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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 mitochondorial KATP 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 KATP 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[1,2], monophosphoryl lipid A[3], whole body heat stress[4,5], and volatile anesthetics[6~9].

Recently, it has been suggested that isoflurane[9] as well as ischemia[10] may actually activate mitochondorial KATP channels and provide protection which is specifically blocked by the selective mito-

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chondorial K_{ATP} channel blocker, 5-hydroxy-decanoate (5-HD)[11,12]. However, it is not known that sevoflurane also exerts such a protective effect *via* opening of mitochondorial K_{ATP} channels, although recent investigations showed that sevoflurane can reduce myocardial infact size by activating sarcolemmal K_{ATP} channels in dog models[13]. To our knowledge, there is no report that sevoflurane exposure before prolonged ischemia can induce infarct size limiting effect *via* opening of mitochondorial K_{ATP} channels *in vivo* rabbit models. This study, therefore, was to determine whether the mitochondrial K_{ATP} 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_2 percentage and end-tidal CO_2 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_2 greater than 100mmHg, $PaCO_2$ 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. 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,

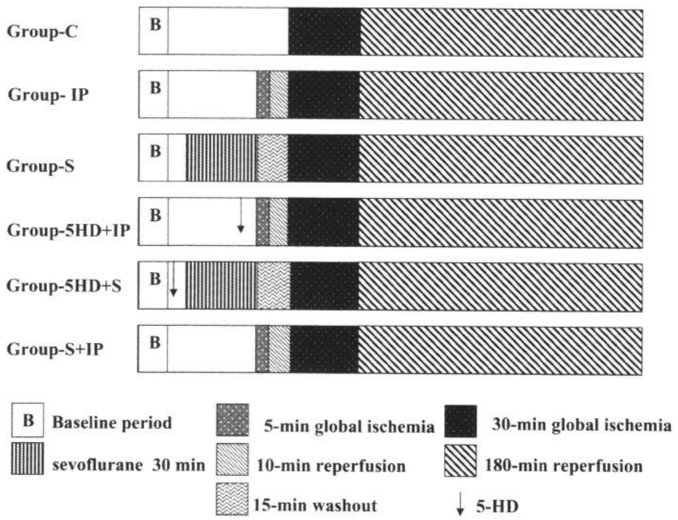

Figure 1 Schematic diagram of the protocol

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_{ATP} 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

Table 1 Rate pressure product during ischemia and reperfusion

	Baseline	Washout/ Reperfusion	Reperfusion		
			30	60	180
kx Control	15426.4±1535.504	15426.4±1535.504	15065.6±1533.942	14870.6±1262.562	12526.6± 458.952
IP	14871.2± 912.505	15047.8± 769.657	13916.2±1027.565	14549.8± 605.913	13210.0± 692.124
Sevo	15365.6±1306.085	13801.2± 856.189	13801.2± 856.189	12227.6±1350.619	11375.4±1754.437
5HD+IP	12018.4± 481.756	11848.2± 689.790	12342.6± 495.115	12019.6± 307.156	11269.0± 717.495
5HD+Sevo	13681.0±1714.820	12267.0±1299.650	11735.4±1312.425	11303.6± 999.027	10750.8±1128.289
Sevo+IP	14067.6± 846.210	13607.8±1086.326	14272.0± 914.499	14141.4±1681.372	12869.0±1267.021

*:P<0.05 *vs.* control (mean±SEM)

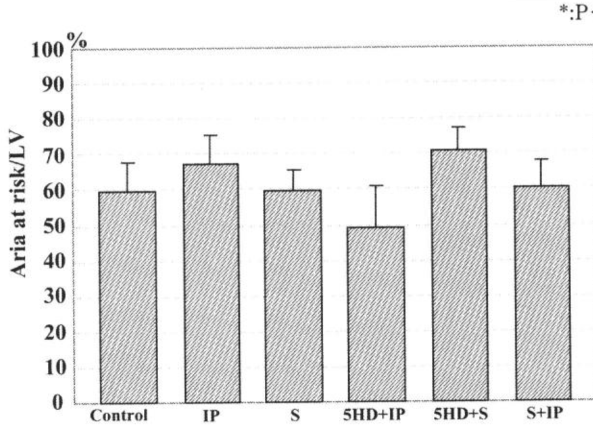

Figure 2 Aria at risk/LV expressed as percentage of anatomic area at left ventricle. Data are expressed as mean±SEM.

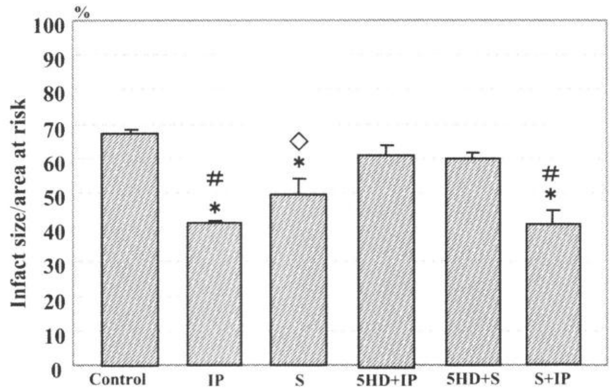

Figure 3 Infarct size expressed as percentage of anatomic area at risk.
Data are expressed as mean±SEM.
* Significantly different ($P<0.05$) from Group-C
◇ Significantly different ($P<0.05$) from Group-5HD+S
Significantly different ($P<0.05$) from Group-5HD+S, Group-5HD+IP

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 KATP 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 preconditions myocardium against infarction *via* activation of mitochondorial KATP 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 mitochondorial KATP 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[14] 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[15,16]. Indeed, heart rate of the pentobarbitone anesthetized rabbit has been reported by several investigators[17,18] 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[19~21]. 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 demand 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 infarct size was determined by staining with triphenyltetrazolium chloride. This method has been found to be an accurate measure of ultimate infarct 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 infarct 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 infarcts 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 infarct 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 mitochondorial *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[28]. 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, infarct size would be augmented by IP. However, in the present study, IP 1ed to a similar strong infarct 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, Piriou V *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 *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 30minutes and 15minute washout. In the present investigation, pretreatment with 30min of sevoflurane exposure at a 1.5% enditidal concentration followed by 15min 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% enditidal 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 mitochondorial K_{ATP} 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

1) Murry CE, Jennings RB, Reimer KA: Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986; 74: 1124-36.

2) Lawson CS, Downey JM: Preconditioning: State of the art myocardial protection. Cardiovasc Res 1993; 27: 542-50.

3) Yoshida K-i, Maaieh MM, Shipley JB, et al: Monophosphoryl lipid A induces pharmacologic 'preconditioning' in rabbit hearts without concomitant expression of 70-kDa heat shock protein. Mol Cell Biochem 1996; 159: 73-80.

4) Yoshida K-i, Sawada H, Furuya M, et al: N-2Mercaptopropionyl Glycine blocks ischemic tolerance and synthesis oh heat shock protein 70 in rabbit hearts. Circ Cont 2003; 24: 46-52.

5) Currie RW, Tanguay RM, Kingma JG Jr., et al: Heat-shock response and limitation of tissue necrosis during occlusion/reperfusion in rabbit hearts. Circulation 1993; 87: 963-71.

6) Chiari P, Bouvet F, Piriou V: Anaesthetic-induced myocardial preconditioning: fundamental basis and clinical implications. Ann Fr Anesth Reanim 2005; 24: 383-96.

7) Riess ML, Kevin LG, Camara AK, et al: Dual exposure to sevoflurane improves anesthetic preconditioning in intact hearts. Anesthesiology 2004; 100: 569-74.

8) Mullenheim J, Ebel D, Bauer M, et al: Sevoflurane confers additional cardioprotection after ischemic late preconditioning in rabbits. Anesthesiology 2003; 99: 624-31.

9) Kersten JR, Schmeling TJ, Pagel PS, et al: Isoflurane mimics ischemic preconditioning via activation of K(ATP) channels: reduction of myocardial infarct size with an acute memory phase. Anesthesiology 1997; 87: 361-70.

10) Rajesh KG, Sasaguri S, Suzuki R, et al: Ischemic preconditioning prevents reperfusion heart injury in cardiac hypertrophy by activation of mitochondrial KATP channels. Int J Cardiol 2004; 96: 41-9.

11) Das B, Sarkar C : Is the sarcolemmal or mitochondrial K(ATP) channel activation important in the antiarrhythmic and cardioprotective effects during acute ischemia/reperfusion in the intact anesthetized rabbit model? Life Sci 2005; 77: 1226-48.

12) Liu Y, Sato T, Seharaseyon J,et al: Mitochondrial ATP-dependent potassium channels. Viable candidate effectors of ischemic preconditioning. Ann N Y Acad Sci 1999; 874: 27-37.

13) Toller WG, Kersten JR, Pagel PS, et al: Sevoflurane reduces myocardial infarct size and decreases the time threshold for ischemic preconditioning in dogs. Anesthesiology 1999; 91: 1437-46.

14) Maxwell MP, Hearse DJ, Yellon DM: Species variation in the coronary collateral circulation during regional myocardial ischemia: a critical determinant of the rate of evolution and extent of myocardial infarction. Cardiovasc Res 1987; 21: 737-46.

15) Haessler R, Kuzume K, Chien GL, et al: Anaesthetics alter the magnitude of infarct limitation by ischaemic preconditioning. Cardiovasc Res. 1994; 28: 1574-80.

16) Jugdutt BI, Rogers MC, Hutchins GM, et al: Increased myocardial infarct size by thiopental after coronary occlusion in the dog. Am Heart J 1986; 112: 485-94.

17) Marber MS, Latchman DS, Walker JM, et al: Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. Circulation. 1993; 88: 1264-72.

18) Cohen MV, Yang XM, Downey JM: Conscious rabbits become tolerant to multiple episodes of ischemic preconditioning. Circ Res 1994; 74: 998-1004.

19) Sawada H, Yoshida K-i, Kukreja RC: Blockade of ischemic preconditioning by glibenclamide in rabbits anesthetized with sevoflurane. The Bulletin of Kanagawa Dental College 1997; 25: 65-71.

20) Furuya M, Yoshida K-i, Ohsawa A: Comparative effects of induced hypotension with nicardipine and nitroglycerin on myocardial ischemia and reperfusion injury. Circ Cont 1999; 20: 281-7.
21) Yoshida K-i, Takano H, Sawada H, et al: Lidocaine protects the ischemic rabbit myocardium from infarction as well as from ventricular arrhythmias. Am J Anesthesiol 2000; 27: 419-23.
22) Stowe DF, Monroe SM, Marijic J, et al: Comparison of halothane, enflurane, and isoflurane with nitrous oxide on contractility and oxygen supply and demand in isolated hearts. Anesthesiology. 1991; 75: 1062--74.
23) Stowe DF, Kevin LG: Cardiac preconditioning by volatile anesthetic agents: a defining role for altered mitochondrial bioenergetics. Antioxid Redox Signal 2004; 6: 439-48.
24) Horneffer PJ, Healy B, Gott VL, et al: The rapid evolution of a myocardial infarction in an end-artery coronary preparation. Circulation 1987; 76: 39-42.
25) Goto M, Miura T, Iliodoromitis EK, et al: Adenosine infusion during early reperfusion failed to limit myocardial infarct size in a collateral deficient species. Cardiovasc Res 1991; 25: 943-9.
26) Kukreja RC, Salloum F, Das A, et al: Pharmacological preconditioning with sildenafil: Basic mechanisms and clinical implications. Vascul Pharmacol 2005; 42: 219-32.
27) Zaugg M, Lucchinetti E, Spahn DR, et al: Volatile anesthetics mimic cardiac preconditioning by priming the activation of mitochondrial K(ATP) channels via multiple signaling pathways. Anesthesiology 2002; 97: 4-14.
28) Toller WG, Gross ER, Kersten JR, et al: Sarcolemmal and mitochondrial adenosine triphosphate- dependent potassium channels: mechanism of desflurane-induced cardioprotection. Anesthesiology 2000; 92: 1731-9.
29) Piriou V, Chiari P, Lhuillier F, et al: Pharmacological preconditioning: comparison of desflurane, sevoflurane, isoflurane and halothane in rabbit myocardium. Br J Anaesth 2002; 89: 486-91.