Dynamically-Induced Spatial Dispersion of Repolarization and the Development of VF in an Animal Model of Sudden Death

ARM Gelzer[1], NF Otani[2], ML Koller[3], MW Enyeart[2], NS Moise[1], RF Gilmour Jr[2]

[1]Department of Clinical Sciences, Cornell University, Ithaca, NY, USA
[2]Department of Biomedical Sciences, Cornell University, Ithaca, NY, USA
[3]Department of Cardiology, Bad-Neustadt, Germany

Abstract

Spatial dispersion of refractoriness and discordant action potential duration (APD) alternans, resulting in local conduction block, have been shown to cause wavebreak that can lead to ventricular fibrillation (VF). Previously, we developed a theory, based on action potential restitution functions, that predicts when the requisite conduction block can be created through a series of premature beats. The theory was applied successfully to normal beagle dogs; however, restitution functions in these animals were similar, both between right and left ventricles in a given animal and across animals. Consequently, for the present study we tested the theory on a population of German shepherds that, due to inherited cardiac abnormalities, presented with a wide variation of APD restitution functions. We found that the theory, when applied to restitution functions determined individually for each animal, reliably generated premature stimulation predictions that frequently resulted in the induction of VF in in vivo experiments.

Evidence from computer modeling [1] and experimental studies [2] suggests that the precipitating event for ventricular fibrillation (VF) is the block and subsequent breakup of action potential wave fronts. A recent theory [3] predicted that the requisite conduction block could be created through a short series of premature beats, with the specific beat intervals determined by the conduction velocity (CV) and action potential duration (APD) "restitution functions"—that is, the dependence of these two quantities on the preceding diastolic interval (DI). The predictive power of this theory was recently confirmed in normal beagle dogs *in vivo* [4]. Specifically, a short-long-short-short (SLSS) sequence of premature beats predictably resulted in VF induction, presumably through maximized spatial dispersion of refractoriness and induced discordant APD alternans, resulting in local conduction block. Differences exist both within a given ventricle and between ventricles of different dogs in a normal dog population [5], but APD restitution relations of the beagle dogs used in our study were remarkably similar between dogs, and resulted in similar beat-interval predictions [4].

We hypothesize that the premature stimulation predictions generated by our computer model to dynamically induce block and subsequent VF in normal dogs are valid also in the setting of underlying cardiac abnormalities. To test this idea, we studied German shepherd dogs (GSD) with an inherited predisposition to ventricular arrhythmias (VA) and sudden death [6]. This dog population exhibits marked alterations in the ionic currents responsible for repolarization [7, 8], spatial heterogeneity of repolarization among different regions of the left ventricle [9], and abnormal heterogeneous sympathetic innervation of the myocardium [10]. Due to these documented abnormalities, we expected that the restitution relations of these GSD would exhibit more variation compared to normal beagles. Thus, we sought to investigate whether the "generic" prediction scheme for dynamical induction of VF developed for the beagles was adequate to make predictions in any dog, or if, instead, a scheme based on our theory, tailored to the specific dog being studied, would enhance the predictability of the VF induction.

1. Materials and methods

We studied 14 intact GSD (9 males, 5 females; median body weight 28 kg, range: 22–31 kg; median age 6 months, (range 5–11 months), raised at Cornell University. Dogs were selected based on the presence of ventricular premature complexes (>6000 VPC/ 24h) on a 24 h Holter acquired at 4 months of age. Two sets of experiments were performed in 2 groups of affected GSD. In Group A (n=8) experiments were performed to test the predictions resulting from the empirical model of Fox *et al.* [1], incorporating normal beagle restitution data. Because the results obtained in Group A dogs did not agree well with the predictions of Fox *et al.*, a second set of experiments was conducted in Group B dogs (n=6), using APD restitution data

acquired in each ventricle of individual GSD at the time of study. These data also were used to generate predictions based on the theoretical model of Otani [3]. Right and left ventricular (RV, LV) monophasic action potential (MAP) recordings were acquired in closed-chest anesthetized GSD. Programmed stimulation was performed to (1) measure the effective refractory periods (ERP) of 1–4 premature stimuli (2) obtain the APD restitution function from a dynamic pacedown protocol and (3) test whether the premature stimulus intervals predicted by the computer model—(a) based on a single, normal beagle APD restitution function or (b) based on ventricle-specific GSD restitution data—would produce conduction block and VF induction *in vivo*. The experimental preparation of both groups was identical and the pacing protocols to determine ERP, APD restitution function and calculation of stimulus intervals theoretically predicted to produce block are described in detail elsewhere [4]. A likelihood ratio test with a generalized estimating equation approach to logistic regression (GENMOD procedure in SAS ($p<0.05$)) was applied to assess the significance of the association between our theoretical predictions and the outcome in the experiments.

2. Results

For the Group A experiments, we tested induction of VF using a SLSS premature stimulation protocol in 8 affected GSD. In 3 of 8 dogs, both the RV and LV were tested (n=11 VF induction trials). VF was inducible in only 4 of the 11 ventricles using the "beagle SLSS" stimulus pattern. Due to this relatively poor inducibility in GSD, we performed subsequent analysis of restitution data in these GSD, which revealed variable APD restitution functions and slopes in the different dogs and ventricles. Post-experiment generated computer predictions for induction of VF based on these restitution data confirmed that the "beagle SLSS stimulation protocol" would have a low probability of inducing VF in 5/7 failed VF inductions. This prompted a systematic testing approach in GSD. For the Group B experiments, we tested induction of VF in 6 affected GSD. Rather than use previously recorded *in vitro* data for the APD restitution relation from beagles, APD restitution was measured from each ventricle *in vivo* at the time of the study. Because CV restitution could not be measured *in vivo*, the CV restitution relation determined previously *in vitro* was used for the modeling [1]. The predictions for this set of experiments were generated by running a coupled maps model (Eqs. (17–19) of [3]) using all possible combinations of ΔDI_2, ΔDI_3, ΔDI_4 and ΔDI_5 between 0 and 70 ms in 1 ms increments, where $\Delta DI_x = DI_x - DI_{min}$, DI_x is the diastolic interval preceding stimulus S_x, for x = 2,3,4 and 5, and DI_{min} is the shortest DI allowing conduction of the subsequent premature beat. Here S_2, S_3, S_4 and S_5 represent up to four premature stimuli used in the simulations and experiments. The simulations generated all combinations of ΔDI_2 and ΔDI_3 that produced block of the S_3 wave, all combinations of ΔDI_2, ΔDI_3 and ΔDI_4 that produced block of the S_4 wave, and the largest value of ΔDI_5 that produced block of the S_5 wave for every combination of ΔDI_2, ΔDI_3 and ΔDI_4.

Because the testing of thousands of stimulus combinations in an *in vivo* experiment is unfeasible, we categorized each premature stimulus from each combination according to whether the preceding DI was short (S) (ΔDI between 0 and 10 ms), intermediate (I) (ΔDI_2, ΔDI_3 and ΔDI_5 between 5 and 50 ms, ΔDI_4 between 5 and 20 ms) or long (L) (ΔDI_2, ΔDI_3 and ΔDI_5 between 50 and 70 ms, ΔDI_4 between 20 and 40 ms). Subsequently, "stimulus combination categories" were formed. Categories contained between 1 and 4 premature stimuli. For example, the "SLSS" category contained all combinations of stimuli for which ΔDI_2, ΔDI_4 and ΔDI_5 were between 0 and 10 ms, and ΔDI_3 was between 50 and 70 ms. We attempted to test as many of these stimulus categories as was experimentally feasible.

Individual APD versus DI analyses from GSD revealed marked variation in APD restitution function and slopes between dogs and between RV and LV. Figure 1(j) shows that the APD restitution function from the LV of one of the 6 GSDs was S-shaped, while the restitution function from the RV of the same dog (Fig. 1(k)) more closely resembled a simple exponential function similar to those obtained previously from normal beagles. Application of the computer algorithm [1] to these two restitution functions yielded strikingly different predictions regarding which patterns of premature stimuli should cause block, as illustrated, for example, by the substantial differences in the shape and location of the multicolored regions appearing in Figs. 1(g) and (h). In particular, the theory predicts that a SLLS combination of premature stimuli, as represented by the Point A in Fig. 1(g), should produce block. In contrast, for the RV, an SLSS pattern of premature stimuli (Point B in Fig. 1(h)) is predicted to create block. The SLLS pattern of premature stimuli induced VF in the LV, but the SLSS combinations did not (Point C), consistent with the theory. Conversely, in the RV, an SLSS combination induced VF, but the SLLS combination did not (Point D), again in agreement with the theory. Some GSD ventricles yielded relatively flat restitution functions (e.g., Fig. 1(l)), which reduced, but did not eliminate, the number of premature stimuli combinations predicted to produce block (Figs. 1(c,f,i)).

Agreement of experimental occurrences and non-occurrences of VF with theoretical predictions were generally excellent. There were two notable classes of disagreement. First, combinations containing the SS sequence in-

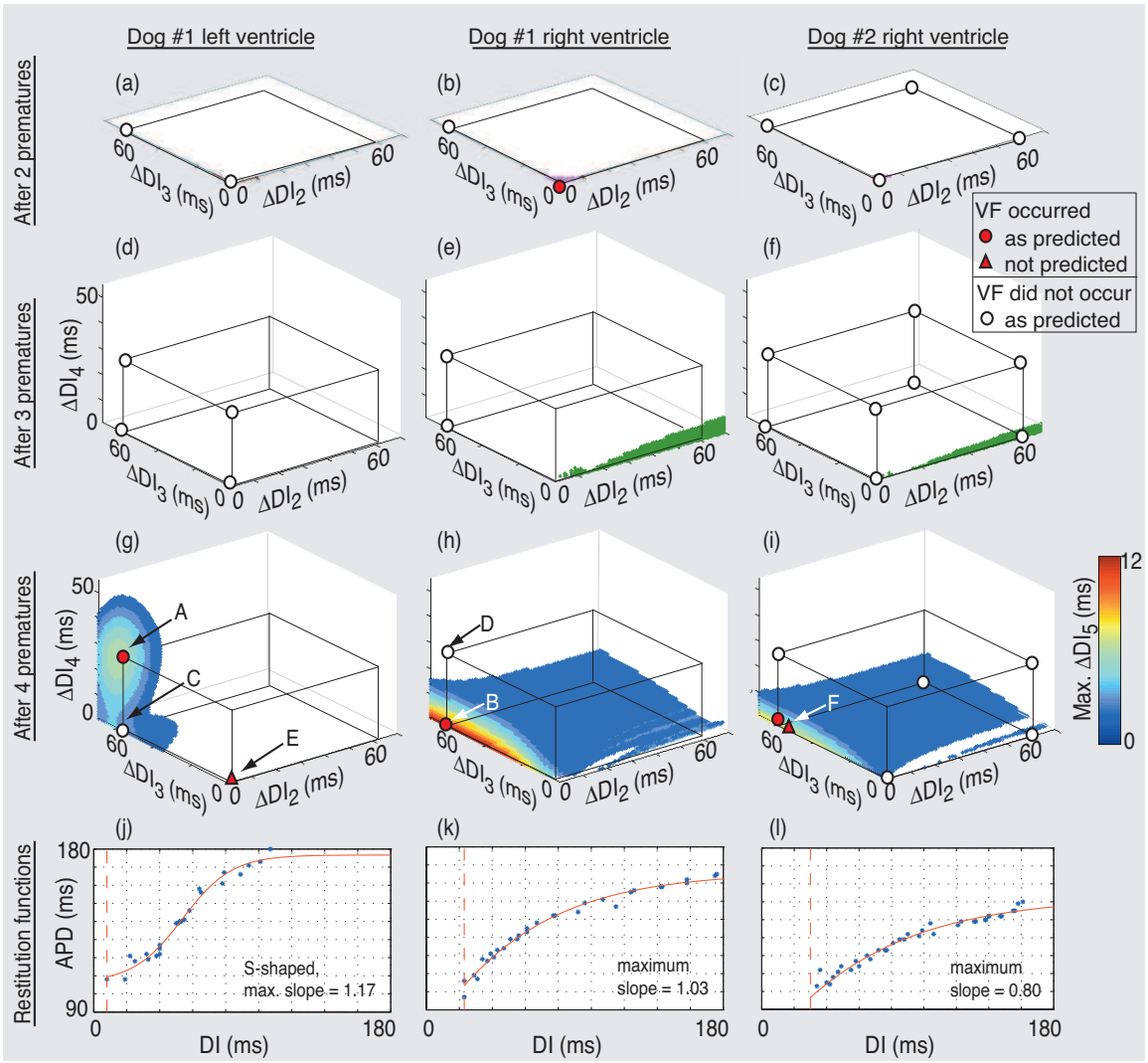

Figure 1. Comparison of experimental occurrences of VF to theoretical prediction. In panels (a–i), the location, shading and shape of the circular and triangular icons indicate, respectively, which categories of pacing interval combinations were tested experimentally, whether VF was inducible or not, and whether the outcome was predicted by theory or not (see legend). Panels (a–c) show the results for 3 of the 11 ventricles for the case of two premature intervals, with the purple (shaded) region of each plot marking those intervals theoretically predicted to produce block, thus theoretically rendering the tissue vulnerable to VF induction. Panels (d–f) show the results for these same 3 ventricles for three premature intervals, with the green (dark) region marking the combinations of the three intervals predicted to produce block. The rectangular lattice was drawn to clarify the perspective. Panels (g–i) show results for four premature intervals, with the location of points within the multi-colored region indicating the values of the first three intervals that can produce block and the color itself representing the maximum value the fourth interval can take and still produce block. Panels (j-l) display the restitution functions fitted using experimental data. The dashed vertical line shows the presumed value of DI_{min}.

duced VF 5 times (out of a total of 182 categories tested) when block was not predicted. One example is the SSSS combination indicated by Point E in Fig. 1(g). Second, combinations containing the SLS pattern yielded VF 6 times when block was not predicted, as was the case for the SLSL sequence shown as Point F. VF was not predicted for this point, despite its location in the VF-predicted region of Fig. 1(i), because blue and green colors of this region imply that the fourth interval must have DI < 6 ms to induce block, whereas the last interval of SLSL is "long." The point adjacent to Point F turns out to be a SLSS point; thus the VF resulting from that sequence was predicted. Other cases of unpredicted VF induction occurred 3 times (1 SLL and 2 LLSLs).

Each attempt to induce VF (n=182) was categorized by (1) the dog and (2) the ventricle in which the attempt was performed, (3) whether less than or greater than 25% of the stimulus combinations in the stimulus combination category used were predicted to produce block, and (4) whether or not VF was actually inducible in the ex-

periment. A generalized estimating equation approach to logistic regression was applied to these data for the event of VF induction using dog, ventricle and theoretical prediction as covariate, and using an exchangeable covariance matrix with dog as a repeated measurement. Applying the likelihood ratio test to this method, neither the effect of dog (p=0.36), nor ventricle (p=0.63) was statistically different from 0. However, the effect of the theoretical prediction was significantly different from 0 (p=0.03). The coefficient of prediction was 2.38.

3. Discussion

This study demonstrates that our computer model reliably generates premature stimulation predictions for induction of VF. The method appears robust not only in normal dogs, but also in the setting of intrinsic cardiac abnormalities, such as exhibited by GSD with spontaneous inherited VA and sudden death, if individual restitution properties are considered.

The high variability in APD functions in GSD explains why the standard "SLSS" stimulation protocol developed for normal beagles used in the Group A GSDs did not have a high probability of inducing VF. Use of individualized APD restitution relations for each of the GSD ventricles tested in the Group B experiments yielded good agreement between the theoretical predictions and the experimental results, as illustrated in Figs. 1(a–i). The ability of SS, SSS and SSSS to induce VF when not predicted may well have been due to the physical proximity of the fronts and backs of the resulting propagating waves. Any intrinsic heterogeneity of CV or repolarization characteristics could cause the wavefronts to block on wavebacks. Such heterogeneity is currently not included in our theory. Similarly, the SLS pattern is inherently an alternans pattern that can easily transform into a 2:1 pattern and thus produce block of every other action potential in the presence of heterogeneities in the APD restitution function. Finally, isolated cases of unpredicted VF induction could occur randomly owing to the propensity of these affected GSDs to generate spontaneous ventricular premature beats, which can then interact with the experimentally induced propagating waves.

In conclusion, the individualized APD restitution functions provide generally good predictions regarding which categories of 1 up to 4 premature stimuli are likely to induce VF. It may be possible to improve the predictive power of the theory by considering the role tissue heterogeneity plays during certain stimulus sequences. Better insight into how these short sequences of premature beats lead to VF may help in our understanding of how VF is initiated and how these malignant sequences might be avoided.

Acknowledgements

The authors acknowledge Dr Giles Hooker for his advice regarding the statistical analyses.

References

[1] Fox JJ, Riccio ML, Drury P, Werthman A, Gilmour Jr RF. Dynamic mechanism for conduction block in heart tissue. New J Phys 2003;5:101.101–101.114.

[2] Fox JJ, Riccio ML, Hua F, Bodenschatz E, Gilmour Jr RF. Spatiotemporal transition to conduction block in canine ventricle. Circ Res 2002;90(3):289–96.

[3] Otani NF. Theory of action potential wave block at-a-distance in the heart. Phys Rev E Stat Nonlin Soft Matter Phys 2007;75:021910.

[4] Gelzer AR, Koller ML, Otani NF, Fox JJ, Enyeart MW, Hooker GJ, Riccio ML, Bartoli CR, Gilmour Jr RF. Dynamic mechanism for initiation of ventricular fibrillation in vivo antifibrillatory effects through modulation of restitution parameters. Circulation 2008;118(11):1123–9.

[5] Cordeiro JM, Greene L, Heilmann C, Antzelevitch D, Antzelevitch C. Transmural heterogeneity of calcium activity and mechanical function in the canine left ventricle. Am J Physiol Heart Circ Physiol 2004;286(4):H1471–9.

[6] Moise NS, Meyers-Wallen V, Flahive WF, Valentine BA, Scarlett JM, Brown CA, Chavkin MJ, Dugger DA, Renaud-Farrell S, Kornreich B. Inherited ventricular arrhythmias and sudden death in german shepherd dogs. J Am Coll Cardiol 1994;24:233–243.

[7] Sosunov EA, Anyukhovsky EP, Hara M, Steinberg SF, Danilo Jr. R, Rosen MR, Moise NS, Merot J, Probst V, Charpentier F, Legeay Y, LeMarec H. Abnormal cardiac repolarization and impulse initiation in german shepherd dogs with inherited ventricular arrhythmias and sudden death. Cardiovasc Res 1999;42:65–79.

[8] Freeman LC, Pacioretty LM, Moise NS, Kass RS, Gilmour Jr RF. Decreased density of i_{to} in left ventricular myocytes from german shepherd dogs with inherited arrhythmias. J Cardiovasc Electrophysiol 1997;8:872–83.

[9] Riccio ML, Moise NS, Otani NF, Belina JC, Gelzer ARM, Gilmour RF. Vector quantization of T wave abnormalities associated with a predisposition to ventricular arrhythmias and sudden death. Ann Noninvas Electrocardiol 1998;3:46–53.

[10] Dae MW, Lee RJ, Ursell PC, Chin MC, Stillson CA, S MN. Heterogeneous sympathetic innervation in german shepherd dogs with inherited ventricular arrhythmia and sudden cardiac death. Circulation 1997;96(4):1337–42.

Address for correspondence:

Anna RM Gelzer, Dr. med. vet
College of Veterinary Medicine
Cornell University, Ithaca, NY 14853
arg9@cornell.edu