Electrophysiological, Anatomical and Histological Remodeling of the Heart to AV Block Enhances Susceptibility to Arrhythmogenic Effects of QT-Prolonging Drugs

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ABSTRACT—The present study was designed to investigate what kinds of adaptation occurred in the canine chronic AV block model, which has been used to study torsade de pointes (TdP). Dogs at 7 – 10 days (acute phase) and 28 – 56 days (chronic phase) after AV block were assessed. Ventricular effective refractory period and monophasic action potential duration were prolonged in chronic animals compared with acute animals; moreover the electrically vulnerable period was prolonged in chronic animals. Non-specific I<sub>Kr</sub> channel blocker cisapride (1 and 10 mg/kg, p.o.) was administered without anesthesia to estimate the feasibility of QT prolongation. In chronic animals, QT prolongation followed by TdP was induced in one dog by the low dose and in all by the high dose, which was not observed in acute animals. MR images indicated increases of diameter and wall thickness of both ventricles in chronic animals. The degree of hypertrophy was prominent in the right ventricular wall and septal wall. Heart weight of the chronic animals was 1.7 times greater than that of normal control subjects. Photo- and electron-micrograph analyses showed myocardial cell hypertrophy with parallel increases of collagen fiber and extracellular space in chronic animals. These electrophysiological, anatomical and histological adaptations may predispose the chronic AV block heart to drug-induced QT prolongation with enhanced risk of re-entry and early afterdepolarization, leading to the onset of TdP.

Keywords: Long QT syndrome, AV block, Monophasic action potential, Holter ECG, MR image

Since drug-induced prolongation of the QT interval is often associated with the onset of torsade de pointes (TdP), the interest in animal models of TdP arrhythmias has considerably increased (1, 2). The dog with chronic AV block has been used as a very suitable large-animal model for the study of TdP, both under conscious (3) and anesthetized circumstances (4 – 10). Previous studies revealed that the functional adaptations predispose the canine AV block heart to acquired TdP (4, 6, 8 – 10). Namely, QT interval and ventricular endocardial monophasic action potential (MAP) duration are much longer than expected from the bradycardia alone largely because of the reduction of I<sub>Ks</sub> and I<sub>Kr</sub> (4 – 6). Moreover, early afterdepolarization and interventricular dispersion of repolarization have been suggested to play major roles in the genesis of acquired TdP (8 – 10). However, it is still difficult to correlate the premature depolarization (trigger) and the dispersion of repolarization (substrate) to the occurrence of TdP.

The present study was designed to investigate electrophysiological, anatomical and histological adaptation possibly related to the facilitated occurrence of TdP in the AV block heart. For this purpose, we first examined the local changes of the final repolarization phase of the action potential by measuring the ventricular MAP and effective refractory period (ERP) from the same site (12 – 17). Next, feasibility of QT prolongation was studied by administering non-specific I<sub>Kr</sub> channel blocker cisapride (1 and 10 mg/kg, p.o.) without anesthesia. In chronic animals, QT prolongation followed by TdP was induced in one dog by the low dose and in all by the high dose, which was not observed in acute animals. MR images indicated increases of diameter and wall thickness of both ventricles in chronic animals. The degree of hypertrophy was prominent in the right ventricular wall and septal wall. Heart weight of the chronic animals was 1.7 times greater than that of normal control subjects. Photo- and electron-micrograph analyses showed myocardial cell hypertrophy with parallel increases of collagen fiber and extracellular space in chronic animals. These electrophysiological, anatomical and histological adaptations may predispose the chronic AV block heart to drug-induced QT prolongation with enhanced risk of re-entry and early afterdepolarization, leading to the onset of TdP.

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prolongation was compared between acute and chronic phase by administering proarhythmic doses of non-specific Ic channel blocker cisapride (13, 18, 19). Finally, structural changes that may explain the electrophysiological adaptation were assessed using ECG gated MR images, in addition to photo- and electron-micrograph examinations.

MATERIALS AND METHODS

All experiments were carried out according to the Guidelines for Animal Experiments of Yamanashi Medical University, which are equivalent to those of the US National Institute of Health (NIH). Beagle dogs of either sex weighing about 10 kg were obtained through the Animal Laboratory of Yamanashi Medical University.

Production of complete AV block

The dogs were anesthetized with pentobarbital sodium (30 mg/kg, i.v.) and artificially ventilated with room air (SN-480-3; Shinano, Tokyo). Tidal volume and respiratory rate were set at 20 ml/kg and 15 strokes/min, respectively. Heparin calcium (200 IU/kg, i.v.) was administered to prevent blood clotting. The surface lead II ECG and the systemic blood pressure at the right femoral artery were continuously monitored using a polygraph system (RM-6000, Nihon Kohden). To prevent blood clotting, heparin calcium (100 IU/kg) was intravenously administered. A bi-directional steerable MAP recording/pacing combination catheter (1675P; EP Technologies Inc., Sunnyvale, CA, USA) was positioned at the endocardium of the interventricular septum of the right ventricle through the right femoral vein to obtain MAP signals. The signals were amplified with a DC pre-amplifier (300, EP Technologies, Inc.). The duration of the MAP signals was measured as an interval, along a line horizontal to the diastolic baseline, from the MAP upstroke to the desired repolarization level. The interval (ms) at 90% repolarization was defined as MAP90, as previously reported (12 – 17).

The right ventricular septal wall was electrically driven using a cardiac stimulator (SEC-3102, Nihon Kohden) through the pacing electrodes of the combination catheter. The stimulation pulses were rectangular in shape, 1 – 2-V amplitude (about twice the threshold voltage) and 1-ms duration, which was kept during the experimental protocol. The MAP90 was measured at a pacing cycle length of 400, 500, 600, 750 or 1000 ms. The ERP was also assessed at the same site by the programmed electrical stimulation through the pacing electrodes of the combination catheter. The pacing protocol consisted of 8 beats of basal stimuli in a cycle length of 400, 500, 600, 750 or 1000 ms followed by an extra stimulus of various coupling intervals. Starting in the late diastole, the coupling interval was shortened in 5–10-ms decrement until refractoriness occurred. The post-repolarization refractoriness (PRR); namely PRR = ERP − MAP90, was calculated at each basal pacing cycle length to estimate the extent of the electrical vulnerability of the heart, as previously described (12 – 17).

Time course of the susceptibility to a QT-prolonging drug (Experiment 2): Preponderance of arrhythmia was also examined in both the acute phase (n = 18) and chronic phase (n = 18) of the complete AV block. In each phase, ECG was assessed using a Holter recording and analyzing

Electrophysiological studies

Time course of the changes in the repolarization process of the AV block heart (Experiment 1): This study was repeated on the same animal group (n = 6) at 7 – 10 days (acute phase) and 28 – 56 days (chronic phase) after the induction of the complete AV block. The AV block dogs were anesthetized with pentobarbital sodium (30 mg/kg, i.v.). After intubation with a cuffed endotracheal tube, the animals were artificially ventilated with room air (SN-480-3, Shinano). Tidal volume and respiratory rate were set at 20 ml/kg and 15 strokes/min, respectively. The surface lead II ECG and the systemic blood pressure at the right femoral artery were continuously monitored using a polygraph system (RM-6000, Nihon Kohden). To prevent blood clotting, heparin calcium (100 IU/kg) was intravenously administered. A bi-directional steerable MAP recording/pacing combination catheter (1675P; EP Technologies Inc., Sunnyvale, CA, USA) was positioned at the endocardium of the interventricular septum of the right ventricle through the right femoral vein to obtain MAP signals. The signals were amplified with a DC pre-amplifier (300, EP Technologies, Inc.). The duration of the MAP signals was measured as an interval, along a line horizontal to the diastolic baseline, from the MAP upstroke to the desired repolarization level. The interval (ms) at 90% repolarization was defined as MAP90, as previously reported (12 – 17).

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2 h after the start of Holter ECG recording, the non-specific
Irvine, CA, USA) as previously described (12, 21). About
system (Model 456A and Model 153; Del Mar Avionics,
22, 23). The ECG parameters at 1 h before the drug admin-
sule was administered to the control AV block animals
the animals without anesthesia. Meanwhile, a vacant cap-
(n low-dose group, n = 6) was orally administered using a gelatin capsule to
between groups were assessed using the unpaired
Statistics
Drugs
Cisapride was extracted from a commercial source (Ace-
nalin fine granules; Janssen-Kyowa, Tokyo), while heparin
calcium (Mitsui, Tokyo) and pentobarbital sodium (Tokyo
Kasei, Tokyo) were purchased.
Statistics
Data are presented as the mean ± S.E.M. Differences
between groups were assessed using the unpaired t-test,
one-way factorial ANOVA, Fisher’s exact method for 2 × 2
cross table data or Chi square test, while those within a
group were examined by either the paired t-test or one-way
repeated-measures ANOVA followed by Contrasts for
mean values comparison. P-value less than 0.05 was
considered significant.
RESULTS
The AV node ablation successfully eliminated AV con-
duction in all animals (n = 30). The number of radio-
frequency ablation trials was 1 – 2 in most of the cases.
Stable idioventricular escaped rhythm always developed
within 1 min after the onset of AV conduction block. This
automaticity is considered to originate from the distal
portion of AV junctional area, because of the relatively
narrow QRS width with the right bundle branch block
pattern. The mean blood pressure just after the AV block
induction was within the physiological range of >80 mmHg
in each animal. Two dogs died from the congestive heart
failure during the 1st week of AV block, while in all other
dogs, the complete AV block was maintained during the
experimental period without any physical sign of decom-
penation.
Experiment 1: Changes in the MAP\text{90}, ERP and PRR of the
right ventricular septal wall
Six dogs were used for this analysis at the acute and
chronic phases. Typical tracings of MAP at a pacing cycle
length of 400 – 1000 ms in both acute and chronic phases
from an animal are depicted in Fig. 1A, and the effects of
basic pacing cycle length on the MAP\text{90}, ERP and PRR are
summarized in Fig. 1B. In the acute phase (n = 6), increas-
ing the pacing cycle length from 400 to 1000 ms prolonged
MAP\text{90} from 202 ± 3 to 232 ± 6 ms and ERP from 213 ± 6
to 230 ± 6 ms, while PRR decreased from +10 ± 7 to
-2 ± 9 ms. Also, in the chronic phase (n = 6), the MAP\text{90}
prolonged from 225 ± 12 to 284 ± 22 ms and ERP from

Morphological studies
MR images (Experiment 3): This study was performed on
the 1st, 45th and 90th day after the complete AV block
to examine morphological changes of the heart with time
(n = 4). The dogs were anesthetized with pentobarbital
sodium (30 mg/kg, i.v.). After intubation with a cuffed
endotracheal tube, the animals were transported to the MRI
suite. T1-weighted images (TR/TE: 440/16) as well as
ECG-gated MR images were obtained using a 0.2-T MR
imager (Signa Profile, General Electric; Milwaukee, WI,
USA). The short-axis view and 4-chamber view were used
for assessment. The wall thickness and diameter of the left
ventricle at end-diastole were measured on the short-axis
view of the ECG-gated images.
Heart and body weights (Experiment 4): To assess the
extent of hypertrophy after AV block, the heart and body
weights of the sacrificed AV block animals in chronic
phase (n = 13) were compared with those of control animals
of normal sinus rhythm (n = 18). Additional data from
another serious of experiments were also used.
Histology (Experiment 5): Histological studies were
performed on the AV block hearts in chronic phase (n = 4)
and the control hearts of normal sinus rhythm (n = 4). The
animals were anesthetized with pentobarbital sodium
(30 mg/kg, i.v.). After the hearts were removed and rinsed
in saline, transmural specimens were obtained from the left
ventricle, septal wall and right ventricle. These specimens
were fixed in 10% neutrally buffered formaline and em-
bedded in paraffin. Paraffin sections in 4-μm thickness
were stained with hematoxylin-eosin (HE) and Azan and
examined microscopically. Moreover, electron-microscopy
was also performed to assess the changes. The formaline-
fixed left ventricle was subjected to electron-microscopic
examination. The tissue was minced into cubes, approxi-
ately 1-mm-thick, re-fixed in glutaraldehyde and osmium
tetroxide, and embedded in Epon. Ultra-thin sections were
stained with uranyl acetate and lead citrate and observed
under a JEM 100CX transmission electron microscope
(JEOL, Tokyo).

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231 ± 7 to 262 ± 14 ms, while PRR changed from +6 ± 7 to −22 ± 9 ms. No early afterdepolarization was detected, but sporadic ventricular premature contractions were induced during the programmed electrical stimulation only in chronic phase. The MAP₉₀ and ERP were longer and the PRR was more negative in the chronic phase as compared to those in the acute phase. Significant differences were detected between the acute and chronic phase animals in the MAP₉₀ and PRR at the pacing cycle length of 750 and 1000 ms, while those were observed at each pacing cycle length in the ERP.

Experiment 2: Change in the response to proarrhythmic effects of cisapride

The proarrhythmic effects of cisapride are summarized in Fig. 2A, and typical tracings of ECG showing the cisapride-induced TdP are depicted in Fig. 2B. In the acute phase (n = 6 for each group), only sporadic ventricular premature contractions (PVCs) of <30 beats/h were induced in 3 animals out of 6 by low dose cisapride and 4 out of 6 by high dose cisapride. Meanwhile, no PVC was observed in the control group animals given vacant capsules. No TdP was induced in the acute phase.

In the chronic phase (n = 6 for each group), the number of animals that showed PVCs increased in both the low- and high-dose group. The number of PVCs was ≥30 beats/h in animals that showed TdP, while it was <30 beats/h in animals without TdP. TdP was induced in 1 animal out of 6 by low-dose cisapride and in all animals by high-dose cisapride. No TdP was observed in the control group. In one animal in the low-dose cisapride group and in 4 in the high-dose cisapride group, ventricular fibrillation finally occurred following TdP.

In animals that showed TdP, the duration of the non-fatal episode was 1 – 5 s and the rate of TdP was >300 beats/min (n = 5). TdP was preceded by a characteristic sequence of short/long/short cycles of R waves. No malignant ventricular arrhythmias were observed before the onset of TdP including sustained and/or monomorphic ventricular tachycardias. The first TdP appeared 7.6 ± 1.8 h after cisapride (n = 7). The elapsed time between the first TdP and the fatal TdP was 2.8 ± 1.4 h (0 – 6.0 h) (n = 5). The number of TdP recorded was 1 – 4 per each animal, and the last ventricular fibrillation was observed 9.4 ± 2.7 h after cisapride (n = 5).

Experiment 2: Change in the effects of cisapride on QTc

The time courses of ECG parameters and the number of surviving animals after cisapride are summarized in Fig. 3. In the acute phase (left panels), the idioventricular escaped rate (VR) (beats/min) and QTc (ms) at the pre-drug control...
Fig. 2. Comparison of the susceptibility for torsade de pointes (TdP) between acute phase and chronic phase animals. A: Summary of the results. B: Compressed and continuous ECG image (lead II) of a dog complicating TdP. Note typical features of prolonged QT interval and short-long-short initiation sequence as shown in the rectangle. PVCs: premature ventricular contractions.

Fig. 3. Comparison of the effects of cisapride on QTc, ventricular rate and the number of surviving animals between acute phase and chronic phase animals. Closed symbols represent significant differences from each control value (C) at $P<0.05$. Data are presented as the mean ± S.E.M. Triangles: 10 mg of cisapride-administered group (n = 6), circles: 1 mg of cisapride-administered group (n = 6) and squares: vacant capsule-administered group (n = 6).
A. Sugiyama et al. (C) were 40 ± 3 and 193 ± 10 in the control group (n = 6), 46 ± 2 and 193 ± 9 in the low dose cisapride group (n = 6) and 44 ± 3 and 197 ± 6 in the high dose cisapride group (n = 6), respectively. There were no differences in the pre-drug control values among the groups. After drug administration, VR decreased at 11 and 15 h in the low-dose cisapride group, but it increased for 2 – 10 h in the high-dose cisapride group, while no significant change was detected in the control group. No significant change was observed in QTc in any of these three groups, although there was a tendency of prolongation in the cisapride groups.

In the chronic phase (right panels), the VR (beats/min) and QTc (ms) at the pre-drug control (C) were 38 ± 4 and 209 ± 9 in the control group (n = 6), 33 ± 2 and 208 ± 5 in the low-dose-cisapride-administered group (n = 6), and 30 ± 1 and 224 ± 7 in the high-dose-cisapride-administered group (n = 6), respectively. There were no differences in the control values among the groups, but basal control QTc was longer and VR was slower in the chronic phase (n = 18) as compared to the acute phase (n = 18). After the drug administration, QTc was prolonged at 2 h, 5 – 17 h and 19 – 20 h in the high-dose-cisapride group, while no significant change was observed in the low-dose-cisapride group and control group, except that QTc was prolonged in one animal with complicating TdP by low dose cisapride. VR increased at 3 – 20 h in the high-dose-cisapride group, while no significant change was observed in the low-dose-cisapride and control groups.

Experiment 3: MR images

The time courses of the typical changes of the short-axis and 4 chamber views are depicted in Fig. 4, while those of the wall thickness and diameter of both ventricles are summarized in Table 1 (n = 4). The wall thickness and diameter of both ventricles increased during the initial 45 days after the induction of AV block. The hypertrophy

<table>
<thead>
<tr>
<th>Days after onset of AVB</th>
<th>1st day</th>
<th>45th day</th>
<th>90th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV Free wall (mm)</td>
<td>6.2 ± 0.6</td>
<td>6.6 ± 0.4**</td>
<td>6.6 ± 0.4**</td>
</tr>
<tr>
<td>Septal wall (mm)</td>
<td>5.7 ± 0.2</td>
<td>7.2 ± 0.1**</td>
<td>7.2 ± 0.1**</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>34.4 ± 0.4</td>
<td>45.9 ± 2.7**</td>
<td>48.3 ± 1.3**</td>
</tr>
<tr>
<td>RV Free wall (mm)</td>
<td>2.7 ± 0.1</td>
<td>3.4 ± 0.2*</td>
<td>4.0 ± 0.3**</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>22.3 ± 1.7</td>
<td>26.8 ± 2.6**</td>
<td>27.8 ± 2.0**</td>
</tr>
</tbody>
</table>

AVB: atrioventricular block, LV: left ventricle, RV: right ventricle (mean ± S.E.M., n = 4). *P<0.05, **P<0.01 compared with the data on the 1st day.

**Table 1.** Time course of the wall thickness and diameter of the ventricles

**Fig. 4.** The time courses of the changes of the short-axis (A, B and C) and 4 chamber views (D, E and F) in T1-weighted MR images. Temporal increase of both the ventricular size and slight increase of the wall thickness were demonstrated, thus showing an eccentric hypertrophy. Scale bar: 30 mm.
Fig. 5. Typical pictures of the AV block heart in the chronic phase. A: Comparison of a chronic AV block heart with a control heart of normal sinus rhythm. B: Right atrium (RA), AV nodal area and right ventricle (RV) of the AV block heart in the chronic phase. Note the white scar region in the AV nodal area where the radiofrequency energy would be applied (Ab).

Fig. 6. Comparison of the body weight (A), heart weight (B) and heart/body weight (C) between control animals of normal sinus rhythm (Cont) or pre-operation animals (AVB Before) and chronic phase animals (AVB Chronic). The summary of the time courses of the heart weight (D) and heart/body weight (E) of the AV block animals. Data are presented as the mean ± S.E.M. AVB: atrioventricular block.

Fig. 7. Photo-micrographs (A and B; Azan stain) and electron-micrographs (C and D) of the left ventricular myocytes of the canine heart. A and C: Myocytes of the control heart of normal sinus rhythm. Few collagen fibers were seen in the intercellular space. B and D: Myocytes of the chronic AV block heart. The myocytes were markedly hypertrophied and collagen fiber was prominent compared with that of the control heart.
was eccentric. The degree of hypertrophy was greater in the right ventricular free wall (+25.9%) and septal wall (+26.3%) compared with that in the left ventricular free wall (+6.5%). On the other hand, no further change was observed during the 45th – 90th days.

**Experiment 4: Changes in the body weight, heart weight and heart/body weight ratio**

A typical AV block heart from a dog sacrificed in the chronic phase is depicted in Fig.5 along with a normal control heart. The time courses of the heart weight and body weight of the AV block animals are summarized in Fig. 6. No significant difference was observed in the body weight between the control (n = 18) and the AV block animals (n = 13) both at the pre-operation period and 28 – 56 days after AV block. Meanwhile, the body weight of the AV block animals slightly increased from 9.9 ± 0.2 kg to 10.3 ± 0.3 kg during these 28 – 56 days after AV block (Fig. 6A). The heart weight and heart/body weight ratio were about 1.7 and 1.6 times greater in the AV block animals in the chronic phase as compared to the control animals, respectively (Fig. 6: B and C). The heart weight and heart/body weight ratio of the AV block animals increased to >100 g and >1% on 17th day after the onset of AV block, respectively (n = 32, Fig. 6: D and E).

**Experiment 5: Histological changes**

Typical photo-micrograph (A and B) and electron-micrograph (C and D) of the left ventricular myocytes of the normal control heart of sinus rhythm (A and C) and AV block heart in the chronic phase (B and D) are depicted in Fig. 7. Few collagen fibers were seen in the intercellular space in the normal control heart, while in the AV block heart, the collagen fibers and extracellular space increased, and each cardiomyocyte hypertrophied as compared to the normal control heart. These changes were confirmed in >90% fields from specimens of both ventricles assessed (n = 4).

**DISCUSSION**

The present electrophysiological and structural changes explain possible arrhythmogenic potential of the chronic AV block heart to QT-prolonging drugs developing to the degeneration of PVC to TdP. In the chronic AV block heart, both the “trigger” and “substrate” for the occurrence of TdP could increase, as will be discussed below.

**Local proarrhythmic substrates**

Previous studies (4, 5, 9) have suggested that interventricular dispersion of action potential duration predisposes the heart to the onset of TdP. The present electrophysiological analysis was designed to explore the local proarrhythmic substrates in the AV block heart. Right ventricular septal wall was assessed for this purpose, since less critical mass has been shown to be required to maintain ventricular fibrillation in the septal wall than in the right and left ventricular free walls (25). The final repolarization phase was analyzed by simultaneously measuring the ventricular MAP and ERP. Both ERP and MAP duration were prolonged in the chronic phase as compared with those of the acute phase, supporting the previous report that delayed rectifier potassium currents are down-regulated in the chronic AV block heart (6). More importantly, the prolongation of the MAP duration was more marked than that of the ERP in the chronic phase, particularly at a slower heart rate, indicating the prolongation of the electrically vulnerable period of the ventricle. These local electrophysiological changes indicate increase in the risk of aberrant excitation, which may provide a “substrate” for re-entry arrhythmias, leading to the onset of TdP (14, 15).

**Induction of TdP by cisapride**

There have been several reports describing that cisapride caused serious cardiac arrhythmias including TdP as a result of QT prolongation (18, 23). In vitro electrophysiological studies showed a possible mechanism of the proarrhythmic effects of cisapride to be its IC50 blocking property (19, 23). In our previous study using the halothane-anesthetized canine in vivo models (13), intravenously administered at 1.0 mg/kg of cisapride prolonged the electrically vulnerable period in addition to prolonging ERP and MAP duration. Moreover, it induced a typical early afterdepolarization. The absolute bioavailability and elimination half-life (T1/2) of oral cisapride are known to be about 40 – 50% and 7 – 10 h in healthy subjects, respectively (22). We administered 1 and 10 mg/kg, p.o. of cisapride to the AV block animals, although there may be potential species-related differences between man and dog in drug metabolism. Neither QTc prolongation nor lethal arrhythmia was initiated in the acute phase by either dose, while TdP following QTc prolongation was induced in one animal by the low dose and in all animals by the high dose in the chronic phase. More importantly, we provided evidence that short/long/short sequence is necessary for the onset of TdP, which has been referred to as the “cascade phenomenon” (10), suggesting that triggered activity due to early afterdepolarization might be occurring. Such premature impulse can be viewed as a “trigger” for re-entrant arrhythmias. Thus, cisapride-induced further prolongation of an already prolonged repolarization period in the chronic AV block heart may not only constitute the “substrate” for maintaining reentry to degenerate to TdP but also provide the “trigger”.
Detection of drug-induced TdP

In a rabbit model for studying acquired long QT syndrome, infusion of cisapride in a dose of 3.0 µmol (= 1.452 mg) · kg⁻¹ · 10 min⁻¹ was associated with a significant lengthening of the QTU interval, but TdP appeared only in two rabbits out of six (23). In the present study, we demonstrated a higher incidence of TdP by p.o. administration of cisapride without anesthesia in the chronic AV block dogs. The higher sensitivity in this model can be explained by pre-drug existence of diminished potassium currents (4 – 6), resulting in the enhanced drug-induced QT prolongation. More importantly, this methodology has an important addition to the drug-induced TdP screening which till now was mostly performed under anesthesia using i.v. drug administration in the chronic AV block dog (4 – 10). Thus, our method in experiment 2 may become an alternative for detecting the torsadeogenic action of drugs. Whether this method is suitable for predicting the occurrence of TdP in patients treated with such drugs has to be determined.

Anatomical adaptation

MR images indicated that significant eccentric hypertrophy of the chronic AV block heart. The degree of the hypertrophy was prominent in the right ventricular free wall and septal wall. These results are in good accordance with a recent report (26), indicating the reproducibility of the currently observed hypertrophic response after AV block induction. Moreover, the previous study (26) has demonstrated that the extent of each portion of the ventricular hypertrophy is closely associated with respective increases of AT1-receptor mRNA. Meanwhile, the heart weight of the chronic animals was 1.7 times greater than that of normal dogs with similar body weight, also confirming a previous publication (4). This type of anatomical remodeling of the heart may allow ventricular cavities to regain a normal wall stress (27), while these morphological changes may increase the risk of macroscopic re-entry in the chronic AV block heart, which could become the "substrate" of TdP. In addition, Fig. 6, D and E, may give the impression that the duration of AV block determines the heart weight, which is only the case in the beginning, but not after the adaptation point has been passed, which will be 2 – 4 weeks after the onset of AV block.

Histological adaptation

Photo- and electron-micrograph analyses showed myocardial cell hypertrophy together with the parallel increases of collagen fiber and extracellular space in the chronic phase. These findings are essentially in accordance with previous observations both in the canine chronic AV block heart (4) and in the right ventricular endomyocardial biopsy specimens from patients with documented AV block without apparent heart disease (28). These histological changes might indicate a physiological adaptation of the contractile muscle and connective tissue network to maintain normal ventricular function (4), which may be the reason for the lack of congestive heart failure in chronic phase animals. However, the myocardial cell hypertrophy may increase action potential duration (5) and the increases of collagen fiber and extracellular space will uncouple the constituent cardiac myocytes (29). Thus, these structural changes can be the “substrate” that may explain the enhanced susceptibility of the chronic stage heart to QT-prolonging drugs and occurrence of TdP.

Conclusions

While electrical and structural changes in the chronic AV block heart might be aimed to maintain cardiac function, they also predispose the heart to the drug-induced QT prolongation with enhanced risk of re-entry and early afterdepolarization, leading to the onset of TdP. Thus, the present canine model of TdP can be used to screen new agents with QT prolonging effects for their proarrhythmic potentials. These results also suggest that patients with ventricular hypertrophy and persistent bradycardia may be more susceptible to proarrhythmic effects of QT-prolonging agents.

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REFERENCES


Bazett HC: An analysis of the time-relations of electrocardiogram. Heart 7, 353 – 370 (1920)

Ikeda T, Kawase A, Nakazawa K, Ashihara TT, Namba TT, Takahashi TK, Sugi K and Yamaguchi T: Role of structural complexities of septal tissue in maintaining ventricular fibrillation in isolated, perfused canine ventricle. J Cardiovasc Electrophysiol 12, 66 – 75 (2001)


