J Anesth (2005) 19:124–130
DOI 10.1007/s00540-004-0290-0

Journal of
Anesthesia

Cardioprotective effects of KB-R7943, a novel inhibitor of Na^+/Ca^{2+} exchanger, on stunned myocardium in anesthetized dogs

Osamu Yoshitomi, Daiji Akiyama, Tetsuya Hara, Sungsam Cho, Shiro Tomiyasu, and Koji Sumikawa

Department of Anesthesiology, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan

Abstract

Purpose. The present study was carried out to determine the cardioprotective effects of KB-R7943 (KBR), a selective inhibitor of the reverse mode of Na^+/Ca^{2+} exchanger (NCX), on stunned myocardium in anesthetized dogs.

Methods. The dogs were allocated to one of three groups ($n = 7$ for each group), and received drug vehicle (group C), low-dose KBR (5 mg·kg^{-1} i.v.) (group L) or high-dose KBR (10 mg·kg^{-1} i.v.) (group H) at 15 min before left anterior descending coronary artery (LAD) occlusion. Stunned myocardium was produced by 15-min occlusion of LAD and 90-min reperfusion in all dogs. Regional myocardial contractility was evaluated with segment shortening (%SS).

Results. Recovery of %SS at 90 min after reperfusion was significantly improved in group H (70.8% ± 3.9% of baseline), whereas the recovery was poor in groups C and L (34.3% ± 2.8% and 36.4% ± 5.4% of baseline, respectively). Regional myocardial blood flow showed no significant difference among groups. KBR had no effect on coronary or systemic hemodynamics.

Conclusion. The results show that preischemic administration of high-dose KBR markedly improves myocardial contractile dysfunction after ischemia-reperfusion in anesthetized dogs, indicating that KBR protects myocardium against the ischemia-reperfusion injury in vivo.

Key words Na^+/Ca^{2+} exchanger · Ca^{2+} overload · KB-R7943 · Stunned myocardium

Introduction

The Na^+/Ca^{2+} exchanger (NCX) is an important Ca^{2+} transporter of the cardiac sarcolemma and a major regulator of intracellular Ca^{2+} concentration in cardiomyocytes [1]. The primary function (forward mode) of NCX in normal cardiac cells is extrusion of Ca^{2+} from myocytes during cardiac relaxation and diastole [2,3]. However, it has been suggested that, during myocardial ischemia-reperfusion, intracellular acidosis drives Na^+ into the cells via Na^+/H^+ exchanger (NHE), and then, the accumulation of intracellular Na^+ activates the reverse mode of NCX, causing Ca^{2+} influx into the cells [4,5]. Intracellular Ca^{2+} overload has been thought to be one of the major causes of myocardial ischemia-reperfusion injury involved myocardial stunning, reperfusion arrhythmia, vascular injury, and myocyte necrosis [6]. Reperfusion injury is well documented in not only animal models but also clinical situations. Elimination of reperfusion injury may further improve the outcome for patients with coronary artery disease [7].

KB-R7943 (2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl] isothiourea methanesulfonate, KBR) (Fig. 1) has been shown to selectively inhibit the reverse mode of NCX and reduce the accumulation of intracellular Ca^{2+} via NCX under pathological conditions, which include cardiac ischemia-reperfusion and hypoxia-reoxygenation, in isolated rat cardiomyocytes [8,9]. Moreover, it has been demonstrated that KBR has lower potency for the forward mode of NCX and other ion transporters such as NHE, L-type Ca^{2+} channels, Ca^{2+}-ATPases, and Na^+/K^+-ATPases [8,10]. Many in vitro studies reported that KBR suppressed ouabain-induced or ischemia-reperfusion-induced arrhythmias and improved myocardial contractile functional recovery after global ischemia-reperfusion through inhibition of the reverse mode of NCX in the isolated heart model of small mammalian species [11–15]. However, the cardioprotective effect of KBR on myocardial ischemia-reperfusion injury of large animals in vivo has not been clarified. This study was conducted to investigate, in anesthetized dogs, whether preischemic administration of KBR improves contractile functional recovery on regional stunned myocardium and whether KBR has effects on the coronary and systemic hemodynamics.

Address correspondence to: O. Yoshitomi
Received: August 18, 2004 / Accepted: November 26, 2004

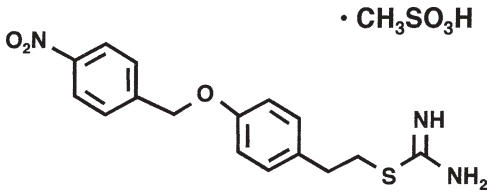

Fig. 1. Chemical structure of KB-R7943

Materials and methods

All experimental procedures used in this investigation were reviewed and approved by the Animal Care Committee of the Nagasaki University School of Medicine.

Instrumentation

Twenty-one mongrel dogs weighing 8–13 kg, free from heartworms, were anesthetized with α-chloralose, 100 mg·kg^{-1} i.v., followed by continuous infusion of α-chloralose, 10 mg·kg^{-1}·h^{-1}, and fentanyl, 5 μg·kg^{-1}·h^{-1}, throughout the study period. After tracheal intubation, mechanical ventilation with a Harvard respiratory pump (Harvard Apparatus, South Natick, MA, USA) was facilitated by an intermittent i.v. infusion of vecuronium, 0.2 mg·kg^{-1}. Tidal volume, respiratory rate, and inspired oxygen concentration were adjusted to maintain the arterial carbon dioxide tension (Pa_{CO_2}) between 35 and 40 mmHg and the arterial oxygen tension (Pa_{O_2}) between 100 and 300 mmHg. End-tidal CO_2 concentration was continuously monitored by using a gas analyzer (Capnomac Ultima; Datex, Helsinki, Finland). Lactated Ringer's solution was infused at a rate of 5 ml·kg^{-1}·h^{-1}. Sodium bicarbonate was administered to maintain the base deficit within 5 mEq·l^{-1}. Arterial blood glucose concentrations were measured before and during ischemia and maintained at baseline values with an i.v. infusion of 10% dextrose as needed throughout the study period. The esophageal temperature was maintained between 36°C and 37°C throughout the study period by using a warmer blanket and a heating lamp.

A heparin-filled catheter was inserted into the left femoral vein to administer fluid and drugs. A pressure transducer-tipped catheter (PC500; Millar Instruments, Houston, TX, USA) was inserted into the thoracic aorta via the left femoral artery to measure arterial blood pressure. A thoracotomy was performed at the left fifth intercostal space. The pericardium was incised while the left lung was gently retracted. An ultrasonic flow probe (HPD 120-10S; Crystal Biotech, Hopkinton, MA, USA) was positioned around the ascending thoracic aorta to measure cardiac output (CO). A 1-cm segment of the left anterior descending coronary artery (LAD) distal to the first diagonal branch was isolated, and a transit-time ultrasonic flow probe (HPD 20-20S; Crystal Biotech) was placed around the vessel to measure coronary blood flow (CBF). A silk ligature was placed around the LAD to produce coronary artery occlusion and reperfusion. A pair of ultrasonic segment length transducers was implanted in the subendocardium of the LAD perfusion territory to measure changes in regional contractile function (percentage segment shortening [%SS]). A pressure transducer-tipped catheter was inserted into the left ventricular (LV) chamber through an incision in the apex for continuous recording of LV pressure. The maximum rate of increase of LV pressure ($LVdP/dt_{max}$) was determined by electric differentiation of the LV pressure waveform. Segment length was monitored by ultrasonic amplifiers (VF-1; Crystal Biotech). End-systolic segment length (ESL) was determined 10 ms before maximum negative LVdP/dt, and end-diastolic segment length (EDL) was determined 10 ms before dP/dt first exceeded 140 mmHg·s^{-1} (immediately before the onset of LV isovolemic contraction). Percentage SS was calculated using the formula $\%SS = (EDL - ESL) \times 100 \times EDL^{-1}$. Relative diastolic coronary vascular resistance was calculated as the ratio of diastolic arterial pressure to diastolic coronary blood flow. All hemodynamic data were continuously monitored on a polygraph and digitized via a computer interfaced with an analog-to-digital converter (HEM; Physio-Tech, Tokyo, Japan).

Colored microspheres (Dye-trak; Triton Technology, San Diego, CA, USA) were used to measure regional myocardial blood flow (RMBF). Immediately before injection, the microspheres were suspended using Voltecs (Scientific Industries, Bohemia, NY, USA). Five million microspheres (15 μm in diameter) were injected into the left atrium as a bolus during a 10-s period and flushed with 10 ml warm (37°C) saline. A timed collection of reference arterial flow was started a few seconds before the microsphere injection using a precalibrated infusion-withdrawal pump through the carotid artery catheter at a constant rate of 6 ml min^{-1} for 3 min. Two milliliters of methylene blue was injected via the LAD immediately distal to the silk ligature, and dyed area was resected as the perfused area after cardiac arrest with KCl injection. After the weight of the resected tissue was measured, the central area of the tissue was isolated and divided into four transmural sections, which were subsequently subdivided into inner, middle, and outer layers. After being weighed, each piece and the remaining myocardial tissue of the dyed region were separately dissolved by 4 mol·l^{-1} KOH, and the colored microspheres were collected by vacuum filtering. These microspheres were dissolved by dimethylformamide, and the photometric absorption of each dye solution was determined by an UV-visible

recording spectrophotometer (UV-160A; Shimazu, Kyoto, Japan).

The composite spectrum of each dye solution was resolved into the spectra of the single constituent by a matrix-inversion technique incorporated into the spectrophotometer. By using calculated photometric absorption, RMBF was determined by using the equation

$$Q_m = (A_m \times Q_r)/A_r$$

where Q_m is blood flow of samples ($ml \cdot min^{-1} \cdot g^{-1}$), Q_r is collection rate of reference blood ($6\,ml \cdot min^{-1}$), A_m is photometric absorption in sample (1 g), and A_r is total photometric absorption of reference blood.

Experimental protocols

Figure 2 shows the experimental design. Dogs were randomly assigned to one of three groups ($n = 7$ for each group). Each group received drug vehicle (group C), low-dose KBR ($5\,mg \cdot kg^{-1}$; group L) or high-dose KBR ($10\,mg \cdot kg^{-1}$; group H). Thirty minutes after the instrumentation was completed, baseline systemic and coronary hemodynamics were recorded. All dogs were subjected to a 15-min period of LAD occlusion followed by a 90-min reperfusion. Drug vehicle and KBR were administered by an intravenous injection at 15 min before LAD occlusion. Hemodynamics and contractile function were monitored continuously throughout the experiment. Colored microspheres to measure RMBF were injected into the left atrium at the three time points during the experiment, i.e., baseline, during ischemia, and during reperfusion.

Materials

KBR (generously donated by Nippon Organon, Osaka, Japan) was first dissolved in dimethylsulphoxide (DMSO) and was diluted with physiological saline. The final concentration of DMSO was 0.1% or less.

Statistical analysis

All data are expressed as mean ± SEM. Data within and among groups were analyzed with analysis of variance for repeated measures followed by Scheffe's F test. A P value < 0.05 was considered statistically significant.

Results

There were no significant differences in demographic data among groups. Arterial blood gas values and blood glucose were maintained within physiological range in all dogs. The range of arterial pH was 7.372–7.415, Pa_{O_2} was 158–266 mmHg, Pa_{CO_2} was 35.6–39.2 mmHg, and blood glucose was 108–172 $mg \cdot dl^{-1}$. RMBF in the ischemic region measured using microspheres is shown in Fig. 3. The RMBF significantly decreased during ischemia, and recovered to the baseline value during reperfusion in each group. There were no significant differences among the layers of myocardium or the groups throughout the time course.

Changes of hemodynamics and %SS are summarized in Table 1. There were no significant differences in the hemodynamics or %SS between pre- and posttreatment of KBR ($5\,mg \cdot kg^{-1}$ or $10\,mg \cdot kg^{-1}$). Mean aortic blood pressure (MAP), heart rate (HR), CO, systemic vascular resistance (SVR), and LV end-diastolic pressure (LVEDP) did not significantly change during ischemia or the reperfusion stage compared with the baseline in any group, and there were no significant differences among the groups throughout the time course.

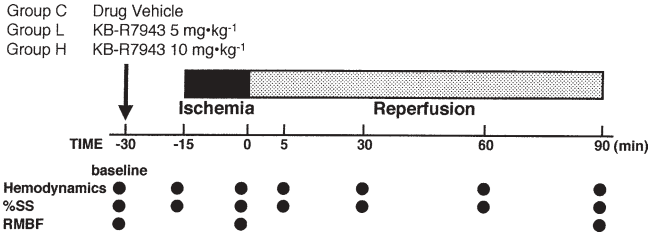

Fig. 2. Time course of the experimental protocol. All dogs were subjected to 15-min occlusion of left anterior descending coronary artery (LAD) and subsequent 90-min reperfusion. Drug vehicle and KB-R7943 were administered by an intravenous injection at 15 min before LAD occlusion. During the experiment, hemodynamics, percentage of segment shortening (%SS), and regional myocardial blood flow (RMBF) were measured at times indicated by the *closed circles*

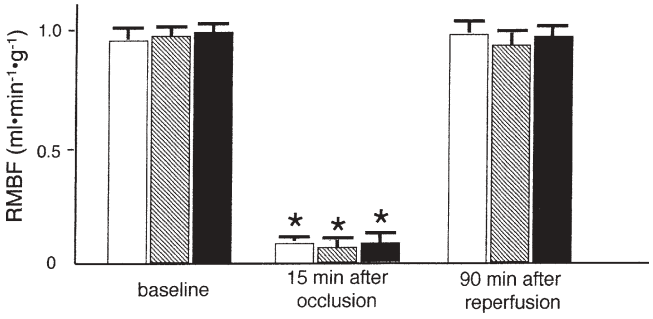

Fig. 3. Regional myocardial blood flow (*RMBF*) in the ischemic region measured using colored microspheres at baseline, 15 min after left anterior coronary artery occlusion and 90 min after reperfusion in all groups. Values are mean ± SEM. Group C, drug vehicle (*open bars*); group L, low-dose KB-R7943 ($5\,mg \cdot kg^{-1}$) (*shaded bars*); group H, high-dose KB-R7943 ($10\,mg \cdot kg^{-1}$) (*black bars*). * $P < 0.05$ vs. baseline

Table 1. Hemodynamic data

	Baseline (pretreatment)	Preischemia (posttreatment)	Ischemia	Reperfusion			
				5 min	30 min	60 min	90 min
HR (beats min^{-1})							
Group C	105 ± 6	106 ± 6	105 ± 6	107 ± 6	105 ± 6	106 ± 8	103 ± 6
Group L	99 ± 7	94 ± 4	106 ± 4	99 ± 4	96 ± 4	94 ± 3	91 ± 2
Group H	99 ± 3	94 ± 2	105 ± 3	101 ± 3	96 ± 3	99 ± 3	101 ± 5
MAP (mmHg)							
Group C	93 ± 5	92 ± 4	89 ± 4	88 ± 4	90 ± 4	86 ± 4	86 ± 4
Group L	90 ± 2	91 ± 3	90 ± 2	91 ± 2	91 ± 2	96 ± 3	92 ± 3
Group H	92 ± 3	95 ± 3	90 ± 5	90 ± 4	92 ± 6	94 ± 4	93 ± 5
CO ($l \cdot min^{-1}$)							
Group C	1.25 ± 0.04	1.25 ± 0.05	1.26 ± 0.04	1.26 ± 0.06	1.26 ± 0.05	1.28 ± 0.05	1.25 ± 0.06
Group L	1.23 ± 0.07	1.18 ± 0.08	1.15 ± 0.08	1.16 ± 0.07	1.14 ± 0.07	1.16 ± 0.06	1.14 ± 0.04
Group H	1.27 ± 0.06	1.25 ± 0.08	1.22 ± 0.07	1.25 ± 0.08	1.22 ± 0.09	1.20 ± 0.07	1.21 ± 0.08
SVP ($dynes \cdot s^{-1} cm^{-5}$)							
Group C	5561 ± 319	5494 ± 314	5264 ± 372	5312 ± 375	5383 ± 390	5109 ± 486	5201 ± 425
Group L	5623 ± 397	5902 ± 426	5949 ± 377	5886 ± 299	6004 ± 308	6222 ± 172	6034 ± 94
Group H	5528 ± 199	5845 ± 325	5519 ± 346	5526 ± 403	5849 ± 502	6019 ± 362	5931 ± 420
CBF ($ml \cdot min^{-1}$)							
Group C	11.1 ± 0.6	11.1 ± 0.7		27.8 ± 2.9*	11.8 ± 0.9	10.9 ± 0.7	11.1 ± 0.6
Group L	12.2 ± 1.0	12.2 ± 1.0		26.4 ± 2.2*	11.7 ± 1.0	11.9 ± 1.0	11.6 ± 1.1
Group H	12.0 ± 1.7	11.9 ± 1.7		22.6 ± 2.2*	11.7 ± 1.7	11.6 ± 1.7	11.7 ± 1.8
CVR ($dynes \cdot s^{-1} cm^{-5}$)							
Group C	493 ± 24	502 ± 27		200 ± 22*	471 ± 50	503 ± 42	452 ± 27
Group L	475 ± 40	495 ± 56		224 ± 22*	510 ± 61	526 ± 52	539 ± 75
Group H	530 ± 62	585 ± 93		259 ± 19*	552 ± 64	564 ± 61	557 ± 66
$LVEDP_{max}$ (mmHg)							
Group C	6.3 ± 0.2	6.1 ± 0.2	7.1 ± 0.4	6.1 ± 0.7	5.9 ± 0.6	6.1 ± 0.4	6.0 ± 0.5
Group L	5.8 ± 0.3	6.4 ± 0.6	6.6 ± 0.2	6.4 ± 0.2	6.3 ± 0.5	6.3 ± 0.4	6.5 ± 0.6
Group H	5.1 ± 0.4	5.7 ± 0.5	6.7 ± 0.3	5.9 ± 0.3	5.4 ± 0.4	5.3 ± 0.4	5.0 ± 0.4
$LVdP/dt_{max}$ ($mmHg \cdot s^{-1}$)							
Group C	2207 ± 77	2201 ± 83	1711 ± 59*	1983 ± 91	2021 ± 63	1929 ± 70	2010 ± 34
Group L	2195 ± 173	2081 ± 190	1876 ± 131*	1901 ± 122	1946 ± 129	1890 ± 112	1927 ± 125
Group H	2097 ± 104	2009 ± 70	1746 ± 116*	1930 ± 119	1938 ± 70	1897 ± 103	1933 ± 91
%SS							
Group C	22.1 ± 1.2	22.0 ± 1.2	−6.4 ± 1.7*	5.4 ± 0.9*	6.6 ± 0.9*	6.8 ± 0.6*	7.6 ± 0.7*
Group L	21.6 ± 1.9	20.5 ± 2.0	−4.7 ± 0.9*	6.6 ± 0.8*	8.0 ± 1.3*	8.3 ± 2.2*	8.2 ± 2.0*
Group H	23.6 ± 2.0	22.5 ± 1.9	−5.8 ± 1.6*	13.0 ± 2.1*,**,***	14.9 ± 1.7*,**,***	16.3 ± 1.6**,***	16.4 ± 1.3**,***

Values are mean ± SEM

HR, heart rate; MAP, mean arterial blood pressure; CO, cardiac output; SVR, systemic vascular resistance; CBF, coronary blood flow; CVR, coronary vascular resistance; LVEDP, left ventricular end-diastolic pressure; LVdp/dt, rate of increase of left ventricular pressure; %SS, percentage of segment shortening; group C, drug vehicle; group L, low-dose KB-R7943 ($5 mg \cdot kg^{-1}$); group H, high-dose KB-R7943 ($10 mg\,kg^{-1}$)

* Significantly different from baseline ($P < 0.05$); ** significantly different from the corresponding value in group C ($P < 0.05$); *** significantly different from the corresponding value in group L ($P < 0.05$)

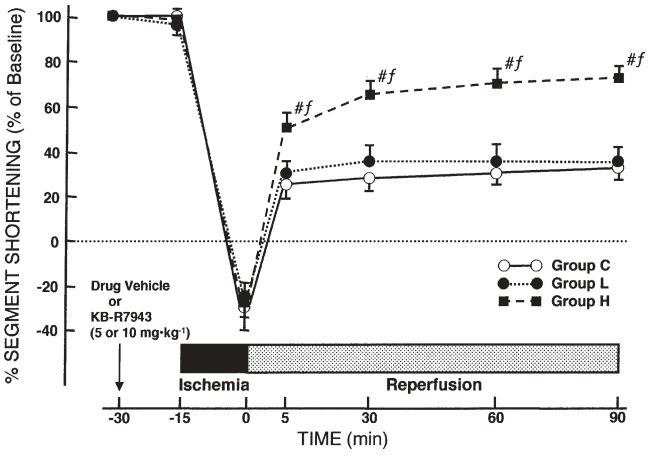

Fig. 4. Percentage segment shortening (%SS) of the ischemic/reperfused area (% of baseline) before left anterior coronary artery occlusion, during occlusion, and at various times during reperfusion in all groups. Values are mean ± SEM. Group C, drug vehicle; group L, low-dose KB-R7943 ($5\,mg{\cdot}kg^{-1}$); group H, high-dose KB-R7943 ($10\,mg{\cdot}kg^{-1}$). # $P < 0.05$ vs. group C; *f* $P < 0.05$ vs. group L

CBF significantly decreased during LAD occlusion and increased 5 min after LAD reperfusion compared with the baseline values in all groups, and there were no significant differences among the groups throughout the time course. Coronary vascular resistance (CVR) significantly decreased 5 min after LAD reperfusion in all groups, and there were no significant differences among the groups throughout the time course. $LVdP/dt_{max}$ significantly decreased 15 min after LAD occlusion compared with the baseline values in all groups, and there were no significant differences among the groups throughout the time course.

Figure 4 shows the percentage changes of %SS from baseline (100%) throughout the time course. In all groups, %SS markedly decreased and fell below 0% during LAD occlusion, indicating bulging. The group C showed poor recovery of %SS during reperfusion, which was no more than 35% of baseline even after 90 min. In group L, the recovery of %SS was incomplete and similar to group C during reperfusion. In contrast, the group H showed a significantly improved recovery of %SS, which reached 70% of baseline 90 min after reperfusion.

Discussion

The present results show that preischemic treatment of KBR, a selective inhibitor of the reverse mode of NCX, significantly improves recovery of regional myocardial contractility after 15-min occlusion and reperfusion of LAD in anesthetized dogs in vivo, and that this effect of KBR is not explained by the change of blood pressure or CBF. In addition, KBR has no effect on the hemodynamics or myocardial contractility in the normal condition.

Reperfusion after a brief period of coronary occlusion shorter than 20 min produces prolonged contractile dysfunction, referred to as "myocardial stunning," in the absence of myocardial necrosis [6]. This contractile dysfunction is reversible, and the dysfunctional myocardium has normal or near-normal coronary flow [6]. Clinically, this phenomenon could occur in a variety of situations in which the myocardium is exposed to transient ischemia, including unstable angina, exercise-induced angina, acute myocardial infarction with early reperfusion, open heart surgery, and cardiac transplantation [16]. The mechanism of myocardial stunning is complex [17]. It has been suggested that the most plausible theories regarding the pathogenesis of myocardial stunning are the oxyradical hypothesis and the calcium hypothesis [17,18].

NHE and NCX have significant relation to intracellular Ca^{2+} overload causing myocardial stunning [6]. NHE is a major regulator of intracellular pH and is one of the major mechanisms for restoring pH after ischemia-induced intracellular acidosis, as a result, causes Na^+ influx into the cells [19,20]. The intracellular Na^+ accumulated via NHE during ischemia cannot be extruded sufficiently by the ATP-dependent Na^+/K^+ pump, because ATP production diminishes due to ischemia [21]. NCX is an electrogenic transporter in the cardiac sarcolemma independent of ATP [1]. Accumulation of intracellular Na^+ activates the reverse mode of NCX and causes intracellular Ca^{2+} overload [21]. KBR has been shown to selectively inhibit the reverse mode of NCX and reduce Ca^{2+} overload [8,9]. Therefore, it has been suggested that KBR would exert cardioprotection against myocardial stunning.

NHE inhibitors have been extensively demonstrated to protect against the myocardial ischemia-reperfusion injury in terms of improved contractile dysfunction, reduction of infarct size, and antiarrhythmic effect in various experimental models and animal species [4,22,23]. Moreover, cariporide, a specific NHE inhibitor, has recently undergone clinical evaluation in high-risk patients with acute coronary syndrome, including those undergoing percutaneous or surgical revascularization [24].

Studies of the physiological roles and molecular aspects of NCX also have been made in various tissues [2]. Recently, although a few NCX inhibitors have been developed, those cardioprotective effects have been still controversial. KBR also was synthesized for the purpose of blocking Ca^{2+} influx via the reverse mode of NCX and reported to be a potent and selective inhibitor in cardiomyocytes [8,10]. KBR suppressed ventricular

arrhythmias and improved LV function after prolonged global ischemia and reperfusion in isolated rat and rabbit hearts [12–15]. Moreover, a recent study reported that KBR more efficiently reduced the infarct size after 30-min ischemia than NHE inhibitor in the isolated rabbit heart [25]. Thus, in vitro studies demonstrated that KBR protected whole hearts against myocardial reperfusion injury after prolonged myocardial ischemia. However, a recent in vivo study reported that KBR did not suppress arrhythmias after 30-min coronary artery occlusion and reperfusion in dogs [26]. The mechanism of myocardial reperfusion injury after prolonged myocardial ischemia might be more or less different from that of myocardial contractile dysfunction after brief myocardial ischemia [17]. Moreover, a previous study reported that reversal of NCX contributed only to myocardial stunning and had no role in reperfusion ventricular fibrillation after 15-min LAD occlusion in open-chest dogs [27]. The present study demonstrates that KBR improves myocardial contractile dysfunction after brief myocardial ischemia and reperfusion in dogs in vivo.

Synthetic compounds previously reported as NCX inhibitors are amiloride derivatives [10]. Among them, dichlorobenzamil (DCB) has been demonstrated to suppress the intracellular accumulation of Na^+ and Ca^{2+} after myocardial ischemia and reperfusion in isolated perfused rat heart [28]. However, DCB has been already reported to be a nonselective NCX inhibitor and also inhibit other ion transporters [10]. DCB markedly inhibits the cardiac mechanical function in normal conditions with a concentration to suppress the accumulation of Na^+ and Ca^{2+} [28]. The present study demonstrates that KBR has no effect on hemodynamics, myocardial contractility, or CBF in normal conditions even at a higher dose. Furthermore, high-dose KBR did not change CBF or RMBF throughout the experimental time course. Therefore, the cardioprotective effect of KBR would be independent of myocardial blood flow.

KBR has been reported to be a potent and selective inhibitor of NCX at low micromolar concentrations [8,10]. It dose-dependently inhibits intracellular Na^+-dependent Ca^{2+} influx with an IC_{50} of 1.2–2.4 $\mu mol \cdot l^{-1}$ in isolated rat cardiomyocytes [8]. Unfortunately, there has been no report to investigate the plasma concentration of KBR in the canine model in vivo. Therefore, the dose used in this study is not comparable with that in the other animal in vitro studies. The high-dose used in this study is maximum among the doses used in previous in vivo studies [11,15,26]. In the present study, low-dose KBR failed to improve the recovery of myocardial contractility after ischemia-reperfusion. Myocardial stunning is a multifactorial process and the interaction of multiple pathogenic mechanisms such as Ca^{2+} overload, oxyradical generation, and impaired Ca^{2+} responsiveness [17]. The present results indicate that a high dose is necessary to suppress intracellular Ca^{2+} overload via NCX and improve the contractile dysfunction completely.

Conclusion

Preischemic administration of KBR, a selective inhibitor of the reverse mode of NCX, enhanced the functional recovery of stunned myocardium in vivo. The protective effect is independent of myocardial blood flow, because KBR has no effect on systemic or coronary hemodynamics.

References

1. Bers DM (2000) Calcium fluxes involved in control of cardiac myocyte contraction. Circ Res 87:275–281
2. Blaustein MP, Lederer WJ (1999) Sodium/calcium exchange: its physiological implications. Physiol Rev 79:763–854
3. Shigekawa M, Iwamoto T (2001) Cardiac Na^+-Ca^{2+} exchange. Molecular and pharmacological aspects. Circ Res 88:864–876
4. Scholz W, Albus U, Lang HJ, Linz W, Martorana PA, Englert HC, Schälkens BA (1993) Hoe 694, a new Na^+/H^+ exchange inhibitor and its effects in cardiac ischemia. Br J Pharmacol 109: 562–568
5. Verdonck L, Borgers M, Verdonck F (1993) Inhibition of sodium and calcium overload pathology in the myocardium: a new cytoprotective principle. Cardiovasc Res 27:349–357
6. Bolli R (1990) Mechanism of myocardial "stunning." Circulation 82:723–738
7. Kloner RA (1993) Does reperfusion injury exist in humans? J Am Coll Cardiol 21:537–545
8. Iwamoto T, Watano T, Shigekawa M (1996) A novel isothiourea derivative selectively inhibits the reverse mode of Na^+/Ca^{2+} exchange in cells expressing NCX1. J Biol Chem 271:22391–22397
9. Ladilov Y, Haffner S, Barser-Schäfer C, Maxeiner H, Piper HM (1999) Cardioprotective effects of KB-R7943: a novel inhibitor of the reverse mode of Na^+/Ca^{2+} exchanger. Am J Physiol 276: H1868–H1876
10. Watano T, Kimura J, Morita T, Nakanishi H (1996) A novel antagonist, no. 7943, of the Na^+/Ca^{2+} exchange current in guinea-pig cardiac ventricular cells. Br J Pharmacol 119:555–563
11. Watano T, Harada Y, Harada K, Nishimura N (1999) Effect of Na^+/Ca^{2+} exchange inhibitor, KB-R7943 on ouabain-induced arrhythmias in guinea-pigs. Br J Pharmacol 127:1846–1850
12. Yamamura K, Tani M, Hasegawa H, Gen W (2001) Very low dose of the Na^+/Ca^{2+} exchange inhibitor, KB-R7943, protects ischemic reperfused aged Fischer 344 rat hearts: considerable strain difference in the sensitivity to KB-R7943. Cardiovasc Res 52:397–406
13. Elias CL, Lukas A, Shurraw S, Scott J, Omelchenko A, Gross GJ, Hnatowich M, Hryshko LV (2001) Inhibition of Na^+/Ca^{2+} exchange by KB-R7943: transport mode selectivity and antiarrhythmic consequences. Am J Physiol Heart Circ Physiol 281:H1334–H1345
14. Schäfer C, Ladilov Y, Inserte J, Schäfer M, Haffner S, Garcia-Dorado D, Piper HM (2001) Role of the reverse mode of the Na^+/Ca^{2+} exchanger in reoxygenation-induced cardiomyocyte injury. Cardiovasc Res 51:241–250
15. Nakamura A, Harada K, Sugimoto H, Nakajima F, Nishimura N (1998) Effects of KB-R7943, a novel Na^+/Ca^{2+} exchange inhibitor,

on myocardial ischemia/reperfusion injury (in Japanese with English abstract). Folia Pharmacol Jpn 111:105–115
16. Bolli R (1992) Myocardial 'stunning' in man. Circulation 86:1671–1691
17. Kloner RA, Bolli R, Marban E, Reinlib L, Braunwald E (1998) Medical and cellular implications of stunning, hibernation, and preconditioning; an NHLBI workshop. Circulation 97:1848–1867
18. Bolli R, Marban E (1999) Molecular and cellular mechanisms of myocardial stunning. Physiol Rev 79:609–634
19. Karmazyn M, Moffat MP (1993) Role of Na^+/H^+ exchange in cardiac physiology and pathophysiology: mediation of myocardial reperfusion injury by the pH paradox. Cardiovasc Res 27:915–924
20. Karmazyn M (1996) The sodium-hydrogen exchange system in the heart: its role in ischemic and reperfusion injury and therapeutic implications. Can J Cardiol 12:1074–1082
21. Karmazyn M, Gan XT, Humphreys RA, Yoshida H, Kusumoto K (1999) The myocardial Na^+-H^+ exchange: structure, regulation, and its role in heart disease. Circ Res 85:777–786
22. Mathur S, Farhangkhgoee P, Karmazyn M (1999) Cardioprotective effects of propofol and sevoflurane in ischemic and reperfused rat hearts. Anesthesiology 91:1349–1360
23. Karmazyn M (1999) Mechanisms of protection of the ischemic and reperfused myocardium by sodium-hydrogen exchange inhibition. J Thromb Thrombolysis 8:33–38
24. Theroux P, Chaitman BR, Danchin N, Erhardt L, Meinertz T, Schroeder JS, Tognoni G, White HD, Willerson JT, Jessel A (2000) Inhibition of the sodium-hydrogen exchanger with cariporide to prevent myocardial infarction in high-risk ischemic situations. Main results of the GUARDIAN trial. Circulation 102:3032–3038
25. Matsumoto T, Miura T, Miki T, Genda S, Shimamoto K (2002) Blockade of the Na^+-Ca^{2+} exchanger is more efficient than blockade of the Na^+-H^+ exchanger for protection of the myocardium from lethal reperfusion injury. Cardiovasc Drugs Ther 16:295–301
26. Miyamoto S, Zhu BM, Kamiya K, Nagasawa Y, Hashimoto K (2002) KB-R7943, a Na^+/Ca^{2+} exchange inhibitor, does not suppress ischemia/reperfusion arrhythmias nor digitalis arrhythmias in dogs. Jpn J Pharmacol 90:229–235
27. Smart SC, Sagar KB, Warltier DC (1997) Differential roles of myocardial Ca^{2+} channels and Na^+/Ca^{2+} exchange in myocardial reperfusion injury in open chest dogs: relative roles during ischemia and reperfusion. Cardiovasc Res 36:337–346
28. Kawada T, Yoshida Y, Sakurai H, Imai S (1992) Myocardial Na^+ during ischemia and accumulation of Ca^+ after reperfusion: a study with monesin and dichlorobenzamil. Jpn J Pharmacol 59: 191–200