Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 523 (2005) 101–108
www.elsevier.com/locate/ejphar

Effects of KR-32570, a new sodium hydrogen exchanger inhibitor, on myocardial infarction and arrhythmias induced by ischemia and reperfusion

Byung Ho Lee *, Kyu Yang Yi, Sunkyung Lee, Sunghou Lee, Sung-eun Yoo

Medicinal Science Division, Korea Research Institute of Chemical Technology, #100, Jang-dong, Yusong, Daejon, 305-343, Republic of Korea

Received 3 March 2005; received in revised form 1 July 2005; accepted 10 August 2005
Available online 14 October 2005

Abstract

The present study was performed to evaluate the cardioprotective effects of [5-(2-methoxy-5-chloro-5-phenyl)furan-2-ylcarbonyl]guanidine (KR-32570) in rat and dog models of coronary artery occlusion and reperfusion. In addition, we sought to clarify the efficacy of KR-32570 on reperfusion-induced fatal ventricular arrhythmia. In anesthetized rats subjected to 45-min coronary occlusion and 90-min reperfusion, KR-32570 (i.v. bolus) dose-dependently reduced myocardial infarct size from 58.0% to 50.7%, 35.3%, 33.5% and 27.0% for 0.03, 0.1, 0.3 and 1.0 mg/kg, respectively ($P<0.05$). In anesthetized beagle dogs that underwent 1.2-h occlusion followed by 3.0-h reperfusion, KR-32570 (3 mg/kg, i.v. bolus) markedly decreased infarct size from 28.9% in vehicle-treated group to 8.0% ($P<0.05$), and reduced the reperfusion-induced release in creatine kinase isoenzyme MB, lactate dehydrogenase, Troponin-I and glutamic-oxaloacetic transaminase. KR-32570 dose-dependently decreased the incidence of premature ventricular contraction, ventricular tachycardia or ventricular fibrillation induced by ischemia and reperfusion in rats. Similar results were obtained in dogs with reperfusion-induced arrhythmia. In separate experiments to assess the effects of timing of treatment, KR-32570 given 10 min before or at reperfusion in rat models also significantly reduced the myocardial infarct size (40.9% and 46.1%, respectively) compared with vehicle-treated group. In all studies, KR-32570 caused no significant changes in any hemodynamic profiles. Taken together, these results indicate that KR-32570 significantly reduced the myocardial infarction and incidence of arrhythmias induced by ischemia and reperfusion in rats and dogs, without affecting hemodynamic profiles. Thus, it could be potentially useful in the prevention and treatment of myocardial injuries and lethal ventricular arrhythmias.

Keywords: KR-32570; Cardioprotection; Antiarrhythmia; Na^+/H^+ exchanger

1. Introduction

In the ischemic myocardium, achieving reperfusion of the myocardium as soon as possible is essential in order to salvage cells and cardiac function. This has been clinically made possible by the coronary reperfusion, such as thrombolytic therapy, percutaneous coronary angioplasty or coronary artery bypass surgery (Weaver et al., 1997; Kloner and Rezkalla, 2004). However, although restoration of blood flow to the jeopardized myocardial area is a prerequisite for myocardial salvage, reperfusion itself may exacerbate the injury sustained during the ischemic period (Braunwald and Kloner, 1985). Recently, a number of experimental evidences indicate that Na^+/H^+ exchanger (NHE) is largely responsible for the myocardial injury induced by ischemia and reperfusion (Gumina et al., 2001; Doggrell and Hancox, 2003; Masereel et al., 2003). With the myocardial ischemia, anaerobic glucose metabolism, ATP breakdown and accumulation of lactate lead to a profound intracellular acidosis. This increase in intracellular H^+ activates the Na^+/H^+ exchanger (NHE), resulting in extrusion of H^+ and the influx of Na^+ (Karmazyn et al., 1999; Gumina et al., 2001; Doggrell and Hancox, 2003; Masereel et al., 2003). The elevation of intracellular Na^+ concentration can subsequently activate Ca^{2+} entry through the Na^+/Ca^{2+} exchanger, causing Ca^{2+} overload and cell damage (Doggrell and Hancox, 2003; Masereel et al., 2003). To date, eight isoforms of NHE have been identified and designated NHE-1 to NHE-8 (Goyal et al., 2003; Doggrell and Hancox, 2003). NHE subtype-1 (NHE-1), although ubiquitously distributed, is the predominant isoform expressed in the heart where it contributes to cardiomyocyte

* Corresponding author. Tel.: +82 42 860 7415; fax: +82 42 861 4246.
E-mail address: bhlee@krict.re.kr (B.H. Lee).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.08.054

pH homeostasis (Doggrell and Hancox, 2003; Masereel et al., 2003). Actually, inhibition of NHE-1 has clearly been shown to be cardioprotective both in vitro and in animal studies (Touret et al., 2003; Tracey et al., 2003; Satoh and Kitada, 2004). Continuously providing patients suffering from coronary artery disease with NHE-1 inhibitors may therefore be beneficial in the case of acute coronary occlusion. This led to the strategy of designing potent and specific NHE-1 inhibitors as cardioprotective compounds.

Recently, in our efforts to discover novel inhibitors of NHE subtype-1 (NHE-1), we have found that a series of analogues of (5-phenylfuran-2-carbonyl)guanidine exhibited potent inhibitory effects on NHE-1 (Lee et al., 2005b). Especially [5-(2-methoxy-5-chloro-5-phenyl)furan-2-ylcarbonyl]guanidine (KR-32570) showed a greatly improved potency on NHE-1 activity compared to cariporide, a potent and selective inhibitor of NHE-1 (Scholz et al., 1995), and a significantly improved cardiac contractile function in isolated rat heart preparations (Lee et al., 2005a). The aim of the present study was to evaluate in vivo cardioprotective effects of KR-32570 in rat and dog models of coronary artery occlusion and reperfusion. The antiarrhythmic effects of KR-32570 were also evaluated in rat and dog. In addition, we sought to clarify the efficacy of KR-32570 on myocardial infarction when administered before and with reperfusion.

2. Materials and methods

2.1. Cardioprotective effects in rat model of ischemic heart

This study conformed with the Guide for the Care and Use of Laboratory animals, published by the U.S. National Institute of Health. Male Sprague–Dawley rats (weighing 380–420 g, Orient Co., Seoul, Korea) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.), placed on a homeothermic blanket control unit at 37 °C, and thoracotomized at the fifth intercostal space under artificial respiration (60 strokes/min, 10 ml/kg). Coronary artery occlusion was produced as previously described (Lee et al., 2001, 2004). After 45 min of occlusion, the coronary artery was reperfused by removal of the polyethylene tube. After 90 min of reperfusion, the coronary artery was reoccluded and 2 ml of a 1% Evans blue was injected via tail vein. The heart was removed, and the left ventricle was dissected free from other structures and sliced transversely into 1-mm thick sections. The image of the sections was captured by Hi-Scope (KH-2200 MD2, HiROX Co., Japan) to determine the area at risk. The sections were then incubated in 1% triphenyltetrazolium chloride for 15 min at 37 °C and then fixed for 20–24 h in a 10% formalin solution to determine the infarct size, which corresponds to the area not stained by triphenyltetrazolium chloride. The image of the sections was captured again, and analyzed with image analyzing program (Image Pro Plus®, Media Cybernetics, Silver Spring, MD, USA).

Arterial blood pressure was continuously monitored via an Isotec pressure transducer (Hugo Sachs Electronic) connected to a physiograph (WR 3300 Linearcorder, Graphtec, Tokyo, Japan). Electrocardiogram and heart rate were measured by Lead II using an electrocardiogram/rate coupler (Type 576; Hugo Sachs Electronic), both being analyzed by the computer program (PONEMAH physiology platform- model P3 Plus, Gould Inc., Cleveland, OH, USA). During an equilibration period rats with spontaneous arrhythmias or a mean arterial pressure below 70 mm Hg or both were not used. KR-32570 or cariporide was intravenously administered by bolus injection at 5 min prior to ischemia. In separate experiments to assess the effects of timing of treatment, KR-32570 (1 mg/kg) was intravenously given by bolus injection 10 min before or simultaneously with reperfusion.

2.2. Antiarrhythmic effects in rat model of ischemic heart

Male Sprague–Dawley rats (weighing 380–420 g, Orient Co.) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and prepared with the same surgical procedure to that used above. The severity of reperfusion-induced arrhythmias is critically dependent on the duration of the proceeding period of ischemia. Thus, we selected a 5-min period of ischemia followed by a 10-min period of reperfusion as it has been demonstrated that the incidence of fibrillation occurring upon reperfusion in vivo anesthetized rat reaches a peak 5 min after occlusion and subsides thereafter (Manning and Hearse, 1984) and our preliminary studies with different time schedule also showed similar result. Definitions of arrhythmias were based on the description of the Lambeth Conventions (Walker et al., 1988). Ectopic ventricular activity was categorized as a single premature ventricular contraction, ventricular tachycardia (4 or more consecutive premature ventricular contraction) or ventricular fibrillation (inability to distinguish individual QRS complexes of electrocardiogram and to measure the rate). Reference was made to the blood pressure tracings to confirm which type of ectopic activity was occurring, particularly to distinguish between the polymorphic ventricular tachycardia and ventricular fibrillation. When the former occurred, the blood pressure was usually still pulsatile, whereas with ventricular fibrillation the blood pressure fell rapidly towards zero and was no longer pulsatile. Ventricular fibrillation may be sustained or may revert spontaneously to a normal sinus rhythm in the rat (Curtis and Hearse, 1989).

2.3. Cardioprotective and antiarrhythmic effects in dog model of ischemic heart

Male beagle dogs (8–10 kg, Marshall Farms Inc., North Rose, NY, USA) were anesthetized with pentobarbital sodium (35 mg/kg, i.v. bolus+3.5 mg/kg/h, i.v. infusion) and prepared with a procedure similar to that used in rats. After tracheal intubation, artificial respiration was performed by a dog ventilator (SAR 830/P ventilator, CWE Inc.) with room air, where the tidal volume and respiratory rate were adjusted to maintain pCO_2 at 30–35 mm Hg (Lee et al., 2001, 2004). To measure the blood gas, the concentrations of electrolytes and glucose in the plasma (Rapidpoint 400, Bayer AG, Germany), about 3.0 ml of blood was withdrawn from the brachial artery. Arterial blood pressure was measured from right femoral artery

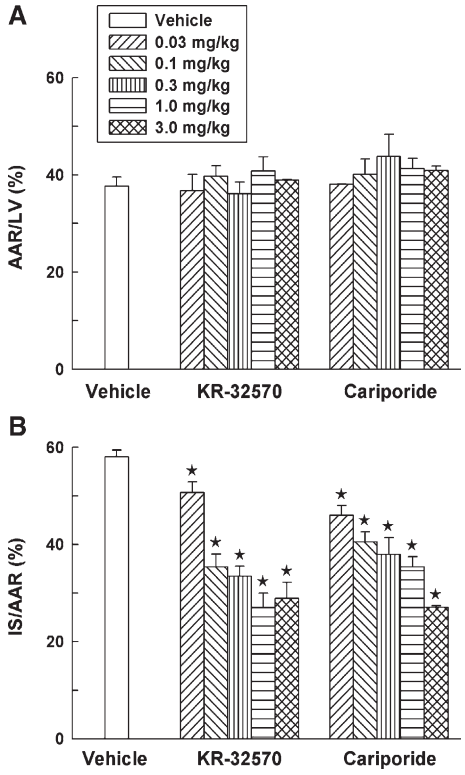

Fig. 1. Effects of KR-32570 and cariporide on myocardial infarct size in rats subjected to 45 min occlusion of left anterior descending coronary artery followed by 90 min reperfusion. Drugs were given by i.v. bolus at 5 min before occlusion. The area at risk (AAR/LV, A) was expressed as a percentage of the left ventricle (LV). The infarct size (IS/AAR, B) was expressed as a percentage of the area at risk. Values are mean percentage±S.E.M. (n=6–14). $^{\star}P<0.05$, significantly different from the vehicle-treated group.

with a Statham P23XL pressure transducer (Grass Inc., Quincy, MA, USA). A Millar Micro-Tip catheter (6F, Millar Ins., Houston, TX, USA) was advanced into the left ventricle for the measurements of left ventricular end-diastolic pressure and left ventricular developed pressure (the difference between the systolic pressure and left ventricular end-diastolic pressure). Lead II electrocardiograms were recorded from sub-dermal platinum electrodes. All data were stored and analyzed by the computer program (PONEMAH physiology platform—model P3 Plus, Gould Inc.) via a Gould 2000 physiograph (Gould Inc., Cleveland, OH, USA). The chest was opened by a left thoracotomy in the fifth intercostal space, and a ligature (4-0 silk) was placed around the left circumflex coronary artery. Rectal temperature was monitored and maintained at 38±1 °C with a heating pad. After stabilization for 30 min, the left circumflex coronary artery was occluded for 1.2 h, followed by release of the occlusion to allow 3.0 h of reperfusion. KR-32570 (3 mg/kg) was administered by i.v. bolus at 10 min before occlusion. At the end of the experiment, the left circumflex coronary artery was cannulated and perfused at the in vivo pressure with Ringer's lactate for determination of the area at risk. Patent blue violet dye (2 mg/0.2 ml/kg) was injected into the left atrium, and then the heart was quickly excised. The left ventricle was cut into 6 transverse slices and traced to determine the area at risk. The heart slices were then incubated in 1% triphenyltetrazolium chloride for 30 min at 37 °C and traced again. The tracing data showing the area at risk and infart size were scanned, fed into a computer, and measured using image analyzing program (Image Pro Plus®, Media Cybernetics).

2.4. Biochemical data in plasma from dog model of ischemic heart

In beagle dogs subjected to ischemia and reperfusion, blood samples were withdrawn by catheter before the administration of vehicle or KR-32570, immediately before reperfusion, and at 1, 2 and 3 h after reperfusion. The blood samples were centrifuged at 1500 g for 15 min. The supernatant plasma was removed and stored in liquid nitrogen until the biochemical analysis was performed. Lactate dehydrogenase, Troponin-I and glutamic-oxaloacetic transaminase were measured by ADVIA-1650 or ACS-180 (Bayer AG, Germany) using commercial kits (Bayer AG). Creatine kinase isoenzyme MB was measured by Cobas Mira-Plus (F. Hoffmann-La Roche Ltd, Switzerland) using kit purchased from F. Hoffmann-La Roche Ltd.

2.5. Statistical analysis

All values are expressed as mean±S.E.M. Data were analyzed by unpaired Student's t-test and one-way analysis of

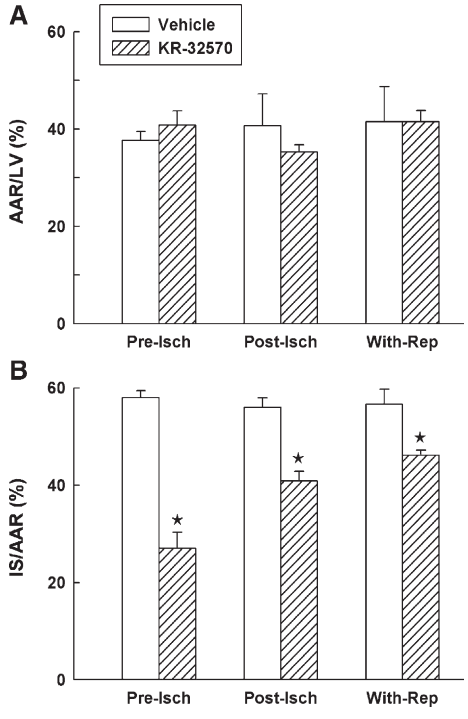

Fig. 2. Cardioprotective effect of KR-32570 at different treatment timing in rats subjected to 45 min occlusion of left anterior descending coronary artery followed by 90 min reperfusion. Drugs were given by i.v. bolus at 5 min before occlusion (Pre-Isch) or 35 min after occlusion (Post-Isch) or reperfusion (With-Rep). The area at risk (AAR/LV, A) was expressed as a percentage of the left ventricle (LV). The infarct size (IS/AAR, B) was expressed as a percentage of the area at risk. Values are mean percentage±S.E.M. (n=6–14). $^{\star}P<0.05$, significantly different from the vehicle-treated group.

Table 1
Effects of KR-32570 on the alterations in mean arterial pressure and heart rate induced by occlusion (45 min) and reperfusion (90 min) of the left anterior descending coronary artery in the anesthetized rat

Parameter	Dose (mg/kg)	Baseline	Occlusion (min)		Reperfusion (min)	
			10	45	30	90
MAP	Vehicle	93±4.8	87±6.8	89±6.0	90±7.1	90±7.1
	0.03	91±8.0	88±8.8	88±6.0	88±5.6	84±3.0
	0.1	91±5.0	86±9.0	81±7.0	81±5.0	87±6.0
	0.3	94±7.2	89±9.7	92±8.8	91±7.0	90±6.6
	1.0	97±7.0	91±7.1	93±7.1	92±8.9	91±5.4
HR	Vehicle	371±17	338±33	362±31	383±28	365±18
	0.03	379±17	351±21	368±27	387±19	389±20
	0.1	361±21	349±20	366±16	368±14	372±7
	0.3	362±16	340±14	345±19	364±14	361±15
	1.0	359±19	345±21	373±23	372±16	371±12

Values are mean±S.E.M. (n=6–14). MAP, mean arterial pressure (mm Hg); HR, heart rate (bpm).

variance (ANOVA) followed by the Dunnett's test for multiple comparisons (Sigma Stat®, Jandel Co., San Rafael, CA, USA). Differences in the incidence of rat arrhythmias (premature ventricular contraction, ventricular tachycardia and ventricular fibrillation) among groups were analyzed by Fisher's exact probability test. In all comparison, the difference was considered to be statistically significant at $P<0.05$.

3. Results

3.1. Cardioprotective effects in rat model of ischemic heart

The effects of KR-32570 and cariporide on myocardial infarct size in anesthetized rats were shown in Fig. 1. The area at risk was similar in all experimental groups (approximately 35–45%; Figs. 1A and 2A), indicating that all groups had the same potential for ischemic damage as a result of left anterior descending coronary artery occlusion. In vehicle-treated group, ischemia (45 min) followed by reperfusion (90 min) resulted in an infarct size of 58.0±1.4% of the area at risk. KR-32570 significantly reduced the myocardial infarct size in a dose-dependent manner (50.7±2.3%, 35.3±2.7%, 33.5±2.0% and 27.0±3.0% at 0.03, 0.1, 0.3 and 1.0 mg/kg, respectively, $P<0.05$) when compared to vehicle-treated group. For reference, cardioprotective effect of cariporide at 0.03, 0.1, 0.3 and 1.0 mg/kg were 46.0±2.0%, 40.5±2.1%, 37.9±3.5% and 35.4±2.1%, respectively.

To correlate the timing of treatment to the effects on myocardial infarct size, KR-32570 was given 10 min before (Post-Isch) or with reperfusion (With-Rep) in anesthetized rats, the results of which were shown in Fig. 2. KR-32570 (1 mg/kg, i.v.) given 10 min before or at reperfusion also significantly reduced the myocardial infarct size (40.9±2.0% and 46.1±1.1%, respectively, $P<0.05$) compared with their

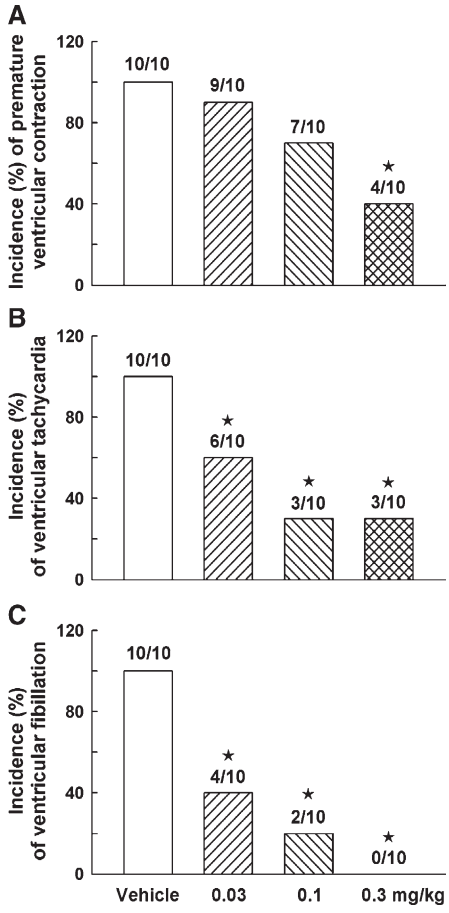

Fig. 3. Dose-dependent prevention of reperfusion-induced premature ventricular contraction (A), ventricular tachycardia (B) and ventricular fibrillation (C) in rats subjected to 5 min coronary artery occlusion followed by 10 min reperfusion. $^{\star}P<0.05$, significantly different from the vehicle-treated group (n=10).

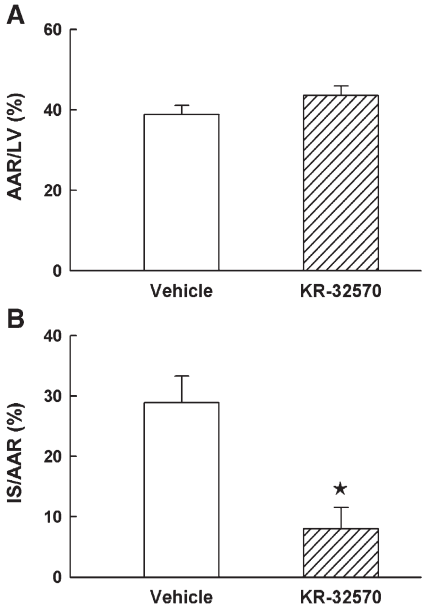

Fig. 4. Effects of KR-32570 (3 mg/kg) on myocardial infarct size in beagle dogs subjected to 1.2 h occlusion of left circumflex coronary artery followed by 3.0 h reperfusion. Drugs were given by bolus i.v. at 10 min before occlusion. The area at risk (AAR/LV, A) was expressed as a percentage of the left ventricle (LV). The infarct size (IS/AAR, B) was expressed as a percentage of the area at risk. Values are mean percentage±S.E.M. (n=6–7). $^{\star}P<0.05$, significantly different from the vehicle-treated group.

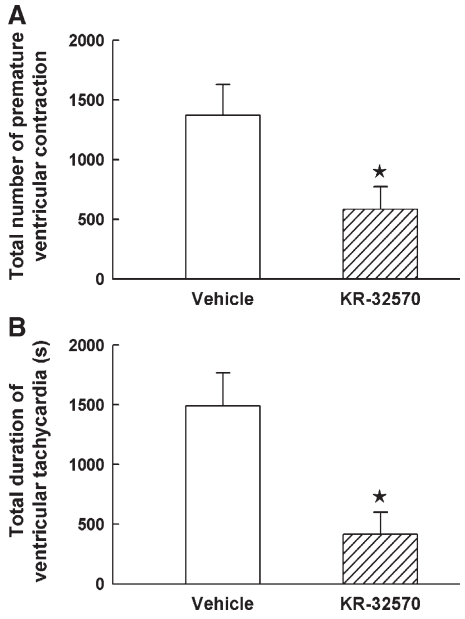

Fig. 5. Effects of KR-32570 on reperfusion-induced premature ventricular contraction (A) and ventricular tachycardia (B) in beagle dogs subjected to 1.2 h occlusion of left circumflex coronary artery followed by 3.0 h reperfusion. Values are expressed as mean±S.E.M. (n=6–7). $^{\star}P<0.05$, significantly different from the vehicle-treated group.

respective vehicle-treated group (55.9±2.0% and 56.6±3.1%, respectively).

The effect of KR-32570 on mean arterial pressure and heart rate was examined before the administration of the compounds, at 10 and 45 min after ischemia, and 30 and 90 min after reperfusion (Table 1). When KR-32570 was administered 5 min before occlusion, the mean arterial pressure of the rats increased transiently, and 4 min later, it returned to the pre-administration value (data not shown). KR-32570 caused no significant changes in the mean arterial pressure and heart rate compared with the vehicle-treated group, although the mean arterial pressure of all groups decreased slightly throughout the experiment.

3.2. Antiarrhythmic effects in rat model of ischemic heart

According to a study on the time-course appearance of ischemia-induced arrhythmias, there was no severe arrhythmia during 5 min of coronary occlusion (data not shown). Thus, the inhibitory effects of KR-32570 on ischemia-induced arrhythmias were not evaluated in this study. The incidence of reperfusion-induced premature ventricular contraction, ventricular tachycardia and ventricular fibrillation in the vehicle-treated group was high (100%, Fig. 3). KR-32570 significantly decreased the incidence of premature ventricular contraction, ventricular tachycardia and ventricular fibrillation in a dose-dependent manner (90%, 70% and 40% for premature ventricular contraction; 60%, 30% and 30% for ventricular tachycardia; 40%, 20% and 0% for ventricular fibrillation at 0.03, 0.1 and 0.3 mg/kg, respectively).

3.3. Cardioprotective and antiarrhythmic effects in dog model of ischemic heart

The effects of KR-32570 (3 mg/kg) on myocardial infarct size in anesthetized dogs were shown in Fig. 4. The size of the area subjected to ischemia (area at risk) of KR-32570-treated group was not significantly different from that of the vehicle-treated group (approximately 40%), indicating that an equivalent degree of myocardial ischemia and reperfusion injury had occurred in each group. In contrast,

Table 2
Hemodynamic measurements in beagle dogs subjected to 1.2-h occlusion of left circumflex coronary artery followed by 3.0-h reperfusion

Parameter	Dose (mg/kg)	Baseline	Occlusion	Reperfusion		
			1.2 h	1 h	2 h	3 h
MAP	Vehicle	121±2.3	111±2.5	105±2.6	117±3.6	119±3.5
	KR-32570	122±3.8	108±5.0	108±6.5	112±5.7	109±5.1
HR	Vehicle	145±5.4	147±5.9	139±7.2	156±4.0	161±4.6
	KR-32570	143±6.6	145±8.1	150±7.4	162±7.3	167±8.9
LVDP	Vehicle	127±2.1	120±2.4	112±3.7	124±4.4	124±3.6
	KR-32570	126±3.3	115±5.4	116±5.6	123±4.4	123±3.6
LV dP/dt_{max}	Vehicle	2900±125	2886±114	2814±103	2900±116	3021±99
	KR-32570	3240±301	2970±208	3020±231	3160±206	3275±148
LVEDP	Vehicle	9.3±0.8	14.1±1.0	12.9±0.9	12.0±0.8	11.1±0.7
	KR-32570	9.8±0.6	11.3±0.7[a]	10.2±0.2[a]	9.9±0.1[a]	9.9±0.4
Glucose	Vehicle	100.5±3.5	102.2±2.0	100.7±5.2	99.0±3.4	98.2±3.4
	KR-32570	99.8±4.4	102.8±2.0	96.0±2.0	98.4±2.5	97.8±1.66
Na^+	Vehicle	147.2±2.0	145.9±0.8	146.2±0.7	145.9±0.5	146.1±0.7
	KR-32570	145.8±0.5	144.8±0.2	145.7±0.5	146.0±0.6	145.8±0.4
K^+	Vehicle	3.12±0.13	3.41±0.11	3.51±0.16	3.74±0.08	3.69±0.10
	KR-32570	3.20±0.02	3.43±0.06	3.45±0.12	3.64±0.14	3.61±0.15
Ca^{2+}	Vehicle	1.34±0.03	1.34±0.02	1.33±0.03	1.33±0.02	1.33±0.03
	KR-32570	1.37±0.02	1.35±0.01	1.31±0.02	1.30±0.02	1.32±0.02

Values are mean±S.E.M. (n=6–7). MAP, mean arterial pressure (mm Hg); HR, heart rate (bpm); LVDP, left ventricular developed pressure (mm Hg); LVEDP, left ventricular end-diastolic pressure (mm Hg); LV dP/dt_{max}, cardiac contractility (mm Hg/s); Na^+ (mM); K^+ (mM); Ca^{2+} (mM).

[a] $P<0.05$, significantly different from the vehicle-treated group.

the infarct size, normalized to the size of the area at risk, was significantly smaller in the KR-32570-treated group (8.0±3.5%, $P<0.05$) than in the vehicle-treated group (28.9±4.4%). The total number of premature ventricular contraction and ventricular tachycardia during reperfusion was only calculated because ventricular fibrillation was not observed in this protocol (Fig. 5). KR-32570 significantly decreased the total number of premature ventricular contraction and ventricular tachycardia compared with that of vehicle-treated group (premature ventricular contraction, 1371±258 and 585±188; ventricular tachycardia, 1493±276 and 416±186, respectively, $P<0.05$).

3.4. Hemodynamic profile in dog model of ischemic heart

The hemodynamic changes in dog models of ischemic heart were examined before the administration of vehicle or KR-32570 (3 mg/kg), immediately before reperfusion, and at 1, 2 and 3 h after reperfusion (Table 2). In the vehicle-treated group, an increase in left ventricular end-diastolic pressure, an indicator of cardiac contracture, was observed immediately after occlusion and persisted throughout the experiments, which was significantly inhibited by treatment of KR-32570. It caused no significant changes in any recorded hemodynamic profiles compared with the vehicle-treated group. There was no demonstrable difference in the concentrations of each electrolyte and glucose among the groups. There were also no significant differences in pH and blood gas values for pO_2 and pCO_2 among groups at the times studied (data not shown). These results demonstrate that KR-32570 does not affect hemodynamic profiles.

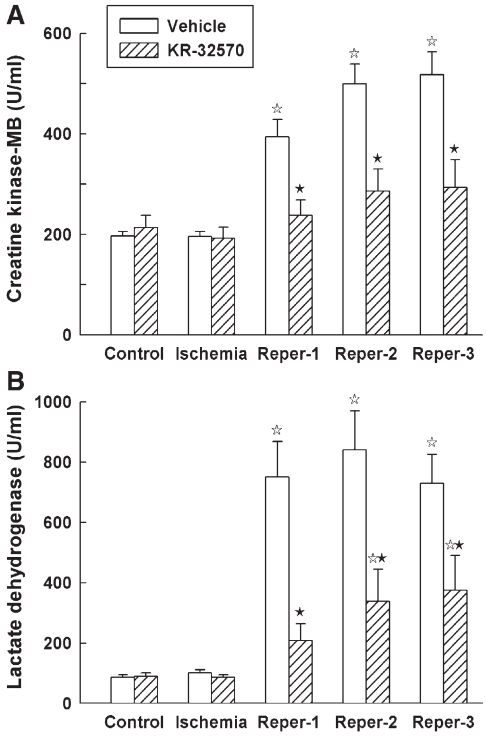

Fig. 6. Effects of KR-32570 on changes in the creatine kinase-MB (A) and lactate dehydrogenase (B) contents induced by occlusion/reperfusion in beagle dogs. Values are expressed as mean±S.E.M. ($n=6$–7). $^{\star}P<0.05$, significantly different from the respective control group. $^{\star}P<0.05$, significantly different from the vehicle-treated group.

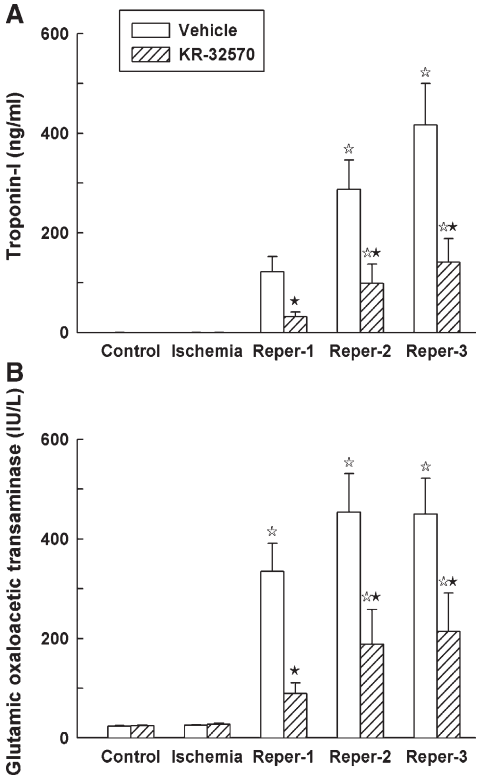

Fig. 7. Effects of KR-32570 on changes in the Troponin-I (A) and glutamic-oxaloacetic transaminase (B) contents induced by occlusion/reperfusion in beagle dogs. Values are expressed as mean±S.E.M. ($n=6$–7). $^{\star}P<0.05$, significantly different from the respective control group. $^{\star}P<0.05$, significantly different from the vehicle-treated group.

3.5. Biochemical data in plasma from dog model of ischemic heart

Figs. 6 and 7 illustrate the changes in plasma concentration or activity of biochemical enzymes in dogs subjected to 1.2-h ischemia and 3.0-h reperfusion. In the vehicle-treated group, the sensitive markers of cardiomyocyte damage, such as CK-MB, LDH, Troponin-I and GOT, were significantly increased during reperfusion, not during ischemia, compared with control values. On the other hand, KR-32570 significantly reduced the release of CK-MB, LDH, Troponin-I and GOT induced by reperfusion.

4. Discussion

The postischemic heart is characterized by contractile dysfunction. Although the pathogenesis of the postischemic contractile dysfunction has not been definitively established, three major theories supported by most experimental evidence suggest that (1) Ca^{2+} overload, (2) the depletion of high energy phosphate stores and (3) the generation of oxygen-derived free radicals (resulted in generation of lipid peroxidation) are

responsible for the postischemic contractile dysfunction (Gross et al., 1999; Wang et al., 2002). In our previous studies, KR-32570 has about 20 times strongly inhibited Na^+/H^+ exchange activity than cariporide in PS120/NHE-1 cells (Lee et al., 2005a). KR-32570 (1–10 μM) also has significantly improved cardiac contractile function and markedly reduced LDH release in isolated rat heart preparations, in conjunction with a preservation of high energy phosphate stores. Importantly, in addition to inhibit the Na^+/H^+ activity and the depletion of high energy phosphate stores, KR-32570 has shown to inhibit the generation of lipid peroxidation (Lee et al., 2005a). These additional effects of KR-32570 on lipid peroxidation might give some advantages in in vivo studies or clinical status, which would be indirectly supported by the study reporting that the lipid peroxidation inhibitor H290/51 itself reduced myocardial infarct size in pigs subjected to occlusion and reperfusion (Shimizu et al., 1998).

As shown in the current study, KR-32570 significantly reduced the myocardial infarct size in rats subject to ischemia and reperfusion in a dose-dependent manner ($P<0.05$ at all doses tested) when compared to vehicle-treated group, and its cardioprotective effect was larger than that of cariporide. KR-32570 (3 mg/kg) has also shown a potent cardioprotective effects in dog models of myocardial infarction. Myocardial enzymes may be released from the injured myocytes induced by ischemia and reperfusion. So, enzyme analysis has proved considerably valuable in the diagnosis of myocardial infarction. In the present study, we found that KR-32570 apparently decreased the plasma concentrations of myocardial enzymes including CK-MB, LDH, Troponin-I and GOT, known markers of cardiomyocyte damage (Huang et al., 2003). Furthermore, the increase in left ventricular end-diastolic pressure, which is an indicator of cardiac contracture during ischemia and reperfusion in dogs, was significantly inhibited by treatment of KR-32570. All of these findings suggest that KR-32570 exerted a beneficial effect on ischemic and reperfused heart from rat and dog.

Cardiac reperfusion after a transient period of ischemia rapidly induces the occurrence of severe ventricular arrhythmia with a high lethality (Manning and Hearse, 1984). KR-32570 significantly and dose-dependently reduced reperfusion-induced fatal ventricular arrhythmia, such as premature ventricular contraction, ventricular tachycardia and ventricular fibrillation, in rats subjected to ischemia and reperfusion. Similar results have been obtained in dog models of ischemia/reperfusion using the KR-32570. Reperfusion-induced arrhythmias are postulated to be related to increased automaticity secondary to the increase of intracellular calcium that occurs during ischemia and reperfusion (Steenbergen et al., 1990) as well as Ca^{2+} oscillations (Ladilov et al., 1995). Therefore, the antiarrhythmia afforded by KR-32570 might be due to suppression of the intracellular Ca^{2+} accumulation and/or oscillations, which results in the observed reduction of reperfusion-induced premature ventricular contraction and ventricular tachycardia.

The efficacy of NHE-1 inhibitors administered just before or at reperfusion still remains controversial, although the timing of administration of NHE-1 inhibitors (pre-ischemia, pre-reperfusion, with-reperfusion) has been extensively studied. Most notably, Klein et al. (1995) failed to demonstrate a cardioprotective effect with HOE694 when intravenously administered at 3 mg/kg, 10 min before reperfusion in a porcine model of myocardial ischemia–reperfusion injury. However, using a porcine model, Rohmann et al. (1995) demonstrated marked cardioprotection with HOE694 when intravenously administered at 7 mg/kg, 15 min before reperfusion. In the present study, KR-32570 (1 mg/kg, i.v.) given 10 min before or at reperfusion also significantly reduced the myocardial infarct size from 56% and 57% in controls to 41% and 46%, respectively ($P<0.05$). The efficacy of KR-32570 was therefore still demonstrable even when drug administration was started before or at reperfusion, suggesting that KR-32570 has good tissue permeability, one of the issues raised regarding this type of cardioprotective agents.

KR-32570 caused no significant changes in any recorded hemodynamic profiles, such as the mean arterial pressure, heart rate, left ventricular developed pressure and left ventricular dP/dt_{max}, compared with the vehicle-treated group. There was no demonstrable difference also in the concentrations of each electrolyte and glucose among the groups. These results demonstrate that KR-32570 does not affect hemodynamic profiles.

In conclusion, results from the present study indicate that KR-32570 significantly reduced the myocardial infarction and incidence of arrhythmias induced by ischemia and reperfusion in rats and dogs. KR-32570 did not show a significant influence on the hemodynamic profiles including blood pressure and heart rate in rats and dogs. These results suggest that KR-32570 could be used to prevent myocardial injuries and lethal ventricular arrhythmias under some clinical conditions such as thrombolytic therapy, percutaneous coronary angioplasty or bypass surgery.

Acknowledgement

This research was supported by a grant (CBM2-A300-001-1-0-0) from the Center for Biological Modulators of the 21st Century Frontier R & D program, the Ministry of Science and Technology, Korea.

References

Braunwald, E., Kloner, R.A., 1985. Myocardial reperfusion: a double-edged sword? J. Clin. Invest. 76, 1713–1719.

Curtis, M.J., Hearse, D.J., 1989. Ischaemia-induced and reperfusion-induced arrhythmias differ in their sensitivity to potassium: implications for mechanisms of initiation and maintenance of ventricular fibrillation. J. Mol. Cell. Cardiol. 21, 21–40.

Doggrell, S.A., Hancox, J.C., 2003. Is timing everything? Therapeutic potential of modulators of cardiac Na(+) transporters. Expert Opin. Investig. Drugs 12, 1123–1142.

Goyal, S., Vanden Heuvel, G., Aronson, P.S., 2003. Renal expression of novel Na^+/H^+ exchanger isoform NHE8. Am. J. Physiol. Renal Physiol. 284, F467–F473.

Gross, G.J., Kersten, J.R., Warltier, D.C., 1999. Mechanisms of postischemic contractile dysfunction. Ann. Thorac. Surg. 68, 1898–1904.

Gumina, R.J., Terzic, A., Gross, G.J., 2001. Do NHE inhibition and ischemic preconditioning convey cardioprotection through a common mechanism? Basic Res. Cardiol. 96, 318–324.

Huang, Z.Q., Shi, G.G., Zheng, J.H., Liu, B., 2003. Effects of *N*-*n*-butyl haloperidol iodide on rat myocardial ischemia and reperfusion injury and L-type calcium current. Acta Pharmacol. Sin. 24, 757–763.

Karmazyn, M., Gan, X.T., Humphreys, R.A., Yoshida, H., Kusumoto, K., 1999. The myocardial Na(+)–H(+) exchange: structure, regulation, and its role in heart disease. Circ. Res. 85, 777–786.

Klein, H.H., Pich, S., Bohle, R.M., Wollenweber, J., Nebendahl, K., 1995. Myocardial protection by Na(+)–H(+) exchange inhibition in ischemic, reperfused porcine hearts. Circulation 15, 912–917.

Kloner, R.A., Rezkalla, S.H., 2004. Cardiac protection during acute myocardial infarction: where do we stand in 2004? J. Am. Coll. Cardiol. 44, 276–286.

Ladilov, Y.V., Siegmund, B., Piper, H.M., 1995. Protection of reoxygenated cardiomyocytes against hypercontracture by inhibition of Na^+/H^+ exchange. Am. J. Physiol. 268, H1531–H1539.

Lee, B.H., Seo, H.W., Yoo, S.E., Kim, S.O., Lim, H., Shin, H.S., 2001. Differential action of KR-31378, a novel potassium channel activator, on cardioprotective and hemodynamic effects. Drug Dev. Res. 54, 182–190.

Lee, B.H., Seo, H.W., Yoo, S.E., 2004. Cardioprotective effects of (2*S*,3*R*,4*S*)-*N*′-benzyl-*N*′′-cyano-*N*-(3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2*H*-benzopyran-4-yl)-guanidine (KR-31372) in rats and dogs. Pharmacology 70, 74–82.

Lee, B.H., Seo, H.W., Yoo, S.E., Yi, K.Y., Lee, S., Lee, S., Yoo, S.-e., 2005a. Effects of KR-32570, a new Na^+/H^+ exchanger inhibitor, on functional and metabolic impairments produced by global ischemia and reperfusion in the perfused rat heart. Eur. J. Pharmacol. 511, 175–182.

Lee, S., Yi, K.Y., Hwang, S.K., Lee, B.H., Yoo, S.-e., Lee, K., 2005b. (5-Arylfuran-2-ylcarbonyl)guanidines as cardioprotectives through the inhibition of Na^+/H^+ exchanger isoform-1. J. Med. Chem. 48, 2882–2891.

Manning, A.S., Hearse, D.J., 1984. Reperfusion-induced arrhythmias: mechanisms and prevention. J. Mol. Cell Cardiol. 16, 497–518.

Masereel, B., Pochet, L., Laeckmann, D., 2003. An overview of inhibitors of Na(+)/H(+) exchanger. Eur. J. Med. Chem. 38, 547–554.

Rohmann, S., Weygandt, H., Minck, K.O., 1995. Preischaemic as well as postischaemic application of a Na^+/H^+ exchange inhibitor reduces infarct size in pigs. Cardiovasc. Res. 30, 945–951.

Satoh, N., Kitada, Y., 2004. Cardioprotective effect of MCC-135 is associated with inhibition of Ca^{2+} overload in ischemic/reperfused hearts. Eur. J. Pharmacol. 499, 179–187.

Scholz, W., Albus, U., Counillon, L., Gogelein, H., Lang, H.J., Linz, W., Weichert, A., Scholkens, B.A., 1995. Protective effects of HOE642, a selective sodium–hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. Cardiovasc. Res. 29, 260–268.

Shimizu, M., Wang, Q.D., Sjoquist, P.O., Ryden, L., 1998. The lipid peroxidation inhibitor indenoindole H290/51 protects myocardium at risk of injury induced by ischemia–reperfusion. Free Radic. Biol. Med. 24, 726–731.

Steenbergen, C., Murphy, E., Watts, J.A., London, R.E., 1990. Correlation between cytosolic free calcium, contracture, ATP, and irreversible ischemic injury in perfused rat heart. Circ. Res. 66, 135–146.

Touret, N., Tanneur, V., Godart, H., Seidler, R., Taki, N., Burger, E., Dammgen, J., Counillon, L., 2003. Characterization of sabiporide, a new specific NHE-1 inhibitor exhibiting slow dissociation kinetics and cardioprotective effects. Eur. J. Pharmacol. 459, 151–158.

Tracey, W.R., Allen, M.C., Frazier, D.E., Fossa, A.A., Johnson, C.G., Marala, R. B., Knight, D.R., Guzman-Perez, A., 2003. Zoniporide: a potent and selective inhibitor of the human sodium–hydrogen exchanger isoform 1 (NHE-1). Cardiovasc. Drug Rev. 21, 17–32.

Walker, M.J., Curtis, M.J., Hearse, D.J., Campbell, R.W., Janse, M.J., Yellon, D. M., Cobbe, S.M., Coker, S.J., Harness, J.B., Harron, D.W., et al., 1988. The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia infarction, and reperfusion. Cardiovasc. Res. 22, 447–455.

Wang, Q.D., Pernow, J., Sjoquist, P.O., Ryden, L., 2002. Pharmacological possibilities for protection against myocardial reperfusion injury. Cardiovasc. Res. 55, 25–37.

Weaver, W.D., Simes, R.J., Betriu, A., Grines, C.L., Zijlstra, F., Garcia, E., Grinfeld, L., Gibbons, R.J., Ribeiro, E.E., DeWood, M.A., Ribichini, F., 1997. Comparison of primary coronary angioplasty and intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review. JAMA 278, 2093–2098.