Therapy-Resistant Ventricular Arrhythmias Developed More Often in Advanced Than in Therapeutic Mild Hypothermic Condition

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Summary
Therapy-resistant ventricular arrhythmias can occur during accidental advanced hypothermic conditions. On the other hand, hypothermic therapy using mild cooling has been successfully accomplished with infrequent ventricular arrhythmia events.

To further clarify the therapeutic-resistant arrhythmogenic substrate which develops in hypothermic conditions, an experimental study was performed using a perfusion wedge preparation model of porcine ventricle, and electrophysiological characteristics, inducibility of ventricular arrhythmias, and effects of therapeutic interventions were assessed at 3 target temperatures (37, 32 and 28°C). As the myocardial temperature decreased, myocardial contractions and the number of spontaneous beats decreased. Depolarization (QRS width, stimulus-QRS interval) and repolarization (QT interval, ERP) parameters progressively increased, and dispersion of the ventricular repolarization increased. At 28°C, VF tended to be inducible more frequently (1/11 at 37°C, 1/11 at 32°C, and 5/11 hearts at 28°C), and some VFs at 28°C required greater defibrillation energy to resume basic rhythm.

An advanced but not a mild hypothermic condition had an enhanced arrhythmogenic potential in our model. In the advanced hypothermic condition, VF with relatively prolonged F-F intervals and a greater defibrillation energy were occasionally inducible based on the arrhythmogenic substrate characterized as slowed conduction and prolonged repolarization of the ventricle.

Key words: Hypothermic arrhythmias, Defibrillation energy, Therapy-resistant VF

Ventricular arrhythmias (VA) sometimes occur in advanced hypothermic conditions (either during natural disasters and/or accidents during snowy mountain climbing, winter sports or snow-removing operations, etc.), and these are usually therapy-resistant and life threatening. On the other hand, hypothermic therapy using mild-cooling has often been successfully applied to patients after cardiopulmonary resuscitation in order to improve neurological outcomes, with infrequent VA events during the treatment. These suggest that the arrhythmogenic potential and electrophysiological characteristics of the myocardium alter as the myocardial cooling is progressing, but this subject has not been well studied in a systematic experimental protocol.

Methods

Experimental preparation: This study, approved by the Animal Studies Subcommittee of our Institutional Review Board, was conducted in compliance with the guidelines of the United States National Institutes of Health for the Care and Use of Laboratory Animals. Porcine hearts (11 hearts in total) were obtained immediately after being sacrificed for food at a meat processing plant in Niigata City, and cardioplegia solution (NaCl = 110 mmol/L, KCl = 16 mmol/L, MgCl2 = 16 mmol/L, NaHCO3 = 10 mmol/L, CaCl2 = 1.2 mmol/L, buffered with 95% O2 and 5% CO2 at 4°C) was quickly injected into both coronary arteries of each heart, on site. The hearts were then transferred to...
our laboratory within one hour, and prepared for right ventricular free wall wedged preparation (approximately 7 × 9 cm) within a container filled with cardioplegia solution warmed to 37°C. The proximal portion of the right coronary artery was exposed and the myocardium was perfused with warmed Tyrode’s solution (NaCl = 129.0 mmol/L, KCl = 0.9 mmol/L, NaH2PO4 = 0.9 mmol/L, NaHCO3 = 20 mmol/L, CaCl2 = 1.8 mmol/L, MgSO4 = 0.5 mmol/L and glucose = 5.5 mmol/L, buffered with 95% O2 and 5% CO2, at 37°C) with a maintenance pressure of 80 mmHg in order to resume spontaneous heart beating.

A bipolar pacing wire was inserted into the myocardium close to the basal portion of the preparation. Limb electrodes of the 12-lead ECG were placed at the 4 corners of the wedged preparation and 3 plunge needle electrodes were inserted into the central area of the preparation with an inter-needle electrode distance of 15 mm (Figure 1). Each needle carried 8 polyimide-coated tungsten wire electrodes, 50 μm in diameter, 1 mm apart, for simultaneous recording of a unipolar transmural electrogram. To simplify the analysis, the proximal electrode (approximately 0.5 mm under the epicardial surface) was used for the epicardial (Epi) recording and the distal electrode for the endocardial (Endo) recording of the myocardium. Either the fourth or fifth distal electrode showing the longer repolarization was used for analysis of midmyocardium (Mid) recordings. A pair of defibrillation electrodes were set to the right and left portions of the preparation, and a temperature monitor probe was inserted into the myocardium in order to monitor the myocardial temperature (Figure 1). In addition, myocardial contraction was estimated by a strain wire placed at the myocardium. In this study, the myocardial contraction at 37°C was set as 100% in each experiment and the myocardial contractions at 32°C and 28°C were described as a percentage in comparison to the 37°C baseline. Using a heat exchanger, the temperature of the Tyrode’s solution was lowered step-wise from 37°C to 32°C and then 28°C. To obtain stable measurements, each temperature was maintained for 20 minutes before taking measurements.

Electrocardiographic Parameters and Their Measurement: The following parameters were measured in the 3 target temperatures. Using a fixed 0.5 ms pulse-width, pacing threshold was measured in 0.1 V steps and the output was programmed for twice as high as those measurements. From the limb ECG leads, stimulus-QRS interval (St-QRS), QRS-width, and QT-interval were measured during constant pacing at 100 ppm. Single extrastimulation was applied after the 8 basic pacings, and the effective refractory periods at the basic cycle lengths of 600 ms (ERP600) and 400 ms (ERP400) were measured in 10 ms steps. From the 3 needle electrodes, the activation-recovery interval (ARI) was measured as the time interval between the minimum first derivative of the intrinsic deflection of the QRS and maximum first derivative of the T wave of the unipolar electrograms. In previous studies, ARI from unipolar electrograms closely approximated the local effective refractory period, regardless of the T-wave morphology. In this study, heterogeneous distribution of myocardial repolarization was assessed as the maximum ARI differences among the 3 needle electrodes.

Induction of Ventricular Arrhythmias (VA): Inducibility of VA was assessed 1) during a 3-minute observation in
the spontaneous beating without pacing and 2) by programmed electrical stimulation including single/double extrastimulation and burst pacing up to 250 ppm. If ventricular fibrillation (VF) was induced, defibrillation shocks were applied incrementally to terminate the VF (10, 20, 30 and then 50 J). If monomorphic ventricular tachycardia (VT) was induced, 5 sequences of antitachycardia pacing (from 20 to 60 ppm faster than the VT) were attempted first. If the antitachycardia pacing failed to terminate the VT, electrical shocks, which was the same for VF termination, were applied to resume the basic rhythm. In this study, VF was defined as tachycardia showing the disorganized excitation wave form without isoelectrical line, and monomorphic VT was defined as tachycardia showing monomorphic QRS configuration.

**Statistical analysis:** The measurements are presented as the mean ± standard deviation. The electrical measurements obtained in the 3 temperatures, including the number of spontaneous beats, pacing threshold, myocardial contractions, St-QRS interval, QRS width, QT interval, ERP600 and ERP400, effective refractory period at 400 ms, 6) QT interval, 7) ERP600 and ERP400, 8) ARI at each transmural zone, and 9) maximum ARI-dispersion, were compared by analysis of variance (ANOVA) and Scheffe’s multiple-range post hoc test, where appropriate. The same statistical method was applied to analyze the ARI-differences among the 3 transmural zones of the myocardium (Epi, Mid and Endo). SPSS statistical software (version 14, SPSS Institute Inc., Chicago, IL) was used for these analyses.

**Results**

**Electrocardiographic parameters:** As the myocardial temperature was lowered, the number of spontaneous beats and strength of the myocardial contractions decreased progressively (from 75 ± 17 bpm and 100% at 37 °C to 56 ± 18 bpm and 85 ± 11% at 32 °C and to 48 ± 22 bpm and 82 ± 17% at 28 °C, respectively, P = 0.001 in ANOVA) (Table I). Pacing threshold increased from 0.68 ± 0.24 V at 37 °C to 0.93 ± 0.35 V at 32 °C and to 1.25 ± 0.59 V at 28 °C, respectively (P < 0.001 in ANOVA). St-QRS interval and QRS-width were prolonged from 31 ± 9 ms and 70 ± 21 ms at 37 °C to 40 ± 11 ms and 86 ± 23 ms at 32 °C and then to 47 ± 13 ms and 106 ± 28 ms at 28 °C, respectively (P < 0.001 in ANOVA) (Figure 2). Similarly, QT interval, ERP600 and ERP400 were lengthened from 330 ± 34 ms, 235 ± 26 ms and 214 ± 16 ms at 37 °C to 390 ± 35 ms, 317 ± 23 ms and 274 ± 15 ms at 32 °C and to 419 ± 33 ms, 360 ± 29 ms and 310 ± 14 ms at 28 °C, respectively (P < 0.001 in ANOVA). Myocardial cooling was also associated with the ARI prolongation in each transmural zone (Endo: 284 ± 28 ms, Mid: 283 ± 28 ms, Epi: 279 ± 28 ms at 37 °C, Endo: 331 ± 27 ms, Mid: 331 ± 28 ms, Epi: 327 ± 28 ms at 32 °C, Endo: 359 ± 27 ms, Mid: 360 ± 25 ms, Epi: 357 ± 26 ms at 28 °C, P < 0.001 in ANOVA) and a mild increase of total ARI-dispersion among the 3 plunge needle electrodes (38 ± 16 ms at 37 °C, 46 ± 22 ms at 32 °C and 54 ± 26 ms at 28 °C, P = 0.007 in ANOVA) (Figure 3), although ARI differences within the 3 transmural zones of the ventricle (Endo, Mid and Epi) were not statistically different in the 3 temperatures (Table II) (Figure 3).

**Induction of VA:** VAs did not occur spontaneously at any measured temperatures but were induced by electrical stimulation in several experiments. VF was induced in one heart at 37 °C and one other heart at 32 °C (Table III). When the myocardial temperature was lowered to 28 °C, VF tended to be inducible more frequently and was observed in 5 hearts (Figure 4). The F-F intervals of the VF induced at 37 °C and 32 °C were 173 and 181 ms, respectively (P < 0.001 in ANOVA) in the 5 VFs induced at 28 °C, showed F-F intervals of 217 ± 38 ms. On the other hand, VF was induced in 4 hearts at 37 °C and in 2 hearts as the myocardial temperature was lowered to 32 °C and 28 °C, respectively (Table III).

The inducibility and activation frequency (F-F interval during VF and cycle length of VT) of the VA seemed to be different for each temperature, but statistical analysis could not be applied to these parameters because of the study design (a comparison of 3 subgroups) and the small number of experiments.

**Effects of ATP and Defibrillation shocks for VA:** Two VFs induced at 37 °C and 32 °C were terminated by 20 J shock. Averaged defibrillation energy for 5 VFs induced at 28 °C was 26±15 J, and greater energy of more than 30 J was required in 2 of them. Since only one VF each was induced at 37 °C and 32 °C, we were unable to analyze the statistical differences in the defibrillation energy. ATP was
Figure 2. Electrocardiogram. At 37°C (A), 32°C (B) and 28°C (C), respectively. As the myocardial cooling was progressing, Stimulus-QRS (St-QRS) interval, width of QRS complex, and QT interval were gradually prolonged. Pacing rate was fixed at 100 ppm.

Figure 3. Transmural unipolar electrograms. Shown are selective transmural electrograms (endocardial: Endo, mid-myocardial: Mid, epicardial: Epi) from 3 needle electrodes. At 37°C (A), 32°C (B), and 28°C (C), respectively. Numbers on each electrogram indicate calculated activation-recovery interval (ARI). As the myocardial temperature was lowered, ARI in each electrogram was prolonged accompanied with an enlargement of ARI dispersion.
Table II. Transmural Distribution of the Ventricular Repolarization Estimated by ARI

<table>
<thead>
<tr>
<th></th>
<th>37 °C</th>
<th>32 °C</th>
<th>28 °C</th>
<th>Temperature differences ANOVA</th>
<th>37 °C versus 32 °C</th>
<th>37 °C versus 28 °C</th>
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<tr>
<td>ARI (ms)</td>
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<td>Endocardium</td>
<td>284 ± 28</td>
<td>331 ± 27</td>
<td>359 ± 27</td>
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<td>Mid-myocardium</td>
<td>283 ± 28</td>
<td>331 ± 27</td>
<td>360 ± 25</td>
<td>&lt; 0.001</td>
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<td>Epicardium</td>
<td>279 ± 28</td>
<td>327 ± 28</td>
<td>357 ± 26</td>
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<td>Transmural differences ANOVA</td>
<td>0.894</td>
<td>0.938</td>
<td>0.964</td>
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Values are mean ± SD. ARI indicates activation-recovery interval; and ANOVA, analysis of variance.

Table III. Inducibility of Ventricular Arrhythmias and Final Intervention for Termination

<table>
<thead>
<tr>
<th>Experimental No.</th>
<th>37 °C</th>
<th>32 °C</th>
<th>28 °C</th>
<th>VT (DC: 10 J)</th>
<th>VF (DC: 50 J)</th>
<th>VT (DC: 30 J)</th>
<th>VT (DC: 20 J)</th>
<th>VF (DC: 10 J)</th>
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<td>VF (DC: 20 J)</td>
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<td>VT (DC: 20 J)</td>
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<td>VF (DC: 10 J)</td>
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Final intervention for termination of the arrhythmias is shown in the closed parenthesis. ATP indicates anti-tachycardia pacing; and DC, direct current shock.

Figure 4. Programmed electrical stimulation. Panels were obtained from one of the experiments (No. 9 in Table III). VF was only induced at an advanced hypothermic condition (C) whereas no VA was induced either at norm-thermic state (A) or a mild hypothermic state (B).

effective in terminating the VT in 50% (2/4) at 37°C, in 50% (1/2) at 32°C, and in 0% (0/2) at 28°C, respectively (Table III).

Discussion

The main results of this study were as follows. 1) Myocardial cooling was associated with slowed conduc-
tion, prolonged repolarization, and suppressed automaticity, and these phenomena became more apparent as the cooling process progressed. These results were consistent with previous experimental studies using different preparations.\(^9,12\)

2) The number of induced VF by programmed electrical stimulation tended to be greater based on our myocardial preparations with heterogeneous distribution of the repolarization, and some of the VFIs required greater defibrillation energy to resume the basic rhythm. These results seem characteristic of VA under advanced hypothermic conditions.

**Myocardial cooling and electrophysiological parameters:** Myocardial cooling in this study was associated with slowed conduction, prolonged repolarization, and suppressed automaticity,\(^9\) and both depolarization (QRS width and ST-QRS interval) and repolarization parameters (QT interval, ERP and ARI) were progressively prolonged as the myocardial temperature decreased from 37°C to 28°C. The number of spontaneous beats also gradually decreased. Several mechanisms have been reported as the causes of these changes in the electrophysiological parameters during hypothermic conditions. The slowed conduction is most likely explained by a decrease of Na\(^+\) current availability via its temperature-dependent slowing of activation/inactivation kinetics.\(^20\) A reduction of gap junction conductance and/or suppression of Na\(^+\)-K\(^+\) pump function by cooling can also contribute to the slowed conduction.\(^11,12\) On the other hand, a decrease in the delayed rectifier K\(^+\) current is considered to be a major factor in the inactivation of L-type Ca\(^{2+}\) current and a decrease of inward rectifier K\(^+\) current may also be associated with this phenomenon.\(^9\) Decreases in the spontaneous beat rate would be a result of the suppression of automaticity from Purkinje fibers. Funny currents,\(^21-23\) which are distributed in the sinus node, atrioventricular node, and Purkinje fibers and are responsible for pacemaking of the myocardium, would be weaker, leading to the decreased spontaneous beats during the hypothermic condition.

As the myocardial cooling was progressing to 28°C, heterogeneous distribution of the myocardial repolarization estimated by ARI dispersion increased. However, this was not caused by the augmentation of transmural ARI dispersion. Previous studies reported that the mid-myocardial M-cell layer was present within the ventricular wall in several species, and that it contributes to creating a large transmural dispersion of ventricular repolarization in some specific conditions.\(^24\) However, the presence of an M-cell layer has not been positively confirmed in porcine right ventricle.\(^25\) Indeed, in this study, ARI distribution was similar among the 3 layers of the right ventricle (Endo, Mid and Epi) at each temperature, suggesting less predominant distribution of myocardial M-cells in the porcine right ventricle. Heterogeneous cooling of the myocardium is also less likely as the reason for the increased ARI dispersion, because we started the measurement 20 minutes after reaching the target temperature, and intramyocardial temperature was continuously monitored using the temperature monitor probe placed into the myocardial wall during the experiments.

Characteristics of VAs induced in the hypothermic condition: It is interesting that the number of induced VF was slightly greater for advanced hypothermia (28°C). Slow conduction and heterogeneous distribution of ventricular repolarization at low temperatures can facilitate inducing reentrant arrhythmias and allow the coexisting multiple waveforms within the myocardium. When the myocardium is activated at a faster rate (like during VF), the prolonged repolarization induced by myocardial cooling is shortened but conduction time is further prolonged. We believe that the relatively longer F-F interval observed in the VF at 28°C represented slower conduction during the VF. Therefore, during the VF in advanced hypothermia, the activation wavelength (estimated by conduction velocity × repolarization) would be shorter. If the activation wavelength is sufficiently short, multiple activation waves can simultaneously be present in the myocardium, which resulted in the requirement of greater defibrillation energy.\(^26,27\) Indeed, greater defibrillation energy was required to resume the basic rhythm in some of the VFs.

Similar results have been reported in several studies.\(^21-25\) In an experimental study using isolated rabbit hearts, Hsieh, et al showed that spatial and temporal dispersion of ventricular repolarization were enhanced at the advanced hypothermic condition and that this was related to frequently induced VF.\(^21\) Piktel, et al also reported in their canine wedge preparation that an advanced but not therapeutic hypothermia-induced reentrant arrhythmia was based on the large transmural dispersion of the ventricular repolarization.\(^25\) In addition, VF that developed in advanced hypothermia has been shown to have a shorter wavelength as compared to that in mild hypothermia.\(^27\)

In contrast, VT tended to be induced infrequently under hypothermic conditions. Indeed, similar results were reported by Harada, et al in their optical mapping study using Langendorff-perfused rabbit hearts.\(^21\) In their study, the spiral wave excitations of VAs at norm-temperature were largely organized (VT configuration), whereas those during mild and advanced hypothermia were characterized by disorganization with frequent breakup (VF configuration). They also demonstrated that the VAs under mild hypothermia were short-lasting as compared with norm-temperature and advanced hypothermia because of the higher incidences of spiral wave collisions during the mild hypothermia. Furthermore, different from the hearts of other species, the ventricular wall of farmed porcine hearts is thick and fatty for the purpose of meat production, and these characteristics can be related to the easy induction of VA under norm-temperature.

**Clinical implications:** Enhanced slowed conduction and prolonged repolarization that developed in advanced hypothermia would be related to the therapy resistance of hypothermic VA. In contrast, hypothermic therapy using mild hypothermia would be safe and less arrhythmogenic. Therapeutic interventions counteracting the slowed conduction and prolonged repolarization (isoproterenol, magnesium, nicorandil, etc.) seem to show beneficial effects for VA developed in advanced hypothermic conditions.\(^24\)

**Limitations:** There are several limitations in this study. First, we used right ventricular wedge preparations of the porcine heart because the left ventricle (usually more than 2 cm) was too thick to maintain a sufficient perfusion and
obtain homogeneous myocardial cooling. Therefore, the role of the left ventricle in creating the arrhythmogenic substrate in the hypothermic condition could not be studied. Second, different from patients in advanced hypothermic conditions, all VAs in this study were induced by electrical stimulation rather than occurring spontaneously. Fukaya, et al reported in their canine experimental model that calcium-mediated premature beats which triggered VF increased in advanced hypothermic conditions.24 The mechanisms of triggered premature beats and maintenance of hypothermic VA could not be studied in this study. Third, using a small number of electrodes placed in the wedge preparation may underestimate the change in the myocardial depolarization and repolarization during the hypothermic condition. Fourth, we obtained the hearts immediately after the animals were killed for food, and created the wedge preparation as quickly as possible in order to minimize myocardial damage. Nevertheless, it is possible that some myocardial damage in the preparation may have affected the results of this study. Finally, any correlation between hypothermic VA and Osborn wave (J-wave) was undetermined,25,26 because we used a wedge preparation model and applied ventricular pacing.

Conclusions

In the advanced hypothermic condition, VF with longer F-F intervals and a greater defibrillation threshold seem to develop more often based on myocardial substrate with enhanced slowed conduction and prolonged ventricular repolarization.

Disclosures

Conflicts of interest: The authors have no potential conflict of interest to disclose.

References