Role of subendocardial Purkinje network in triggering torsade de pointes arrhythmia in experimental long QT syndrome

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Aims The present study addresses the controversy regarding the ‘primary’ role of the subendocardial Purkinje network in triggering torsade de pointes (TdP) ventricular tachyarrhythmia (VAs) in the long QT syndrome (LQTS).

Methods and results We investigated the well-established canine anthopleurin-A (AP-A) surrogate model of LQT3 to study the role of the subendocardial Purkinje network in triggering VAs. Three-dimensional activation and repolarization patterns were analysed from unipolar extracellular electrograms utilizing 64 plunge needle electrodes. In 6 dogs, the animals were placed on cardiopulmonary bypass and chemical ablation of the endocardial Purkinje network was obtained using Lugol’s solution. Spontaneous VAs consistently developed in response to AP-A infusion and were triggered by a subendocardial focal activity acting on a substrate of spatial three-dimensional dispersion of repolarization. Endocardial ablation was considered successful by the development of complete atrioventricular block in the absence of ventricular escape rhythm. Following endocardial ablation spontaneous VAs were no longer observed. However, an appropriately coupled premature stimulus consistently induced re-entrant VAs.

Conclusion The present study strongly suggests that in the LQTS, focal activity generated in subendocardial Purkinje tissue is the primary, if not the only, trigger for TdP VAs by acting on a substrate of three-dimensional dispersion of myocardial repolarization to induce re-entrant excitation.

KEYWORDS Arrhythmias; Long QT syndrome; Mapping; Repolarization; Early afterdepolarizations; Re-entry; Ablation

Introduction Both congenital and acquired long QT syndrome (LQTS) are caused by abnormalities (intrinsic, acquired, or both) of the various ionic currents underlying ventricular repolarization.¹ Prolongation of repolarization is a key priming step for the generation of early afterdepolarizations (EADs). Prolonged repolarization is also associated with increased dispersion of myocardial repolarization. The focal EAD-triggered wavefront can infringe on the underlying substrate of inhomogeneous repolarization to initiate re-entrant excitation in the form of rotating scrolls.²

The exact role that EADs play in the torsade de pointes (TdP) tachyarrhythmia has been controversial. One view contends that EADs generated primarily in the Purkinje network provided the first one or ‘few’ triggering beats of TdP, whereas the rest of the tachyarrhythmia is because of re-entrant excitation.² Another view suggests that TdP is sustained by repetitive rapid firing of EADs from several foci.³,⁴ Further, some authors maintain that EADs could arise from both Purkinje and myocardial fibres in vivo.⁵

The present study was planned to address the role of the Purkinje network in triggering TdP arrhythmia. The study utilized the original canine anthopleurin-A (AP-A) surrogate model of LQT3 and investigated the effects of chemical ablation of the endocardium on the arrhythmia and the three-dimensional dispersion of repolarization.

Methods Experimental model

The investigation conformed 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 surrogate canine experimental model of LQT3 utilized the neurotoxin AP-A that delays Na-channel inactivation and results in marked prolongation...
of action potential duration of both Purkinje and myocardial fibres. The experimental model was described several years before the first description of the clinical congenital LQT3 syndrome. The Na channelopathy of the congenital disease is almost identical to that of the acquired experimental model.

Surgical preparation
The present study was approved by the Animal Studies Subcommittee of the local institutional review board and conformed to the guiding principles of the Declaration of Helsinki. Experiments were performed on nine purpose-bred mongrel puppies 10–12 weeks old and weighing 3.5–5.0 kg. Puppies were pre-anesthetized with sodium thiopental (17.5 mg/kg IV) via the cephalic vein. Puppies were then intubated and anesthetized with 1.0–2.0% isoflurane (vaporized in 100% O2) via a positive ventilation anesthesia machine (F500; The Forreger Co.). Catheters were inserted into the femoral vein for administration of fluids and drugs and into the femoral artery to monitor the blood pressure. ECG leads I, aVF, and V1 and blood pressure (Statham transducer; Gould) were continuously monitored with a physiological recorder (VR12; PPG Industries). The heart was exposed through a midsternotomy.

Endocardial ablation
After systemic heparinization, the aygos vein was ligated and both venae cavae were cannulated individually for cardiopulmonary bypass. The femoral artery was cannulated for arterial perfusion. The animals were placed on total cardiopulmonary bypass, and bypass. The femoral artery was cannulated for arterial perfusion. After systemic heparinization, the aygos vein was ligated and both

Active drug dosing
AP-A was administered as an intravenous bolus of 25 μg/kg followed by a maintenance dose of 10 μg/kg per minute. Wild-type AP-A produced through a bacterial expression system was used in this study.

Data acquisition and isochronal mapping
Details of the mapping technique, calculation of activation–recovery intervals (ARIs), and construction of activation and repolarization isochronal maps have been previously reported. Sixty-four plunge needle electrodes, each consisting of four to eight unipolar electrodes with 1 to 2 mm inter-electrode distance, were used for three-dimensional mapping of both ventricles. Right ventricle plunge needles usually had four to six electrodes spaced 1 mm apart. On the other hand, interventricular septum plunge needles had eight electrodes spaced 2 mm apart. Unipolar electrograms were acquired using 2- or 3-variable gain 128-channel multiplexed data acquisition systems (DSC2000, INET Corp.), allowing simultaneous recording of up to 384 channels. The timing of selected landmarks in each activation and recovery complex was automatically computed and stored for later analysis. At the end of the experiment, the exact position of the electrodes was identified as previously described.

Stimulation protocol
Programmed ventricular stimulation was performed using a digital programmable stimulator (Medtronic 5325, Medtronic, Inc.) delivering square pulses (3 ms duration) through a bipolar plunge electrode placed in the right ventricle outflow tract.

Results
In all six dogs, complete endocardial ablation was considered successful by the development of complete atrio-ventricular block (AVB) in the absence of ventricular escape rhythm. Ventricular pacing was consistently maintained at a cycle length (CL) of 1000 ms. In all six experiments, one or more spontaneous polymorphic ventricular tachyarrhythmias (VAs) consistently developed in response to AP-A infusion prior to endocardial ablation. Consistent with previous observations, activation maps revealed VAs to be due to a premature subendocardial focal activity acting on a substrate of spatial three-dimensional dispersion of repolarization (i.e. ARIs) to initiate re-entrant excitation. Following endocardial ablation no VAs was observed. This was in contrast to the three dogs that served as control where no spontaneous VAs developed before or after endocardial saline painting.

Table 1A shows the average overall dispersion of ARIs from 10 different needles across the LV wall in three control experiments before and after saline painting. Table 1B shows data from the six animals that underwent endocardial ablation. The average overall dispersion from 10 different needles across the LV wall is listed during control, following AP-A administration and after endocardial ablation. AP-A resulted in significant increase in three-dimensional dispersion of ARIs. On the other hand, endocardial ablation had no significant effect on ARIs across the ventricular wall.
Table 1  Dispersion of activation-recovery intervals across left ventricle free wall at cycle length of 1000 ms

<table>
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<th>Dog no.</th>
<th>Pre-saline (ms)</th>
<th>Post-saline (ms)</th>
<th>Pre-saline (ms)</th>
<th>Post-saline (ms)</th>
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<td>Control experiments</td>
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<tr>
<td>1</td>
<td>25 ± 3</td>
<td>26 ± 5</td>
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<td>21 ± 7</td>
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<td>2</td>
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<td>3</td>
<td>28 ± 7</td>
<td>31 ± 5</td>
<td>24 ± 6</td>
<td>26 ± 6</td>
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<tr>
<td>Endocardial ablation experiments</td>
<td></td>
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</tr>
<tr>
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<td>85 ± 12</td>
<td>80 ± 11</td>
<td>80 ± 9</td>
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<td>26 ± 3</td>
<td>82 ± 9</td>
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</table>

AP-A, antipiotephrin-A; ARI, activation-recovery interval; LV, left ventricle. *Mean ± SD.

Figure 2 illustrates the results from one of the experiments following AP-A infusion. The figure illustrates eight unipolar electrograms recorded from one of the needles in the free LV wall. Figure 2A and B is consecutive and illustrate the spontaneous development of a short run (A) and a long run (B) of VA following AP-A infusion. The numbers in Figure 2B represent ARIs across the LV wall. All ARIs were markedly prolonged compared with control prior to AP-A administration (not shown). The longest ARIs were in mid myocardial wall and shortest in epicardial and subepicardial regions. The overall dispersion of ARIs across the LV wall was 80 ms. Of perhaps greater importance than the overall dispersion of ARIs across the wall was the marked difference of ARIs in two contiguous sites spaced 1 mm apart. For example, there was a 41 ms difference between myocardial sites #3 and #4. Figure 2C shows recording from the same needle following endocardial ablation and demonstrates the absence of any significant changes in ARIs following ablation. Spontaneous VAs were no longer observed.

Figure 3 from the same experiment illustrates the three-dimensional activation map of the initiating beat of the VA in Figure 2B. The initiating beat arose as a focal activity from an endocardial site and initiated re-entrant excitation. This is further illustrated by the recording in the left panel of Figure 3 showing selected electrograms along the re-entrant pathway.

Following endocardial ablation, we investigated if VAs could still be induced by programmed premature ventricular stimulation. In all six experiments, programmed stimulation succeeded in inducing VAs. Three-dimensional mapping showed that the arrhythmias were the result of the premature stimulus acting on the AP-A-induced substrate of three-dimensional dispersion of repolarization to initiate re-entrant excitation.

Figure 4 from the same experiment shown in Figures 2 and 3 illustrates electrocardiographic recordings following endocardial ablation. Figure 4A illustrates control S1 ventricular pacing at CL of 1000 ms. An S2 stimulus was introduced at CL of 350 ms and failed to induce VAs. Note that following S2, there was complete AVB and no ventricular escape rhythm occurred prior to the resumption of ventricular pacing at the end of the recording. In Figure 4B, the S2 stimulus was introduced at a shorter CL of 340 ms and resulted in the induction of VAs.

Discussion

There is a wide consensus that TdP is initiated by EADs. However, there is some controversy as to whether the tachyarrhythmia is sustained by repetitive rapid firing of EADs from several foci or EADs account for the initiation of the arrhythmia. In the latter case, the tachyarrhythmia will be because of interaction of the EAD-triggered premature activation with an underlying substrate of dispersion of repolarization. The later mechanism was shown in simulation studies and was documented in the canine LQT3 model utilizing three-dimensional mapping of activation.

The original experimental study of the canine surrogate model of LQT3 has addressed this issue. Utilizing a Purkinje-muscle preparation, it was shown that EADs arose...
from Purkinje fibre, and conducted to overlying myocardium with varying degrees of conduction delay through Purkinje-muscle junctions. In two more recent studies, the role of Purkinje network in TdP was investigated in experimental models of LQT-2 and LQT-3 by chemically ablating the endocardium. The two studies came to different conclusions. In one study, ablating the endocardium did not abolish the ‘spontaneous’ development of TdP. In the other study, ablating the endocardium abolished the ‘spontaneous’ development of TdP, but the arrhythmia could still be induced by premature stimulation acting on a substrate of dispersion of repolarization to initiate re-entrant excitation. The two studies utilized epicardial mapping of optical action potentials. Optical mapping techniques provide more direct and possibly more accurate data on evaluation of the spatial changes in cardiac repolarization compared with extracellular electrograms. However, because of its essentially two-dimensional recording nature, optical mapping is currently incapable of analyzing three-dimensional properties of cardiac repolarization. The different results could be attributed to the difference

Figure 3 Right panel illustrates the three-dimensional activation map of the initiating beat of the ventricular tachyarrhythmia shown in Figure 2B. In this and subsequent figures, the isochrones were drawn as closed contour at 20-ms intervals and labelled as 1, 2, 3, and so on to make it easier to follow the activation pattern. Functional conduction block is represented in the maps by thick solid lines. The initiating beat arose as a focal activity from a sub-endocardial site and initiated re-entrant excitation. The left panel illustrates selective electrograms along the re-entrant pathway.

Figure 4 Recordings from the same experiment shown in Figures 2 and 3 that illustrate electrocardiographic tracings following endocardial ablation. Although spontaneous ventricular tachyarrhythmia (VA) did not occur following ablation, VA could be induced by a critically coupled premature stimulus, S2 (B) introduced during S1, ventricular pacing at CL of 1000 ms. Note that in (A) there is a complete AV block and no ventricular escape rhythm in the absence of ventricular stimulation at the end of the recording.
to either triggered activity (early or delayed afterdepolarizations) or abnormal automaticity under β-adrenergic stimulation, ischemic conditions, and calcium overload. There is evidence to suggest that ectopic activity is more easily generated in Purkinje rather than myocardial fibres. Although Purkinje tissue may overlie ventricular muscle throughout large regions of the ventricles, electrical connections between Purkinje and ventricular cells exist only in restricted regions of Purkinje-muscle junctions. The margin of safety for anterograde conduction at the Purkinje-muscle junction is low and differential conduction delays at two different junctions and/or anterograde block at one junction may create the necessary conditions for a one-dimensional ring-like circuit.

The relative ease by which focal activity initiated at the Purkinje network could conduct to capture myocardium is contrasted with the difficulty of the induction and propagation of focal activity in myocardium. A major problem in considering a myocardial focus as the genesis of TdP arrhythmia is that while the presence of a coupling conductance between the automatic focus and the surrounding cells is necessary for propagation out of the focus region, this coupling conductance may also suppress the activity of the focus region by electrotonic interactions during the diastolic depolarization phase of the focus cells. This loading effect depends on many factors including the size (number of cells) of the focus region, the intercellular coupling among the focus cells, the input resistance of the surrounding quiescent cells, and the value and spatial orientation of the coupling conductances among the surrounding cells. Further, the inhomogeneity of the focus region both in terms of membrane properties as well as the distribution of cellular coupling may play an important part.

Limitations

Although it is quite likely that the initiating beats of TdP, characterized in the present mapping technique as subendocardial in origin, were generated from the Purkinje network there was no actual recordings from Purkinje. Because the initiating beat of TdP could arise from anywhere within the Purkinje network, it would be very difficult to consistently record Purkinje potential preceding such beats even with a high-resolution mapping system. Also, because Logol’s solution could ablate both Purkinje fibres and endocardial tissue we cannot role out the contribution of endocardial cardiomyocyte. On the other hand, the study does not exclude the possibility that successive EADs from multiple Purkinje foci may be both the initiators as well as the perpetuators of TdP. In a previous report from this laboratory, this mechanism was considered unlikely based on both theoretical and experimental data. Although it is customary for rapid succession of EADs to be generated from phase 2/early phase 3 of action potentials in isolated Purkinje strands subsequent to manipulations that prolong AP duration, the situation is different in the in vivo heart. EADs induced in Purkinje fibres in the LQTS are bradycardia-dependent. The initiation of TdP arrhythmia is implicitly associated with shortening of successive cardiac cycle lengths that should mitigate against the induction of further EADs unless some Purkinje foci have entrance block but no exit block. This situation is probably conceivable only for one or very few subsequent beats.
this was occasionally demonstrated in mapping studies of this model, but in the majority of TdP only the first beat was of subendocardial origin, whereas successive beats were the result of re-entrant excitation.

Conclusions

The present study strongly suggests that in the LQTS, the focal activity generated in subendocardial Purkinje tissue is the primary, if not the only, trigger for TdP by acting on an underlying substrate of three-dimensional dispersion of myocardial repolarization to induce re-entrant excitation. This mechanism is further documented by the ability to induce re-entrant excitation by an appropriately coupled premature stimulus following complete ablation of endocardium.

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Conflict of interest: none declared.

References