Asynchronous development of electrical remodeling and cardiac hypertrophy in the complete AV block dog

Marieke Schoenmakers, Christian Ramakers, Jurren M. van Opstal, Jet D.M. Leunissen, Camila Londoño, Marc A. Vos*

Department of Cardiology, Cardiovascular Research Institute Maastricht, PO Box 5800, 6202 AZ Maastricht, The Netherlands

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Abstract

Objective: Left ventricular hypertrophy has been associated with the prolongation of QT-time, and an increased risk of ventricular arrhythmias. The renin angiotensin system has been implicated in the development of ventricular hypertrophy. At 5 weeks complete AV block (CAVB) in the dog, there is: (1) biventricular hypertrophy associated with a transient activation of components of the renin angiotensin system, (2) increased APD, more pronounced in the left than in the right ventricle leading to spatial dispersion of repolarization, and (3) enhanced susceptibility to drug-induced torsade de pointes arrhythmias. To investigate whether these remodeling processes develop in parallel, time dependency was assessed in absence or presence of the AT1 receptor-blocker Irbesartan.

Methods and results: Dogs in sinus rhythm, 2 and 5 weeks CAVB were compared to dogs chronically treated with Irbesartan (30 mg/kg BID). Endocardial monophasic APD of left and right ventricle was measured and susceptibility to torsade de pointes was tested by infusing Dofetilide (0.025 mg/kg/5'). Hypertrophy was determined by relating heart-to-body weight at sacrifice. Left ventricular APD had increased more than right ventricular APD at 2 and 5 weeks CAVB, leading to an increase in spatial dispersion. At that time torsade de pointes were evocable in the majority of the dogs. Hypertrophy had only developed completely at 5 weeks CAVB. Irbesartan had no effect on electrical and structural parameters or on arrhythmogenicity. Conclusions: In the CAVB dog ventricular hypertrophy is not a prerequisite for electrical remodeling or drug-induced torsade de pointes, and the AT1-receptor has no dominant role in the completion of these remodeling processes.

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Keywords: Repolarization; Ventricular arrhythmias; Renin angiotensin system

1. Introduction

Left ventricular hypertrophy is a common finding in the adult population and associated with an increased QT-time and enhanced risk of ventricular arrhythmias and sudden cardiac death [1,2]. In numerous animal models the relation between ventricular hypertrophy and the occurrence of electrophysiological changes, like the prolongation of ventricular repolarization, has been confirmed [3]. In the chronic complete AV block (CAVB) dog, electrical remodeling is further characterized by an increase in spatial dispersion and a higher propensity to (drug-induced) early afterdepolarizations, ectopic beats and Torsade de Pointes-arrhythmias (TdP) [4,5]. Although the association between the presence of ventricular hypertrophy and the related electrical remodeling and arrhythmogenicity seems firm, the synchronicity in their
course of development has never been studied. Therefore we determined the temporal behaviour of these processes after creation of AV-block.

Secondly, we attempted to dissociate these remodeling processes pharmacologically using Irbesartan (I), an angiotensin II type-1 receptor (AT1)-blocker. This approach was selected since in the CAVB dog-model: the renin angiotensin system (RAS) is transiently activated [4], and AT1 mRNA expression levels relate to the degree of ventricular hypertrophy [6].

2. Methods

2.1. General

Thirty mongrel dogs (body weight 21–33 kg, 15 females) underwent experimental testing. Irbesartan (Sanofi-Synthélabo, Montpellier, France) was administered orally at a dose of 30 mg/kg BID [7] to 10 dogs, starting 1 week prior to AV block creation. Serial measurements were performed under premedication (ECG) or during complete anesthesia. Surface ECGs were recorded during regular sinus rhythm (SR) before and during Irbesartan (I), and repetitively after creation of AV-block until 2 weeks AV-block. Invasive measurements were performed at different time points: SR, acute AV-block (AAVB), CAVB2, and/or CAVB5 in which 2 and 5 indicates 2 or 5 weeks CAVB respectively. At the moment of sacrifice, dogs belonged to the following groups: SR (n = 5), CAVB2 (n = 8), CAVB5 (n = 7), CAVB2I (n = 5), and CAVB5I (n = 5). Since dogs were tested serially the numbers depicted in the tables often exceed the group size.

Animal handling was in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The ‘Committee for Experiments on Animals (DEC)’ of the University of Maastricht, Netherlands approved the experiments.

2.2. Experimental design of the studies

After overnight fasting premedication consisted of acepromazine (0.6 mg/kg i.m.) and buprenorphine (0.009 mg/kg i.m.). The anesthesia, ventilation, preoperative, and postoperative care have been described in previous articles [4,8]. In 25 dogs complete AV-block was induced by radiofrequency ablation. A 7 French steerable catheter with a 4-mm tip (RF Marin™, Medtronic CardioRhythm, San Jose, CA, USA) was positioned across the tricuspid valve to record a large low atrial and a small His bundle potential, guided by fluoroscopic views from the right and left anterior oblique angles. The temperature controlled radiofrequency energy, with a power limit of 35–50 W and a target temperature of 70 °C was delivered from a 500 Hz generator (Atak™, Medtronic CardioRhythm, San Jose, CA, USA) for 2 min, between the large tip thermocouple electrode of the ablation catheter and an adhesive pad applied on the back of the dog. For electrophysiological parameters (EP), the regular 6-lead surface ECG was accompanied by 2 monophasic action potentials (MAPs, EP Technologies) placed at the endocardium of the left and right ventricle (LV and RV, respectively). The EP measurements at AAVB and CAVB were performed at two basic rates: (1) the idioventricular rate (IVR), and (2) a fixed pacing rate (FRP) with an interstimulus interval of 500 ms. During FRP, which was performed for 2 min from the RV MAP, the ventricular effective refractory period (VERP) was determined incrementally (steps of 5 ms) at twice diastolic threshold. For hemodynamics, pressure (P) signals were recorded with a Sentron catheter (Sentron Europe), which was positioned in the abdominal aorta for a short period (only during SR) and advanced to the LV cavity where it remained thereafter. All hemodynamic data were gathered during IVR. In the CAVB5 group, no hemodynamic data were collected since an extensive data set already existed [9]. Definitions, amplifications and filter settings were described in previous publications [4,8,10].

An arrhythmogenic challenge was performed at CAVB by applying 0.025 mg/kg dofetilide intravenous for 5 min [8]. The occurrence of TdP was scored for 20 min. When TdP was seen 3 or more times, the dog was considered susceptible.

At sacrifice, the heart was excised and weighed. The ventricles were isolated from the atria, RV was removed, and the septum was taken as part of the LV.

2.3. Data analysis

The signals of EP and hemodynamics were sampled at 1 kHz and stored on hard disk. With a custom made computer program (ECG View) with a resolution of 2 ms and adjustable gain and time scale, the following parameters were measured offline: PP-time, RR-time, QRS-duration, QT-time, duration of the MAP (MAPD) at 100% repolarization, aortic P, LV end diastolic pressure (EDP), LV systolic pressure (SP), and LV peak rate of pressure rise (+dP/dtmax). Corrected (c) QT-time [11] was calculated, and spatial dispersion (ΔMAPD) was defined as the difference between LV MAPD and RV MAPD. All data presented are the mean of 5 consecutive beats. An independent observer (CL) checked all measurements.

2.4. Analysis of ACE and chymase mRNA

At sacrifice LV and RV tissue of untreated SR and CAVB dogs was immediately frozen in liquid nitrogen and stored at −80 °C until mRNA analysis. ACE and chymase mRNA levels were assessed at SR, CAVB2, and CAVB5 using quantitative real-time PCR (LightCycler, Roche Diagnostics, Almere, The Netherlands). Total RNA was isolated using the Qiagen RNeasy kit according to the
manufacturer’s protocol. Contamination of genomic DNA was further eliminated by digestion with DNAsel (Promega Benelux, Leiden, The Netherlands). First strand cDNA was synthesised using an optimised reverse transcription protocol [12]. Real time PCR was performed using the FastStart DNA Master Sybr Green 1 kit (Roche Diagnostics, Almere, The Netherlands) and dog specific ACE, chymase [13] and GAPDH primers. Data of both ventricles were combined to increase sample size.

2.5. Angiotensin II dose–response study

During SR the pharmacologic activity of Irbesartan was characterized in preliminary experiments. The response of the aortic systolic pressure to intravenous incremental boluses of angiotensin II was measured in 3 dogs before and 1 week after the administration of Irbesartan.

2.6. Statistics

Pooled data are expressed as mean±S.D. Serial comparisons were performed by paired student’s t-test and single parameters between independent groups by a 2-way ANOVA with a post-hoc Bonferroni t-test. Temporal measurements were tested using ANOVA for repeated measures. Values of \( P < 0.05 \) were considered significant.

3. Results

3.1. Ventricular remodeling in the CAVB dog (control)

3.1.1. Time course of electrical remodeling

In this study, focus was on the electrophysiological changes taking place in the first 2 weeks after creation of AV-block. In the left panel of Fig. 1 changes in PP, RR, and QT-time are depicted at different time points under sedation. Acute AV-block resulted in a significant atrial cycle length decrease, while RR and QT did not significantly increase (left panels). Initially, this lack of severe ventricular bradycardia came as a surprise. From personal observations we learned that this was only the case during the first 24 h after creation of AV-block, thereafter the RR-time increased significantly. This lack of bradycardia

![Figure 1](image-url)
could well be related to the severe decrease in PP-time (increased adrenergic state). In time (1 week CAVB: CAVB1) the evolving ventricular bradycardia led to a further lengthening of QT-time, while the PP-time was returning to normal. At CAVB2 there was a further increase in QT-time while the RR remained stable (electrical remodeling). The presence of electrical remodeling was confirmed at CAVB2 when the heart rate was controlled at 500 ms fixed rate pacing (FRP) under complete anesthesia (right panel Fig. 1). The QT-time, LV MAPD, RV MAPD, and VERP were significantly increased at CAVB2. Moreover, the increase of MAPD was more pronounced in LV than in RV, leading to an increase in spatial dispersion of repolarization (ΔMAPD: 14±10 ms at AA VB and 26±23 ms at CAVB2).

From 2 to 5 weeks CAVB, all electrophysiological parameters remained stable (Table 1). Dofetilide administration resulted in the induction of reproducible TdP in the majority of the dogs at both times: 5 out of 6 dogs at CAVB2 and 5 out of 7 at CAVB5.

3.1.2. Development of cardiac hypertrophy

The presence of cardiac hypertrophy at CAVB5 was demonstrated by a significant increase in heart weight-to-body weight ratio (H/Bw) as compared to SR, 11.6±1.1 vs. 7.9±0.7 g/kg (Fig. 2). In contrast, the CAVB2 group did not show cardiac hypertrophy (H/Bw: 8.8±0.9 g/kg). When comparing the individual H/Bw ratios of 3 dogs in the CAVB2 group is out with the range observed in the SR group but within the range of the CAVB5 group. This indicates the presence of cardiac hypertrophy in these dogs.

3.1.3. Hemodynamic parameters

The LVSP and LVEDP showed comparable values at SR and CAVB2. LVSP 110±23 and 103±20 mmHg, respectively, and LVEDP 8±5 and 8±1 mmHg. Contractility of the LV however was significantly higher at CAVB2 as seen by an increased +dP/dtmax as compared to SR (2543±1054 vs. 1447±396 mmHg/s respectively). None of the dogs showed physical signs of congestive heart failure either during the experimental period or at autopsy.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Electrophysiology in control groups</th>
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<tbody>
<tr>
<td>IVR</td>
<td>CAVB2 (n=8)</td>
</tr>
<tr>
<td>RR-time, ms</td>
<td>1144±419</td>
</tr>
<tr>
<td>QT-time, ms</td>
<td>384±44</td>
</tr>
<tr>
<td>QTc-time, ms</td>
<td>372±26</td>
</tr>
<tr>
<td>LV MAPD</td>
<td>336±50</td>
</tr>
<tr>
<td>RV MAPD</td>
<td>296±40</td>
</tr>
<tr>
<td>ΔMAPD</td>
<td>40±22</td>
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</table>

3.1.4. Time dependent changes in mRNA expression levels of ACE and chymase

The two components of the cardiac tissue RAS showed a change in mRNA expression after creation of AV-block. Ventricular ACE mRNA expression was unchanged at CAVB2 as compared to SR, and decreased at CAVB5. The mRNA expression of chymase however was increased at both CAVB2 and CAVB5 (Fig. 3).

3.2. Effect of Irbesartan on ventricular remodeling

3.2.1. Effectiveness of AT1-blockade

During SR the pharmacologic activity of Irbesartan was characterized in preliminary experiments. Daily administration of Irbesartan resulted in (1) a similar mean aortic pressure (96±9 vs. 92±13 mmHg during Irbesartan), and (2) a downward shift of the dose–response curve (Fig. 4). At a subdepressor dosage regimen, effective blockade of angiotensin II was reached.

3.2.2. Effect of Irbesartan on electrophysiological parameters during SR

Daily chronic administration of Irbesartan during SR did not alter EP. Serially (n=10) the heart rhythm (PP-time: 726±198 ms before and 722±213 ms during Irbesartan), conduction (QRS-width: 59±9 ms vs. 64±12 ms) and ventricular repolarization (QT-time: 234±16 ms vs. 227±28 ms) were not influenced after 1 week of treatment. In the EP group comparison, where control (n=12) and Irbesartan (n=10) were compared, this lack of effect was confirmed by determination of LV MAPD (213±16 ms vs. 204±22 ms respectively), RV MAPD (203±14 ms vs. 196±19 ms), and ΔMAPD (12±9 ms vs. 11±8 ms).
3.2.3. AT1-blockade and ventricular remodeling

Within 2 weeks after creation of AV-block, electrical remodeling occurred in the Irbesartan treated animals. The changes were similar to those seen in the control groups. In Table 2, QT, LV MAPD, RV MAPD, ΔMAPD, and VERP during fixed rate pacing (FRP), serially conducted at AAVB and CAVB2 are depicted. All electrophysiological parameters increased significantly at CAVB2. From 2 to 5 weeks AV-block all parameters remained stable, e.g., QTc is shown in Fig. 5. Thus, Irbesartan did not prevent the occurrence of electrical remodeling. Dofetilide administration resulted in the reproducible induction of TdP in the majority of the dogs: 2 out of 4 at CAVB2I, and 3 out of 5 at CAVB5I. Both the induction and duration of the TdPs were comparable in the Irbesartan groups, and comparable
to the arrhythmias in the control groups. A representative
example is shown in Fig. 6.

LVSP and LVEDP were unaltered at CAVB2I and CAVB5I when compared to the SR group. The LVSP was
103±4 mmHg at CAVB2I, 98±20 mmHg at CAVB5I and
95±13 mmHg at SR, and the LVEDP was 9±3, 5±2 and
8±3 mmHg, respectively. Contractility of the LV had
significantly increased at CAVB2I (LV \( +\)<nolabel>\( \frac{dP}{dt}\)max: 2463±512 mmHg/s), and CAVB5I (2338±564 mmHg/s)
as compared to SR (1570±188 mmHg/s). The hemo-
dynamic parameters were comparable to the control group.
None of the Irbesartan treated animals showed signs of
congestive heart failure.

Observations concerning the development of cardiac
hypertrophy were similar in the Irbesartan groups as
compared to control (Fig. 5). Hypertrophy was present at
CAVB5I (10.7±1.0 g/kg) but not at CAVB2I (8.3±0.5
g/kg). A subanalysis revealed that the LV weight-to-body
weight ratio at CAVB2I (4.7±0.3 g/kg) was significantly
less than at CAVB5I (6.1±0.6 g/kg). The same was true
for the RV weight-to-body weight ratio: 1.8±0.2 g/kg at
CAVB2I vs. 2.3±0.4 g/kg at CAVB5I.

3.3. Different time course of electrical remodeling and
cardiac hypertrophy

When plotting the values of the control and Irbesartan
groups at similar time points (Fig. 5), the distinction in
temporal behaviour of the two remodeling processes
can be appreciated. Electrical remodeling is complete at
CAVB2, while cardiac hypertrophy has only developed at
CAVB5.

4. Discussion

In the CAVB dog, ventricular electrical remodeling and
the arrhythmia-prone condition, i.e., Dofetilide-induced
TdP, are already present at CAVB2, while the development
of cardiac hypertrophy seems to follow a slower time
course. Irbesartan, the AT1-blocker, does not prevent
ventricular remodeling in this model, regardless of the fact
that components of the renin angiotensin system have been
implicated in hypertrophy.

4.1. Temporal dissociation of electrical remodeling from
cardiac hypertrophy

The long-term (>5 weeks) adaptations of the CAVB
dog have been the subject of many publications [4–
6,8,9,14,15]. Electrical remodeling can be characterized by
a non-homogeneous lengthening of MAPD (LV > RV)
leading to spatial dispersion of repolarization. The inci-
dence of class III antiarrhythmic-induced TdP is high, and
the incidence of sudden cardiac death is 10–15%. There is
eccentric biventricular hypertrophy, not accompanied by
excessive collagen depositions or changes in capillary–
fiber ratio. At 5–6 weeks CAVB, mechanical adaptations
have led to a hemodynamically compensated state: LV and
RV \( +\)<nolabel>\( \frac{dP}{dt}\)max have increased, cardiac output is main-
tained, and ventricular EDP is unchanged in comparison to SR.

This study is the first one to describe the temporal dissociation of electrical remodeling and related proarrhythmia from cardiac hypertrophy in an in vivo dog model. An increased QT, ventricular MAPD prolongation, spatial dispersion of repolarization, and susceptibility to drug-induced TdP were uniformly present at CAVB2. Cardiac hypertrophy on the other hand developed more slowly: at CAVB2 hypertrophy was absent in the majority of the dogs, while at CAVB5 it was fully present. LV and RV weights showed comparable results. Lack of synchrony has been indicated in the first days after myocardial infarction in the rat, where downregulation of K⁺-channel genes and currents was present while no hypertrophy was detectable yet [16]. In two papers pharmacological separation of these processes during their developmental phase was reported. In a murine model of pressure overload, cyclosporin A was able to prevent myocyte hypertrophy, while the prolongation of the action potential was only attenuated [17]. In aortic-banded rats changes in gene products of Iᵢ, Iₛ, and Iᵢₛ were prevented by treatment with a Ca²⁺-antagonist, while LV hypertrophy developed [18]. However none of these pharmacological studies described the arrhythmogenic consequences of the dissociation. These studies and the present one, where electrical remodeling and hypertrophy develop asynchronously, indicate the presence of different signalling routes. More recently, it was suggested that the reduction of ion channel gene products might even be the starting point of hypertrophic signalling [19].

4.2. No sign of heart failure in the CAVB dog

Another important finding is that during short-term volume overload, when hypertrophy is not yet present (CAVB2), there are no signs of congestive heart failure: LVEDP is not elevated, LV +dP/dt max is significantly increased, and there are no physical signs of pump failure. This suggests that during the early time course of mechanical overload mechanisms other than hypertrophy come into play to compensate for the increased workload of the heart. Mouse models of pressure overload, either transgenic or treated with cyclosporin A, confirm that a preserved cardiac function is not necessarily accompanied by cardiac hypertrophy [20,21]. Whether contractile improvements can be maintained in the long run without the involvement of cardiac hypertrophy is unclear. In this study, where mechanical adaptations and cardiac hypertrophy are both present at CAVB5, hypertrophy may be necessary for the long-term compensation.

4.3. Lack of effect of Irbesartan

For several reasons we selected AT1-inhibition: (1) tissue and plasma components of the RAS are (transiently) elevated in the CAVB dog [4,6], (2) chymase mRNA was elevated at CAVB while ACE-expression was unchanged or even decreased, possibly suggesting a predominance of chymase in tissue angiotensin II production after AV-block, (3) other pathways, like bradykinin, influenced by ACE-inhibitors are left untouched by AT1-blockade, and (4) Irbesartan can be applied effectively at a dosage not influencing blood pressure thereby preserving hemodynamics.

Though the activation of AT1 in the mammalian heart mainly results in the development of myocyte hypertrophy in vitro [22], conflicting data have been published concerning the effect of blockade in vivo. Most prevention studies have focused on the effect of AT1-blockade during pressure overload. For example, in some studies concerning aorta banding in rats, hypertrophy was attenuated, while in others no effect was seen [18,23,24]. AT1-blockers failed to prevent cardiac hypertrophy in constrictive pulmonary artery-banded rabbits, cats, and fetal sheep [25–27]. Prevention studies in volume overload models are scarce. In rat models of volume overload AT1-blockade attenuated hypertrophy [28,29] Perry et al. described the presence of myocyte hypertrophy after mitral valve regurgitation despite AT1-blockade in the dog [30]. In the CAVB dog no prevention of hypertrophy by Irbesartan was seen at CAVB5I as compared to CAVB5. However, a blunted response cannot be ruled out. This all, leads us to believe that AT1 may not be the dominant factor in the development of mechanical overload-induced hypertrophy, but that other signaling pathways are of importance. Moreover, Irbesartan was not able to prevent electrical remodeling in this model. Furthermore, the relation between the presence of electrical remodeling and the susceptibility to Dofetilide-induced TdP remained present.

4.4. Clinical implications

In clinical studies an increased QT-time and the presence of ventricular arrhythmias has been documented in patients with left ventricular hypertrophy [1,2]. A positive correlation between the increase in LV mass and the QT duration has been implicated [2]. This experimental study suggests that electrical remodeling and proarrhythmia are likely to precede the presence of hypertrophy.

So far, no clinical study has addressed temporal aspects of electrical remodeling, proarrhythmia and hypertrophy. We realize that conducting such a study will merely be impossible due to ethical and practical constraints. Therefore the CAVB dog is a very suitable model to study these aspects in detail.

4.5. Limitations

We do not know whether Irbesartan actually reached the AT1 on the membrane of the cardiomyocyte, though we assume this was the case because its vascular counterpart...
was blocked as checked by angiotensin II dose–response studies. Since we did not measure angiotensin II levels in the cardiac tissue, we have to be careful in relating the changed levels of ACE and chymase mRNA after AV block to the RAS in general. We are aware of the fact that groups are too small in order to compare the incidence of Dofetilide-induced TdP between controls and Irbesartan treated dogs, however we want to emphasize that Irbesartan did not influence electrical remodeling. We realize that since LV hemodynamics were only measured during IVR contractility of the LV was as such not fully characterized.

5. Conclusions

In the complete AV block dog, ventricular hypertrophy is not a prerequisite for electrical remodeling or drug-induced torsade de pointes. In the model, the AT1 receptor has no dominant role in the completion of these ventricular remodeling processes.

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References


