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Lidocaine protects against myocardial ischemia-reperfusion injury in anesthetized rabbits

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In order to investigate the myocardial protective effect of lidocaine, we used a model of myocardial ischemia in anesthetized rabbits to study the effect on infarct size of lidocaine infusion during the first 30 minutes of reperfusion after myocardial ischemia. Japanese white rabbits anesthetized with nitrogen oxide, oxygen and pancuronium were placed on their sides. The left anterior descending coronary artery was exposed via left thoracotomy. Following a 30-minute ischemia to the coronary artery, a 30-minute infusion of lidocaine at 1.0 mg/kg/h or physiological saline was started immediately after the initiation of 120-minute reperfusion. During the experiment, systemic blood pressure was continuously monitored using a catheter placed in the femoral artery, and serum lidocaine concentration was determined periodically. At the end of the experiment, the heart was isolated and treated with Evans blue dye to identify the area at risk (AAR) and the non-risk area. The areas of infarction and the risk area were determined using 1% 2,3,5-triphenyltetrazolium chloride (TTC).

Although the systemic arterial pressure at 10 and 20 minutes after the coronary ligation was lower than baseline, no significant differences were observed between the lidocaine and saline groups. The AAR and non-risk areas did not differ significantly between the lidocaine and saline groups. The percentage of infarct size per AAR was 14.4% lower in animals receiving lidocaine infusion during reperfusion than in those receiving saline. These findings indicate that high-dose lidocaine infusion protects the myocardium against ischemia-reperfusion injury without affecting blood pressure during reperfusion. (J Osaka Dent Univ 2013 ; 47 : 201–207)

Key words : Lidocaine ; Myocardial protection ; Myocardial infarction ; Reperfusion injury ; Rabbit

INTRODUCTION

Perioperative myocardial infarction is a major complication that significantly affects patient prognosis. Surgical invasion and anesthesia significantly change perioperative hemodynamics, and increases the risk of myocardial ischemia. Lidocaine, an anesthetic agent that has been commonly used for local anesthesia during dental procedures, is often used for the treatment of ventricular arrhythmias associated with acute myocardial infarction. It has been reported that lidocaine exerts a pharmacological preconditioning effect similar to that of volatile anesthetics, and that administration of lidocaine before and during myocardial ischemia decreases infarct size after reperfusion and lessens myocardial injury.[1–4] However, although many studies on myocardial ischemic preconditioning and pharmacological preconditioning mainly with volatile anesthetic agents have been conducted as *ex vivo* studies using isolated hearts,[5, 6] the number of *in vivo* studies is limited. It has been suggested that lidocaine exerts its myocardial protective effect only when administered at high doses prior to ischemia, and it is largely unknown whether lidocaine given after the initiation of reperfusion protects myocardium.[2]

In the present study, lidocaine was continuously ad-

ministered immediately after the initiation of reperfusion after myocardial ischemia to investigate the effect of the drug on infarct size after ischemia-reperfusion.

MATERIALS AND METHODS

Animals

Male Japanese white rabbits weighing between 2.3 and 3.1 kg (JW/CSK ; Shimizu Laboratory Supplies, Kyoto, Japan) were used in this study. All procedures were carried out under auspices of the Guidelines for Animal Research at Osaka Dental University and with the approval of the Animal Experiment Committee of Osaka Dental University (No.11–03032), Osaka, Japan. These guidelines were in accordance with the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Science. The rabbits were allowed access to food and tap water *ad libitum.*

Induction of myocardial ischemia

All animals were anesthetized by slowly injecting 25 mg/kg pentobarbital sodium (Kyoritsu Seiyaku, Tokyo, Japan) into an ear vein. Each animal was intubated with an endotracheal tube (Portex ; Smiths Medical Japan, Tokyo, Japan) of inner diameter 4.0 mm through a midline tracheal incision, which was immediately connected to a positive pressure respirator for small animals (Model 680 ; Harvard Apparatus, South Natick, MA, USA). Using a capnometer (Capnocheck plus ; BCI International, Waukesha, WI, USA), tidal volume and respiratory frequency were adjusted to maintain normocapnia. During experiments, anesthesia was maintained with a mixture of 50% nitrogen oxide, 50% oxygen. Ringer's acetate solution containing pancuronium bromide was administered through a 22G polyethylene catheter inserted into the left ear vein at a rate of 10 mg/kg/h to immobilize each animal and maintain its fluid volume.

A 22G polyethylene catheter inserted into the right femoral artery was connected to a pressure transducer (P23XL ; Spectramed, Oxnard, CA, USA) to monitor arterial blood pressure continuously and check for the occurrence of arrhythmia based on the arterial pressure wave. It also served to analyze blood sampling for determination of serum lidocaine concentrations. A heating mat (ATC–101 ; Unique Medical, Tokyo, Japan) was used to maintain rectal temperature at 37.0–38.0°C. The chest was opened via left thoracotomy in the fourth intercostal space and the beating heart was exposed after pericardiotomy. A 3–0 silk thread was passed through the myocardium around the left anterior descending coronary artery (LAD). Ischemia was induced by pulling the ends of the suture through a small segment of a soft tube, which was firmly attached against the artery with a clamp.

The successful induction of ischemia was verified by visual inspection (cyanosis) of the heart. The occurrence of ventricular fibrillation (VF) during ischemia was identified based on visual inspection and arterial pressure wave, and VF was defibrillated by applying pressure to the left ventricle with a cotton swab. Reperfusion was achieved by releasing the clamp and verified by refilling of the artery.

Experimental protocols

The animals were randomly divided in two groups : the controls (CTL, $n = 8$) and the lidocaine-pharmacological post conditioning group (L-PPC, $n = 6$). Both groups were exposed to 30 min ischemia followed by 120 min reperfusion and infarct size was estimated. Immediately after reperfusion, saline was given intravenously at a rate of 0.1 mL/kg/h for 30 min in the CTL animals and lidocaine was given at the rate of 1.0 mg/kg/h for 30 min in the L-PPC group. Experimental protocols are presented schematically in Fig. 1. Serum lidocaine concentrations were measured immediately after lidocaine disconnection and at the end of 120 min reperfusion. Mean arterial blood pressure was measured before sustained ischemia (baseline), at 15 min after occlusion, immediately after reperfusion, at 10, 20 and 30 min after infusion of saline and lidocaine, and at 60 and 120 min after reperfusion.

Risk area and infarct size measurement

At the end of 120 min reperfusion, the hearts were quickly removed and mounted on a reperfusion apparatus and perfused retrogradely via the aorta with nor-

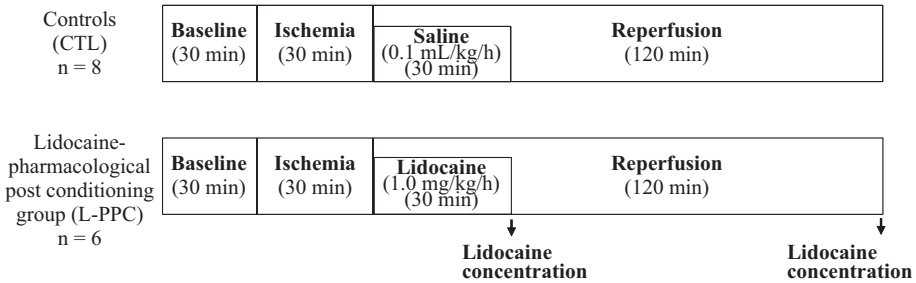

Fig. 1 Experimental protocol. After a stabilization period of 30 min (baseline), all rabbit hearts were subjected to 30 min of ischemia followed by 120 min of reperfusion. In the controls (CTL), saline was infused intravenously at 0.1 mL/kg /h for 30 min immediately after reperfusion. In the lidocaine-pharmacological post conditioning group (L-PPC), lidocaine was infused intravenously at 1 mg/kg/h for 30 min immediately after reperfusion. Serum lidocaine concentration was determined immediately after conclusion of lidocaine infusion and at the end of the 120-minute reperfusion period.

mal saline (10 mL/min) for 2 min. When all residual blood had been removed from the coronary arteries, the coronary ligature was retightened at the same site and 5 mL of 10% Evans blue dye (Sigma, St. Louis, MO, USA) was infused over 5 min for the delineation of the normally perfused tissue from the area at risk (AAR) zone. The hearts were then frozen at –80°C for 15 min, and sliced into 2-mm-thick sections from the apex to base (six slices/heart).

After removing the right ventricle and defrosting, each slice was weighed and incubated in 1% 2,3,5-triphenyl tetrazolium chloride (TTC) (Sigma) in isotonic phosphate buffer solution at pH 7.4 for 10 min at 37°C. The slices were immersed in 10% formaldehyde solution for at least 5 h to clearly delineate the infarcted areas. To identify the borders between the risk area and the normal area, slices were examined under UV light. Each slice was photographed (EOS ; Canon, Tokyo, Japan) and the area of necrotic myocardium determined using digital imaging software (Adobe Photoshop CS ; Adobe, CA, USA). The area was then multiplied by the weight of the slice, and the infarct and risk area volumes were expressed in cm^3 and the percent of infarct to risk area (%I/R) was calculated.

Statistical analysis

Results are reported as the mean and standard deviation (SD). Data of mean arterial pressure (MAP) were analyzed by a two-way repeated measures ANOVA for time and drug. When significant differences were detected, comparisons were performed as one-way ANOVA followed by post hoc tests. Analysis of infarct size was performed using one-way ANOVA followed by post hoc tests. A probability of $p < 0.05$ was considered significant.

RESULTS

Sixteen rabbits were always used in fourteen experiments ; two rabbits with VF were excluded for hemodynamic reasons. Eight animals were used for the CTL group and six for the L-PPC group. There was no significant difference in body weight among the groups. Heart weights were not different among the groups.

Changes in mean arterial pressure (MAP)

Baseline MAP was similar between the groups. At 15 min after occlusion and immediately after reperfusion, MAP decreased in both groups compared with

Table 1 Changes in mean arterial blood pressure

Baseline	101 ± 12	
15 min after occlusion	81 ± 12*	
Immediately after reperfusion	78 ± 14*	
	Lidocaine	Saline
During infusion :		
at 10 min	88 ± 21	87 ± 10
at 20 min	88 ± 20	89 ± 12
at 30 min	85 ± 19	87 ± 11
During reperfusion :		
60 min after reperfusion	87 ± 12	91 ± 14
120 min after reperfusion	89 ± 8	97 ± 10

Mean ± SD, *$p < 0.05$ vs baseline. (mmHg)

Table 2 Time to occurrence of arrhythmia and ventricular fibrillation after occlusion of the left anterior descending coronary artery (LAD)

	Arrhythmia	VF	Arrhythmia + VF
Cases (n)	6/16	2/16	8/16
Time (sec)	418 ± 130	540 ± 254	–

Mean ± SD

Table 3 Serum lidocaine concentration in animals in the L-PPC group immediately after completion of lidocaine infusion and at the end of the 120-minute reperfusion period

	Immediately after 30 min of lidocaine infusion	120 min after reperfusion
L-PPC (n = 6)	25.1 ± 2.6	2.3 ± 0.5

Mean ± SD (μg/mL)

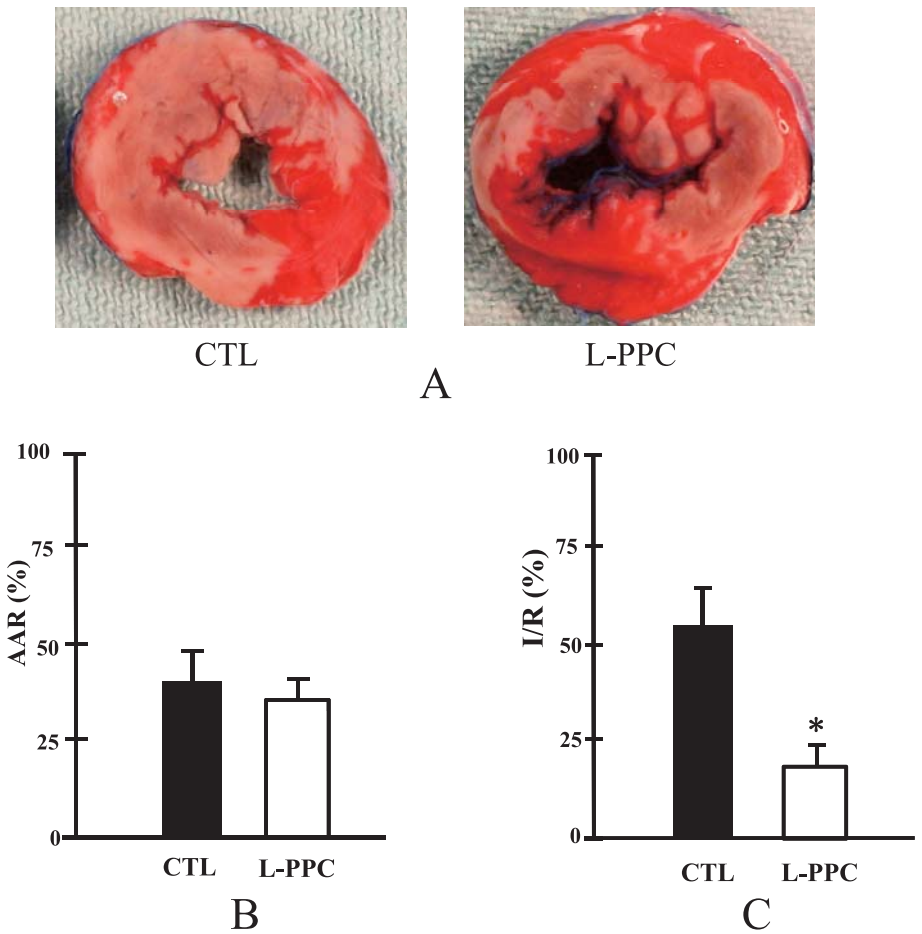

Fig. 2 Infarct lesion. (A) The white areas indicate the lesions in the left ventricles of animals in the CTL and L-PPC groups. (B) No significant difference was observed in the percentage of area at risk (AAR) between the CTL and L-PPC groups. (C) The infarct size as a percentage of AAR was significantly smaller in the L-PPC group (*$p < 0.01$ vs CTL).

the baseline ($p < 0.05$). MAP during saline or lidocaine infusion and the reperfusion period was similar to baseline values. There was no significant difference in MAP between groups throughout the experiment (Table 1).

Expression of arrhythmia or VF

During the 30-minute ischemia, arrhythmia without VF developed in 6 of the 16 rabbits, and the mean time to onset of arrhythmia was 418 ± 310 seconds. VF developed abruptly in 2 of the 16 rabbits, and 8 rabbits had arrhythmia followed by VF. The mean time to onset of arrhythmia was 540 ± 254 seconds. Two of the 16 rabbits developed VF that was not controllable with manual defibrillation and died (Table 2).

Infarct size

Figure 2 shows photographs of typical TTC staining after reperfusion in the CTL and L-PPC groups (A). L-PPC reduced infarct size. There was no significant difference in AAR as a percentage of the left ventricle between the CTL and L-PPC groups (B). In the CTL group, the infarct size was 57.3 ± 10.4 of AAR, while in the L-PPC group it was decreased to 14.4 ± 8.9% ($p < 0.01$ compared with the CTL group) (C).

Serum lidocaine concentrations

Serum lidocaine concentration immediately after 30 min of lidocaine was 25.1 ± 2.6 μg/mL, and it was 2.3 ± 0.5 μg/mL at 120 min after reperfusion ($p < 0.01$) (Table 3).

DISCUSSION

Perioperative myocardial ischemia may threaten the life of patients undergoing surgery, including oral surgery.[7] Although reperfusion therapy is commonly performed in patients with myocardial infarction, no more effective measures have been established to protect the myocardium after ischemia. After Murry *et al.* reported that a brief episode of ischemia and reperfusion limits infarct size by enhancing myocardial resistance to infarction, and termed this phenomenon as ischemic preconditioning,[8] many studies on pharmacological preconditioning using anesthetic agents and postconditioning have been reported.[9–10]

The results of the present study indicate that administration of lidocaine, a drug exerting an antiarrhythmic effect, during reperfusion after myocardial ischemia does not affect hemodynamics during reperfusion and significantly decreases infarct size without inducing arrhythmia or ventricular fibrillation. In an *ex vivo* study using an isolated rat heart perfused in a Langendorff perfusion system, Ebel *et al.* demonstrated that the presence of lidocaine during ischemia decreased myocardial injury and improved functional recovery. In an *in vivo* study similar to the present study, Nasser *et al.* reported that lidocaine administered before ischemia decreased infarct size in dogs.[3] Lee *et al.* reported that retrograde infusion of lidocaine through the coronary sinus before reperfusion decreased myocardial injury in a porcine model of myocardial ischemia, while systemic administration of lidocaine was not effective.[11]

The myocardial protective effect of lidocaine may be dependent on the timing (before vs. after ischemia), the method of administration and dose, and may also be affected by activity of the autonomic nervous system, hormonal balance, and hemodynamics. In the present study, all rabbits had arrhythmia after coronary occlusion, and many of them had VF, which was successfully defibrillated. Ischemia also decreased systemic blood pressure. It is well known that reperfusion after myocardial ischemia induces arrhythmias, especially ventricular fibrillation. The present study demonstrated that lidocaine administered immediately after reperfusion prevents the occurrence of VF and maintains blood pressure at a stable level.

When the effects of preconditioning are evaluated, it is difficult to determine whether the myocardial injury is associated with ischemia itself or reperfusion injury. It is believed that ischemia produces proinflammatory stimuli to the myocardium, and that reperfusion promotes aerobic metabolism in viable myocardium during reperfusion and causes reactive hyperemia in coronary arteries.[12, 13] The significant decrease in infarct size by administration of lidocaine during reperfusion suggests that the anti-inflammatory effect of lidocaine and other local anesthetic agents may promote the myocardial protection by these drugs.

It has been proposed that lidocaine protects the myocardium against ischemia via antiapoptotic mechanisms, or via mechanisms involving reactive oxygen species (ROS), protein kinase C and nitric oxide (NO).[1, 2, 14] Moreover, an increase in ROS has been noted as a factor in ischemia-reperfusion injury. In addition, increased accumulation of calcium ions in myocardial cells leads to disruption of ion pumps and channels, as well as contractile proteins.[15] We did not investigate the role of single pathways in the myocardial protection of lidocaine in the present study. However, further studies should be conducted to clarify the mechanism by which lidocaine protects the myocardium against ischemia-reperfusion injury.

Volatile anesthetic agents should be used in high

concentrations to elicit pharmacological preconditioning or postconditioning effects. However, the use of these agents in high concentrations is detrimental to systolic and diastolic heart functions.[16–18] In this study we observed no significant changes in hemodynamics during and after lidocaine infusion. Serum lidocaine concentration immediately after the completion of lidocaine infusion exceeded 20 μg/mL. This concentration of lidocaine was higher than the concentration that elicits antiarrhythmic effects in humans after systemic administration.[19]

In our previous study,[20] we administered lidocaine to rabbits under general anesthesia at a rate of 4 mg/kg/h to investigate in detail the effects of lidocaine on the time to onset of convulsions, its effect on cerebral blood flow and its effect on systemic blood pressure. We found that lidocaine induced four phases of EEG changes before the onset of burst suppression, and that convulsions developed and systemic blood pressure increased 10 minutes after the increase in cerebral blood flow. Since none of the animals showed significant changes in systemic blood pressure during lidocaine infusion at 1 mg/kg/h, we think that the dose that we used in the present study is lower than that required to induce lethal convulsions in rabbits.

Kaczmarek *et al.*[2] reported that infarct size per area-at-risk was reduced by 27% in mice treated with a lidocaine bolus (1 mg/kg) before a continuous infusion (0.6 mg/kg/h) during 40 min of ischemia, and that plasma lidocaine concentration 180 min after reperfusion became 13.4 $\pm$ 1 μg. Since the lidocaine concentration 120 min after reperfusion in this study was 2.3 $\pm$ 0.5 μg/mL, our lidocaine infusion rate was considered to be one that exerts an antiarrhythmic effect in humans in a clinical setting.

When administered at cytotoxic concentrations, lidocaine abolishes the cardioprotection induced by ischemic preconditioning and volatile anesthetic post-conditioning.[21, 22] Lidocaine may affect mitochondrial bioenergetics in a concentration-dependent manner, leading to improved tolerance for ischemia and reperfusion at low concentrations. However, lidocaine may cause a mitochondrial dysfunction at high concentrations.[2] It has been reported that one minute after intravenous administration, 70% of lidocaine is distributed in organs that have high blood flow, while the plasma lidocaine concentration decreases to 2% after 15 minutes.[23] In the present study, where lidocaine was administered during reperfusion, serum lidocaine concentration was higher than the toxicity threshold at the conclusion of lidocaine infusion. However, it decreased to a clinical concentration 90 minutes later. These findings suggest that lidocaine should be administered at high concentrations to protect the myocardium against ischemia-reperfusion injury. Further studies should be performed to determine whether lidocaine protects the myocardium against ischemia-reperfusion injury when administered continuously at the clinically optimal concentration.

In conclusion, lidocaine infusion at 1 mg/kg/h during reperfusion after myocardial ischemia decreased the infarct size in a model of myocardial ischemia in anesthetized rabbits, which indicated that lidocaine protects the myocardium from ischemia-reperfusion injury. Our findings suggest that lidocaine at high concentrations during the early phase of reperfusion after myocardial ischemia may play a role in protecting the myocardium.

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