflurane exposure at a 1.5% endtidal concentration, as with isoflurane[5], mimics the cardioprotective effects of ischemic preconditioning.

In myocardial infarctions with ST-segment elevation, ischemia followed by reperfusion leads to arrhythmias. A variety of mechanisms have been proposed to explain the genesis of ischemia/reperfusion arrhythmias[6,23~25].

It was reported that ischemic preconditioning reduced incidence of ventricular arrhythmias *in vivo* rat and dog models[26~28] as well as *in vitro*[21].

As far as we have investigated, there have been no references that preconditioning reduces arrhythmias in *in vivo* rabbit model. In this study, ischemic or anesthetic preconditioning did not have anti-arrhythmic effect in spite of infarct size limiting effect. Although we can not clearly explain the discrepancy from their results, it might be due to little collateral flow in rabbits[11,12].

Conclusion

Continuous sevoflurane exposure might confer additive infarct limiting effect on ischemic preconditioning. Also, ischemic preconditioning and sevoflurane preconditioning do not have anti-arrhythmic effect, though sevoflurane exposure has anti-arrhythmic effects against ischemia and reperfusion-induced arrhythmia.

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