Intramural conduction system gradients and electrogram regularity during ventricular fibrillation

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ABSTRACT

Introduction: The His-Purkinje system has been shown to harbor triggers for ventricular fibrillation (VF) initiation. However, the substrate responsible for VF maintenance remains elusive. We hypothesized that standard, electrode-based, point-to-point mapping would yield meaningful insight into site-specific patterns and organization which may shed light on the critical substrate for maintenance of VF.
Methods: VF was induced under general anesthesia by direct current (DC) application to the right ventricle in 7 acute canines. A standard EPT Blazer mapping catheter (Boston Scientific, Natuck, MA) was used for mapping in conjunction with a Prucka recording system. We collected 30 consecutive electrograms at 24 distinct sites, confirmed by fluoroscopy and intracardiac echo. These sites included both endocardial and epicardial locations throughout the ventricles and conduction system.
Results: A total of 5040 individual data points were collected in 7 separate canine studies. During VF mapping, a transmural disparity was found between the epicardium (average cycle length [CL] of 1136 m s) and the endocardium (average CL of 123 m s) with a p value of <0.01. An additional, intramural gradient was found when comparing the proximal, insulated conduction system to the distal, non-insulated conduction system (average CL 218 versus 111 m s [p = 0.03]).
Conclusion: Our data are supportive of a novel observation of intramural difference between insulated and non-insulated regions of the His-Purkinje network in canines. In addition, certain areas exhibited periods of regular electrogram characteristics; this was despite the heart remaining in terminal VF. These early canine data merit further study to investigate if specific ablation of the distal conduction system can perturb or extinguish VF.

1. Introduction

Ventricular fibrillation (VF) affects as many as 400,000 people per year, making it the number one cause of death in the United States [1]. At present, there is no cure for VF. This is largely due to a lack of complete understanding of the mechanism of this dysrhythmia [2]. Currently, the most effective treatment option is the implantable cardioverter-defibrillator (ICD), which, although lifesaving, does not prevent future VF [3–5]. Medications, including Amiodarone, Lidocaine, and Epinephrine, are utilized most commonly in shock-resistant VF occurring in-hospital, but these medications have not demonstrated significant long-term benefit outside of the initial resuscitation period [4,6,7]. Radiofrequency (RF) ablation has been explored; however, it is currently sub-

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optimal for curative purposes, as the critical substrate necessary for VF maintenance remains elusive [8—10].

Research identifying the triggers for VF has shown premature ventricular contractions (PVCs) that originate in the His-Purkinje network as being the potential culprit [8,9,11,12]. However, these findings are difficult to implicate clinically due to the extensive and pervasive nature of the His-Purkinje system [13—15]. Meanwhile, even less is known about the substrate for the maintenance of this lethal arrhythmia [16]. While Jalife et al. have used optical mapping in Langendorff-perfused hearts to demonstrate that the His-Purkinje system plays a role in VF sustainability, this area still remains largely under-investigated. Studies have revealed areas of organization in the heart during VF and suggested regions of relative periodicity at the epicardial surface of the heart; however, regional differences within the endocardium have not been researched to date [16,17].

As such, we sought to analyze differences in VF organization throughout the heart. We hypothesized that standard, electrode-based, point-to-point mapping would yield meaningful insight into site-specific patterns during VF, thereby indicating potential areas of critical substrate required for VF maintenance. We also hypothesized that areas most critical for VF arrhythmogenesis would demonstrate more rapid, more regular, and more near-field signals than other areas, given that drivers of other arrhythmias tend to display these characteristics [18].

2. Methods

2.1. Model for studying inducible VF

Studies were conducted in acute canines that had undergone electrophysiologic experimentation in the Mayo Clinic Innovations Laboratory. These canines were from previously completed, IACUC-approved experiments, at the end of which humane euthanasia was performed under general anesthesia with the application of direct current to the endocardium or epicardium. After the application of direct current, no attempt was made to revive or sustain the heart. VF was allowed to continue until the heart stopped completely. Immediately after VF induction, an electrode catheter was placed in various locations as outlined below to collect electrograms for mapping purposes.

2.2. Electrophysiologic study

We used a bipolar, 5-mm tip, electrical mapping catheter (EPT Boston Scientific ™ Marlborough, MA) for point-to-point mapping. The catheter was placed in multiple sites throughout the ventricular endocardium and epicardium. The epicardial surface was accessed through a sub-xiphoid puncture as previously described by Sosa et al. [19]. A summary of the categorization and characteristics of these sites is found in Table 1. After mapping was completed, the recorded electrograms were then stored digitally on

a multi-channel recording system (Prucka Cardiolab system, GE Healthcare, Wauwatosa, WI). Electrograms were recorded with band pass filters between 0.5 and 1000 Hz. The notch filter was programmed off. These filter settings were optimized to allow for the detection of Purkinje-related signals and to prevent signal clipping that hindered measurement of amplitude. Stored signals were analyzed manually using the software's digitized measurement tools.

Because this experiment was conducted in hearts undergoing long-duration VF, changes in signal characteristics over time were anticipated. To reduce bias across studies, the order of acquisition was randomly varied between experiments. In 3 canines, signal collection began in right-sided endocardial locations, in 2 experiments data was first collected from left-sided sites, and in 2 canines epicardial sites were measured first. The site of VF induction was also varied, with VF originating endocardially in 4 canines and epicardially in 3.

2.3. Signal analysis and measurements

Electrograms were analyzed in blocks of 30 consecutive signals at each of 24 distinct sites. Amplitude (mV) was evaluated on the Cardiolab system using automated calipers to manually measure from the peak to the trough of each signal. Cycle length was determined by taking the peak of one electrogram waveform to the peak of the next. We assumed that longer cycle lengths would be more indicative of regular rhythm in the heart, and shorter cycle lengths would be more likely to be characteristic of VF. Thus, the slew, or slope, of the electrograms was analyzed as the change in voltage (dV) over the change in time (dt), in mV/ms. The dV was assumed to be the same value as the amplitude measured earlier, while the dt was measured as the distance between the beginning of the wave and its peak.

Regularity of rhythm was defined using the standard deviation of 30 consecutive cycle lengths and was interpreted in our study as a representation of the level of organization in the heart. After a standard deviation was calculated, regularity was defined as the percentage of measured signals within one standard deviation of the mean cycle length value. Regularity was analyzed in the heart by separating study locations into cardiac sites with the top 10% highest slew and all other sites, and comparing the two groups. Two authors worked simultaneously on data extraction and achieved consensus on collection technique as well as measured values.

3. Results

Data collection in seven acute canine experiments resulted in a total of 5040 unique and individual data points. Overall, VF was sustained for an average of 37 min following induction [range; 20 min—67 min]. The average cycle length from all sites was 256 m s ($\pm$292.7); the average amplitude was 1.53 mV ($\pm$0.98); and

Table 1
Anatomical sites studied during Ventricular Fibrillation in acute canine model.
Various sites included in this study were categorized according to these 4 parameters. The sites measured included a variety of regions within the heart, including conduction and muscle tissue, and both endocardial and epicardial surfaces. Some categorizations, such as Purkinje and epicardial sites, contained several distinct locations within the category.

Endocardium(Right and left ventricle)	Endocavitary	Conduction system	Epicardium
Anterior Wall	Moderator band	Purkinje	Left Ventricle
Septum	Papillary muscle	Left Bundle	Right Ventricle
Apex		Right Bundle	
Free Wall		His Bundle	
Annulus			

the average slew was 0.06 mV/ms (±0.01). In addition, the average regularity was determined to be 90.04% (±18.5).

We observed site-specific variations across all parameters. A gradient, or regional variation in electrogram characteristics, could be seen between the endocardium and epicardium (Fig. 3). Noticeable organization was seen in the epicardium as compared to the rest of the heart, which continued to display fibrillatory rhythms on the surface ECG. The difference in average cycle length between endocardial and epicardial sites was found to be 123 m s vs. 1136 m s, respectively, p = 0.00009).

Within the His-Purkinje network, a difference was detected between the proximal and distal conduction system in terms of electrogram characteristics. The average cycle length of the proximal system was 218.2 m s versus 111.3 m s for the distal conduction system sites (p = 0.03). Notably, the amplitude was lower in the proximal conduction system at 0.714 mV, as opposed to 1.53 mV in the distal (p = 0.0005).

Electrogram characteristics were also compared between right and left ventricular endocardial sites, although no statistically significant difference in any of the parameters were found. The cycle length in the right ventricle was found to be approximately 117.8 m s versus 130 m s in the left ventricle. The same was found for amplitude (1.5 mV vs. 1.7 mv) and slew (0.04 mV/ms vs. 0.06 mV/ms) (p > 0.05 for all).

Regularity of separate sites was compared in the top 10% of sites with the highest slew versus the rest of the sites. This analysis indicated that sites with higher slew had an average regularity of 85.96%, while the sites with lowest slews had an average of 65.24%, suggesting a correlation between higher slew and higher regularity.

4. Discussion

We have demonstrated 3 salient findings in this work: 1) establishment and testing of a "real life" large animal model for examining VF, 2) a gradient between the endocardium and the epicardium during VF, and 3) a difference between the proximal and distal conduction systems during VF.

4.1. Euthanized canine model for evaluation of VF

Our study supports that a euthanized canine model for VF using electrode-tipped catheter mapping is viable for investigation of VF. One of the standard methods for animal experiment termination is euthanasia through the induction of ventricular fibrillation. It is important to note that the animals had undergone a prior study so we are not able to exclude any inherent effects related to the experiments on VF. However, as these were varied in a non-uniform manner, it is equally unlikely to cause the same effect in VF mapping.

This study model is more reflective of actual clinical VF when compared to previous models, which often involve studying tissue post-mortem which can induce changes in tissue and cellular conduction characteristics. Some previously used techniques, such as the elimination of the conduction system with Lugol's iodine or the use of voltage-sensitive dyes in optical mapping, are toxic to the heart and therefore can only be used in isolated heart models [20,21]. Others have utilized Langendorff-perfused hearts and VF of shorter duration than seen in this experiment [2,16,17,22,23]. In addition, tissue procurement procedures can also make it difficult to interpret studies and more importantly, translate this to "real-life" clinical VF. Our experimental model is novel in this regard and could serve as a strong option for future studies as we move towards a better understanding of VF. Further investigation may benefit from alternate methods of VF induction, such as coronary artery occlusion, to more fully simulate naturally occurring fibrillation in patients.

4.2. Periodicity

Previous models of this arrhythmia have often regarded VF as completely chaotic or turbulent electrical activity in the heart [24]. However, our data lend support to the idea that the heart, even while in VF, has regions that are much more organized than previously suspected. The electrograms we observed seemed to suggest near-sinus rhythm in certain areas, especially at epicardial sites, whereas very rapid but somewhat regular waveforms in endocardium were found (especially at sites involving the His-Purkinje conduction tissue (Fig. 1)). These patterns and the existence of significant organization during fibrillation may help support more recent theories regarding VF, including rotors, spirals, scroll waves, and relatively organized myocardial activity [25]. Some authors, including Latcu et al. and Thomas et al. have suggested previously that the mechanism of VF is dependent on relatively stable or periodic sources, with specific emphasis on intracardiac and endocardial sites [34,35].

4.3. Transmural gradient

A transmural gradient was detected between the epicardial and endocardial surfaces of the heart (Fig. 2). Previous research has suggested the possibility of such a difference, although it has never been definitively confirmed with ventricular electrograms [26,27]. In some studies, it should be noted that the gradient was seen more significantly in canines than it was in swine. Our findings are in line with this already existing data in that the epicardium is more regular and displays fewer characteristics of an arrhythmogenic substrate than the endocardial surface. This suggests that the epicardium plays a non-essential role during VF and may therefore not contribute to its maintenance. Therefore, the substrate for VF may likely be located within the endocardium. Key differences between the endocardium and epicardial surfaces that could contribute to this phenomenon include action potential differences between the two layers, relative ischemia because of contractile forces, wall tension, epicardially located coronary arteries, and significantly more pervasive presence of the Purkinje on and in the endocardium [28,29]. Our use of point-to-point mapping at known conduction tissue sites and continued observation of conduction tissue-like signals cause us to favor the latter as a putative culprit arrhythmogenic difference.
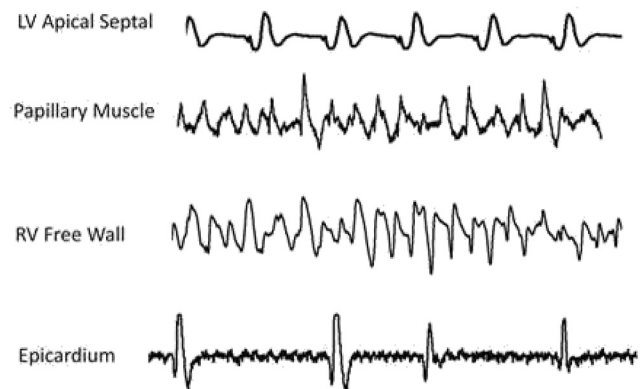

Fig. 1. Example of Electrogram showing site-specific differences during VF.
Examples of ventricular electrograms during ventricular fibrillation throughout the study period. Notable differences include the regularity of endocardial sites versus endocardial sites. The relatively fast but somewhat regular sites near the conduction system are shown for comparison.
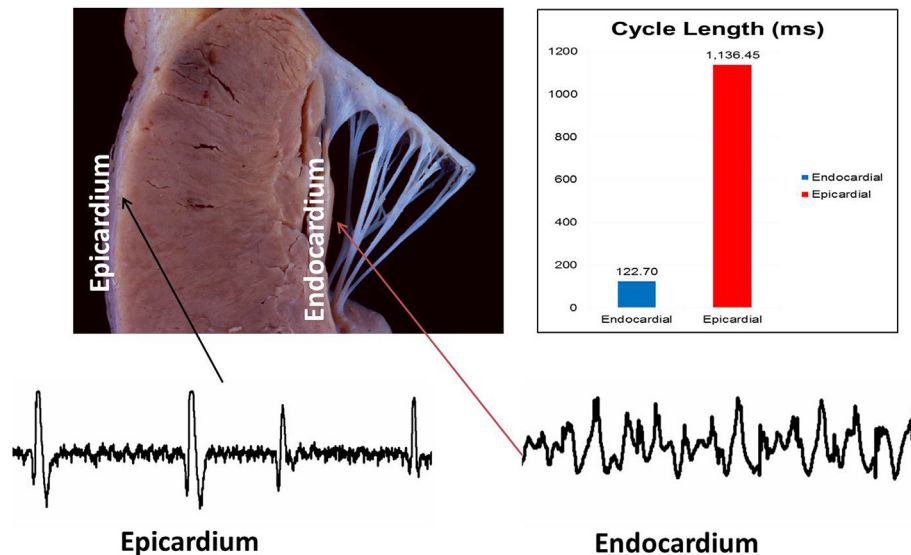
Fig. 2. Transmural gradient exists between endocardium and epicardium during VF.
Examples of electrograms from the epicardium and endocardium are shown for comparison. Panel one shows bar graph that displays the significant difference in cycle length between the two surfaces.
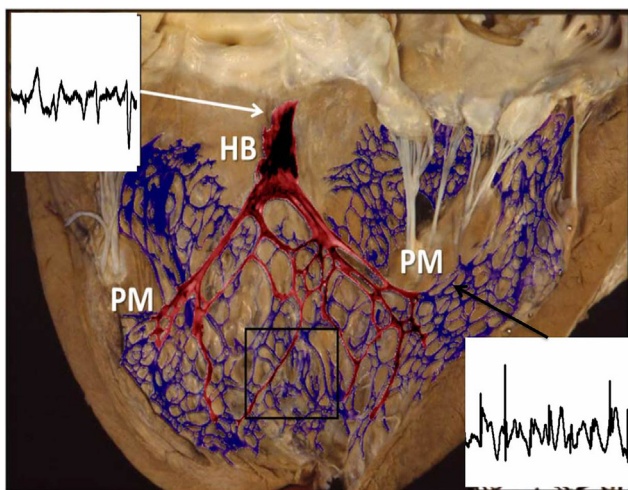
Fig. 3. Intramural gradient exists between the proximal and distal conduction system during VF.
A calf heart is shown with colored staining to define the regions of the His-Purkinje network. The His-Purkinje network using stained using India ink. The area stained in red highlights the proximal conduction system (His bundle and bundle branches), while areas stained blue comprise the distal conduction system. Representative electrograms from these regions of the conduction system are shown for comparison.
(*Adapted from*: Sebastian et al., IEEE TRANSACTIONS ON MEDICAL IMAGING, VOL. 32, NO. 1, JANUARY, 2013).

4.4. Intramural gradient

An intramural gradient was noted between the distal and proximal conduction system in our study. The proximal conduction system, which is made up of the His bundle, right and left bundles, and bundle branches, is covered by a sleeve of fibrous insulation that prevents electrical activity from reaching the muscle (Fig. 3). Shorter cycle lengths detected in the distal conduction system could mean that the distal system comprises the culprit substrate behind VF. Although the mechanism behind this association requires further study, a possible explanation is that multiple simultaneous reentrant circuits are readily sustainable and can be

created in the meshwork of non-insulated Purkinje fibers but are impossible at sites where the Purkinje is insulated. This finding could have major clinical significance for future innovative therapies for VF. With this new insight into the more specific mechanisms of VF, ablation therapy could be used to eliminate the distal conduction system while keeping the proximal conduction system intact and minimizing risk of conduction block for the patient.

Further evidence for the endocardial substrate specifically being Purkinje was presented by the relationship between regularity and slew. Areas with higher slew in the endocardium were assumed to be associated with Purkinje firing, and these sites also showed greater amounts of organization. This could be analogous to studies that have been conducted in atrial fibrillation, where rapid, regular triggers in the pulmonary vein created irregular atrial fibrillation [30]. Similarly, in VF, fast but organized activity in the distal conduction system could result in irregularity in other sites.

We noted little to no disparity between right and left ventricular endocardial sites. Though past research has suggested the possibility of more active conduction in the left ventricle, our research seemed to indicate no significant difference between the two regions [12–14,31]. This is in keeping with the other results of this study, given that Purkinje fibers are present in both ventricles [15,32].

4.5. Incremental knowledge of VF arrhythmogenesis

Previous research, largely completed in extracted and perfused hearts, has yielded similar results. Jalife et al. used voltage-sensitive dyes and optical mapping techniques to identify spatial and temporal organization during VF. These findings were supported by our study's detection of high levels of periodicity even when the heart appeared to be undergoing a chaotic rhythm via surface ECG. A study by Tabereaux et al. completed in Langendorff hearts, detected Purkinje activation during VF and suggested an important role of the His-Purkinje network in VF maintenance [33]. Both of these methods differ from our own because of their use of isolated hearts and VF durations of less than 10 min, while our model mapped intact hearts undergoing long-duration post-mortem VF, as well as ischemia as the method of VF as opposed to direct current [6,12,33]. An additional study by Angel et al. determined that activation

patterns could be seen in the proximal conduction system during VF, an observation consistent with our own findings [23]. However, no comparison was made between the distal and proximal portions of the conduction system, which when completed in our study revealed a significant disparity in all parameters.

Future steps to build upon the existing understanding of VF include the development of better targeted therapies for this dysrhythmia. Ongoing studies are being completed to investigate the efficacy of electroporation ablation of the distal conduction system. We also intend to co-validate our findings with those suggested by studies utilizing Langendorff models. Promising trends have been observed when mapping VF in human patients with left-ventricular assist devices (LVADs).

4.6. Limitations

This model utilized a direct current application method for the induction of VF, which may differ from that which occurs in normal patients. However, studies done previously in perfused hearts have suggested similar findings, which may demonstrate validity [2,16,17,22,23]. In addition, experiments could not always be conducted over the same time interval, due to variance in the duration of VF after induction, ranging from 20 min to over an hour. It was observed that amplitude and slew decreased significantly as ventricular fibrillation continued, although cycle length stayed within the same range. To attempt to reduce bias in our studies, we randomized the order in which locations were measured between experiments. This helped eliminate the possibility that observed gradients were the result of certain sites simply being measured earlier than others. Lastly, it is important to note that our findings were in canines and thus cannot be extrapolated directly to human VF without further study, tempering the conclusions presented here.

5. Conclusion

In this study, we demonstrate the feasibility of an acute canine model in catheter-based mapping of VF. Our data suggest that a transmural gradient exists between the epicardium and the endocardium during VF. In addition, we also found an intramural gradient between the proximal and distal conduction systems. These findings, in conjunction with the ever-growing body of VF literature, may provide additional insight for elucidating the mechanism of VF, especially the potential role of the distal conduction system in its maintenance.

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