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Antiarrhythmic effects of HOE642, a novel Na^+-H^+ exchange inhibitor, on ventricular arrhythmias in animal hearts

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Abstract

HOE642 (4-isopropyl-3-methylsulphonylbenzoyl-guanidine methanesulphonate), a novel Na^+-H^+ exchange subtype 1 inhibitor, was investigated for its possible antiarrhythmic effects on coronary artery ligation/reperfusion and ouabain-induced arrhythmias in the canine heart which may occur after intracellular Ca^{2+} overload. Also, the effects of HOE642 on coronary artery ligation/reperfusion of the left coronary artery were tested in rat hearts. HOE642 (1 mg/kg) significantly suppressed the occurrence of fatal ventricular fibrillation during coronary artery ligation and after reperfusion in dogs (2 out of 8 dogs in the treated group compared to 7 out of 8 dogs in the control group, $P < 0.05$), but did not suppress ventricular premature contractions and ventricular tachycardia during ischemia in the canine hearts. HOE642 at the same dose markedly reduced the total duration and the incidence of reperfusion-induced ventricular tachycardia, and the incidence and mortality of reperfusion-induced ventricular fibrillation in rats (ventricular tachycardia duration, 159 ± 12 s to 21 ± 8 s, $P < 0.01$; ventricular tachycardia, 100% to 69%; ventricular fibrillation, 89% to 0%, $P < 0.01$; mortality, 89% to 11%, $P < 0.01$). The heart rate, blood pressure, QT interval and ST segment did not change in the canine and rat hearts. HOE642 slightly decreased the arrhythmic ratio of the ouabain-induced arrhythmia only at two time points (28 and 35 min after injection) in the canine hearts. In conclusion, HOE642 has obvious antifibrillatory effects on ischemia/reperfusion arrhythmias and, in addition, has a weak suppressing effect on the ouabain-induced arrhythmia.

Keywords: HOE642; Na^+/H^+ exchange; Na^+/Ca^{2+} exchange; Arrhythmia ischemia; Arrhythmia reperfusion; Digitalis arrhythmia

1. Introduction

The pathophysiology of coronary ischemia and reperfusion is complex, and different mechanisms have been proposed for the ischemia-induced arrhythmias and reperfusion-induced arrhythmias (Corbalan et al., 1976), but these are still controversial. Recently, a cascade of ionic alterations has been proposed to explain the cardiac damage and arrhythmias that occur in the process of ischemia/reperfusion (Meng and Pierce, 1990). An excess increase in the intracellular Ca^{2+} concentration in myocardial cells appears to be one of the major causative factors in myocardial contractile dysfunction and necrosis during the ischemia/reperfusion process (Opie, 1989). One of the major mechanisms of Ca^{2+} overload involves the Na^+-H^+ exchange pathway. A decrease in the intracellular pH in response to ischemia (Poole-Wilson, 1978; Couper et al., 1984) promotes extrusion of H^+ in exchange for Na^+ and upon reperfusion the H^+ is washed out rapidly from the extracellular space, resulting in a sudden increase in the H^+ gradient across the sarcolemmal membrane. This will further stimulate the sarcolemmel membrane Na^+-H^+ exchange to remove H^+ from the cell in exchange for extracellular Na^+ into the cell. The increase in intracellular Na^+ leads to a rapid accumulation of intracellular Na^+ (Tosaki et al., 1989). The increase in intracellular Na^+ decreases the gradient required for Na^+-Ca^{2+} exchange, resulting in accumulation of intracellular Ca^{2+}. This may finally cause Ca^{2+} overload and promote subsequent cellular damage and cardiac arrhythmias (Duff, 1995). Likewise, toxic doses of ouabain inhibit Na^+ efflux via Na^+ pump inhibition, resulting in an increase in the concentration of Na^+ within the cell. The resulting Ca^{2+} overload via Na^+-Ca^{2+} exchange is believed to be reponsible for the occurrence of digitalis arrhythmias (Lee et al., 1985).

Therefore it seems that inhibitors of Na^+-H^+ exchange should interrupt the vicious circle that causes Ca^{2+} excess, especially in the ischemia/reperfusion condition, but not

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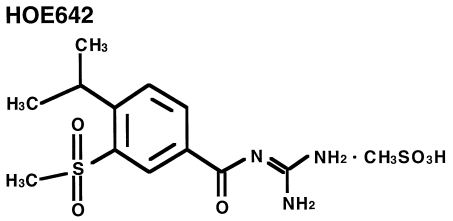

Fig. 1. Chemical structure of HOE642 (4-isopropyl-3-methylsulphonyl-benzoyl-guanidine methanesulphonate).

in digitalis toxicity, and restore normal ionic gradients on both sides of the cell, and hopefully diminish the occurrence or severity of arrhythmias induced by ischemia/reperfusion.

HOE642 (4-isopropyl-3-methylsulphonylbenzoyl-guanidine methanesulphonate) (Fig. 1) is a recently developed selective Na^+-H^+ exchange subtype 1 inhibitor. HOE642 concentration dependently inhibited the amiloride-sensitive sodium influx in rabbit erythrocytes, reduced the swelling of human platelets induced by intracellular acidification, and delayed pH recovery in rat cardiomyocytes (Scholz et al., 1995). It also dose dependently reduced the incidence and the duration of reperfusion arrhythmias in the isolated working rat heart, and reduced or prevented ischemia-induced ventricular premature contraction, ventricular tachycardia and ventricular fibrillation in anesthetized rats (Scholz et al., 1995). Since there have been no reports of HOE642 in more clinically relevant animal arrhythmia models, we examined the in vivo antiarrhythmic effects of HOE642 in dogs and rats, using arrhythmia models produced by either coronary ischemia/reperfusion, and in dogs, using ouabain-induced arrhythmia.

2. Materials and methods

The animal experiments were approved by the Yamanashi Medical University Animal Experimentation Committee and animals were obtained through the Animal Laboratory for Research of Yamanashi Medical University.

2.1. Production of coronary ligation and reperfusion arrhythmia in dogs

Sixteen Beagle dogs of either sex, weighing 7–13 kg, were anesthetized with intravenous pentobarbital sodium, 30 mg/kg, and intubated. Anesthesia was maintained by intravenous pentobarbital sodium, 5 mg/kg. As reported earlier (Hashimoto et al., 1991a; Hashimoto, 1992), the chest was opened and the left anterior descending coronary artery was isolated just proximal to the first diagonal branch of the left anterior descending coronary artery. Since the incidence of coronary ligation/reperfusion arrhythmia is known to be quite variable, experiments were randomized using a pair of beagles (by coin-flip); one received HOE642 (1 mg/kg, intravenous bolus) and the other received 5% dimethyl sulfoxide (DMSO), the solvent of HOE642. Thirty minutes after injection of HOE642 or 5% DMSO, the left anterior descending artery was ligated using a silk thread and 30 min later the ligation was released to examine reperfusion responses.

A pair of epicardial electrodes was sutured on to the border zone of the ischemic area of the left ventricle for continuous recording of the ventricular electrograms. The QT interval was assessed from the lead II electrocardiogram and the ventricular surface electrogram. The QTc interval was calculated using Bazett's formula, $QTc = QT/\sqrt{RR}$. The heart rate and the changes in the ST segment were measured from the lead II ECG and the blood pressure was continuously monitored through a double lumen arterial cannula in the femoral artery. Arterial blood samples were obtained through another lumen of the cannula at 0, 1, 3, 5, 7, 10, 15 min after the start of injection, and at 29 min (just before left anterior descending artery ligation) and 59 min (just before left anterior descending artery reperfusion).

2.2. Production of coronary reperfusion model in rats

As reported earlier (Komori et al., 1994), the male Sprague-Dawley rats (220–300 g) were anesthetized with 60 mg/kg pentobarbital sodium injected intraperitoneally. The femoral vein was cannulated for drug administration, and the trachea was cannulated for artificial respiration. Systemic arterial pressure via a catheter inserted into the carotid artery and a standard limb lead II electrocardiogram were continuously monitored on a recorder (Nihon Kohden, RM-62001, Tokyo, Japan). The chest was opened by a left thoracotomy, followed by sectioning of the 4th and 5th ribs, approximately 2 mm to the left of the sternum. Artificial respiration was started immediately with room air (volume 1.5 ml/100 g, rate 54 strokes/min) in order to maintain arterial blood gases and pH within the normal range. After incision of the pericardium, the heart was exteriorized using gentle pressure on the rib cage. A 5/0 nylon suture attached to a 14-mm micropoint reverse cutting needle was placed under the left coronary artery. The heart was replaced in the chest, and the rat was allowed to recover for 15 min. The rats that had arrhythmias and/or had a mean systemic blood pressure less than 70 mmHg were discarded.

Regional myocardial ischemia was produced by pulling the two ends of the suture through a plastic tube and pressing the tube down against the surface of the myocardium and then clamping the tube together with the suture. According to our preliminary experiment, we chose a short (5 min) ligation time (i.e., the duration of ischemia), which barely induced coronary ligation arrhythmias.

Reperfusion could be initiated by declamping and removing the tube.

HOE642 (1 mg/kg) and 5% DMSO were given 5 min before the coronary ligation. The heart rate and QT interval were measured from electrocardiogram lead II just before the drug or 5% DMSO injection (0 min) and 1, 2, 3, 4, 5 min after the start of drug or 5% DMSO injection. The QT interval was measured at the point of 90% repolarization and is referred to as QT90. Following the Lambeth Conventions (Walker et al., 1988), ventricular tachycardia was defined as a run of four or more ventricular premature beats, and ventricular fibrillation was defined as an ECG signal where individual QRS deflections could no longer be distinguished from one another. The total duration of ventricular tachycardia was defined as the sum of the ventricular tachycardia time occurring after reperfusion and expressed in seconds. Because ventricular fibrillation is not necessarily a terminal event in this rat model, we defined ventricular fibrillation which lasted more than 3 min as a fatal one.

2.3. Production of digitalis-induced arrhythmia in dogs

Six beagle dogs of either sex, weighing 8–11 kg, were anesthetized with intravenous pentobarbital sodium, 30 mg/kg. As reported earlier (Hashimoto et al., 1985), 40 μg/kg ouabain was injected intravenously and then followed by an additional 10 μg/kg every 20 min until stable ventricular tachycardia was produced. In the absence of drug administration, the resulting arrhythmia remained stable for more than 60 min, as reported previously (Awaji et al., 1995). HOE642 was administered as an intravenous bolus at a dose of 1 mg/kg. Arterial blood samples were drawn from one lumen of the arterial double lumen cannula at 0, 1, 3, 5, 7, 10, 15, 30 and 60 min after the injection.

The lead II ECG, atrial electrogram from catheter tip electrodes in the right atrium, and the instantaneous and mean blood pressure were continuously recorded.

2.4. Determination of HOE642 plasma levels

The arterial blood samples were collected into heparinized syringes at predetermined times and centrifuged at $3000 \times g$ for 5 min. The plasma was stored at about -80°C until the assay. Plasma samples were analysed at the Research Laboratory of Hoechst (Frankfurt, Germany) by reversed phase HPLC after solid phase extraction on a cation exchanger. In brief, S90 2604 was added as an internal standard, giving a final concentration of 1 μg/ml. Plasma samples were subsequently subjected to solid phase extraction on Varian Bond Elut CBA (100 mg columns) at pH 5.5. Elution was accomplished with 1% TFA in 50% MeOH. For reversed phase analysis the eluates were diluted with one volume of water and injected on to a Lichrospher 60RP-select B (Merck EcoCART 125-3, Merck, Darmstadt, Germany) cartridge. Separation was achieved with a gradient of 15–45% B against (A + B) over 20 min at a flow rate of 0.6 ml/min, A being 5 mM heptanesulfonic acid in 10% acetonitrile and B being 5 mM heptanesulfonic acid in 80% acetonitrile.

2.5. Evaluation of antiarrhythmic effects

The severity of digitalis-induced ventricular arrhythmia was expressed by the arrhythmic ratio: the number of ventricular ectopic beats divided by the total heart rate, which is the number of all beats counted from the 5-s strip of the electrocardiogram (i.e., the number of ventricular ectopic beats plus the number of conducted beats), and the ventricular beats were judged by the different shape of the ventricular complex from the normal QRS complex. The arrhythmic ratio before drug injection was almost 1, as shown in the control values of the figures, and there was no spontaneous improvement in this ratio.

2.6. Drugs

The drugs used in the present study were HOE642 (Hoechst Japan, Tokyo, Japan), dimethyl sulfoxide (DMSO, Wako, Osaka, Japan), pentobarbital sodium (Tokyo Kasei Kogyo, Tokyo, Japan) and ouabain octahydrate (Aldrich, Milwaukee, WI, USA).

2.7. Statistical analysis

Analysis of variance (ANOVA) was performed to evaluate drug-induced changes in the blood pressure, heart rate and electrocardiogram parameters. When a statistical difference was detected, Dunnett's multiple comparison test was used to determine the difference between the 0 time value and the other values. Differences in the incidence of arrhythmias between HOE642 groups and 5% DMSO groups were analysed with Fisher's exact probability test. All data are expressed as means ± S.E.M. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Coronary ligation and reperfusion arrhythmia in dogs

In the 8 DMSO control beagles, the average heart rate, mean blood pressure and QTc interval at the start of injection were 161 beats/min, 119 mmHg and 0.36 $s^{1/2}$, respectively. These parameters barely changed during the whole experimental period. The average number of ventricular premature contractions including beats occurring as ventricular tachycardia was 183 beats/30 min of coronary ligation ($n = 8$) (Table 1). However, 3 out of 8 dogs fibrillated during the 30 min of ischemia, and a further 4 out of 5 dogs who survived myocardical ischemia fibrillated immediately after reperfusion.

Table 1
Effects of HOE642 on coronary ligation and reperfusion arrhythmias in dogs

	5% DMSO ($n=8$)	HOE642 ($n=8$)
Before treatment		
HR (beats/min)	161 ± 5	167 ± 9
QTc ($s^{1/2}$)	0.36 ± 0.01	0.37 ± 0.01
mBP (mmHg)	119 ± 7	121 ± 7
30 min after treatment		
HR (beats/min)	156 ± 5	165 ± 11
QTc ($s^{1/2}$)	0.36 ± 0.01	0.37 ± 0.01
mBP (mmHg)	119 ± 9	117 ± 6
VPC (during 30 min ligation)	183 ± 91	147 ± 61
VF (during ischemia/reperfusion)	7/8	2/8 [a]

VPCs: ventricular premature contractions; VF: ventricular fibrillation.
[a] $P < 0.05$ when compared with 5% DMSO group.

In the HOE642-treated group ($n = 8$), the heart rate, mean blood pressure and QTc interval between the start of injection and just before coronary ligation and reperfusion also did not change, as in the control group. The average number of total ventricular premature contractions was 147 beats/30 min of coronary ligation (Table 1). The difference in the number of total ventricular premature contractions between the control and drug-treated groups was not significant. HOE642 had a significant antiarrhythmic effect on the fatal ventricular fibrillation during coronary ligation/after reperfusion (2 out of 8 dogs in the treated group as compared to 7 out of 8 dogs in the control group). HOE642 tended to improve the ST segment changes during ischemia, which did not reach a significant level. The plasma concentrations at 0, 1, 3, 5, 7, 10, 15, 29 and 59 min after HOE642 administration were 0, 2634 ± 280, 1158 ± 117, 802 ± 20, 713 ± 18, 620 ± 14, 522 ± 12, 397 ± 9 and 270 ± 17 ng/ml ($n = 7$).

3.2. Coronary reperfusion arrhythmia in rats

In the 5% DMSO control group, the total duration of reperfusion-induced ventricular tachycardia was 159 ± 12 s (Fig. 2, $n = 9$), but HOE642 significantly reduced the duration of ventricular tachycardia to 21 ± 8 s ($P < 0.01$). The incidence of reperfusion-induced ventricular tachycardia, ventricular fibrillation and mortality in the control group was high (ventricular tachycardia 100%, ventricular fibrillation 89% and mortality 89%, Fig. 3), but HOE642 markedly decreased the incidence of ventricular tachycardia to 69%, ventricular fibrillation to 0% ($P < 0.01$) and mortality to 11% ($P < 0.01$). One rat showed atrioventricular block. HOE642 did not change the heart rate, blood pressure or QT90 during ischemia (Table 2).

3.3. Digitalis-induced arrhythmia in dogs

After a total intravenous injection of about 60–70 μg/kg ouabain, almost all the beats were of ventricular

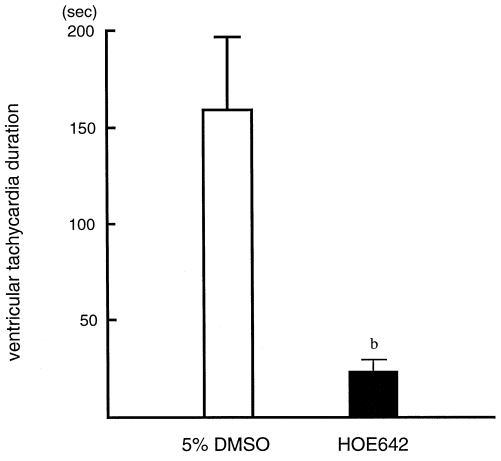

Fig. 2. Effects of intravenous injection of HOE642, 1 mg/kg, on the total duration of ventricular tachycardia after reperfusion in rat hearts. Results are the means ± S.E.M for 9 rats. [b] $P < 0.01$, statistically significant difference from the 5% DMSO-treated control group.

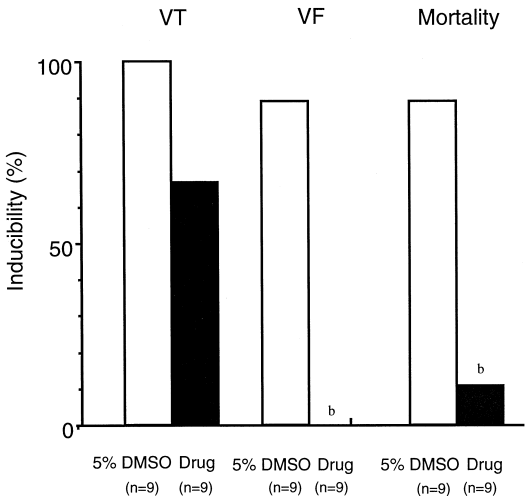

Fig. 3. Effects of intravenous injection of HOE642, 1 mg/kg, on the inducibility of VT, VF and mortality after reperfusion in rat hearts. [b] $P < 0.01$, statistically significant difference from the 5% DMSO-treated control group.

Table 2
Effects of HOE642 on the hemodynamic indexes of rat hearts

	5% DMSO ($n=9$)	HOE642 ($n=9$)
Before treatment		
HR (beats/min)	449 ± 12	469 ± 9
QT90 (ms)	45 ± 4	51 ± 4
SBP (mmHg)	106 ± 10	117 ± 6
DBP (mmHg)	78 ± 8	99 ± 6
5 min after treatment		
HR (beats/min)	449 ± 12	477 ± 10
QT90 (ms)	43 ± 2	53 ± 3
SBP (mmHg)	96 ± 8	85 ± 9
DBP (mmHg)	62 ± 7	73 ± 7

HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure.

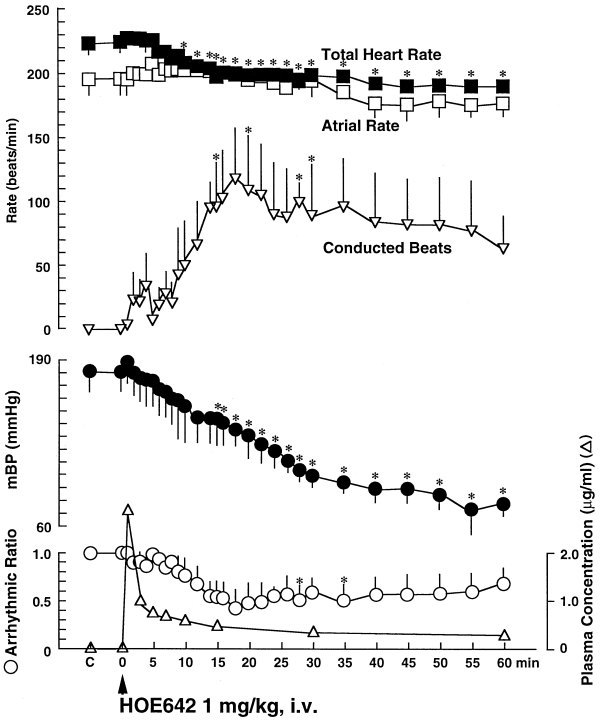

Fig. 4. Effects of intravenous injection of HOE642, 1 mg/kg, on ouabain-induced arrhythmia in dogs. * $P < 0.05$, compared with the value at 0 time. S.E.M are shown at each time point. (■) Total heart rate; (□) atrial rate; (▽) conducted beats; (●) mean blood pressure; (○) arrhythmic ratio; (△) plasma concentration of HOE642.

origin, namely the arrhythmic ratio was 1.00 ± 0.00 ($n = 6$). The same dose of HOE642 as that used in the coronary ligation and reperfusion experiments was used. As shown in Fig. 4, HOE642 significantly decreased the total heart rate. No effect was observed on the atrial rate. The mean blood pressure after 15 min was significantly decreased. The arrhythmic ratio gradually decreased after the injection, but a significant decrease of the arrhythmic ratio was observed only at 28 and 35 min after injection. The significant decrease of the mean blood pressure after 15 min was probably due to the disappearance of the ouabain effect. The plasma concentrations of HOE642 decreased double exponentially, fitting the two-compartment model, as shown in Fig. 4.

4. Discussion

In the present study we demonstrated in dogs that HOE642, a novel Na^+-H^+ exchange inhibitor, is effective in suppressing the occurrence of fatal ventricular fibrillation during coronary ligation and after reperfusion, without changing the heart rate and blood pressure. However, it was not effective in decreasing the number of ventricular premature contractions and did not significantly improve the changes in the ST segment during ischemia. Also, in the reperfusion arrhythmia model with rats, HOE642 significantly suppressed the total duration of ventricular tachycardia, the incidence of ventricular fibrillation and mortality. These results suggest that HOE642 has a marked antiarrhythmic effect during cardiac ischemia/reperfusion, and thus the Na^+-H^+ exchange pathway may be involved in the cardiac ischemia/reperfusion arrhythmias.

A recent paper reported antiarrhythmic effects of HOE642 in rats, showing a dose-dependent reduction of ventricular premature contractions and in the incidence of ventricular tachycardia and ventricular fibrillation during 30 min of coronary artery ligation, whereas such effects were not observed with nifedipine (Scholz et al., 1995). Different from this paper, our present results for dogs showed that HOE642 was not effective in decreasing the numbers of ventricular premature contractions during ischemia, but instead showed that HOE642 had antifibrillatory effects, irrespective of whether ventricular fibrillation occurred during ischemia or just after reperfusion. This antifibrillatory effect deserves some comment, because the mechanisms for ischemia and reperfusion arrhythmias are known to be different. Corbalan et al. reported that an adrenergic mechanism plays a key role in ischemia-induced arrhythmias, whereas reperfusion-induced arrhythmias may be due to the washout of products of cellular ischemia (Corbalan et al., 1976). However, Ca^{2+} overload may be the final common pathway leading to ischemia/reperfusion arrhythmias that may be partly related to induction of delayed afterdepolarization (Ferrier et al., 1985). The different results for ischemia in dogs and rats are hard to explain. It is easy to speculate that complex electrophysiologic changes occur during ischemia/reperfusion associated with ionic, metabolic and energy state changes produced by cellular K^+ loss, opening of ATP-sensitive K^+ channels, excessively generated H^+ and intracellular increases in Na^+, Ca^{2+}, and free radicals. However, there is a lack of results to explain the different time course, magnitude, and mechanisms of arrhythmias during ischemia/reperfusion in dogs and rats. There are studies on the protective effect of inhibiting Na^+-H^+ exchange on cardiac performance during ischemia (Meng and Pierce, 1990), and also amiloride, a prototype Na^+-H^+ exchange inhibitor, was reported to enhance postischemic recovery of ventricular function in the isolated rat heart (Karmazyn, 1988). As for arrhythmias, amiloride has been reported to suppress reperfusion ventricular arrhythmias in isolated rat hearts (Meng and Pierce, 1991), and to inhibit triggered arrhythmias induced in isolated canine Purkinje fibers during simulated ischemia/reperfusion (Moffat, 1989). These studies suggest that amiloride produces antiarrhythmic effects mainly though inhibition of the Na^+-H^+ exchange mechanism.

Concerning antiarrhythmic drug therapy, the CAST study emphasized the limitations of class I antiarrhythmic drugs (Echt et al., 1991), and new K^+ channel blocking class III drugs have emerged, some of which have been

proven to be effective in the canine ventricular arrhythmia models (Hashimoto et al., 1991a,1995; Hashimoto, 1992), but class III drugs also have proarrhythmic effects (Awaji et al., 1995; Xue et al., 1996; Hashimoto et al., 1996). So novel and different approaches from blocking K^+ or other ionic channels are required for antiarrhythmic therapy. A Na^+-H^+ exchange inhibitor, amiloride, has been reported to have antiarrhythmic effects in a limited number of in vivo and clinical studies. Amiloride suppressed the incidence of ventricular tachycardia in approximately 50% of dogs with myocardial infarction (Duff et al., 1988). Amiloride suppressed ventricular tachycardia in 6 of 31 patients who showed symptomatic and inducible ventricular tachycardia (Duff et al., 1989). Another clinical study reported that amiloride can suppress spontaneous ventricular premature contractions (Myers, 1990; Holland et al., 1988). Although amiloride is not a selective Na^+-H^+ exchange inhibitor and simultaneously blocks Na^+-Ca^{2+} exchange, a recent study by Karmazyn et al. with congeners of amiloride (Karmazyn et al., 1990), which produce more specific blockade of the Na^+-H^+ exchange, confirmed that blockade of this exchange is the likely mechanism for myocardial protection against ischemia/reperfusion arrhythmias.

Thus the antiarrhythmic effect or protective effect of HOE642 on ischemia/reperfusion may be primarily due to an inhibition of Na^+-H^+ exchange. It has been reported that HOE642 is a very selective Na^+-H^+ exchange subtype 1 inhibitor which has almost no influence on other ion transport systems compared with the less selective inhibitors such as amiloride (Scholz et al., 1995). HOE642 actually had no effect on QTc, unlike class III drugs. The mechanism of ischemia/reperfusion damage may involve enhanced Na^+-H^+ exchange, which leads to intracellular Na^+ overload and concomitant intracellular Ca^{2+} overload (Murphy et al., 1988). An increase in intracellular Ca^{2+} can generate arrhythmias by activating the transient inward current, I_{ti}, or by causing electrical uncoupling of cardiac myocytes (Benndorf et al., 1991). The favorable properties of Na^+-H^+ exchange inhibitors in cardiac ischemia/reperfusion are probably involved in the reduction of Na^+ and Ca^{2+} overload (Anderson et al., 1990; Pike et al., 1993). The present observation that HOE642 was effective against ventricular fibrillation during ischemia and after reperfusion is consistent with the above hypothesis. The presence of Na^+-H^+ exchange activity was identified in purified canine cardiac sarcolemmal vesicles (Seiler et al., 1985). The range of plasma concentrations of HOE642 attained in the present canine cardiac ischemia/reperfusion experiment was between 6.9 and 0.7 μM, and under this condition the concentration of HOE642 would be enough to inhibit Na^+-H^+ exchange, because the IC_{50} of HOE642 for suppressing Na^+-H^+ exchange is between 0.1 and 1 μM in several tissues, e.g., rabbit erythrocytes, human platelets, and rat cardiac myocytes (Scholz et al., 1995).

With regard to ouabain-induced arrhythmias, HOE642 decreased the total heart rate and increased the number of conducted beats, and gradually decreased the arrhythmic ratio, but the significant decrease in the arrhythmic ratio was only seen at two time points (28 and 35 min) after injection. The significant decrease in blood pressure 15 min after the administration of HOE642 was probably due to the decrease in the plasma ouabain concentration because ouabain was not given after the administration of test drugs. In addition, it is clear that there was no linear relationship between the plasma concentration of intravenous HOE642 and the decrease in the arrhythmic ratio. We have reported that Na^+ channel blocking drugs are quite effective against digitalis-induced arrhythmia in dogs and easily eliminate ventricular premature contractions (Hashimoto et al., 1991b; Wu et al., 1993), but in the case of HOE642 it has been reported that it has no Na^+ channel blocking effect. Ca^{2+} overload induced by ouabain toxicity is thought to be due to intracellular Na^+ accumulation caused by a Na^+ pump inhibition, which results in Ca^{2+} overload via Na^+-Ca^{2+} exchange (Eisner et al., 1984; Frelin et al., 1984; Kass et al., 1978). Since the Na^+-H^+ exchange system is not involved in the mechanism of this type of Ca^{2+} overload, then it is natural to speculate that Na^+-H^+ exchange inhibitors (e.g., DMA) cannot directly antagonize ouabain toxicity (Lazdunski et al., 1985). Nevertheless, the present results showed that HOE642 has beneficial effects on ouabain-induced arrhythmias. In addition, amiloride has been reported to have beneficial effects on the heart as an adjunct to digitalis therapy in humans (Greeff and Kohler, 1975; Jounella and Pyorala, 1975; Waldorff et al., 1981).

In conclusion, the present experiments, for the first time, show that HOE642 protected against severe ischemia/reperfusion-induced ventricular fibrillation in the canine heart, and significantly suppressed the reperfusion-induced total duration of ventricular tachycardia, ventricular fibrillation and mortality in rats. Since HOE642 is a selective Na^+-H^+ exchange subtype 1 inhibitor and has no significant cardiovascular, antiischemic and electrophysiological effects, as judged by the ST segment changes and other ECG intervals, it is possible that Na^+-H^+ exchange plays an important role in modulating the cardiac response to ischemia/reperfusion. Since in vivo canine and rat ischemia/reperfusion arrhythmia models mimic clinical myocardial infarction or intracoronary thrombolysis or angioplasty situations, it is expected that Na^+-H^+ exchange inhibitors may become effective antiarrhythmic drugs.

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