Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 521 (2005) 115–123
www.elsevier.com/locate/ejphar

Reduction of myocardial infarct size by SM-197378, a novel Na^+/H^+ exchange inhibitor, in rabbits

Kazuto Yamada [a,*], Kazuki Matsui [a], Shoji Ogawa [a], Setsuko Yamamoto [a], Masaya Mori [a], Masafumi Kitano [a], Naohito Ohashi [b]

[a] *Sumitomo Pharmaceuticals Co., Ltd., Research Division, Japan*
[b] *Corporate Planning and Coordination Office, 1-98 Kasugadenaka 3-chome, Konohana-ku, Osaka 554-0022, Japan*

Received 25 April 2005; received in revised form 4 August 2005; accepted 8 August 2005
Available online 23 September 2005

Abstract

The effects of SM-197378, 2-[[[amino(imino)methyl]amino]carbonyl]-1-methyl-4-trifluoromethyl-1*H*-indol-7-yl=hydrogen=sulfate monohydrate, a novel potent Na^+/H^+exchange inhibitor, on heart injury were studied using a rabbit model involving 30 min of myocardial ischemia and 5 h of reperfusion. Intravenous administration of SM-197378 before ischemia reduced the infarct size by approximately 30–50% in a dose-dependent manner. This anti-necrotic effect was achieved without significant hemodynamic changes. Moreover, administration of SM-197378 before reperfusion also resulted in a significant, approximately 30–40%, reduction in the infarct size. The anti-necrotic effect of pre-ischemic bolus treatment with SM-197378 was compared with that of nicorandil, a K^+channel opener with nitrate-like activity, and ischemic preconditioning. With 30 and 60 min of ischemia, the anti-necrotic effects of SM-197378 and ischemic preconditioning were similar and superior to that of nicorandil. With 90 min of ischemia, the anti-necrotic effect of SM-197378 was superior to that of ischemic preconditioning.

Keywords: Na^+/H^+exchange; Myocardial ischemia and reperfusion injury; Ischemic preconditioning

1. Introduction

The Na^+/H^+exchange system is believed to play an important role in the pathophysiology of ischemic reperfusion injury in the heart. Recent findings using Na^+/H^+exchange-deficient mice support this notion directly (Wang et al., 2003). The stimulation of Na^+/H^+exchange activity in cardiac ischemia followed by reperfusion has been proposed as a potential causative factor for post-ischemic reperfusion injury, because the activation of this exchanger may result in increased Na^+influx followed by intracellular Ca^{2+}overload through the Na^+/Ca^{2+}exchange pathway. Intracellular Ca^{2+}overload has a variety of detrimental effects, such as cell damage, generation of arrhythmia, and contractile dysfunction (Scholz and Albus, 1993; Avkiran, 1996; Piper et al., 1996). In fact, we have already demonstrated that a Na^+/H^+exchange inhibitor, SM-20550, reduces the increase in $Na^+$and Ca^{2+}concentration, leading to an improvement of cardiac function in the reperfused rat heart (Yamamoto et al., 2000) and similarly reduces myocardial necrosis in rabbit myocardial ischemia and reperfusion injury (Yamada et al., 2000).

We recently identified a novel potent and highly selective inhibitor of the Na^+/H^+exchanger, SM-197378 (2-[[[amino(imino)methyl]amino]carbonyl]-1-methyl-4-trifluoromethyl-1*H*-indol-7-yl=hydrogen=sulfate monohydrate), which has an IC_{50} of 37 nM for Na^+/H^+exchange activity in rat cardiomyocytes (Hotta et al., 2004). In addition, SM-197378 has a cardioprotective effect in isolated perfused guinea-pig heart (Hotta et al., 2004). The present study focuses on the effect of SM-197378 on myocardial ischemia reperfusion injury in a rabbit model. SM-197378 was administered before or after ischemia. Pre-ischemic treatment with the Na^+/H^+exchange inhibitors ethylisopropyl–amiloride (Bugge et al., 1996), cariporide (Garcia-Dorado et al., 1997; Klein et al., 1997; Miura et al., 1997), EMD85131 (Gumina et al., 1998), and SM-20550 (Yamada et al., 2000) is cardioprotective. However, the effects of treatment before reperfusion with these inhibitors have been

* Corresponding author. Tel.: +81 6 6466 5205; fax: +81 6 6466 5182.
E-mail address: yamadak@sumitomopharm.co.jp (K. Yamada).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.08.018

less clear, with several groups reporting contradictory results. Ethylisopropyl–amiloride, administered after ischemia, failed to reduce myocardial necrosis in a rabbit myocardial ischemia and reperfusion injury model (Bugge et al., 1996). Similarly, cariporide treated before reperfusion did not reduce necrosis in a rabbit and a porcine model (Garcia-Dorado et al., 1997; Miura et al., 1997). However, EMD85131 (Gumina et al., 1998) and SM-20550 (Yamada et al., 2000) administered before reperfusion, limited the infarct size.

On the other hand, since pre-ischemic treatment with Na^+/H^+exchange inhibitors such as ethylisopropyl–amiloride (Bugge et al., 1996), cariporide (Garcia-Dorado et al., 1997; Klein et al., 1997; Miura et al., 1997), BIIB513 (Gumina et al., 1999), EMD85131 (Gumina et al., 1998), and SM-20550 (Yamada et al., 2000) reduced the infarct size in several animal models much like ischemic preconditioning, Gumia et al. compared the effect of BIIB513 and that of ischemic preconditioning on infarct size in a rabbit myocardial ischemia and reperfusion injury model (Gumina et al., 1999). We also demonstrated that SM-20550, a Na^+/H^+exchange inhibitor, did not diminish the cardioprotective effect of ischemic preconditioning (Yamada et al., 2002). Moreover, the combined cardioprotective effect of SM-20550 and sub-threshold ischemic preconditioning was greater than additive (Yamada et al., 2002).

The objectives of the present study were to clarify the efficacy of SM-197378 for preventing myocardial infarction, not only when administered before ischemia but also before reperfusion, and to compare the anti-necrotic effect of SM-197378 as a pre-ischemic treatment with that of ischemic preconditioning and nicorandil, a K^+channel opener with nitrate-like activity. Nicorandil has been shown to have cardioprotective effects in patients with anterior wall myocardial infarction (Ito et al., 1999).

2. Materials and methods

2.1. Surgical preparation

The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985). All procedures involving the use of animals were reviewed and approved by the Institutional Animal Care and Use Committee at Sumitomo Pharmaceuticals Research Center (Osaka, Japan).

Animal preparation was based on a previously reported procedure (Yamada et al., 1998). Briefly, male New Zealand White rabbits (2.5–3.5 kg, Kitayama Labes Co. Ltd., Ina, Japan) were anesthetized with sodium pentobarbital (40 mg/kg, i.v.). The rabbits were intubated through a tracheotomy and ventilated with a mechanical respirator (SN-480-6 or SN-480-5, Shinano Co. Ltd., Tokyo, Japan). A catheter was inserted into the right carotid artery to allow measurement of the mean arterial blood pressure by a pressure transducer (TP-300T or DX-100, Nihon Koden Co. Ltd., Tokyo, Japan). Another catheter was inserted into the right jugular vein for the administration of vehicle or compounds. Additional anesthesia was administered through the right femoral vein as needed. A thoracotomy was performed through the fourth intercostal space and the heart was suspended in a pericardial cradle. A catheter was placed in the left atrium through the left atrial appendage. A 4-0 silk suture was tied around the large marginal branch of the left circumflex coronary artery. The electrocardiogram lead II was used to monitor the heart rate and ST-segment elevation. The mean arterial blood pressure was continuously monitored by a blood pressure meter (AP-621G and AP-611G or AP-641G, Nihon Koden Co. Ltd., Tokyo, Japan), and the electrocardiogram was monitored by electrocardiogram amplifier (AC-601G, Nihon Koden Co. Ltd., Tokyo, Japan) during the experiment. The heart rate was also measured using a heart-rate counter (AT-600G or AT-601G, Nihon Koden Co. Ltd., Tokyo, Japan).

After surgery, the rabbits were allowed to stabilize for at least 30 min. The arterial blood pH, pO_2, and pCO_2 were measured by a blood gas system 248 (Ciba-Corning, Tokyo, Japan) and maintained within the physiological range by adjusting the respiration rate and oxygen flow. After a baseline recording of the mean arterial blood pressure, the heart rate and an electrocardiogram were taken, and myocardial ischemia was induced by tightening the silk suture to occlude the vessel completely. Coronary artery occlusion was maintained for 30 min (in one experiment, the ischemic period was 60 or 90 min), and myocardial ischemia was confirmed by the presence of regional cyanosis and ST-segment elevation. The occlusion was then released and reperfusion was allowed for 5 h. For the sham-operated group, the coronary artery was not occluded, but all other experimental procedures were performed in the same way as for the group that underwent occlusion.

2.2. Experimental protocol

2.2.1. Treatment before ischemia or before reperfusion

Vehicle or SM-197378 (Sumitomo Pharmaceutical Co. Ltd., Osaka, Japan) was administered intravenously by bolus injection 10 min prior to ischemia in the first set of experiments, and 10 min prior to reperfusion in the second set of experiments. After the bolus injection, the infusion of vehicle or SM-197378 was sustained until the end of the experiment. The rabbits in the SM-197378 experiments were divided into four groups in the first and second set of experiments. These groups received (1) saline (Otuka Pharamaceutical Co., Ltd., Tokyo, Japan) as the vehicle, in the same volume as the solutions containing inhibitor, (2) 0.00051 mg/kg by bolus injection and 0.00084 mg/kg/h by continuous infusion as the “1/3 Low”-dose SM-197378 group, (3) 0.0017 mg/kg by bolus injection and 0.0028 mg/kg/h by continuous infusion as the “Low”-dose SM-197378 group, (4) 0.0051 mg/kg by bolus injection and 0.0084 mg/kg/h by continuous infusion as the “Mid”-dose SM-197378 group, and (5) 0.017 mg/kg by bolus injection and 0.028 mg/kg/h by continuous infusion as the “High”-dose SM-197378 group.

2.2.2. Infusion period

SM-197378 was administered intravenously 10 min prior to reperfusion by bolus injection (0.017 mg/kg), followed by infusion (0.028 mg/kg/h) until 1, 2, or 5 h after the start of reperfusion.

2.2.3. Effect of pre-ischemic SM-197378 treatment with several ischemic periods

In this set of experiments, the ischemic periods were 30, 60, and 90 min and the reperfusion period was held at 5 h. SM-197378 was administered intravenously 10 min prior to ischemia by bolus injection (0.3 mg/kg). The anti-necrotic effect of SM-197378 was compared with that of ischemic preconditioning and nicorandil (SIGMART®$_{Injection}$, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan). Ischemic preconditioning was induced with 5 min of ischemia and 10 min of reperfusion prior to the 30–90 min of sustained ischemia. This ischemic preconditioning procedure showed a 72% reduction in infarct size in a rabbit model of 30 min ischemia and 5 h reperfusion (Yamada et al., 2002). Nicorandil was administered intravenously 10 min prior to ischemia by infusion (0.1 mg/kg/min) for 5 min. This dose of nicorandil has been reported to show an anti-necrotic effect in a rabbit model of 30 min ischemia and 48 h reperfusion (Ohno et al., 1997).

2.3. Quantification of myocardial area-at-risk and necrotic area

At the end of the 5 h of reperfusion, the silk suture was tightened again, and 1% Evans blue solution was infused quickly via the catheter into the left atrium. The heart was then isolated and soaked in ice-cold saline. The left ventricle was dissected away from all other structures and cut into 13 slices, parallel to the atrioventricular groove. The slices were classed as to whether they contained areas that were positively or negatively stained, then they were traced onto transparent plastic sheets. Evans blue-negative staining defined the area-at-risk. The slices were then incubated in a 1% solution of 2,3,5-triphenyltetrazolium chloride (Wako Pure Chemical Industries Ltd., Osaka, Japan) for 10 min at 37 °C and soaked in a neutral pH, buffered 10% formaldehyde solution overnight to enhance the contrast of the staining. The areas that were positive or negative for triphenyltetrazolium chloride staining were traced onto transparent plastic sheets. Areas that were negative for triphenyltetrazolium chloride were defined as necrotic.

The total left ventricle area, the area-at-risk, and the necrotic area were measured using image-analyzing software (NIH Image, Wayne Rasband, National Institutes of Health, USA).

2.4. Measurement of serum creatine phosphokinase

Blood samples (2.5 ml) were withdrawn by catheter before the administration of vehicle or SM-197378, immediately before ischemia, immediately before reperfusion, and at 1, 3, and 5 h after reperfusion. Whole-blood samples were transferred to collection tubes and spun at 1000 ×g for 20 min at 4 °C. The serum samples were collected and stored at −20 °C until they were used for the creatine phosphokinase measurement, which was performed by the colorimetric method of Allain (Allain et al., 1973) using a CPK?-test kit (Wako Pure Chemical Industries Ltd., Osaka, Japan).

2.5. Statistical analysis

All values in the figures and tables are presented as means ± S.E.M. Statistical comparisons among the groups for areas-at-risk, serum creatine phosphokinase activity before administration of compounds, and hemodynamic parameters before ischemia were performed using analysis of variance (ANOVA). Statistical comparisons between the vehicle and sham group after ischemia were performed using Student's *t*-test or Welch's test. Statistical comparisons among the groups for infarct size, serum creatine phosphokinase activity after the administration of compounds, and hemodynamic parameters after ischemia were performed using the Williams' test, Shirley–Williams' test, or Dunnett's test. *P*-values less than 0.05 were considered to be statistically significant.

3. Results

Summary of experimental design is listed in Table 1.

Table 1
Summary of experimental design

(1) Administered by bolus plus infusion in 30 min ischemia and 5 h reperfusion

Group	Animal number
Treatment before ischemia	
Sham	4
Vehicle	8
SM-197378 1/3 Low	8
SM-197378 Low	8
SM-197378 Mid	8
SM-197378 High	8
Treatment before reperfusion	
Vehicle	7
SM-197378 Low	7
SM-197378 Mid	8
SM-197378 High	6
Infusion period (treated before reperfusion)	
Vehicle	8
SM-197378 High until 1 h after reperfusion	8
SM-197378 High until 2 h after reperfusion	8
SM-197378 High until 5 h after reperfusion	8

(2) 30, 60, 90 min ischemia and 5 h reperfusion

Ischemic period	Group	Animal number
30 min	Vehicle	7
	Nicorandil 0.1 mg/kg/min for 5 min	8
	Ischemic preconditioning	7
	SM-197378 0.3mg/kg bolus	6
60 min	Vehicle	8
	Nicorandil 0.1 mg/kg/min for 5 min	8
	Ischemic preconditioning	9
	SM-197378 0.3mg/kg bolus	8
90 min	Vehicle	7
	Nicorandil 0.1 mg/kg/min for 5 min	6
	Ischemic preconditioning	8
	SM-197378 0.3 mg/kg bolus	7

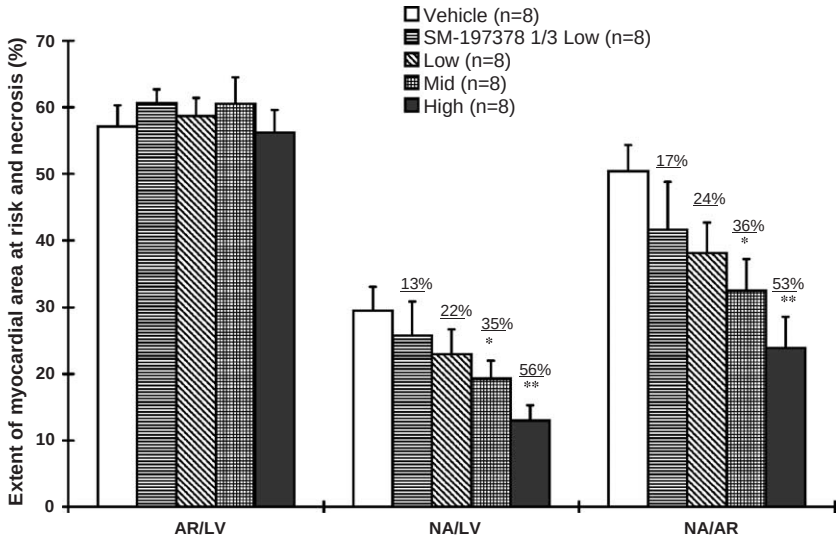

Fig. 1. Effect of pre-ischemic treatment with SM-197378 on myocardial infarct size. Rabbits were subjected to 30 min of coronary artery occlusion (CAO) followed by 5 h of reperfusion. AR, LV, and NA indicate the area at risk, left ventricle area, and necrotic area, respectively. The number of animals is indicated in parenthesis. Each bar represents the mean and S.E.M. The underlined values give the percentage of inhibition. (A) Vehicle or SM-197378 was administered intravenously 10 min prior to ischemia by bolus injection. The dosages (mg/kg) were: 1/3 Low, 0.00051; Low, 0.0017; Mid, 0.0051; High, 0.017. Following the ischemic period, drug infusion was continued during the reperfusion, until the end of the experiment. The infusion dosages (mg/kg/h) were: 1/3 Low, 0.00084; Low, 0.0028; Mid, 0.0084; High, 0.028. There was no significant difference among the groups for AR/LV by ANOVA. Statistical significance: *, $P<0.05$; **, $P<0.01$ vs. vehicle by Williams' test.

3.1. Treatment before ischemia or before reperfusion

The effect on infarct size of administering SM-197378 before ischemia is illustrated in Fig. 1. The size of the area subjected to ischemia (i.e., the area-at-risk) among the groups was not significantly different, indicating that an equivalent degree of myocardial ischemia and reperfusion injury had occurred in each group. In contrast, the infarct size in the SM-197378-treated animals, normalized to the size of the area-at-risk, was significantly smaller in the Mid-and High-dose groups than in the vehicle-treated group. Moreover, the reduction in infarct size by SM-197378 was dose-dependent: 17% reduction (1/3 Low-dose group), 24% (Low-dose group), 36% (Mid-dose group), and 53% (High-dose group). Similarly, the infarct size, expressed as a percentage of the left ventricle area, was smaller in the SM-197378-treated groups than in the vehicle-treated group; again, this was dose-dependent, and statistical significance was achieved in the Mid-and High-dose groups.

Fig. 2 illustrates the changes in serum creatine phosphokinase activity during ischemia and reperfusion. After ischemia, the serum creatine phosphokinase was significantly higher in the vehicle-treated group than in the sham-operation

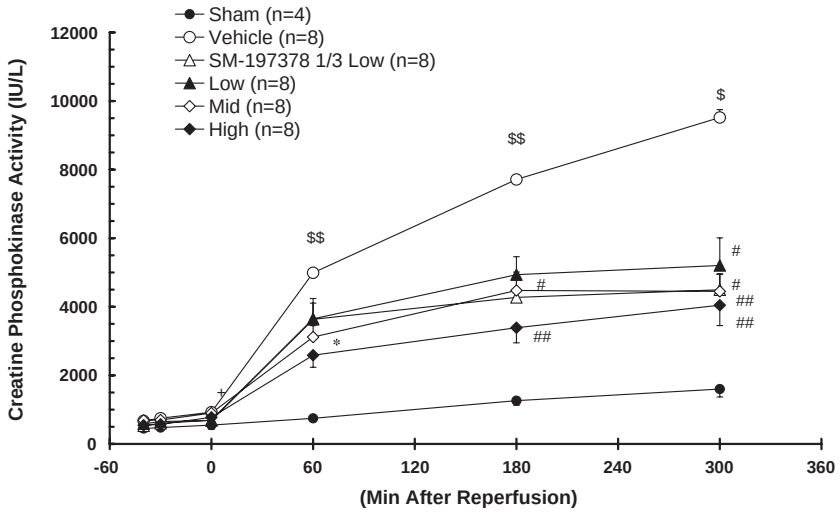

Fig. 2. Effect of pre-ischemic treatment with SM-197378 on creatine phosphokinase activity. Blood samples were collected at the indicated time points, and the creatine phosphokinase (CPK) activity in the serum was measured. Rabbits in the sham group were not subjected to coronary artery occlusion, but were otherwise treated the same as the other groups. Each point represents the mean and S.E.M. See the legend for Fig. 1 for details. There was no significant difference among the groups for values of before ischemia by ANOVA. Statistical significance: +, $P<0.05$ vs. Sham by Student's t-test. $, $P<0.05$; $$, $P<0.01$ vs. Sham by Welch's test. *, $P<0.05$; **, $P<0.01$ vs. Vehicle by Williams' test. #, $P<0.05$; ##, $P<0.01$ vs. Vehicle by Shirley–Williams' test.

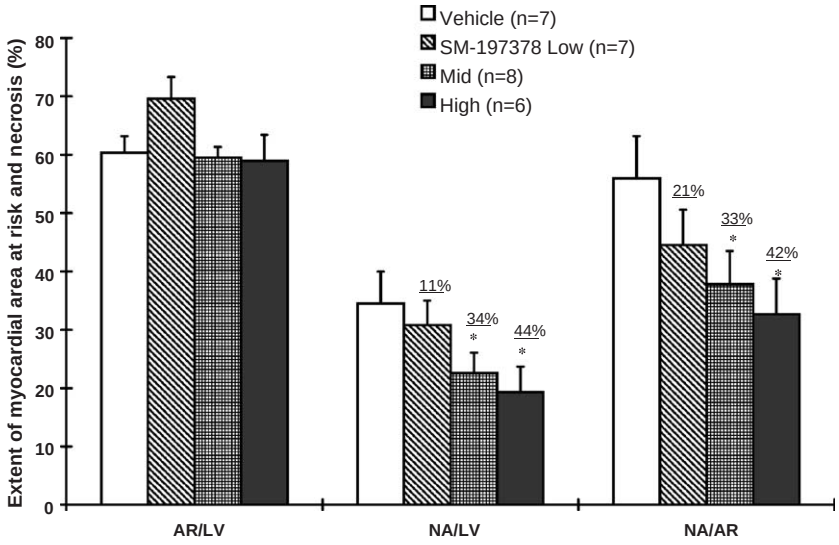

Fig. 3. Effect of SM-197378 treated before reperfusion on myocardial infarct size. SM-197378 was administered intravenously 10 min prior to reperfusion, by bolus injection. Dosages (mg/kg): Low, 0.0017; Mid, 0.0051; High, 0.017. Drug infusion followed, until the end of the experiment. Infusion dosages (mg/kg/h): Low, 0.0028; Mid, 0.0084; High, 0.028. See the legend for Fig. 1 for details. There was no significant difference among the groups for AR/LV by ANOVA. Statistical significance: **, $P<0.01$ vs. vehicle by Williams' test.

group. Administration of SM-197378 before ischemia reduced the serum creatine phosphokinase in a dose-dependent manner. Statistical significance was achieved 60 and 180 min after reperfusion for the Mid-and High-dose groups, and 300 min after reperfusion for all the SM-197378-treated groups.

Fig. 3 shows the effect on infarct size of administering SM-197378 before reperfusion. The area-at-risk was similar among all groups. The infarct size with SM-197378, normalized to the area-at-risk, was significantly smaller in the Mid-and High-dose groups than in the vehicle-treated group, and the effect was dose-dependent. The reduction in infarct size was 21% (Low-dose group), 33% (Mid-dose group), and 42% (High-dose group). Similarly, when expressed as a percentage of the left ventricle area, the infarct size was significantly smaller in the Mid-and High-dose group than in the vehicle-treated group.

The effect of SM-197378 on hemodynamic changes (mean arterial blood pressure and heart rate) was examined before ischemia, during the ischemic period (30 min), and during the reperfusion period (5 h). The mean arterial blood pressure and heart rate of all groups decreased gradually throughout the experiment. SM-197378, when administered before ischemia, caused no significant changes in any recorded parameters, compared with the vehicle-treated group (Table 2). These results demonstrate that SM-197378 does not affect hemodynamic parameters.

Table 2
Summary of hemodynamic parameters

Hemodynamic parameters	Treatment group	Pre-treatment	Pre-ischemia	Post-ischemia	Post-reperfusion				
				30 min	30 min	60 min	120 min	180 min	300 min
Mean arterial blood pressue (mmHg)	Sham (4)	71.5±0.9	69.5±1.8	71.3±3.9	65.0±4.0	63.5±4.8	55.8±4.9	51.0±4.7	49.0±3.7
	Vehicle (8)	71.8±2.3	66.1±3.3	48.6±6.8	[a]47.9±3.6	49.8±3.7	44.9±3.6	44.5±2.7	43.5±2.8
	SM-197378								
	1/3 Low (8)	71.1±2.7	66.8±2.4	60.3±6.9	54.3±4.8	53.4±4.2	48.5±3.1	50.5±3.4	48.5±4.5
	Low (7–8)	70.0±2.9	63.8±3.1	56.6±5.2	49.8±3.7	50.9±2.7	48.8±2.5	47.6±3.4	43.8±4.8
	Mid (8)	70.4±2.3	67.6±3.1	59.3±3.8	52.9±3.8	52.1±4.2	48.0±2.8	50.4±1.9	46.6±2.2
	High (8)	71.0±3.1	64.6±2.8	58.6±3.1	49.1±4.9	49.3±3.0	47.4±2.2	51.4±1.9	50.4±1.9
Heart rate (beats/min)	Sham (4)	272.8±8.6	269.0±9.0	260.8±9.3	257.5±7.8	259.3±8.8	254.5±10.2	237.8±12.9	209.0±6.6
	Vehicle (8)	281.8±6.1	277.0±5.2	258.5±12.6	258.0±7.8	261.8±7.9	243.5±9.2	221.3±12.8	198.6±12.7
	SM-197378								
	1/3 Low (8)	270.0±8.9	263.0±8.0	266.5±10.2	257.6±9.8	255.1±10.2	247.4±9.3	234.5±6.5	220.3±8.2
	Low (7–8)	274.1±6.0	271.6±6.8	270.3±13.5	256.4±8.1	255.5±8.5	241.9±5.1	226.9±5.7	204.5±9.4
	Mid (8)	283.9±11.7	279.4±9.4	276.1±10.8	261.5±8.6	264.1±10.1	252.9±8.6	244.5±7.6	213.1±11.8
	High (8)	272.1±5.4	268.9±7.4	262.3±8.1	238.5±12.1	245.8±10.1	243.9±11.8	236.1±9.6	221.8±8.2

Each value represents mean±S.E.M.

Mean arterial blood pressure and heart rate were measured at the indicated time points. See the legend for Fig. 1 for details. There was no significant difference among 6 groups for values before treatment and before ischemia by ANOVA. There was no significant difference between Vehicle and each SM-198110 treated-group for values after ischemia by Dunnet's test.

a; $p<0.05$ vs. sham by Student's t-test.

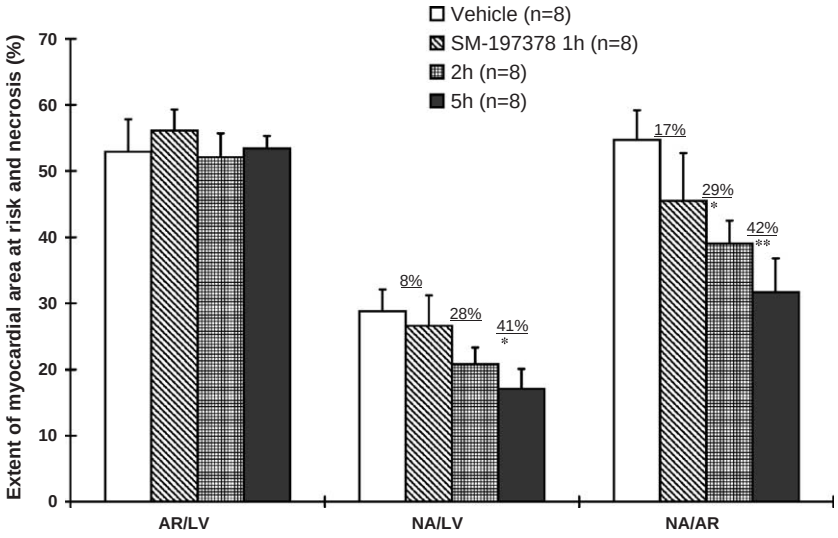

Fig. 4. Effect of SM-197378 treated before reperfusion given for different infusion periods on myocardial infarct size. SM-197378 was administered intravenously 10 min prior to reperfusion (Rep) by bolus injection (0.017 mg/kg), followed by infusion (0.028 mg/kg/h) until 1 h after Rep, 2 h after Rep, or 5 h after Rep. See the legend for Fig. 1 for details. There was no significant difference among the groups for AR/LV by ANOVA. Statistical significance: *, $P<0.05$; **, $P<0.01$ vs. vehicle by Williams' test.

3.2. Infusion period

The effect on infarct size of the duration of the post-ischemic infusion period of SM-197378 is illustrated in Fig. 4. The area-at-risk was similar among all groups. The infarct size, normalized to the area-at-risk, was significantly smaller in the SM-197378-treated groups than in the vehicle-treated group when SM-197378 was given for 2 h or more after the start of reperfusion. The reduction in infarct size was 17% (treatment for 1 h after reperfusion), 29% (2 h), and 42% (5 h).

Fig. 5 illustrates the changes in serum creatine phosphokinase activity during ischemia and reperfusion. Administration of SM-197378 after ischemia also reduced the serum creatine phosphokinase in a manner dependent on the infusion period. Statistical significance was achieved 60 min or more after reperfusion for the SM-197378-treated group until 5 h after reperfusion.

3.3. Effect of pre-ischemic SM-197378 treatment in several ischemic periods

Fig. 6 illustrates the effect of pre-ischemic treatment with SM-197378, nicorandil, or ischemic preconditioning on infarct size with 30, 60, or 90 min of ischemia and 5 h of reperfusion. The size of the area-at-risk among the 4 groups was not significantly different (data not shown), indicating that an equivalent degree of myocardial ischemia and reperfusion injury

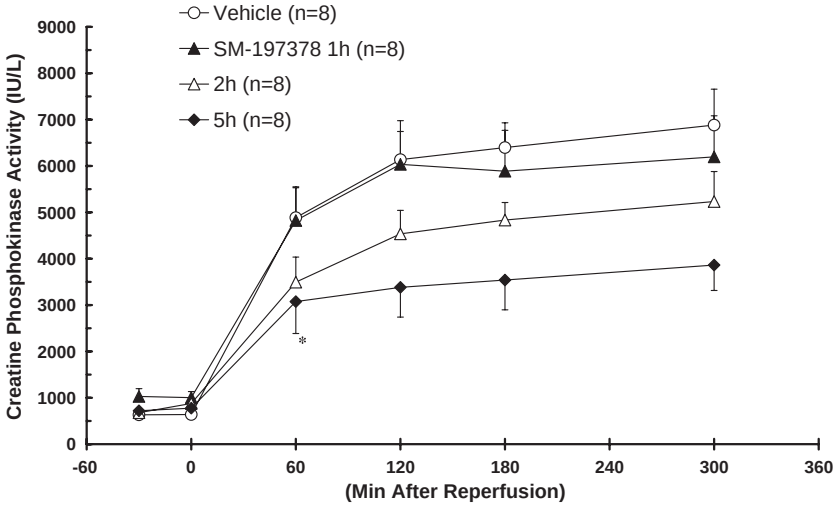

Fig. 5. Effect of SM-197378 treated before reperfusion for different infusion periods on creatine phosphokinase activity. See the legends for Figs. 4 and 2 for details. There was no significant difference among the groups in creatine phosphokinase values before ischemia by ANOVA. Statistical significance: *, $P<0.05$; **, $P<0.01$ vs. vehicle by Williams' test.

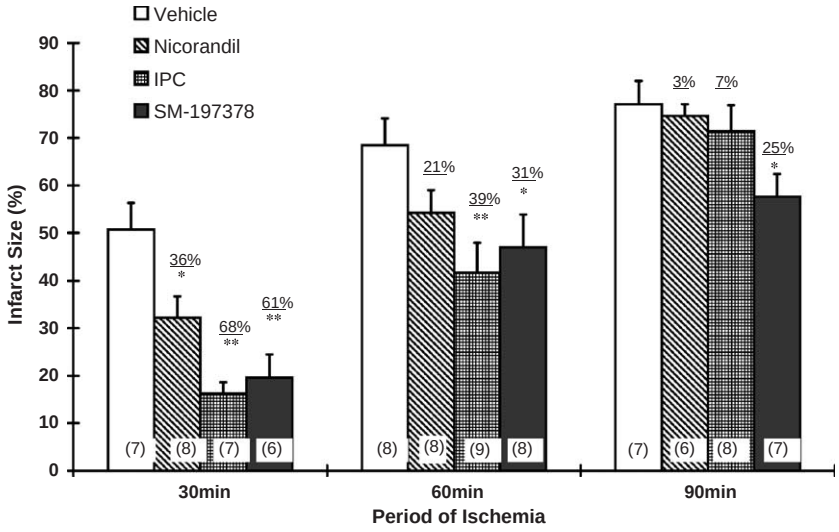

Fig.6. Effect of pre-ischemic SM-197378 treatment in several ischemic period. Rabbits were subjected to 30, 60, or 90 min of coronary artery occlusion (CAO) followed by 5 h of reperfusion. Infarct size is given as necrotic area/area-at-risk. Nicorandil was administered intravenously 10 min prior to ischemia by infusion (0.1 mg/kg/min) for 5 min. Ischemic preconditioning was induced with 5 min of ischemia and 10 min of reperfusion prior to the sustained ischemia. SM-197378 was administered intravenously 10 min prior to ischemia by bolus injection (0.3 mg/kg). See the legend for Fig. 1 for details. There was no significant difference among the groups for AR/LV by ANOVA. Statistical significance: *, $P<0.05$; **, $P<0.01$ vs. Vehicle by Dunnett's test.

had occurred in each group. The infarct size, normalized to the size of the area-at-risk, increased with the ischemic period. The infarct size with 30 min of ischemia was significantly smaller in all the drug-or ischemic preconditioning-treated groups than in the vehicle-treated group. The reduction in infarct size was 36% (nicorandil), 68% (ischemic preconditioning), and 61% (SM-197378). However, the infarct size with 60 min of ischemia was significantly smaller than in the vehicle-treated group in the ischemic preconditioning (39%) and SM-197378-treated (31%) groups but not in the nicorandil-treated group (21%). With 90 min of ischemia, the infarct size was only significantly smaller in the SM-197378-treated group (25%), and not in either nicorandil-treated (3%) or in ischemic preconditioning (7%) group.

4. Discussion

In myocardial ischemia and reperfusion injury, inhibition of the Na^+/H^+exchanger prevents stunning, reperfusion arrhythmia, and myocardial infarction in several animal models. Recently, a novel, potent, and highly selective inhibitor of the Na^+/H^+exchanger, SM-197378, was demonstrated to inhibit Na^+/H^+exchange activity in rat cardiomyocytes, with an IC_{50} of 83 nM (Hotta et al., 2004). In the present study, pre-ischemic treatment with SM-197378 in a rabbit heart injury model reduced the infarct size (Fig. 1) and serum creatine phosphokinase activity (Fig. 2) in a dose-dependent manner, without affecting hemodynamic parameters (Table 2). Moreover, SM-197378 treated even before reperfusion dose-dependently decreased myocardial necrosis by 42% (Fig. 3). The anti-necrotic effect of SM-197378 appears to be similar to that of SM-20550 (Yamada et al., 2000). While SM-197378 and SM-20550 (Yamada et al., 2000) reduced the infarct size when treated both before ischemia and before reperfusion, other Na^+/H^+exchange inhibitors, such as Ethylisopropyl-amiloride (Bugge et al., 1996) and cariporide (Miura et al., 1997; Garcia-Dorado et al., 1997; Klein et al., 1998), have been demonstrated to limit the infarct size only in pre-ischemic treatment.

In the course of ischemia, the Na^+/H^+exchanger is activated as a result of cellular acidosis (Tani and Neely, 1989; Imahashi et al., 1998), and upon reperfusion, it is activated again by the washing out of acidic extracellular fluid, which induces an intracellular and extracellular pH imbalance (Tani and Neely, 1989). The activation of the Na^+/H^+exchanger during myocardial ischemia and reperfusion induces an increase in the intracellular Na^+concentration, which ultimately leads to Ca^{2+}overload due to activation of the Na^+/Ca^{2+}exchanger (Scholz and Albus, 1993; Avkiran, 1996; Piper et al., 1996). In fact, the increases in $Na^+$and Ca^{2+}levels in the heart were reduced and cardiac function was improved when SM-197378 was treated during global ischemia and reperfusion in the isolated perfused guinea-pig heart (Hotta et al., 2004). The inhibitory effect of SM-197378 on the activation of the Na^+/H^+exchanger during ischemia and reperfusion could lead to a reduced Ca^{2+}overload and thus the inhibition of myocardial necrosis.

Tani and Neely showed that there is an overshoot of intracellular Na^+concentration upon reperfusion, which reactivates the Na^+/H^+exchanger, in isolated perfused rat heart (Tani and Neely, 1989), leading to the idea that Na^+/H^+exchange inhibitors might reduce the Na^+influx to some extent, even if given just before reperfusion. Treatment with Na^+/H^+exchanger inhibitors before ischemia reduces the increase in intracellular Na^+concentration during ischemia (Tani and Neely, 1989; Imahashi et al., 1998) and should also reduce the increased Na^+upon reperfusion, while treatment with such inhibitors just before reperfusion should reduce only the increase in intracellular Na^+concentration that is associated with reperfusion.

This could explain why the pre-ischemic treatment with SM-197378 showed a greater inhibitory effect on myocardial necrosis (53%) than did the treatment before reperfusion (42%). The anti-necrotic effect of SM-197378 treated before reperfusion was confirmed by using different infusion periods. SM-197378 significantly reduced the infarct size during an infusion period of 2 to 5 h after reperfusion (Figs. 4 and 5). Although several Na^+/H^+exchange inhibitors have been demonstrated to be effective for reducing myocardial necrosis when administered as a pre-ischemic treatment, there has been no clear demonstration of their anti-necrotic effects when they are administered before reperfusion. The anti-necrotic effect of SM-197378 demonstrates that the Na^+/H^+exchanger is still involved at least 2 h after reperfusion in myocardial reperfusion injury in vivo.

In Fig. 6, we compared the anti-ischemic effect of SM-197378 with that of nicorandil and ischemic preconditioning, by bolus injection to eliminate the effect of SM-197378 on reperfusion. With 30 or 60 min of ischemia, SM-197378 and ischemic preconditioning reduced the infarct size significantly and by almost the same amount, but the anti-necrotic effect of nicorandil was smaller than with these treatments. We have already shown that a Na^+/H^+exchange inhibitor, SM-20550, reduces the infarct size more than nicorandil does in pre-ischemic treatment (Yamada et al., 2000), and the anti-necrotic effect of SM-20550 is similar to that of ischemic preconditioning (Yamada et al., 2002) in rabbits with 30 min of ischemia and 5 h of reperfusion. The results of the present study support our previous results (Yamada et al., 2000, 2002) and indicate that the anti-ischemic effect of Na^+/H^+exchange inhibitors is superior to that of nicorandil, a clinically used anti-ischemic drug. With 90 min of ischemia, SM-197378 reduced the infarct size significantly (by 25%) but ischemic preconditioning did not (7%). Klein et al. reported that pre-ischemic treatment with cariporide increases the tolerance to ischemia by 20–25 min in a pig model (Klein et al., 1997). Gumia et al. showed pre-ischemic treatment with BIIB513 exerts an anti-necrotic effect greater than that of ischemic preconditioning on infarct size in a canine myocardial ischemia and reperfusion injury model (Gumina et al., 1999). Our results support the previous findings (Gumina et al., 1999) and indicate that the anti-ischemic effect of Na^+/H^+exchange inhibitors is superior to that of ischemic preconditioning.

In our opinion, Na^+/H^+exchange inhibitors can be classified into 2 types: the first shows an anti-necrotic effect when given before reperfusion, and includes SM-20550 and SM-197378, and the second does not exert an anti-necrotic effect treated before reperfusion. However, the pre-ischemic effect of both types is similar and superior to ischemic preconditioning. The reasons for this difference among Na^+/H^+exchange inhibitors are not clear at present. Further studies to clarify the cardioprotective mechanism of Na^+/H^+exchange inhibition are needed.

In summary, we demonstrated that SM-197378, a novel Na^+/H^+exchanger inhibitor, reduced myocardial infarction when given before ischemia and before reperfusion, without affecting hemodynamic parameters. The pre-ischemic anti-necrotic effect of SM-197378 was superior to those of nicorandil and ischemic preconditioning.

Acknowledgments

We thank Ms. Masako Toda and Ms. Yuka Yamana for their excellent technical assistance during the course of this study. We are grateful to other members of the Cardiovascular Research Group for their contribution to this work and for valuable discussion.

References

Allain, C.C., Henson, C.P., Nadel, M.K., Knoblesdorff, A.J., 1973. Rapid single-step kinetic colorimetric assay for lactate dehydrogenase in serum. Clin. Chem. 19, 223–227.

Avkiran, M., 1996. Sodium-hydrogen exchange in myocardial ischemia and reperfusion: a critical determinant of injury? In: Karmazyn, M. (Ed.), Myocardial Ischemia: Mechanism, Reperfusion, Protection. Birkhäuser Verlag, Basel, Switzerland, pp. 299–311.

Bugge, E., Munch-Ellingsen, J., Ytrehus, K., 1996. Reduced infarct size in the rabbit heart in vivo by ethylisopropyl-amiloride. A role for Na^+/H^+exchange. Basic Res. Cardiol. 91, 203–209.

Garcia-Dorado, D., Gonzaláz, M.A., Barrabés, J.A., Ruiz-Meana, M., Solares, J., Lidon, R.-M., Blanco, J., Puigfel, Y., Piper, H.M., Soler-Soler, J., 1997. Prevention of ischemic rigor contracture during coronary occlusion by inhibition of Na^+–H^+exchange. Cardiovasc. Res. 35, 80–89.

Gumina, R.J., Mizumura, T., Beier, N., Schelling, P., Schultz, J.J., Gross, G.J., 1998. A new sodium/hydrogen exchange inhibitor, EMD 85131, limits infarct size in dogs when administered before or after coronary artery occlusion. J. Pharmacol. Exp. Ther. 286, 175–183.

Gumina, R.J., Buerger, E., Eickmeier, C., Moore, J., Daemmgen, J., Gross, G.J., 1999. Inhibition of the Na^+/H^+exchanger confers greater cardioprotection against 90 min of myocardial ischemia than ischemic preconditioning in dogs. Circulation 100, 2519–2526.

Hotta, Y., Nishimaki, H., Takeo, T., Itoh, G., Yajima, M., Otsuka-Murakami, H., Ishikawa, N., Kawai, N., Huang, L., Yamada, K., Yamamoto, S., Matsui, K., Ohashi, N., 2004. Differences in the effects of Na^+–H^+exchange inhibitors on cardiac function and apoptosis in guinea-pig ischemia-reperfused hearts. Eur. J. Pharmacol. 503, 109–122.

Imahashi, K., Hashimoto, K., Yamaguchi, H., Nishimura, T., Kusuoka, H., 1998. Alteration of intracellular Na^+during ischemia in diabetic rat hearts: the role of reduced activity in Na^+/H^+exchange against stunning. J. Mol. Cell Cardiol. 30, 509–517.

Ito, H., Taniyama, Y., Iwakura, K., Nishikawa, N., Masuyama, T., Kuzuya, T., Hori, M., Higashino, Y., Fujii, K., Minamino, T., 1999. Intravenous Nicorandil can preserve microvascular integrity and myocardial viability in patients with reperfused anterior wall myocardial infarction. J. Am. Coll. Cardiol. 33, 654–660.

Klein, H.H., Bohle, R.M., Pich, S., Lindert-Heimberg, S., Wollenweber, J., Nebendahl, K., 1997. Time delay of cell death by Na^+/H^+-exchange inhibition in regionally ischemic, reperfused porcine hearts. J. Cardiovasc. Pharmacol. 30, 235–240.

Klein, H.H., Bohle, R.M., Pich, S., Lindert-Heimberg, S., Wollenweber, J., Schade-Brittinger, C., Nebendahl, K., 1998. Time-dependent protection by Na^+/H^+exchange inhibition in a regionally ischemic, reperfused porcine heart preparation with low residual blood flow. J. Mol. Cell Cardiol. 30, 795–801.

Miura, T., Ogawa, T., Suzuki, K., Goto, M., Simamoto, K., 1997. Infarct size limitation by a new Na^+–H^+exchange inhibitor, Hoe642: difference from preconditioning in the role of protein kinase C. J. Am. Coll. Cardiol. 29, 693–701.

Ohno, Y., Minatoguchi, S., Uno, Y., Kariya, T., Arai, M., Yamashita, K., Fujiwara, T., Fujiwara, H., 1997. Nicorandil reduces myocardial infarct size by opening the K(ATP) channel in rabbits. Int. J. Cardiol. 623, 181–190.

Piper, H.M., Basler, C., Ladilov, Y.V., Schäfer, M., Siegmund, B., Ruiz-Meana, M., Garcia-Dorado, D., 1996. The role of Na^+/H^+exchange in ischemia-reperfusion. Basic Res. Cardiol. 91, 191–202.

Scholz, W., Albus, U., 1993. Na^+/H^+exchange and its inhibition in cardiac ischemia and reperfusion. Basic Res. Cardiol. 88, 443–455.

Tani, M., Neely, J.R., 1989. Role of intracellular Na^+ in $Ca2^+$ overload and depressed recovery of ventricular function of reperfused ischemic rat hearts: Possible involvement of H^+–$Na^+$and Na^+–$Ca2^+$exchange. Circ. Res. 65, 1045–1056.

Wang, Y., Meyer, J.W., Ashraf, M., Shull, G.E., 2003. Mice with a null mutation in the NHE1 Na^+–H^+exchanger are resistant to cardiac ischemia-reperfusion injury. Circ. Res. 93, 776–782.

Yamada, K., Tojo, S.J., Hayashi, M., Morooka, S., 1998. The role of P-selectin, sialyl Lewis X and sulfatide in myocardial ischemia and reperfusion injury. Eur. J. Pharmacol. 346, 217–225.

Yamada, K., Matsui, K., Satoh, K., Kitano, M., Yamamoto, S., Ohashi, N., 2000. Reduction of myocardial infarct size by SM-20550, a novel Na^+/H^+exchange inhibitor, in rabbits. Eur. J. Pharmacol. 404, 201–212.

Yamada, K., Matsui, K., Yamamoto, S., Kitano, M., Ohashi, S., 2002. Effect of an Na^+/H^+exchange inhibitor, SM-20550, on ischemic preconditioning in rabbits. Int. J. Tissue React. 24, 1–10.

Yamamoto, S., Matsui, K., Kitano, M., Ohashi, N., 2000. SM-20550, a new Na^+/H^+exchange inhibitor and its cardioprotective effect in ischemic-reperfused isolated rat hearts by preventing $Ca2^+$-overload. J. Cardiovasc. Pharmacol. 35, 855–862.