



## Original Article

# Hydralazine inhibits ventricular tachyarrhythmias in an acquired long QT rabbit model

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## ABSTRACT

**Background:** Some cardioactive vasodilating agents inhibit ventricular tachyarrhythmias (VT) associated with acquired long QT syndrome (LQT). We tested whether a vasodilator without direct cardiac effect can eliminate abnormal repolarization-related VT.

**Methods:** The effect of hydralazine on the occurrence of VT was assessed in a methoxamine-sensitized rabbit model of acquired LQT. To verify that VTs in this animal model are triggered by early afterdepolarization (EAD), monophasic action potential (MAP) on the left ventricular surface was recorded in open-chest rabbits. **Results:** In control rabbits, combined administration of methoxamine and nifekalant frequently induced VTs (16/20, 80%). In contrast, VT occurred only in 2 out of 14 rabbits treated with hydralazine (14.3%,  $P < 0.0001$  vs. control). After the treatment, blood pressure was lower in the hydralazine group than in the control group (systolic pressure,  $146 \pm 19$  vs.  $165 \pm 16$  mmHg,  $P < 0.0001$ ; diastolic pressure,  $54 \pm 10$  vs.  $101 \pm 11$  mmHg,  $P < 0.0001$ ). EAD-like hump was less frequently detected in hydralazine-treated rabbits (2/10) than in saline-treated rabbits (9/10,  $P < 0.005$ ). Presence of a hump was significantly related to the appearance of VTs ( $P < 0.05$ ).

**Conclusion:** Hydralazine inhibited VT in a rabbit LQT model. Vasodilation may have a therapeutic effect on abnormal repolarization-related VT.

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## 1. Introduction

Abnormal repolarization facilitates the advent of ventricular tachyarrhythmias (VT). Bradycardia, ischemia, hypokalemia, and some cardiotoxic drugs increase the cardiac vulnerability to abnormal repolarization-related VT. Vasodilating agents such as carperitide [1] and clonidine [2] inhibit VT in an experimental model of acquired long QT syndrome (LQT). However, most vasodilating agents concomitantly have a certain cardioactive effect to some extent. In an earlier study, physiologically conceivable fluctuation of volume load did not markedly influence the electrophysiological properties or arrhythmogenicity of the canine ventricle [3]. Thus, it still seems disputable if vasodilation can have an appreciable impact on VT associated with acquired LQT.

In the present study, we tested whether hydralazine, a “direct acting vasodilator” with little action on the myocardium, could decrease the incidence of VT in an established rabbit LQT model but with attenuated arrhythmogenic potential. The goal of this study was to validate the view that vasodilatation itself has a therapeutic implication for acquired LQT-related VT.

## 2. Methods

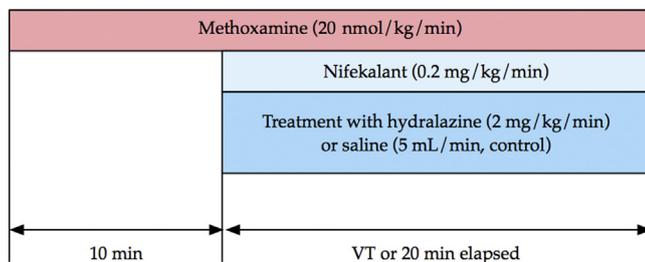
### 2.1. Animal preparation

This experiment was approved by the local institutional review board on November 1, 2007 (approval number: 07-012). All procedures were carried out in accordance with the guidelines by the Committee of Animal Care and Experiments of Teikyo University.

Fifty-eight Japanese white rabbits (2.4–2.7 kg) were anesthetized with intravenous isozol (62.5 mg/body). Additional doses were given if necessary to maintain an appropriate level of anesthesia. Rabbits were ventilated with room air through an artificial respirator (model 6025, Ugo, Basile, Italy) via a tracheal cannula. Arterial blood pressure was monitored using a right femoral artery cannula attached to a Statham pressure transducer (Amplifier AP621G, Nihon Koden, Tokyo, Japan). Body temperature was maintained at about 37 °C with an electrical blanket. Arterial blood gases and electrolytes were measured with a portable clinical analyzer (i-STAT 200A, i-STAT Corporation, Princeton, NJ, USA). Tidal volume and respiratory rate were adjusted to maintain arterial blood gases and pH within physiological ranges. Two surface electrocardiograms, lead I and II, were continuously monitored, and the data were stored in a personal computer

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**Fig. 1.** Experimental protocols for each group. In all rabbits, methoxamine and nifekalant were given to induce ventricular tachyarrhythmias (VTs). After a 10-min stabilization period, treatment using saline or hydralazine was started simultaneously with the administration of nifekalant until VT or until 20 min elapsed.

together with that for arterial blood pressure for subsequent analysis (PowerLab 8-channel System, ADInstruments Pty Ltd, Sydney, NSW, Australia).

## 2.2. Experimental protocol

### 2.2.1. Study 1: effect of hydralazine on VT in closed-chest rabbits ( $n=34$ )

Referring to an *in vivo* animal model of torsade de pointes established by Carlsson et al. [4], we used 0.2 mg/kg/min of nifekalant chloride concomitantly with methoxamine, an  $\alpha$ -1 stimulant, at a rate of 70 nmol/kg/min. This combination of the agents almost consistently induced VT in the baseline state, namely, in 14 out of 15 rabbits. VT developed in 10 out of 15 rabbits treated with hydralazine [1]. Thus, hydralazine did not fully eliminate VT. Taking these observations into consideration, we assumed that attenuation of arrhythmogenic potential of the model might be essential to detect the antiarrhythmic effect of hydralazine. Consequently, we adopted slower infusion rate of methoxamine.

The protocol of this part is schematically shown in Fig. 1. After a 10-min period of stabilization, methoxamine was intravenously administered at 20 nmol/kg/min. A 10-min infusion of methoxamine was followed by nifekalant chloride (0.2 mg/kg/min). Then, intravenous infusion of either hydralazine at 2 mg/kg/min or 0.5 mL/min of saline solution (control group) was introduced simultaneously with the administration of nifekalant. Fourteen rabbits were assigned to the hydralazine group. As a control, 20 rabbits were given 0.5 mL/min of saline solution. We compared the incidence of VT between the 2 groups. Systolic and diastolic blood pressure (SBP and DBP), heart rate (HR), number of premature ventricular contractions (PVCs) and QTc intervals were recorded. For the rabbits that developed VT, time to VT was measured.

### 2.2.2. Study 2: recording of monophasic action potential (MAP) in open-chest rabbits ( $n=20$ )

This part was performed to assess whether ventricular arrhythmias in this model were actually caused by early afterdepolarization (EAD).

MAP was recorded in 20 open-chest rabbits. The heart was exposed through a midsternal incision and suspended in a pericardial cradle. MAP was recorded on the left ventricular surface using a contact electrode with an interelectrode distance of 3 mm. MAP signals were amplified with a frequency range of 0.1–10 kHz (AP-621G; Nihon Kohden, Tokyo, Japan), and were performed in the baseline state and during treatment with hydralazine ( $n=10$ ) or saline ( $n=10$ ) at the same dose as in Study 1. MAP was accepted when the amplitude was stable and  $> 10$  mV.

## 2.3. Definition and measurement

VT was defined as an episode of at least 6 coupled ventricular complexes. Heart rates and QT intervals were obtained from the measurements of 3 consecutive RR intervals or those of 3 beats in

either lead with more prominent T waves, respectively. The QT interval was corrected using the Bazett formula (QTc). The measurements of variables in the baseline state were made prior to the administration of methoxamine. Measurements during the treatment with each agent were performed 5 min from the onset of administration of nifekalant.

Frequent premature beats was defined as more than 10 PVCs occurring in a minute.

## 2.4. Statistical analysis

All continuous data are expressed as mean  $\pm$  SD. Comparisons of variables among the groups were performed using 2-way analysis of variance. In the presence of a significant  $P$  value, further comparison between each pair of variables was performed using the Bonferroni method. Intergroup difference in the incidence of VT in Study 1 and the association between EAD-like hump and the appearance of VT in Study 2 were tested using Fisher's exact test. Probability values  $< 0.05$  were considered to indicate significance.

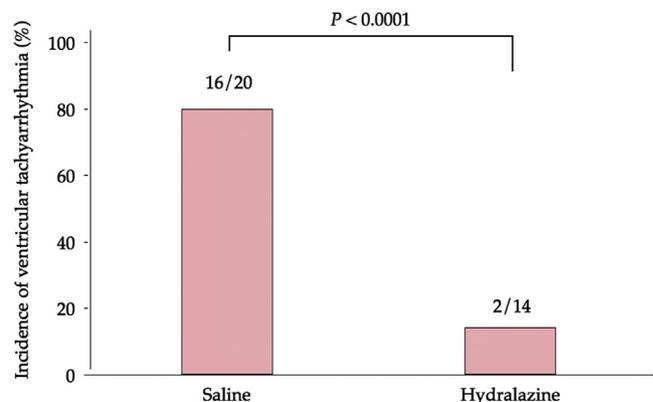
## 3. Results

### 3.1. Part 1: incidence of VT in rabbits with or without hydralazine, changes in hemodynamic and electrocardiographic variables, and time to develop VT

VT appeared in 16 out of 20 control rabbits. In rabbits treated with hydralazine, incidence of VT was significantly lower than that in the control group (2/14,  $P < 0.0001$ , Fig. 2).

Hemodynamic parameters and QTc before and after the treatment are shown in Table 1. SBP, DBP, and HR in the 2 groups were similar at baseline. QTc intervals during saline infusion were not consistently available because of frequent PVCs and VTs. After the treatment, SBP was elevated and HR slowed down in all rabbits, whereas DBP in the control group was not altered; hydralazine even decreased DBP after the treatment. Compared with saline, hydralazine was associated with less conspicuous changes ( $P < 0.0001$  for both SBP and HR). Among the rabbits that developed VT, time to VT in the control group was longer than that in the hydralazine group ( $10.9 \pm 5.7$ ,  $n=12$  vs.  $7.3 \pm 3.3$ ,  $n=4$ ,  $P < 0.05$ ).

Hydralazine was associated with lower incidence of frequent premature beats (2/14 vs. 14/20,  $P < 0.005$ ) as compared with that of the control group.



**Fig. 2.** Comparison of the incidence of ventricular tachyarrhythmia (VT). In control rabbits treated with saline, VT was almost consistently induced by the administration of methoxamine and nifekalant. Hydralazine reduced the incidence of VT.

**Table 1**  
Results in Study 1.

Treatment	Control (n=20)	Hydralazine (n=14)	P value
VT	16/20	2/14	< 0.0001
SBP-Pre (mmHg)	133 ± 17	135 ± 19	n.s.
SBP-Post (mmHg)	165 ± 16	146 ± 19	< 0.0001
DBP-Pre (mmHg)	100 ± 9	94 ± 9	n.s.
DBP-Post (mmHg)	101 ± 11	54 ± 10	< 0.0001
HR-Pre (bpm)	292 ± 35	327 ± 49	n.s.
HR-Post (bpm)	221 ± 39	285 ± 55	< 0.0001
QTc-Pre	225 ± 56	240 ± 40	n.s.
QTc-Post	N.A.	452 ± 80	

All values except VT are presented as mean ± SD. VT=ventricular tachyarrhythmia, SBP=systolic blood pressure, DBP=diastolic blood pressure, HR=heart rate, n.s.=not significant, and N.A.=not available.

**Table 2**  
Incidence of EAD-like humps in MAP recordings and VT.

	Hump(+)/VT (+)	Hump(+)/VT (-)	Hump(-)/VT (+)	Hump(-)/VT (-)
Saline (n=10)	9	0	0	1
Hydralazine (n=10)	0	2	2	6
Total no.	9	2	2	7

EAD=early afterdepolarization, MAP=monophasic action potential, and VT=ventricular tachyarrhythmia.

than in saline-treated rabbits (9/10,  $P < 0.005$ ). Presence of a hump was significantly related to the advent of VTs ( $P < 0.05$ , Table 2).

#### 4. Discussion

The major findings of the present study were as follows: (1) hydralazine decreased the incidence of VT in a rabbit acquired LQT model and (2) EAD-like hump was associated with the appearance of VTs.

Because of its little cardiac effect, hydralazine is known as a “direct vasodilator.” The main action of hydralazine is to inhibit the IP<sub>3</sub>-induced release of Ca<sup>2+</sup> from the sarcoplasmic reticulum in vascular smooth muscle cells [5]. The present results demonstrated that vasodilation has a therapeutic implication for abnormal repolarization-related VT.

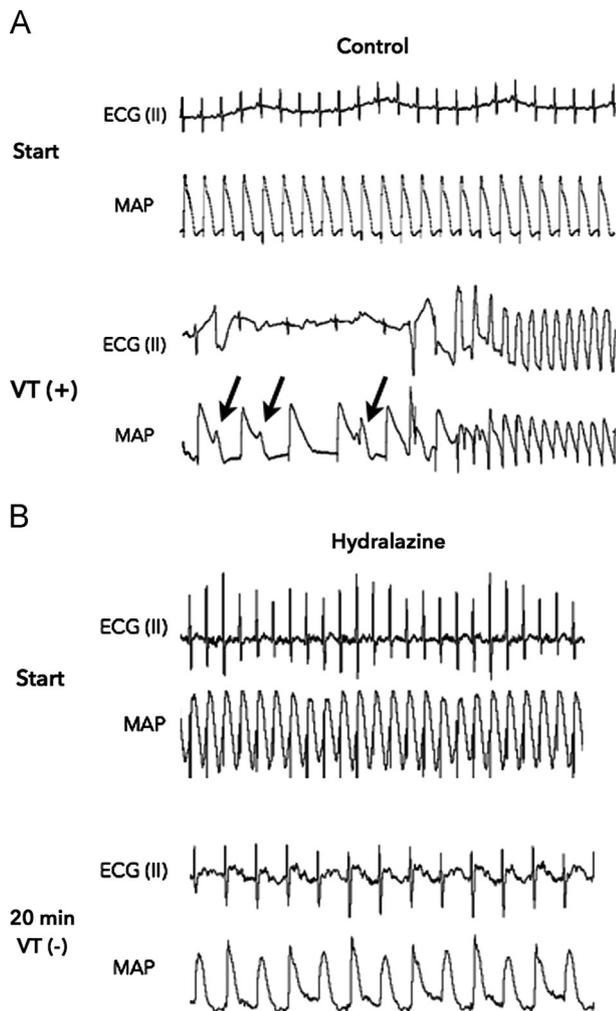
We observed that hydralazine decreased the incidence of EAD-like hump in the MAP study, and that the vasodilator also suppressed the frequency of PVCs. These findings indicated that the anti-VT effect of hydralazine should be associated with modification in abnormal repolarization. Because the direct vasodilator has minimal electrophysiological effect, this alteration is to be derived from mechanical unloading and/or hemodynamic reaction.

Mechanical stretch is one of the modulating factors of myocardial electrophysiological property, i.e., “mechano-electrical feedback,” and presumably its arrhythmogenicity. Ventricular loading causes the following: (1) reduction of action potential duration and refractoriness, (2) development of EAD, and (3) ectopic beats originating from the afterdepolarization [6,7].

Calkins et al. demonstrated that volume loading has a greater electrophysiological significance under pathologic conditions such as chronic infarction [8]. Some vasodilators can counteract such electrophysiological changes and suppresses VT. Captopril prolonged the ventricular refractory period in patients with ventricular dysfunction and inducible VT [9]. hANP and hydralazine hampered VT in a cesium-induced rabbit model [10]. Our observation in the present study is in good agreement with this earlier finding in that modification of pressure and/or volume load attenuates the ventricular arrhythmogenicity.

Prazosin (an alpha-1 blocker), clonidine (an alpha-2 agonist), and hANP reduced VTs in a similar rabbit LQT model. Pinacidil, a potassium channel opener, also decreased both arterial pressure and the incidence of VT. All these pharmacological interventions share direct cardiac effects. Therefore, their favorable effect on abnormal repolarization-related VT may be attributable to direct cardio-active action, and is not explained solely by indirect action via attenuated pressure and/or volume load.

Hydralazine has a positive inotropic effect on isolated mammalian myocardium [11]. Additionally, it increases HR and cardiac output, and enhances cardiac conduction in patients with cardiovascular diseases [11]. The cardiac effects are considered to be due to resultant sympathetic drive to the heart, because beta blockers abolished these phenomena [12]. Relatively higher HR during



**Fig. 3.** (A) and (B) Illustrative electrocardiograms (ECGs) and epicardial monophasic action potential (MAP). (A) In the saline group, early afterdepolarization (EAD)-like humps, as indicated by arrows, were observed on MAP recordings during the administration of methoxamine and nifekalant. Then a ventricular ectopic beat triggered ventricular tachyarrhythmia (VT). (B) In contrast, both EAD-like humps and VTs were abolished in most of the hydralazine-treated rabbits.

#### 3.2. Part 2: EAD-like hump on MAP recordings

Fig. 3A shows demonstrable electrocardiogram (ECG) and MAP recordings from one of the control rabbits. Those of a rabbit treated with hydralazine are shown in Fig. 3B. EAD-like hump was less frequently detected in hydralazine-treated rabbits (2/10)

hydralazine infusion may be relevant to, if partly, its antiarrhythmic action in this rabbit model because bradycardia is one of the exacerbating factors of acquired LQT.

Patients with advanced heart failure, who naturally suffer from continuous cardiac loading, are often confronted with life-threatening VT. The use of a vasodilator is a primary choice for the treatment of heart failure. The present study supports the view that vasodilation itself is therapeutic for a certain type of VT. Clinically, in some patients, volume and/or pressure load enhance the risk of serious ventricular arrhythmias. If conventional antiarrhythmic treatment fails to decrease life-threatening VTs, reconsideration of the hemodynamic condition may be desirable.

## 5. Limitations

Spatial and temporal dispersion of repolarization are considered as determinants of initiation and maintenance of VT. We do not know whether site-to-site heterogeneity of repolarization has a relevance to VT in the model because MAP was recorded only from a single epicardial surface.

Extrapolation of the finding in an animal LQT model to a clinical setting is inevitably limited. Clinically, it is essential to ameliorate triggering conditions of LQT such as hypokalemia and bradycardia. It is not clear how the favorable effect of hydralazine is related with autonomic and humoral changes.

Because the presence or absence of EAD-like hump was subjectively judged, the result of Study 2 was not free from bias. We do not deny that some other mechanism may have been involved in the genesis of arrhythmias in the model.

The faster HR produced by hydralazine has a possible therapeutic role in the VTs in this model. Bradycardia prolongs APD and is known as one of the risk factors for Torsade de Pointes. However, sustained sympathetic tone is not always therapeutic on such VTs because it also deteriorates intracellular  $Ca^{2+}$  handling. In the present study, hydralazine also suppressed EAD-like hump and PVCs, suggesting that the vasodilation was supposed to produce electrophysiological changes in the cardiomyocyte. Therefore, accelerating HR is not the sole reason why hydralazine provided an anti-VT effect. In other words, hydralazine may have additional therapeutic effects beyond the resultant tachycardia.

## 6. Conclusions

Hydralazine, which is considered to have minimal direct electrophysiological influence on the ventricular myocardium, significantly inhibited VT. Although it is not clear how hemodynamic

alterations produced by hydralazine affect the electrophysiological properties of the myocardium, this result was consistent with the view that vasodilation has a therapeutic effect on VT in acquired LQT.

## 7. Conflict of interest

None.

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## References

- [1] Inaba H, Hayami N, Ajiki K, et al. Human atrial natriuretic peptide suppresses torsades de pointes in rabbits. *Circ J* 2008;72:820–4.
- [2] Tsutsui K, Hayami N, Kunishima T, et al. Dexmedetomidine and clonidine inhibit ventricular tachyarrhythmias in a rabbit model of acquired long QT syndrome. *Circ J* 2012;76:2343–7.
- [3] Calkins H, Maughan WL, Kass DA, et al. Electrophysiological effect of volume load in isolated canine hearts. *Am J Physiol* 1989;256:1697–706.
- [4] Carlsson L, Abrahamsson C, Drews L, et al. Antiarrhythmic effects of potassium channel openers in rhythm abnormalities related to delayed repolarization. *Circulation* 1992;85:1491–500.
- [5] Eilershaw DC, Gurney AM. Mechanisms of hydralazine induced vasodilation in rabbit aorta and pulmonary artery. *Br J Pharmacol* 2001;134:621–31.
- [6] Eckardt L, Kirchhof P, Breithardt G, et al. Load-induced changes in repolarization: evidence from experimental and clinical data. *Basic Res Cardiol* 2001;96:369–80.
- [7] Greve G, Lab MJ, Chen R, et al. Right ventricular distension alters monophasic action potential duration during pulmonary arterial occlusion in anaesthetised lambs: evidence for arrhythmogenic right ventricular mechano-electrical feedback. *Exp Physiol* 2001;86:651–7.
- [8] Calkins H, Maughan WL, Weisman HF, et al. Effect of acute volume load on refractoriness and arrhythmia development in isolated, chronically infarcted canine hearts. *Circulation* 1989;79:687–97.
- [9] Bashir Y, Sneddon JF, O'Nunain S, et al. Comparative electrophysiological effects of captopril or hydralazine combined with nitrate in patients with left ventricular dysfunction and inducible ventricular tachycardia. *Br Heart J* 1992;67:355–60.
- [10] Ooie T, Takahashi N, Saikawa T, et al. Suppression of cesium-induced ventricular tachyarrhythmias by atrial natriuretic peptide in rabbits. *J Card Fail* 2000;6:250–6.
- [11] Gould L, Reddy CV, Zen B, et al. Electrophysiologic properties of hydralazine in man. *Pacing Clin Electrophysiol* 1980;3:548–54.
- [12] Brunner H, Hedwall PR, Meier M. Influence of adrenergic beta-receptor blockade on the acute cardiovascular effects of hydralazine. *Brit J Pharmacol* 1967;30:123–33.