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Effects of SEA0400, a Na^+/Ca^{2+} exchange inhibitor, on ventricular arrhythmias in the in vivo dogs

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Abstract

SEA0400 (2-[4-[(2,5-difluorophenyl)methoxy]phenoxy]-5-ethoxyaniline), a novel and selective inhibitor of Na^+/Ca^{2+} exchanger, was investigated for its possible antiarrhythmic effects on arrhythmias of Ca^{2+} overload induced by coronary ligation/reperfusion and by digitalis in the dog. SEA0400 (1.0 mg/kg) did not change the hemodynamics but slightly prolonged the QRS duration ($P<0.05$). Pre-ischemic administration (10 min before coronary occlusion) of SEA0400 (1.0 mg/kg) and post-ischemic administration (1 min before reperfusion) of SEA0400 (0.3, 1.0 and 3.0 mg/kg) had no effects on the incidence of ventricular fibrillation induced by coronary ligation/reperfusion. On the other hand, SEA0400 (3.0 mg/kg) decreased the arrhythmic ratio in the digitalis arrhythmias ($P<0.01$). However, atrioventricular block and cardiac standstill were induced in two digitalized dogs. In conclusion, SEA0400 has no significant antiarrhythmic effect on arrhythmias induced by coronary ligation/reperfusion, but has an obvious suppressing effect on tachyarrhythmias induced by digitalis in in vivo canine models.

Keywords: Ischemia/reperfusion; Arrhythmia; Na^+/Ca^{2+} exchange; Digitalis

1. Introduction

The Na^+/Ca^{2+} exchanger is an important transporter for the regulation of intracellular Ca^{2+} concentration (Shigekawa and Iwamoto, 2001). Cardiac Na^+/Ca^{2+} exchanger normally extrudes Ca^{2+} from the cytoplasm (the forward mode) to maintain the intracellular Ca^{2+} concentration at a very low level. However, under pathophysiological conditions such as coronary ligation/reperfusion and digitalis toxicity, Ca^{2+} influx via Na^+/Ca^{2+} exchange (the reverse mode) is enhanced by the increase of intracellular Na^+ concentration, leading to intracellular Ca^{2+} overload. This Ca^{2+} overload is thought to be a major cause of cell injury and tachyarrhythmias induced by coronary ligation/reperfusion and digitalis toxicity (Tani, 1990). Although the inhibition of Na^+/Ca^{2+} exchanger had been thought as a possible target for antiarrhythmic treatment, the investigations of this possibility had not been done because of the lack of useful Na^+/Ca^{2+} exchange inhibitors.

Recently, selective Na^+/Ca^{2+} exchange inhibitors have been synthesized, i.e. KB-R7943 (2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl]isothiourea methanesulfonate), SEA0400 (2-[4-[(2,5-difluorophenyl)methoxy]phenoxy]-5-ethoxyaniline) and SN-6 [2-[4-(4-nitrobenzyloxy)benzyl]thiazolidine-4-carboxylic acid ethyl ester] (Watano et al., 1996; Matsuda et al., 2001; Iwamoto et al., 2004). Some investigators reported beneficial effects of KB-R7943 on ischemia- or reperfusion-induced injury in in vivo animal models. Nakamura et al. (1998) reported that pre-ischemic administration of KB-R7943 at 10 mg/kg suppressed ventricular

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fibrillation and cardiac arrest induced by coronary reperfusion in in vivo rat models. Watano et al. (1999) also reported that KB-R7943 (1 and 3 mg/kg) increased the doses of digitalis required to induce premature ventricular beats, ventricular tachycardia, ventricular fibrillation and cardiac arrest in in vivo guinea-pig models. However, our previous data in in vivo dog models showed that pre-ischemic administration of KB-R7943 (5 and 10 mg/kg i.v.) did not suppress arrhythmias induced by coronary ligation/reperfusion and post-digitalized administration of KB-R7943 (5 mg/kg) did not suppress arrhythmias induced by digitalis (Miyamoto et al., 2002). These discrepancies might be partly due to the differences of the study protocols and animals used, and also due to the multi-channel blocking actions, and/or incomplete selectivity of Na^+/Ca^{2+} exchange inhibition of KB-R7943. To examine the pure effects of Na^+/Ca^{2+} exchange inhibitors, the use of more selective and more potent Na^+/Ca^{2+} exchange inhibitors is necessary.

Recently, SEA0400 was developed and showed more selective and potent inhibition of Na^+/Ca^{2+} exchanger compared with that of KB-R7943 (Matsuda et al., 2001; Tanaka et al., 2002). Takahashi et al. (2003) reported that post-ischemic administration of SEA0400 at 0.3 and 1 mg/kg suppressed the incidence of ventricular fibrillation and mortality induced by coronary ligation/reperfusion in in vivo rat models. There is no report clarifying antiarrhythmic effects of SEA0400 on the in vivo larger animals.

In this study, we examined the hypotheses that the selective inhibition of Na^+/Ca^{2+} exchanger by SEA0400 to reduce Ca^{2+} overload suppresses the arrhythmias induced by coronary ligation/reperfusion and by digitalis on the in vivo dog arrhythmia models. Until now, we had examined various antiarrhythmic agents with these in vivo dog models (Hashimoto et al., 1991). We compared antiarrhythmic efficacy of SEA0400 with other antiarrhythmic agents, especially with Na^+/H^+ exchange inhibitors, which showed effectiveness on arrhythmias induced by coronary ligation/reperfusion.

2. Materials and methods

This study was performed in accordance with the *Guideline for Animal Experiment, University of Yamanashi*. Animals were obtained through the Animal Laboratory for Research of University of Yamanashi.

2.1. Arrhythmias induced by coronary ligation/reperfusion

Forty-eight adult female beagle dogs, weighing 8.0–11.5 kg, were anesthetized with pentobarbital sodium (30 mg/kg i.v.) and artificially ventilated with room air (Sinano, SN-480-3, Tokyo, Japan). Tidal volume and respiratory rate were set at 20 ml/kg and 15 stroke/min, respectively. Anesthesia was maintained by continuous intravenous infusion of pentobarbital sodium (5.0 mg/kg/h). As reported earlier (Miyamoto et al., 2002), the left chest was opened and the left ventricle was exposed. The left anterior descending coronary artery was isolated just proximal to the first branch of left anterior descending coronary artery. The left anterior descending coronary artery was ligated using a 2–0 nylon thread for 30 min and then released to observe reperfusion response. The surface lead II electrocardiogram (ECG) and systemic blood pressure at the right femoral artery were continuously monitored using a polygraph system (Nihondenki San-ei, Tokyo, Japan).

On the basis of previous studies reported by Matsuda et al. (2001) and Takahashi et al. (2003), we chose the present study protocols and the doses of SEA0400, as follows. In the pre-ischemic administration protocol, 1.0 mg/kg of SEA0400 or its vehicle, a lipid emulsion containing 20% soybean oil, was administered 10 min before coronary ligation. In the post-ischemic administration protocol, a wider range to higher doses of 0.3, 1.0 and 3.0 mg/kg of SEA0400 or its vehicle were administered 1 min before coronary reperfusion because Takahashi et al. (2003) reported the efficacy of SEA0400 given just prior to reperfusion, and 1.0 mg/kg pre-ischemic administration was not effective. Six groups with eight dogs for each group were assigned.

2.2. Arrhythmias induced by digitalis

Eight adult female beagle dogs, weighing 7.0–12.0 kg, were anesthetized with pentobarbital sodium (30 mg/kg i.v.) and artificially ventilated with room air (Sinano, SN-480-3, Tokyo, Japan). Tidal volume and respiratory rate were set at 20 ml/kg and 15 strokes/min, respectively. Anesthesia was maintained by continuous intravenous infusion of pentobarbital sodium (5.0 mg/kg/h). Both left and right vagi were cut at the midcervical level. As reported previously (Miyamoto et al., 2002), 40 μg/kg of digitalis was injected intravenously (i.v.) and then followed by an additional 10 μg/kg i.v. every 20 min until stable ventricular tachycardia was induced, usually at cumulative doses of 60–70 μg/kg. In the absence of drug administration, the resulting arrhythmias remained stable for more than 60 min, as reported by us (Awaji et al., 1995). After the induction of stable ventricular tachycardia (ventricular tachycardia maintained for more than 5 min), SEA0400 was administered as an intravenous bolus injection at a high dose of 3 mg/kg, as SEA0400 did not show antiarrhythmic effects on arrhythmias induced by the above coronary ligation/reperfusion in this study, we selected the highest dose of SEA0400 in the digitalis study. The effects were observed for 60 min after administration of SEA0400. The surface lead II ECG and blood pressure at the right femoral artery were continuously monitored using a polygraph system (Nihondenki San-ei).

The severity of arrhythmias induced by digitalis was evaluated by the arrhythmic ratio, which was calculated by dividing the number of premature ventricular beats by the number of total heart rates, i.e., the number of premature ventricular beats plus the number of conducted beats. The

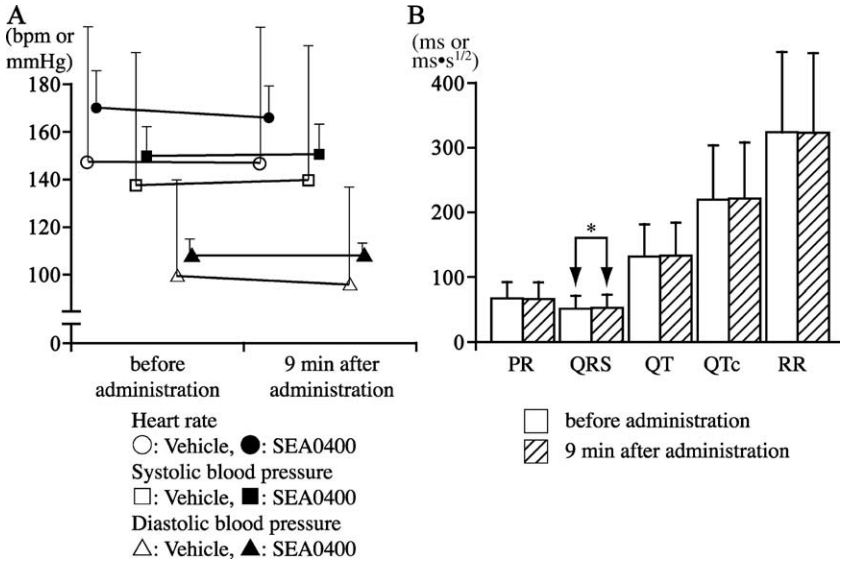

Fig. 1. Effects of intravenous administration of SEA0400 (1.0 mg/kg) on hemodynamics and ECG parameters. (A) Hemodynamics. The vertical bar represents the mean±S.D. (n=8) bpm–beats per minute. (B) ECG parameters. The corrected QT interval (QTc) was calculated using Bazett's formula, QTc=QT/$\sqrt{}$(RR). The vertical bar represents the mean±S.D. (n=8). *P<0.05 vs. Pre.

different shape of the ventricular complex from the normal QRS complex was the criteria for judging the ventricular beats. Reduction of arrhythmic ratio signifies antiarrhythmic effect. As reported earlier, without drugs, there was no spontaneous improvement in this ratio of digitalis induced arrhythmias (Awaji et al., 1995).

2.3. Measurement of free plasma concentration of SEA0400

To measure the free plasma concentration of SEA0400, blood samples were obtained 10, 20 and 40 min after intravenous administration of SEA0400 (1.0 mg/kg) in the pre-ischemic administration protocols. Blood samples were stored at −80 °C until the measurement. Sensitive and specific determinations of the free plasma concentration of SEA0400 were performed at the laboratory of Taisho Pharmaceutical (Saitama, Japan) using a liquid chromatography mass spectrometric/tandem mass spectrometric method (LC-MS/MS).

2.4. Drug

SEA0400 and its vehicle were kindly provided by Taisho Pharmaceutical. Because of the hydrophobic properties of SEA0400, SEA0400 was administered as a lipid emulsion containing 20% soybean oil. Pentobarbital sodium was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Digitalis (ouabain octahydrate) was purchased from Aldrich (Milwaukee, WI, USA).

2.5. Statistical analysis

Data are presented as the mean±S.D. The statistical comparisons of mean values within a group were evaluated by one-way repeated measures analysis of variance (ANOVA) followed by contrasts, or assessed by paired t-test, and those between the groups were examined by unpaired t-test. The incidence of arrhythmias was compared between the groups

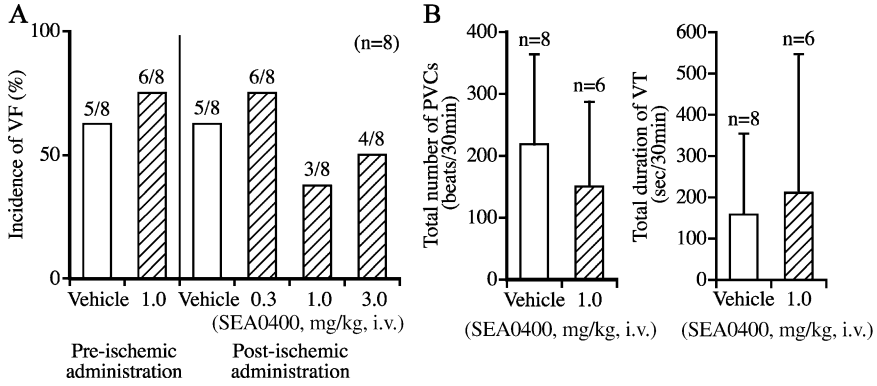

Fig. 2. Effects of intravenous administration of SEA0400 on arrhythmias induced by coronary ligation/reperfusion. (A) The incidence of ventricular fibrillation (VF) induced by coronary ligation/reperfusion. The number shown above each bar represents the number of dogs in which fibrillation occurred. (B) Total number of premature ventricular constrictions (PVCs) and total duration of ventricular tachycardia (VT) during ischemic period in the pre-ischemic protocol. The vertical bar represents the mean±S.D.

by Fisher's exact probability test. The difference was regarded as significant if the P-value was less than 0.05.

3. Results

3.1. Free plasma concentration of SEA0400

At 10, 20 and 40 min after the administration of SEA0400 (1.0 mg/kg), the free plasma concentration of SEA0400 were 1.10±0.10, 0.56±0.05 and 0.33±0.03 μg/ml, respectively.

3.2. Effects of SEA0400 on hemodynamics and ECG parameters before coronary occlusion

SEA0400 (1.0 mg/kg i.v.) and its vehicle did not change the heart rate and blood pressure before coronary occlusion (Fig. 1A). Although SEA0400 did not change the PR interval, QT interval, QTc and RR interval, only the QRS duration was slightly increased 9 min after administration of SEA0400 (59±4 ms in the treated group vs. 57±3 ms in the control group, $P<0.05$, as shown in Fig. 1B). The vehicle of SEA0400 did not change any of the ECG parameters (data not shown).

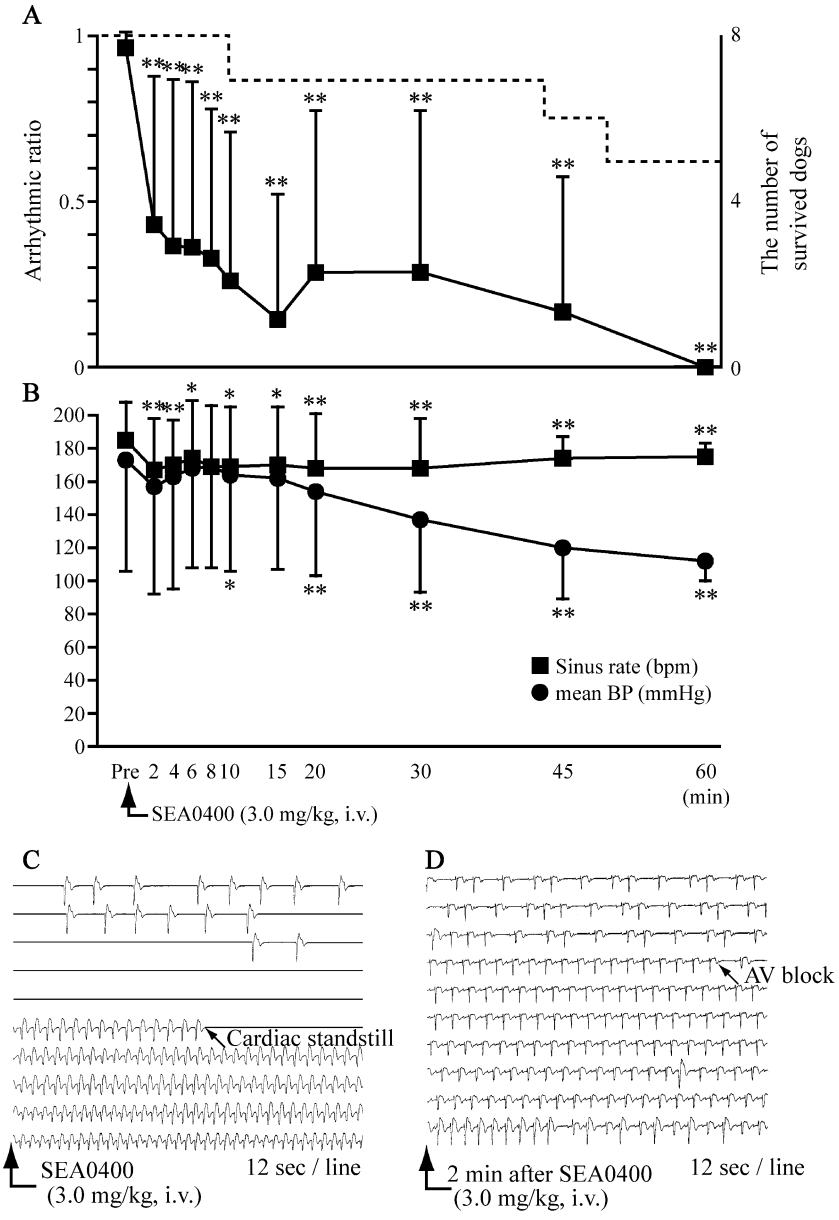

Fig. 3. Effects of SEA0400 on arrhythmias induced by digitalis. (A) Arrhythmic ratio (solid line) and the number of survived animals (dashed line). **$P<0.01$ vs. Pre. Pre: just before administration of SEA0400 (3.0 mg/kg i.v.). The vertical bar represents the mean±S.D. (B) Sinus rate and mean blood pressure (mBP). *$P<0.05$, **$P<0.01$ vs. Pre. The vertical bar represents the mean±S.D. bpm–beats per minute. (C) The ECG recorded from the dog, in which cardiac standstill occurred after administration of SEA0400. ECG was recorded from the bottom of the chart to the top. (D) The ECG recorded from the dog, in which atrioventricular (AV) block occurred after administration of SEA0400. ECG was recorded from the bottom of the chart to the top.

3.3. Effects of SEA0400 on arrhythmias induced by coronary ligation/reperfusion

In the pre-ischemic administration, SEA0400 at 1.0 mg/kg did not change: (1) the incidence of ventricular fibrillation induced by coronary ligation/reperfusion (six out of eight dogs in the treated group vs. five out of eight dogs in the control group; no statistically significant difference, n.s., Fig. 2A); (2) the number of premature ventricular beats during 30 min of ischemic period (150±137 beats in the treated group vs. 219±145 beats in the control group, n.s., Fig. 2B); (3) the total duration of ventricular tachycardia during the ischemic period (212±336 s in the treated group vs. 159±196 s in the control group, n.s., Fig. 2B). In the post-ischemic administration, SEA0400 even at higher dose did not change the incidence of ventricular fibrillation (six out of eight dogs, three out of eight dogs and four out of eight dogs at 0.3, 1.0 and 3.0 mg/kg of SEA0400, respectively, in the treated group as compared to the control group of five out of eight dogs, n.s., Fig. 2A).

3.4. Effects of SEA0400 on arrhythmias induced by digitalis

The administration of SEA0400 at 3.0 mg/kg decreased the arrhythmic ratio (shown in Fig. 3A, $P<0.01$) and sinus rates (Fig. 3B, $P<0.01$), and its antiarrhythmic effects lasted for the entire 60 min of observation without attenuation. Mean blood pressure was decreased 20 min after administration of SEA0400 (Fig. 3B, $P<0.01$). In two of eight dogs, however, atrioventricular block and cardiac standstill were induced after the administration of SEA0400 (Fig. 3C,D). Two of eight dogs died due to ventricular fibrillation and one of six remaining dogs died due to cardiac standstill.

4. Discussion

In the present study, intravenous administration of SEA0400 at 1.0 mg/kg slightly prolonged the QRS duration, but did not change any other ECG and hemodynamic parameters. Both pre-ischemic administration of SEA0400 at 1.0 mg/kg and post-ischemic administration of SEA0400 at 0.3, 1.0 and 3.0 mg/kg had no effects on the incidence of ventricular tachycardia and ventricular fibrillation induced by coronary ligation/reperfusion. On the other hand, SEA0400 attenuated tachyarrhythmias induced by digitalis, simultaneously inducing atrioventricular block and cardiac standstill after administration of SEA0400 in two digitalized dogs. The sinus rate decreased slightly but significantly after the administration of SEA0400 in the digitalized dogs. These results suggest that SEA0400 has no significant antiarrhythmic effect on arrhythmias induced by coronary ligation/reperfusion, but has an obvious suppressing effect on tachyarrhythmias induced by digitalis in in vivo canine models.

It has been well known that during the ischemic period, ATP depletion changes metabolic processes, leading to intracellular acidosis. The gradient of H^+ concentration across sarcolemmal membrane stimulates the Na^+/H^+ exchanger to remove the H^+ from the cell in exchange for Na^+, which leads to the increase of intracellular Na^+ concentration (Tani, 1990). In digitalis toxicity, inhibition of Na^+/K^+ ATPase by digitalis is the cause of increase in the intracellular Na^+ concentration (Mohammadi et al., 2003). Increased intracellular Na^+ concentration activates Na^+/Ca^{2+} exchanger to remove three intracellular Na^+ in exchange of one extracellular Ca^{2+} via a reverse mode, which leads to intracellular Ca^{2+} overload (Tani, 1990). The intracellular Ca^{2+} overload is thought to be a major cause of cell injury and triggered tachyarrhythmias in coronary ligation/reperfusion and digitalis toxicity (Tani, 1990; Satoh et al., 2003). Therefore, inhibition of Na^+/H^+ exchange or Na^+/Ca^{2+} exchange is expected to be effective against coronary ligation/reperfusion-induced arrhythmias and digitalis toxicity-induced arrhythmias, respectively.

In the present study, both the pre-ischemic and post-ischemic administration of SEA0400 did not suppress arrhythmias induced by coronary ligation/reperfusion. In our previous report using another inhibitor of Na^+/Ca^{2+} exchanger, KB-R7943 at 5 and 10 mg/kg, its pre-ischemic administration also did not suppress the incidence of ventricular fibrillation induced by coronary ligation/reperfusion in the canine model (Miyamoto et al., 2002). On the basis of our studies, we think that Na^+/Ca^{2+} exchange inhibitors have no common suppressing effects on the arrhythmias induced by coronary ligation/reperfusion at least in in vivo canine models. In the in vivo rat model, Takahashi et al. (2003) reported on the antiarrhythmic effects of SEA0400 on arrhythmias induced by coronary ligation/reperfusion. As for KB-R7943, Nakamura et al. (1998) reported that pre-ischemic administration of KB-R7943 at 10 mg/kg suppressed ventricular fibrillation; on the other hand, Lu et al. (1999) also reported that pre-ischemic treatment of KB-R7943 at 1.25 mg/kg did not suppress arrhythmias induced by coronary ligation/reperfusion. Therefore, the views on antiarrhythmic efficacy of Na^+/Ca^{2+} exchange inhibitors in rat ischemia/reperfusion models remain controversial.

In contrast to Na^+/Ca^{2+} exchange inhibitors, all Na^+/H^+ exchange inhibitors showed common antiarrhythmic effects on coronary ischemia/reperfusion, as shown in our previous dogs and rats experiments (Xue et al., 1996; Aye et al., 1997, 1999; Yamada et al., 1998; Sugiyama et al., 1999; Hashimoto et al., 1999; Zhu et al., 2002). Although inhibitors of both Na^+/Ca^{2+} exchanger and Na^+/H^+ exchanger showed improve intracellular Ca^{2+} overload under coronary ligation/reperfusion conditions, the presented results showed different effects on arrhythmia induced by coronary ligation/reperfusion. These results may suggest that antiarrhythmic mechanisms of Na^+/H^+ exchange inhibitors are not wholly explained by the prevention of intracellular Ca^{2+} overload. In previous reports, Schäfer et al. (2000) reported that inhibition of Na^+/H^+ exchanger protects reoxygenated cardiomyocytes independently of anoxic Ca^{2+} overload and acidosis. One possible reason is that Na^+/H^+ exchange inhibitors improve both Na^+

and Ca^{2+} overload during coronary ligation/reperfusion, though Na^+/Ca^{2+} exchange inhibitors aggravate Na^+ overload. Iwai et al. (2002) reported that intracellular Na^+ accumulation during ischemia itself induces irreversible damage to mitochondria through mechanisms such as depolarization of the mitochondrial membrane potential and a depression of the mitochondrial state 3 respiration. Recently, Ruiz-Meana et al. (2003) reported that cariporide, a Na^+/H^+ exchange inhibitor, may act at the mitochondrial level to delay mitochondrial matrix acidification and to slow the ATP depletion during ischemia, and these effects may contribute to suppress cell death secondary to ischemia–reperfusion. These results indicate that the prevention of Na^+ overload may be more important for cardioprotection than the prevention of Ca^{2+} overload. This may also explain the reason why Na^+/Ca^{2+} exchange inhibitors were ineffective against the arrhythmias induced by coronary ligation/reperfusion.

We have reported effects of various antiarrhythmic agents on tachyarrhythmias induced by digitalis, and suggested that Na^+ channel blockers suppress the tachyarrhythmias in a plasma concentration dependent manner (Hashimoto et al., 1988, 1989, 1991; Akiyama and Hashimoto, 1989). In the present study, SEA0400 suppressed tachyarrhythmias induced by digitalis and decreased the sinus rate, and its antiarrhythmic effect lasted for the entire 60 min of the observation period, probably independent of the plasma concentration changes. Although SEA0400 at 1.0 mg/kg prolonged QRS duration slightly in this study, we think that this prolongation has no physiological significance and there is no electrophysiological data to support the possibility that SEA0400 has a Na^+ channel blocking action. Thus the antiarrhythmic effects of SEA0400 on tachyarrhythmias induced by digitalis must be due to the inhibition of Na^+/Ca^{2+} exchanger. Although SEA0400 did not suppress the mortality rate in the present study (three out of eight dogs died), we think that SEA0400 is effective against tachyarrhythmias induced by digitalis, because the alterations of rhythms (arrhythmias) were observed immediately after the administration of SEA0400 in seven out of eight dogs, and the deaths of two dogs resulted from bradyarrhythmia late after the administration of SEA0400. These results are inconsistent with our previous reports on KB-R7943 (Miyamoto et al., 2002). The opposite effects of the two Na^+/Ca^{2+} exchange inhibitors on tachyarrhythmias induced by digitalis may be explained by other independent effects of the two agents on cardiac channels or transporters, but it was difficult to identify the specific causes of the ineffectiveness of KB-R7943 from our studies.

It may be worth noting that atrioventricular block and cardiac standstill were induced after SEA0400 in two cases of the digitalized dogs. These phenomena were not observed in KB-R7943 (Miyamoto et al., 2002). One of the possible mechanisms for these phenomena is the suppression of Ca^{2+} channel by SEA0400. Tanaka et al. reported that SEA0400 did not influence cardiac L-type Ca^{2+} currents at 1 μM, a concentration enough to inhibit Na^+/Ca^{2+} exchanger by more than 80% in the isolated guinea-pig ventricular myocytes (Tanaka et al., 2002). However, because the free plasma concentration of SEA0400 at the dose of 1.0 mg/kg was 1.1 μg/ml at 10 min after the administration, the free plasma concentration of SEA0400 at the dose of 3.0 mg/kg in the digitalis study was expected to be high enough to suppress Ca^{2+} channels immediately after the administration. However, it is still unclear why these phenomena were not observed for KB-R7943, which suppresses Ca^{2+} channels more potently than SEA0400 (Tanaka et al., 2002). Further experiments are required to clarify the mechanisms.

There are some limitations in this study. First, our coronary ligation/reperfusion arrhythmia model in open-chested and a pentobarbital-anesthetized dogs may differ from the pathophysiological conditions in the human. Second, the functions of Na^+/Ca^{2+} exchanger are different among species (Shigekawa and Iwamoto, 2001), and thus the results of the present study cannot be directly applied to other species. Third, in this canine model, it is difficult to compare many drugs, because the incidence of ventricular fibrillation induced by the coronary ligation/reperfusion in the control group is only about 70% on average, probably due to well developed collateral coronary circulation (Hashimoto et al., 1999), and there is a possibility of underestimating the antiarrhythmic efficacy of the drugs.

In conclusion, the selective inhibition of Na^+/Ca^{2+} exchanger by SEA0400 did not suppress the arrhythmias induced by coronary ligation/reperfusion, but obviously suppressed the tachyarrhythmias induced by digitalis in in vivo dogs. In our previous studies, while Na^+/H^+ exchange inhibitors suppressed the arrhythmias induced by coronary ligation/reperfusion, on the other hand, another Na^+/Ca^{2+} exchange inhibitor, KB-R7943, also did not suppress the arrhythmias (Miyamoto et al., 2002). On the basis of our studies, we think that antiarrhythmic efficacy of the Na^+/Ca^{2+} exchange inhibition is lower than that of the Na^+/H^+ exchange inhibition, probably because of the lack of the prevention of Na^+ overload.

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