原 著

Differential Effects of Halogenated Volatile Anesthetics on Myocardial Ischemia/Reperfusion Injury in *In Vivo* Rabbit Model

Mitsuhiro Ito*, Uno Imaizumi*, Hisashi Beppu*, Hitoshi Yui*, Munetaka Furuya*, Hirofumi Arisaka*, Kazu-ichi Yoshida*

Abstract

This study evaluated the comparative effects of three inhalational anesthetic agents on myocardial infarction and arrhythmias in rabbit hearts subjected to a regional ischemia/reperfusion insult. Rabbits received regional ischemia by 30 min of left anterior descending coronary artery (LAD) occlusion and followed by 3 hours of reperfusion under general anesthesia. The anesthetics studied were: ketamine/xylazine (35 mg/kg/hr and 5 mg/kg/hr respectively, Control), halothane (1.0%, H), isoflurane (1.4%, I), and sevoflurane (2.1%, S). At the end of the 3 hrs reperfusion, the area at risk was delineated by Evans blue staining and the infarct size was determined by tetrazolium staining. The area at risk showed no significant differences among the groups. The mean infarct size was 59.3±1.9% of the risk area in Control, and it was significantly greater than those in H, I and S: 36.9±3.3%, 39.1±4.8%, and 23.0±3.2%, respectively ($p < 0.05$ vs. control). The incidence of arrhythmia during myocardial ischemia was 66.7% in Control, 16.7% in H, I, and S. The incidence of arrhythmias during reperfusion was 50.0% in Control, 33.3% in H, 16.7% in I and S. The volatile anesthetics tested in this study could protect the ischemic rabbit heart from infarction, as compared with ketamine/xylazine anesthesia. Sevoflurane may have the most powerful cardioprotection among these volatile anesthetics.

Key words; halothane, isoflurane, sevoflurane, ischemia/reperfusion injury, myocardial protection

*Division of Anesthesiology, Department of Clinical Care Medicine, Kanagawa Dental College, Kanagawa, Japan

Introduction

All halogenated volatile anesthetics have cardiac depressant effects that decrease myocardial oxygen demand and may thus improve the myocardial oxygen balance during ischemia. Recent experimental evidence has clearly demonstrated that, in addition to these indirect effects, volatile anesthetic agents also directly protect from ischemic myocardial damage[1].

Halogenated volatile anesthetic agents mimic ischemic preconditioning, a powerful cardioprotective phenomenon first described in 1986[2], which represents an adaptive response to brief sublethal episodes of ischemia leading to an enhanced protection against subsequent lethal ischemia. The potential cardioprotective effects of volatile anesthetics were actually discovered before the concept of anesthetic preconditioning had been investigated. In 1988, Warltier et al.[3] demonstrated that pretreatment with halothane or isoflurane improved left ventricular systolic function after a 15 min left anterior descending coronary artery (LAD) occlusion.

The reperfusion of coronary arteries is arrhythmogenic and may result in ventricular arrhythmias[4].

Several studies have suggested the protective effect of halogenated volatile anesthetics on ischemic hearts[5~8], but little is known about the effects on ischemia and reperfusion-induced arrhythmias.

In the present study, we examined differential effects of three halogenated volatile anesthetics on ischemia and reperfusion-induced arrhythmias as well as on myocardial infarction.

Materials and methods

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

A. General Surgical Preparation and Experimental Protocol

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. 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 3.5 mm). The animals were ventilated with room air supplemented with additional oxygen using a 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 100 mmHg, $PaCO_2$ at 35~45 mmHg, and pH 7.35~7.45. 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.

Electrocardiogram was recorded throughout the experiment *via* lead II. Ischemia or reperfusion-induced arrhythmias included premature ventricular contraction (PVC) and ventricular tachycardia (VT). Rabbits were randomly assigned to the following 4 groups (n=6, respectively) by maintenance anesthetics: ketamine and xylazine (ketamine 35 mg/kg/hr, xylazine 5 mg/kg/hr i.m.; K/X) (Control), halothane 1.0% (H), isoflurane 1.4% (I), and sevoflurane 2.1% (S) groups.

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. After all the surgical procedures had been performed, a 15 min period was allowed for stabilization. Then, the LAD was occluded by pulling the snare, which was 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 received regional ischemia by 30 min of the LAD occlusion followed by 3 hrs of reperfusion.

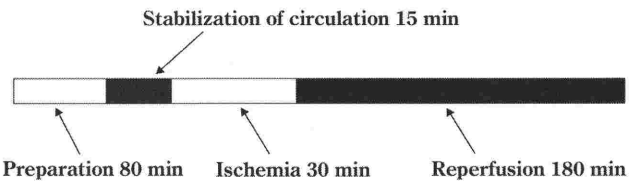


Figure 1 Schematic diagram of the protocol

B. Determination of area at risk and infarct size

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 area at risk (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. The infarct size was expressed both as a percentage of total left ventricular mass (L) and as a percentage of the ischemic risk area.

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 29 min of ischemia and 30 and 60 min of reperfusion.

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

Results

Rate pressure product (RPP) in each group is shown in **Table 1**. RPPs were not significantly different between Control and S groups. However, RPPs in H and I were significantly less as compared with Control.

The ratio of areas at risk to left ventricular mass ranged from 47.9±4.8% to 58.7±6.2% with no significant difference among all the groups (**Fig. 2**). **Fig. 3** shows the infarct size expressed as percentage of the area at risk in four groups. Mean infarct size of the area at risk was significantly greater in Control (59.3±1.9%), than those in H (36.9±3.3%), I (39.1±4.8%), and S (23.0±3.2%) ($p<0.05$). The infarction size in S was significantly less than those of H and I.

The incidence of arrhythmias during myocardial ischemia was 66.7% (4/6) in Control, 16.7% (1/6) in H and I, and S. The incidence of arrhythmias during reperfusion was 50.0% (3/6) in Control, 33.3% (2/6) in H, 16.7% (1/6) in I and S.

Table 1 Rate Pressure Products (RPP) at Pre-ischemia, 29 min after ischemia and 60 min after reperfusion (mmHg-beats per min)

	Pre-ischemia	29 min after ischemia	60 min after reperfusion
Control	13,528±262	12,588±341	12,965±478
Hal	9,653±869#	10,397±1,570#	9,209±407#
Iso	10,740±874#	10,762±950	10,348±1,210#
Sev	14,055±341	13,413±1,004	15,506±1,336

# $p<0.05$ vs Control

Hal: halothane, Iso: isoflurane, Sev: sevoflurane

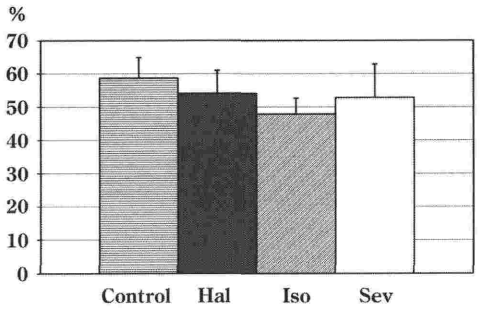
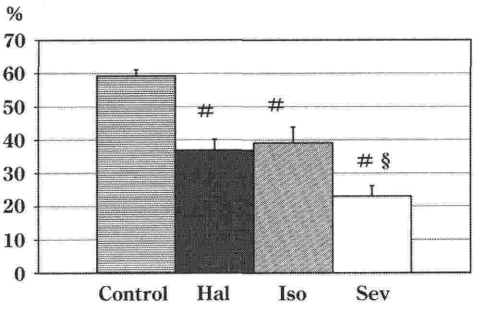


Figure 2

The figure shows ratio of areas at risk to left ventricular mass value, which means the area at risk expressed as percentage of left ventricle. The area at risk revealed no significant difference among all groups.
Hal; halothane, Iso; isoflurane, Sev; sevoflurane.



 # $p<0.05$ vs. Control
§ $p<0.05$ vs. Hal, Iso

Figure 3

The figure shows the infarct size expressed as percentage of the area at risk in four groups. Mean infarct size of the area at risk was significantly less in H, I, and S as compared with Control.
Hal; halothane, Iso; isoflurane, Sev; sevoflurane.

Discussion

The present study shows that the halogenated volatile anesthetic agents halothane, isoflurane and sevoflurane could protect the heart from infarction as compared with ketamine/xylazine anesthesia. In *in vivo* rabbit hearts, continuous administration of a volatile anesthetic before and during ischemia reduced the extent of infarction by about 38%, 34%, and 61% in H, I, and S, respectively. Sevoflurane, therefore, may have the most powerful cardioprotection.

Several studies demonstrated that volatile anesthetics reduced myocardial oxygen demand during ischemia, resulting in a reduced ischemic damage[9~11]. As mechanisms behind this protection, the negative inotropic and negative chronotropic action of the anesthetics is suggested[12]. Moreover, volatile anesthetics maintain myocardial energy stores and might increase collateral blood flow towards the ischemic area, thereby reducing the severity of ischemia[12]. However, it should be taken into account that collateral blood flow in rabbits is almost zero, which is similar to that of human heart. There has been a report that the myocardial protective effects of halothane may be caused, at least in part, to a decrease in oxygen demand relative to oxygen supply[13,14].

Since the RPP, which is one index of myocardial oxygen demand, significantly less in H and I as compared with Control, our study also suggests that the infarct size limiting effect by halothane and isoflurane may partly depend on myocardial oxygen demand and supply. The direct mechanisms underlying protec-

tion from ischemia/reperfusion injury by halothane remain unclear, although beneficial effects of halothane anesthesia appear to be involved in inhibition of intracellular calcium accumulation and improved recovery of voltage-sensitive calcium channels after ischemia and reperfusion[15].

In the present study, 1.4% isoflurane reduced the extent of infarction to almost the same degree as halothane. These results are consistent with the report that 1.7% isoflurane exposure reduced infarct size *in situ* rabbit model[15]. However, a previous investigation from our laboratory demonstrated that 1.2% isoflurane exposure failed to limit infarct size[16]. This indicates that the ability of isoflurane to reduce infarct size depends on the concentration of its exposure.

On the contrary, RPP did not significantly alter among Control and S, though sevoflurane exposure significantly reduced infarct size. This suggests that cardioprotection by sevoflurane was not directly related to a decrease in myocardial oxygen consumption. The results of our study were similar to those reported previously in our laboratory[16]. Treatment with glibenclamide, ATP-sensitive potassium channel blocker, of preconditioned rabbits resulted in a significant increase in the infarct size[17]. These studies indicate that myocardial protection afforded by sevoflurane during ischemia and reperfusion, at least, may be due to K_{ATP} activation.

The cellular mechanisms responsible for volatile anesthetic-induced cardioprotection are not fully understood, but many studies have indirectly suggested that the cardioprotection depend on both adenosine receptors and protein kinase C, and thus is similar to the mechanism of protection seen with ischemic preconditioning[15]. The study supports that the choice of anesthetic regiments affects the outcome of patients following cardiac surgery[2].

Halothane, isoflurane and sevoflurane reduced incidence of arrhythmias during ischemia and reperfusion, but the data did not reach statistical significance. Halothane has been reported to decrease the incidence and duration of ischemia-induced arrhythmia in a dose-dependent manner in dogs and rats models[5,18]. On the contrary, MacLeod et al. reported that halothane pre-treatment had no appreciable effect upon ischemia-induced arrhythmias in pigs[6]. In the rabbit, in which collateral flow is negligible, halothane anesthesia was associated with a significantly lower incidence of ventricular dysrhythmias and less regional postischemic systolic dysfunction after reperfusion. It is probably due to the differences of timing and concentration of halothane exposure, species, length of ischemia, and extent of collateral blood flow[19].

In conclusion, the volatile anesthetics tested in this study could protect the ischemic rabbit heart from infarction, in contrast to ketamine/xylazine anesthesia. Sevoflurane has the most powerful cardioprotection regardless of myocardial oxygen consumption, though cardioprotection by halothane and isoflurane might be in part related to a decrease in myocardial oxygen consumption. Halothane, sevoflurane and isoflurane have antiarrhythmic effects during ischemia (halothane = isoflurane = sevoflurane) and reperfusion (isoflurane = sevoflurane > halothane), as well as infarct limiting effects.

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