Sevoflurane does not confer additive cardioprotection on early ischemic preconditioning in rabbit hearts

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Abstract

The present study aimed to compare the cardioprotective potencies of sevoflurane and ischemic preconditioning (IP) in vivo rabbit hearts. All anesthetized open-chest rabbits underwent 30 min of left anterior descending coronary artery (LAD) occlusion followed by 3 h of reperfusion. Before this, rabbits were randomized into one of six groups. Control rabbits received no intervention for 45 min before LAD occlusion and reperfusion (control; n=5). The ischemia-preconditioned (IP) rabbits underwent 5 min LAD occlusion followed by 10 min of reperfusion (IP; n=5). In the sevoflurane (S) group, 30 min of sevoflurane exposure at a 1.5% end-tidal concentration was followed by 15 min of washout (S, n=5). The selective mitochondrial K\textsubscript{ATP} channel blocker, 5-hydroxydecanoate (5-HD, 5 mg/kg) was given intravenously 10 min before ischemic preconditioning and sevoflurane exposure, respectively, (5-HD+IP; n=5, 5-HD+S; n=5). In the sevoflurane-plus-IP group, rabbits received 30 min of sevoflurane exposure at a 1.5% end-tidal concentration followed by 15 min of washout before 5 min LAD occlusion and 10 min of reperfusion (S+IP; n=5). At the end of the 3-h reperfusion period, area at risk and infarct size were measured. There were no differences in systemic hemodynamics among 6 groups. The area at risk showed no significant differences during baseline conditions among experimental groups. Mean infarct size was 67.4 ± 1.5% (mean±SD) of the risk area in untreated controls. The mean infarct size was significantly smaller in the IP, S, and S+IP groups: 41.2±0.9%, 49.7±4.6%, and 40.9±3.6%, respectively (P<0.05 vs. control). In contrast, mean infarct size was 56.7±2.1% in the 5-HD+IP group, and 61.6±2.8% in the 5-HD+S group. Sevoflurane-induced preconditioning as well as IP exerts infarct size limiting effect through opening of mitochondrial K\textsubscript{ATP} channels. Our data suggest that there is no additive effect of sevoflurane on IP induced cardioprotection.

Key words: Sevoflurane, Anesthetic-induced preconditioning, Ischemic preconditioning, Cardioprotection, Heart infarct size

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\(^1\). There have been many reports on cardiac preconditioning as multiple brief periods of ischemia\(^2\), monophosphoryl lipid A\(^3\), whole body heat stress\(^4\),\(^5\), and volatile anesthetics\(^6\)–\(^9\).

Recently, it has been suggested that isoflurane\(^10\) as well as ischemia\(^11\) may actually activate mitochondrial K\textsubscript{ATP} channels and provide protection which is specifically blocked by the selective mito-
chondrial K<sub>ATP</sub> channel blocker, 5-hydroxy-decanoate (5-HD)<sup>11,12</sup>. However, it is not known that sevoflurane also exerts such a protective effect via opening of mitochondrial K<sub>ATP</sub> channels, although recent investigations showed that sevoflurane can reduce myocardial infarct size by activating sarcolemmal K<sub>ATP</sub> channels in dog models<sup>31</sup>. To our knowledge, there is no report that sevoflurane exposure before prolonged ischemia can induce infarct size limiting effect via opening of mitochondrial K<sub>ATP</sub> channels in vivo rabbit models. This study, therefore, was to determine whether the mitochondrial K<sub>ATP</sub> channel blocker, 5-HD (5mg/kg) abrogates the protection afforded by sevoflurane and/or IP. Also, the potential interaction between anesthetic-induced preconditioning and IP is still unknown. It would be interesting to know whether anesthetic-induced preconditioning may confer additional cardioprotection on IP when the myocardium is already in a protected state. The second goal of this study was to investigate a possible interaction of IP and sevoflurane-induced preconditioning in the rabbit hearts in vivo during anesthesia with ketamine and xylazine. Moreover, we investigated if there is an additive effect of sevoflurane on IP induced cardioprotection.

Methods and Materials

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

A. General Surgical Preparation

Male New Zealand White rabbits weighing 2.7~3.2kg were allowed ad libitum access to standard laboratory stock diet and water. Animals were initially anesthetized with ketamine (35mg/kg) and xylazine (5mg/kg) given intramuscularly. Five ml of 1% lidocaine was subcutaneously injected as an additional local anesthetic during the initial surgical procedures. Tracheotomy was performed and rabbits were intubated with an uncuffed endotracheal tube (ID 3.5mm). The animals were ventilated with room air supplemented with additional oxygen using mechanical ventilator (Shinano, SN-480-5, Tokyo, Japan) and a semi-closed breathing circuit (Shinano, SN-487, Tokyo, Japan). Inspired and expired anesthetic concentration, inspiratory O<sub>2</sub> percentage and end-tidal CO<sub>2</sub> partial pressures were continuously monitored using a multigas anesthetic monitor (Datex, Capnomac, Helsinki, Finland). Ventilator rate was 30~35 breaths per minute and tidal volume was between 30~35 ml. The respiratory rate was frequently adjusted to maintain PaO<sub>2</sub> greater than 100mmHg, PaCO<sub>2</sub> 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. 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. The rabbits were given 500units of heparin for preventing thrombus formation in the coronary artery after reperfusion. 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.

B. Study Groups and Experimental Protocol

Fig. 1 presents study groups and experimental protocol. Anesthesia was maintained with ketamine and xylazine solution (ketamine 35mg/kg/hr,
xylazine 5 mg/kg/hr i.m.; KX) with room air supplemented with additional pure oxygen. Anesthetic and respiration were frequently adjusted to maintain steady hemodynamics throughout the experiments in all groups of animals. After all the surgical procedures had been performed, a 15 min period was allowed for stabilization. All anaesthetized open-chest rabbits underwent 30 min of left anterior descending coronary artery (LAD) occlusion followed by 3h of reperfusion. Before this, the animals were randomized into one of the following experimental protocols:

Control rabbits received no intervention for 45 min before LAD occlusion and reperfusion (control; n=5). The ischemia-preconditioned (IP) rabbits underwent 5 min coronary artery occlusion followed by 10 min of reperfusion (IP; n=5). In the sevoflurane (S) group, 30 min of sevoflurane exposure at a 1.5% end-tidal concentration was followed by 15 min of washout (S, n=5). The K<sub>ATP</sub> channel blocker, 5-hydroxydecanoate (5-HD, 5mg/kg) was given intravenously 10 min before ischemic preconditioning (IP) and sevoflurane exposure, respectively, (5-HD+IP; n=5, 5-HD+S; n=5). In the sevoflurane-plus-IP group, rabbits received 30 min of sevoflurane exposure at a 1.5% end-tidal concentration followed by 15 min of washout before 5 min LAD occlusion and 10 min of reperfusion (S+IP; n=5). All rabbits that did not receive 5-hydroxydecanoate were given a control injection of vehicle. At the end of the 3-h reperfusion period, area at risk and infarct size were measured.

C. Hemodynamic measurements

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.

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 30sec 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 area at risk, infarcted area and left ventricle were made with computer aided morphometry. From each section, the ischemic risk area (unstained by blue dye) and the infarcted area (unstained by TTC) 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 the ischemic risk area.

**Statistical analysis**

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 ± SEM, and were considered statistically significant at a probability value (P) less than 0.05.

**Results**

**A. Hemodynamic parameters**

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

**B. Infarct Size and Area at Risk**

The areas at risk ranged from 49.3 ± 11.5% to 70.7 ± 6.7% with no significant difference among all the groups (Fig. 2), suggesting that changes in the size of infarct observed between the groups were not related to the percentage of area of left ventricle

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<th>Table 1 Rate pressure product during ischemia and reperfusion</th>
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*: P < 0.05 vs. control (mean ± SEM)

![Figure 2](image_url) Aria at risk/LV expressed as percentage of anatomic area at left ventricle. Data are expressed as mean±SEM.
occluded by our technique.  Fig. 3 shows the infarct size expressed as percentage of area at risk in eight groups. Mean infarct size was 67.4±1.5% of the risk area in untreated controls and mean infarct size was decreased significantly in IP, S, and S+IP groups: 41.2±0.9%, 49.7±4.6%, and 40.9±3.6%, respectively (P < 0.05 vs. control). In contrast, mean infarct size was 56.7±2.1% in the 5-HD+IP group, and 61.6±2.8% in the 5-HD+S group.

From these results, sevoflurane did not enhance the infarct limitation effect of ischemic preconditioning. The K<sub>ATP</sub> channel blocker, 5-HD by itself had no apparent effect on infarct size in non-preconditioned rabbits (data not shown), but elicited complete protection in both sevoflurane and ischemia preconditioned hearts. The vehicle solution for 5-HD did not have a significant effect on infarct size as compared to the non-treated controls.

Discussion

The major finding of our study is that sevoflurane-induced preconditioning does not confer additional cardioprotection on early ischemic preconditioning in vivo rabbit model. In addition, the data obtained from the present study suggest that sevoflurane exposure before prolonged ischemic insult directly precondition myocardium against infarction via activation of mitochondrial K<sub>ATP</sub> channels in the absence of hemodynamic effects and exhibits acute memory of preconditioning, and sevoflurane-induced preconditioning shares similar mechanism as ischemic preconditioning, which induced activation of mitochondrial K<sub>ATP</sub> channels.

The first explanation for the infarct-limiting effect with brief sevoflurane exposure is that this agent might as a trigger to increase collateral blood flow to the ischemic area during coronary occlusion. Maxwell and colleagues<sup>14</sup> determined there is species variation in the coronary circulation during regional myocardial ischemia. They also found that coronary collateral flow in rabbits is almost zero, which is similar to human hearts. Thus, the beneficial effects of sevoflurane exposure in our models are not explained by the improved myocardial oxygen demand. Second, it has been discussed as to the possibility that the choice of anesthetics may affect hemodynamic data as well as the severity of ischemia<sup>15,16</sup>. Indeed, heart rate of the pentobarbitone anesthetized rabbit has been reported by several investigators<sup>17,18</sup> to range between 240 and 290 beat/min, whereas that of ketamine-xylazine anesthetized rabbit is reportedly less than 200 beat/min. including our data<sup>19–21</sup>. In spite of the difference of heart rate, blood pressure appears similar among different reports, indicating that the difference of rate pressure product would affect the oxygen de-
mand of the heart, although heart rate itself has no effect on infarct size in the rabbit model. The rate pressure product, which is one index of myocardial oxygen demand, was not decreased in sevoflurane preconditioned group. Thus, the protective effects of sevoflurane exposure on ischemia and reperfusion injury were probably not mediated by reduced contractility with a decreased oxygen demand, although it has been proposed as a potential mechanism of myocardial protection by volatile anesthetics\(^{22,23}\).

In the present investigation, the infract size was determined by staining with triphenyltetrazolium chloride. This method has been found to be an accurate measure of ultimate infract size at 2 to 48 hr of reperfusion when compared with subsequent histologic analysis in animals not receiving further treatment\(^{24}\). Tetrazolium staining has been demonstrated to reveal equivalent infract size values when compared with histologic determination in rabbits after 2 to 3 hr of reperfusion\(^{25}\). Since similar conditions of ischemia and reperfusion were used in the present study, the lower infracts observed in our results do not appear to be attributable to factors other than the anesthetic; thus sevoflurane seems to possess preconditioning-mimicked effect.

Recently, it has been suggested that preconditioning of the heart by means of pharmacological agents, such as monophosphoryl lipid A\(^3\) and sildenafil\(^{26}\) and volatile anesthetics\(^{6,8,13}\), produces a marked decrease in infract size followed by a prolonged ischemic insult. Mitochondrial K\(_{ATP}\) channels are thought to play a central role in mediating these phenomena\(^{13,27}\). An important piece of evidence for implicating mitochondrial K\(_{ATP}\) channels as mediators of preconditioning is the consistent inhibitory effect of 5-HD. Of importance in the present study is the observation that 5-HD abolished cardioprotection by sevoflurane induced preconditioning. These data suggest that the protective effect due to sevoflurane may be mediated, at least in part, by mitochondrial K\(_{ATP}\) channel. The relative contributions of cardiomyocyte mitochondrial versus sarcolemmal K\(_{ATP}\) channels in the cardioprotection remain to be known, though the mitochondrial K\(_{ATP}\) channels have been proposed to be involved as a subcellular mediator in cardioprotection afforded by ischemic preconditioning\(^{11}\) and anesthetic induced preconditioning\(^{29}\). Further, there is considerable evidence that 5-HD is a selective inhibitor of the mitochondrial K\(_{ATP}\) channels\(^{12}\). Thus, the protective effects by sevoflurane as well as brief ischemia may be mediated by mitochondrial rather than sarcolemmal K\(_{ATP}\) channel opening.

Since simultaneous administration of sevoflurane and IP does not induce additional protection over that provided by each intervention alone, cardioprotection by sevoflurane exposure is thought to be mediated by the same end effector as ischemic preconditioning. If there were an incomplete activation of K\(_{ATP}\) channels by sevoflurane exposure, infract size would be augmented by IP. However, in the present study, IP 1ed to a similar strong infract size reduction, as sevoflurane induced preconditioning. Probably, sevoflurane exposure already induces maximal cardioprotection, or at least over threshold for preconditioning. Although the exact mechanisms underlying the cardioprotective effects of sevoflurane-induced preconditioning are unknown, opening of K\(_{ATP}\) channels plays a pivotal role in the signal transduction cascade.

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, Pirioiu V \textit{et al.}\(^{29}\) reported that halothane, isoflurane and desflurane induced pharmacological preconditioning, whereas 3.7% of sevoflurane had no significant preconditioning-like effect in the same rabbit model as in this study. It is apparent that high concentration of sevoflurane cannot be administered \textit{in vivo} models because of decrease of systemic circulation. In preliminary experiments, we have administered a variety of dose and duration of sevoflurane exposure and found sevoflurane does
not always produce more profound reduction in infarct size in concentration-dependent manner, and optimal concentration and time period of sevoflurane for obtaining infarct size limiting effect was 1.5% and 30 minutes and 15 minute washout. 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 by 26.3% in comparison with controls. Thus, we could confirm the results of the present studies that pretreatment with sevoflurane exposure at a 1.5% endtidal concentration, as with other volatile anesthetics, mimics the cardioprotective effects of ischemic preconditioning.

In conclusion, sevoflurane exposure as well as IP exerts infarct size limiting effect through opening of mitochondrial KATP channels. Our data suggest that there is no additive effect of sevoflurane on IP induced cardioprotection. Further studies will be necessary to determine the time, period, and concentration of sevoflurane to elicit the maximal cardioprotection.

References


