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ORIGINAL RESEARCH

Effect of Amiodarone and Hypothermia on Arrhythmia Substrates During Resuscitation

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BACKGROUND: Amiodarone is administered during resuscitation, but its antiarrhythmic effects during targeted temperature management are unknown. The purpose of this study was to determine the effect of both therapeutic hypothermia and amiodarone on arrhythmia substrates during resuscitation from cardiac arrest.

METHODS AND RESULTS: We utilized 2 complementary models: (1) In vitro no-flow global ischemia canine left ventricular transmural wedge preparation. Wedges at different temperatures (36°C or 32°C) were given 5 μmol/L amiodarone (36-Amio or 32-Amio, each n=8) and subsequently underwent ischemia and reperfusion. Results were compared with previous controls. Optical mapping was used to measure action potential duration, dispersion of repolarization (DOR), and conduction velocity (CV). (2) In vivo pig model of resuscitation. Pigs (control or targeted temperature management, 32–34°C) underwent ischemic cardiac arrest and were administered amiodarone (or not) after 8 minutes of ventricular fibrillation. In vitro: therapeutic hypothermia but not amiodarone prolonged action potential duration. During ischemia, DOR increased in the 32-Amio group versus 32-Alone (84±7 ms versus 40±7 ms, $P<0.05$) while CV slowed in the 32-Amio group. Amiodarone did not affect CV, DOR, or action potential duration during ischemia at 36°C. Conduction block was only observed at 36°C (5/8 36-Amio versus 6/7 36-Alone, 0/8 32-Amio, versus 0/7 32-Alone). In vivo: QTc decreased upon reperfusion from ischemia that was ameliorated by targeted temperature management. Amiodarone did not worsen DOR or CV. Amiodarone suppressed rearrest caused by ventricular fibrillation (7/8 without amiodarone, 2/7 with amiodarone, $P=0.041$), but not pulseless electrical activity (2/8 without amiodarone, 5/7 with amiodarone, $P=0.13$).

CONCLUSIONS: Although amiodarone abolishes a beneficial effect of therapeutic hypothermia on ischemia-induced DOR and CV, it did not worsen susceptibility to ventricular tachycardia/ventricular fibrillation during resuscitation.

Key Words: amiodarone ■ hypothermia ■ ischemia-reperfusion ■ optical mapping ■ resuscitation ■ ventricular fibrillation

Targeted temperature management (cooling to 32–36°C, TTM) is recommended for patients with return of spontaneous circulation (ROSC) after resuscitation from cardiac arrest because it is neuroprotective and improves survival.[1–4] But surprisingly little is known about the electrophysiologic effects of hypothermia during acute cardiac ischemia and reperfusion, and the effect of temperature on pharmacologic management of ventricular arrhythmias during advanced cardiac life support (ACLS) is incompletely understood.[5,6] Amiodarone is recommended for refractory ventricular fibrillation (VF) during ACLS. It has been shown to improve survival to hospital admission, but improved survival to hospital discharge and an effect on neurologically intact survival is less clear.[7–9] Furthermore, the interaction between amiodarone and hypothermia, specifically at temperatures used in TTM, has not been extensively studied.[5,6]

Acute ischemia induces profound and complex effects on electrophysiologic function, including a decrease in conduction velocity (CV), shortening of

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CLINICAL PERSPECTIVE

What Is New?

- The effect of hypothermia on the antiarrhythmic effects of amiodarone during resuscitation has not been established.
- During ischemia, amiodarone worsened ischemia-induced dispersion of repolarization and conduction slowing during hypothermia without worsening arrhythmia susceptibility.

What Are the Clinical Implications?

- While targeted temperature management alters the arrhythmia substrates during ischemia in the presence of amiodarone, there appears to be no difference in arrhythmias in our in vitro or in vivo models of ischemic cardiac arrest.

Nonstandard Abbreviations and Acronyms

APD	action potential duration
CV	conduction velocity
DOR	dispersion of repolarization
I/R	ischemia and reperfusion
RG	repolarization gradient
ROSC	return of spontaneous circulation
TH	therapeutic hypothermia
TTM	targeted temperature management

the action potential duration (APD), and an increase in transmural dispersion of repolarization (DOR).[10] Severe hypothermia (temperatures <30°C) also causes heterogeneities in CV and DOR.[11–17] Clinically, TTM has been shown to prolong the QT interval,[18] but the effect of TTM on arrhythmias is unclear.[19,20] Our investigations in an experimental model of global ischemia suggest TTM may in fact be *antiarrhythmic* by attenuating ischemia-induced transmural CV slowing and block, while limiting ischemia-induced increases in transmural DOR.[21] However, in a translational model of resuscitation from cardiac arrest, additional proarrhythmic effects of TTM were also identified.[22]

Amiodarone is the most common antiarrhythmic drug used during resuscitation, with effects on repolarizing potassium (class III) and fast sodium currents (class I) as well as effects similar to beta blockade (class II, although through a different mechanism than direct beta receptor blockade). During resuscitation, amiodarone's exact antiarrhythmic mechanism is unknown.[23,24] Acute intravenous administration of amiodarone inhibits depolarization through blockade of fast sodium and/or L-type calcium channels producing conduction slowing, while having minimal effects on repolarization, as defined by minimal changes in APD, QT intervals, or the effective refractory period (ERP).[23,25] Transmurally, epicardial and endocardial APD are unchanged by amiodarone, but APD may shorten in the midmyocardial region.[25] None of these effects have been studied under hypothermic conditions, and there have been no clinical studies investigating the effect of amiodarone for refractory VF while undergoing TTM. As both hypothermia and amiodarone affect conduction and repolarization, it is entirely possible that colder temperatures can alter amiodarone's antiarrhythmic effects in undesirable ways, specifically increasing ischemia-induced conduction slowing and potentially altering the repolarization currents that may increase DOR.

In this study we use 2 complementary models of resuscitation to investigate how temperature used during TTM modulates the effect of amiodarone on arrhythmia substrates. In order to evaluate the interaction of temperature and amiodarone during acute global myocardial ischemia and reperfusion (I/R), we used our previously developed canine ventricular wedge preparation, which allows determination of transmural conduction, heterogeneities of repolarization, and arrhythmia induction. We next utilized our previously developed translational porcine model of resuscitation from ischemia-induced VF arrest to determine whether hypothermia modulated amiodarone's electrophysiological effects. Our hypothesis was that amiodarone would increase I/R-induced CV slowing and increase DOR during hypothermia, thereby worsening arrhythmia substrates, compared with warmer temperatures.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request. All experiments were carried out in accordance with Public Health Service guidelines for the care and use of laboratory animals and approved by our Institutional Animal Care and Use Committee from 2013 to 2015 for ex vivo and 2017–2018 for in vivo experiments.

Optical Mapping in the Canine Wedge Preparation

Study Design

We previously developed a model of global ischemia in the canine wedge preparations to evaluate the electrophysiologic effects of global ischemia.[21] Canine left ventricular transmural wedges were used from 9 male mongrel dogs (≈15–20 kg) and

compared with 12 previous controls. The number of wedges for experimental analysis from each canine heart can vary, and pairing is not always possible. Therefore, both amiodarone groups (n=8 per group) and previous controls (n=7 normal temperature and 6 hypothermia), were treated as unpaired for analysis.[21] Briefly, the intact heart was rapidly excised by right lateral thoracotomy performed with animals under pentobarbital (50 mg/mL IV) anesthesia. Transmural wedges of left ventricular myocardium were isolated, and the branch of the corresponding coronary artery was cannulated and perfused with Tyrode's solution (140 mmol/L NaCl, 4.03 mmol/L KCl, 1.8 mmol/L $CaCl_2$, 5.5 mmol/L dextrose, 0.5 mmol/L $MgSO_4$, 0.9 mmol/L NaH_2PO_4, 10 mmol/L HEPES, NaOH titrated to pH 7.41, oxygenated at 100% O_2). The wedge was then placed in the chamber with the transmural surface against the glass imaging plate and perfused with a voltage-sensitive dye (di-4-ANEPPS, 8 μmol/L). We have previously described our high-resolution transmural optical mapping system.[21,26,27] Action potentials were recorded from 256 sites simultaneously with high spatial (0.89–1.1 mm), temporal (0.1 ms), and voltage (0.5 mV) resolution. Blebbistatin (5 μmol/L) was used to eliminate motion artifact. The imaging chamber was insulated and temperature closely regulated by an insulated water circuit and measured using a digital temperature probe (Omega) in the water bath, allowing for temperature precision of ±0.1°C. Temperature was controlled at 36°C or 32°C before inducing ischemia to achieve and maintain specific temperature during the ischemia protocol.[28] Amiodarone (5 μmol/L) was then administered via the perfusion system before ischemia for 15 minutes. This dose was used previously in optical mapping and is similar to the standard plasma concentration of amiodarone (1–1.5 mg/L, or 1.6–2.3 μmol/L).[29] In order to induce global ischemia, flow of oxygenated Tyrode's solution was stopped. Measurements were made at 5, 10, and 15 minutes of no-flow ischemia. Flow was then resumed and measurements were made at 5 and 10 minutes of reperfusion.

Groups and Methods of Measurement

APD was measured in any 1 transmural layer by averaging 5 epicardial, midmyocardial, and endocardial cell APDs, respectively. Transmural cell types were defined by previously validated anatomic and functional criterion.[30] Dispersion of repolarization (DOR) was defined as the difference between the APD of the longest and shortest cell type. CV and mean repolarization gradient (RG) were determined by a previously validated vector analysis technique.[31,32] Briefly, CV and RG vectors between nearby sites of the 256 site array were calculated and averaged across the maximal direction of depolarization (CV) and repolarization (RG) to obtain the average CV and RG per wedge. ERPs were determined as the shortest coupled cycle length before stimulation failed to capture. For arrhythmia analysis, programmed electrical stimulation (PES) protocol was used. During endocardial pacing (to reproduce normal endocardial to epicardial activation in the heart (S1), up to 2 premature beats were delivered (S2 and S3), until failure to capture the preparation or an arrhythmia was induced. PES was considered positive for an inducible arrhythmia if there were >3 beats of ventricular tachycardia induced from baseline or a spontaneous arrhythmia was observed.

Four groups were analyzed. The major comparison in this study was to identify differences between arrhythmia substrates when amiodarone was added under different temperatures. Therefore, wedges given amiodarone were compared with wedges without amiodarone at 36°C (36-Alone, n=7 versus 36-Amio, n=8) and wedges given amiodarone were compared with wedges not given amiodarone at 32°C (32-Alone, n=7 versus 32-Amio, n=8). Both temperature groups were compared with previously published controls.[21]

Translational Model of Resuscitation in Pig

Although the canine wedge model provides significant insight into the interaction between amiodarone and temperature during global I/R, as occurs after successful resuscitation, it does not provide in vivo data modeling the interaction between temperature and amiodarone during clinical resuscitation. Therefore, we utilized a complementary translational model of resuscitation from ischemic cardiac arrest with amiodarone given and cooling started upon ACLS initiation (Figure 1). Details of the experimental design have been described elsewhere, with some modifications.[22] Briefly, closed chest, anesthetized (isoflurane, 0.5%–2%) adult female pigs (≈35 kg, n=21) were fully instrumented for hemodynamic and electrophysiological assessments. Three animals died without initial ROSC and were not included in the study. Animals were included in electrophysiologic analysis if they completed the experimental timeline. Animals were included in the outcome analysis if they achieved initial ROSC. Temperature, heart rate, arterial blood pressure, end-tidal CO_2, and oxygen saturation were continuously assessed throughout the experimental protocol. Electrophysiological assessments were made using a continuous 12-lead ECG and using percutaneous placement of electrophysiological catheters to measure local electrograms.

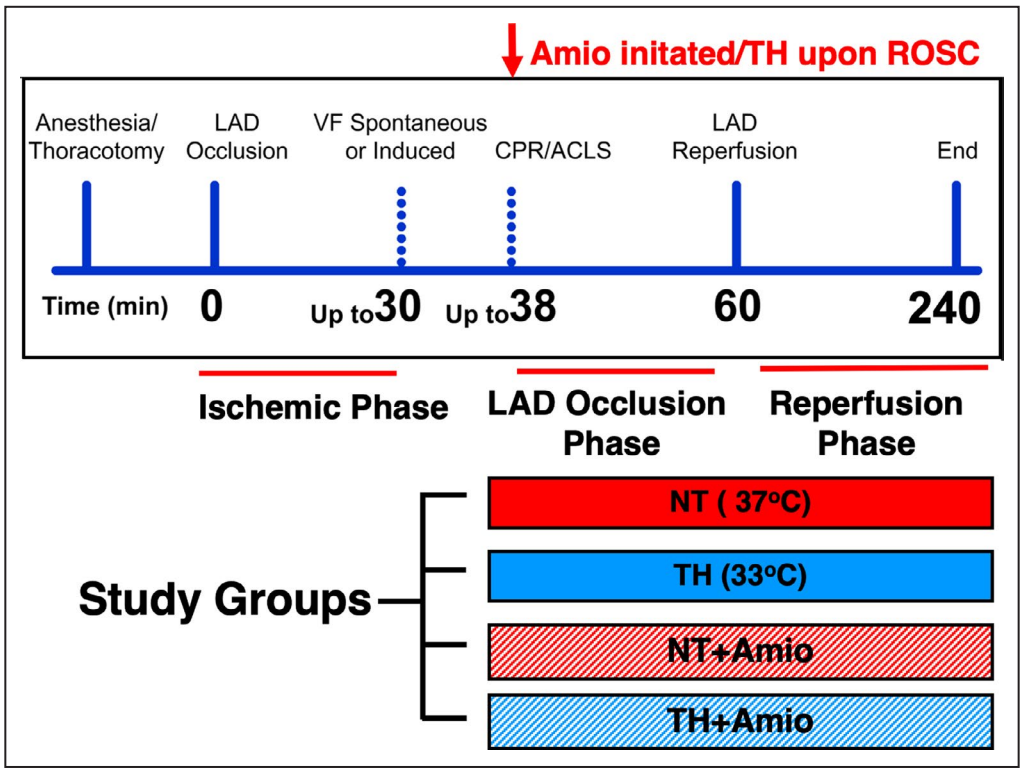

Figure 1. In vivo experimental timeline and study groups.
Amio indicates amiodarone; CPR/ACLS, cardiopulmonary resuscitation and advanced cardiac life support; LAD, left anterior descending; NT, normal temperature; TH, therapeutic hypothermia; ROSC, return of spontaneous circulation; and VF, ventricular fibrillation.

Catheters were placed in the RV septum, left ventricle, and great cardiac vein. These allowed measurements of electrograms in the infarct, border, and normal zones relative to an apical myocardial infarction created by reversible occlusion of the proximal left anterior descending coronary artery (LAD) between the first and second diagonal branch using an angioplasty balloon. Experimental timeline is shown in Figure 1. The groups were not blinded or randomized. Baseline electrophysiological measurements and hemodynamic measurements were performed. Ischemia continued for a total of 60 minutes (acute myocardial infarction phase of left anterior descending coronary artery Occlusion). After 30 minutes of ischemia, if spontaneous VF did not occur then VF was induced using 9V direct current applied through the left ventricle electrophysiology catheter.

VF continued without compressions or pharmacologic intervention for 8 minutes to simulate out-of-hospital cardiac arrest (VF phase of left anterior descending coronary artery occlusion). After 8 minutes, subjects were then resuscitated under ACLS.[4] Manual compressions were started with a goal to maintain a rate of >100 and <140 (using the invasive pressure monitoring). Quality of cardiopulmonary resuscitation was gauged by a rise in end-tidal CO_2 and an adequate invasive blood pressure (BP) rise. Defibrillation was achieved using a Physio-Control Lifepak 9 delivering a monophasic 360 J single shock followed by 2 minutes of cardiopulmonary resuscitation. Epinephrine (0.01 mg/kg of the 1:1000 IV) was used during cardiopulmonary resuscitation as standard therapy. Amiodarone was administered after first shock, 5 mg/kg over 5 to 20 minutes (slower if there was hypotension after ROSC). ACLS was continued until ROSC. Subjects in the therapeutic hypothermia (TH) groups were cooled at the beginning of ACLS using an esophageal cooling device, cooling blanket, ice packs, and cold saline (bolus 4°C NS [30 mg/kg]) for a goal of 32° to 34°C and maintained with a cooling blanket and ice packs as needed. If VF recurred, ACLS was resumed. We chose a range of 32° to 34°C in order to not overshoot our temperature goal into a more severe hypothermia range, and because of the in vivo nature of the study, core temperature may have a wider variance than the ex vivo preparation. Pulseless electrical activity (PEA) was defined as systolic BP <50% of baseline BP. If PEA occurred, then ACLS was started. Arrhythmias were recorded without intervention only if BP remained 50% of baseline. Pressure support after ROSC was achieved was used when needed using epinephrine

infusion at a beginning rage of 0.2 μg/kg per minute and titrated to ≈75% of baseline BP. Left anterior descending coronary artery occlusion was continued for a total of 60 minutes. The left anterior descending coronary artery balloon was then removed and the heart was reperfused for a total of 180 minutes (left anterior descending coronary artery reperfusion phase). If VF/pulseless ventricular tachycardia or PEA recurred, ACLS was resumed. Protocols ended after 3 hours of reperfusion, or 20 minutes of arrest without reperfusion. All events (ex. VF initiation, PEA, defibrillation) were recorded during the entire protocol.

Groups and Methods of Measurement

Pigs were assigned to 1 of 4 groups: Normal Temperature without amiodarone (NT, no cooling or amiodarone, N=4), TTM without amiodarone (TTM, N=4), No TTM with amiodarone (NT+Amio, N=3), and TH with amiodarone (TTM+Amio N=3). Seven pigs did not achieve ROSC and were not included in the final groups. The groups were not randomized because of the outcome measures being electrophysiologic parameters and the need for having the appropriate numbers for survival. The studies were not blinded because of the nature of the cooling required during the experiments.

Local electrograms were used to determine activation recovery intervals to measure ventricular conduction and repolarization. Unipolar electrograms (filtered between 0.05 and 300 Hz) and bipolar electrograms (filtered 10 to 300 Hz) were recorded at baseline and every 2 minutes until protocol end, using standard, previously validated methods.[22,33] Global DOR was determined by the difference between the longest and shortest recorded action recovery intervals across the left ventricle. Left ventricular activation times were assessed by the difference between the earliest and latest activation time of the electrograms relative to the earliest ventricular activation recorded from the beginning of the QRS on the ECG.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 24 and 27 (IBM Corp). For ex vivo experiments, repeated-measures ANOVA was used to analyze the differences in mean APD, CV, DOR, ERP, and maximal repolarization gradients (RG), before and after amiodarone, at baseline, before any ischemia. Comparisons were made for each temperature. To analyze the trend from baseline to ischemia and then reperfusion, repeated-measure ANOVA was again performed with the baseline as a covariant for DOR and CV. Group effects and differences between specific means were considered significant with a $P \leq 0.05$. For in vivo experiments, categorical variables were compared using Fisher exact test. Repeated-measures ANOVA was performed for regional and global DOR, CV, and action recovery intervals as these were continuous variables determined by the electrograms. Statistical significance for the figures is represented by an asterisk (*) or as otherwise stated in the figure legends. All mean data are represented with the value and SEM. Previous power analyses suggested n=5 per group to detect a significant difference in DOR in the canine wedge with a power of 0.8 and an error of 0.05.[28] A priori power analysis was not performed in the in vivo model as this was used for comparison of effects seen in the ex vivo model.

RESULTS

Effect of Amiodarone and Temperature on APD and DOR Ex Vivo

Figure 2A shows the effect of amiodarone on APD and DOR during ischemia at 36°C. The addition of amiodarone alone before ischemia had no significant effect on APD, QT interval, or DOR (before versus after drug: APD 225±7 versus 224±7 ms, QT interval 263±12 versus 259±11 ms, DOR 29±3 versus 33±4 ms, all *P*=ns). During ischemia, APD and QT interval shortened while DOR increased with or without amiodarone. Figure 2B shows the effect of amiodarone on APD and DOR at 32°C. Colder temperature increased both APD and QT interval before ischemia (as shown previously)[21] and the addition of amiodarone did not further lengthen APD or DOR, although QT interval was nonsignificantly increased (before versus after drug: APD 286±4 versus 295±6 ms, *P*=ns, DOR 37±2 versus 34±3 ms, *P*=ns, QT interval 320±2 versus 336±5 ms, *P*=0.06). During ischemia, the addition of amiodarone to 32°C significantly worsened DOR (see summary data, Figure 3). APD and QT shortened from baseline, but without a significant difference with the addition of amiodarone during ischemia.

Figure 3 is a summary of the DOR during ischemia and reperfusion at both temperatures. At 36°C (Figure 3A), DOR worsens during ischemia and returns to baseline, although no difference is seen with the addition of amiodarone. At 32°C (Figure 3B), DOR is significantly worsened during ischemia with amiodarone (15 minutes ischemia, 32-Alone=37±7 ms, 32-Amio=83±7 ms) and improved to baseline during reperfusion, despite a small baseline difference between 32-Amio and historic control group (repeated measures accounting for baseline difference *P*=0.002). In summary, amiodarone together with TH worsens DOR compared with TH alone. Under control conditions, amiodarone had no effect on DOR or APD.

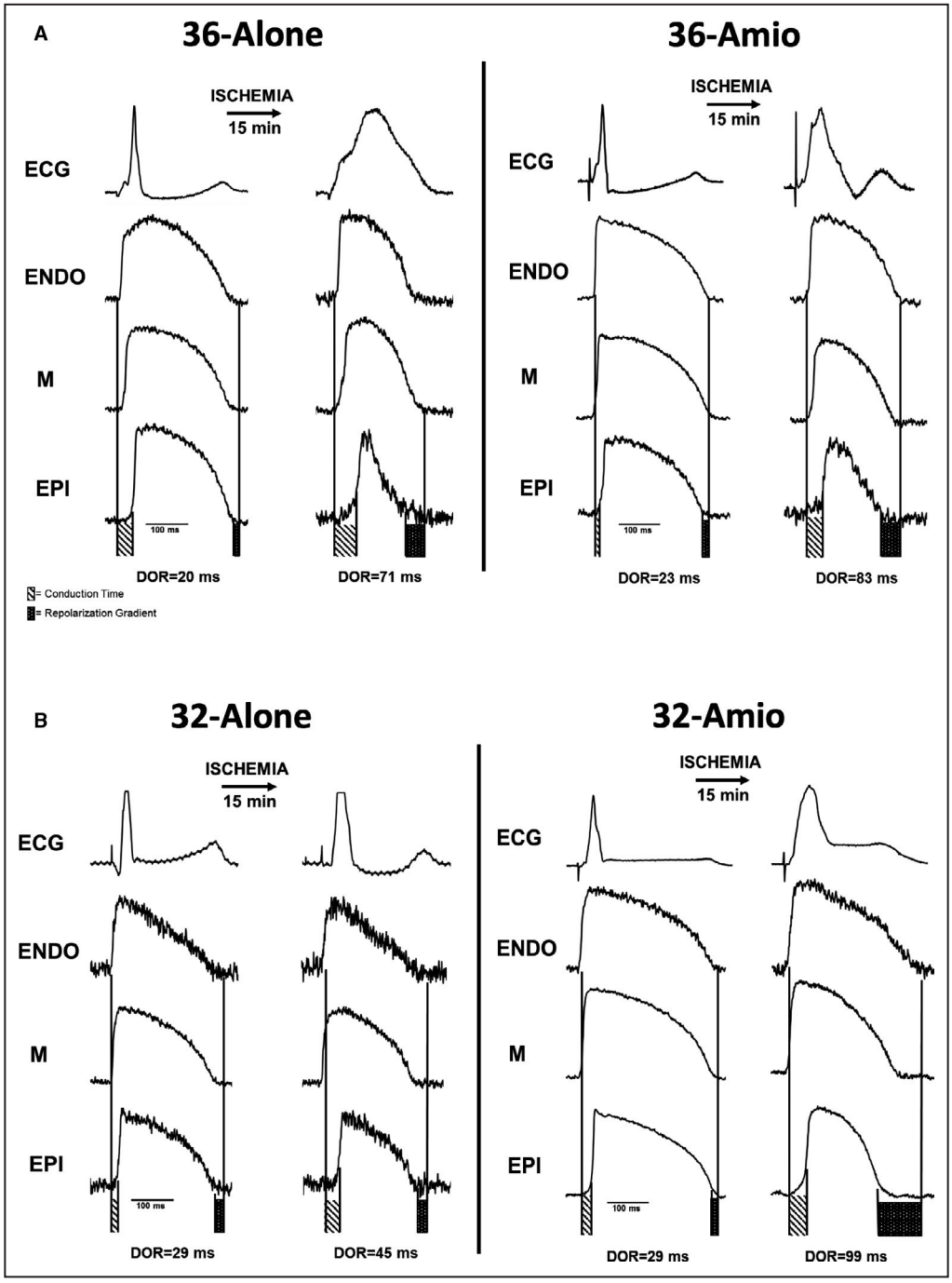

Figure 2. Effect of amiodarone on action potential duration and dispersion of repolarization. **A**, 36°C. Representative ECG and action potentials from endocardial (ENDO), midmyocardial (M), and epicardial (EPI) cells at control temperatures (36°C) are shown. *Left, 36-Alone*. After 15 minutes of ischemia, dispersion increases secondary to relatively greater shortening of epicardial action potential duration (APD). *Right, 36-Amio*. Amiodarone (Amio) does not affect APD or dispersion at baseline or during ischemia. **B**, 32°C. *Left, 32-Alone*. At 32°C, there is less APD shortening during ischemia and therefore a decrease in dispersion of repolarization (DOR) during ischemia compared with 36°C. *Right, 32-Amio*. The addition of amiodarone worsens APD shortening during ischemia and increases DOR.

Effect of Amiodarone on CV and Refractoriness With Temperature

Figure 4A, top panel, is a representative isochrone map showing the effect of amiodarone on CV at 36°C. Amiodarone had no significant effect on CV at baseline (CV 36-Alone=43±3 cm/s, versus 36-Amio=40±3 ms *P*=ns). Ischemia slowed CV, but this was not affected by the addition of amiodarone (decrease in CV by

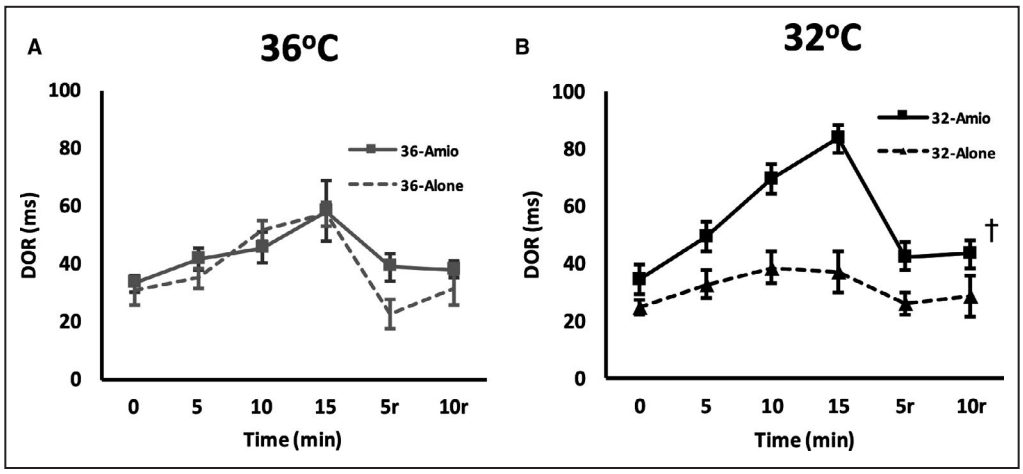

Figure 3. Summary effect of amiodarone on dispersion of repolarization during ischemia. **A**, At 36°C, no difference was seen between dispersion of repolarization (DOR) with the addition of amiodarone (Amio). **B**, At 32°C, DOR increased in the 32-Amio group compared with the 32-Alone group at 15 minutes of ischemia. (†P<0.05 by repeated measures).

36-Amio=47±5% versus 36-Alone=50±6%, P=ns). In Figure 4A, there is similar conduction slowing (crowding of isochrones) seen in both 36-Alone and 36-Amio during ischemia (right panels), and both have transmural conduction block. Figure 4A bottom panel shows the effect of amiodarone on CV at 32°C. Amiodarone did not alter CV at baseline before ischemia (32-Alone=35±3 cm/s, 32-Amio=34±3 cm/s, P=ns). However, amiodarone significantly slowed CV during ischemia compared with control (decrease in CV by 32-Amio=39±5% versus 32-Alone=19±5%, P=0.03). In Figure 4A lower, there is more conduction slowing (crowding of isochrones) 32-Amio as compared with 32-Alone, where conduction is relatively preserved. Importantly, neither demonstrate conduction block. Figure 4B shows summary data on CV slowing from baseline during ischemia and reperfusion at 36 (left) and 32 (right).

At 36°C, the addition of amiodarone did not affect the ERP (ERP a measure of refractoriness) at baseline (36-Alone=240±8 ms, 36-Amio=244±5 ms, P=ns). At 10 minutes of ischemia, ERP increased, which was ameliorated in the amiodarone group (36-Alone=276±17 ms versus 36-Amio=230±18 ms, P=0.035). While decreased temperature to 32°C prolonged ERP at baseline, the addition of amiodarone did not affect the ERP (32-Alone=320±3 ms, 32-Amio=334±9 ms, P=ns). At 10 minutes of ischemia, ERP was unchanged between groups (32-Alone=320±12 ms versus 32-Amio=310±10 ms, P=ns). At both 36°C and 32°C, maximal RG increased during ischemia but were unaffected by the addition of amiodarone (Figure 5). Importantly, although DOR increased significantly during 32-Amio, the slowing of CV induced by amiodarone prevented significant increases in RG and therefore, in part, ERP.

Taken together, amiodarone and TH produce more conduction slowing during ischemia than TH alone, suggesting that TH enhances the class I effect of amiodarone. Amiodarone also enhanced DOR during TH; however, there were no significant differences during TH in refractoriness or local RG, which would be expected to enhance susceptibility to reentrant arrhythmias.

Effect of Amiodarone on Conduction Block and Inducible Arrhythmias With Temperature

At 36°C amiodarone did not prevent conduction block (36-Alone=6/7, 36-Amio=5/8). Similarly, the addition of amiodarone did not worsen arrhythmia susceptibility at 36°C (36-Alone=3/5, 36-Amio=3/8, one 36-Alone experiment did not undergo PES). At 32°C, CV slowing and worsening of DOR with the addition of amiodarone did not promote transmural conduction block (32-Alone=0/7, 32-Amio=0/8) and also did not affect arrhythmias susceptibility (32-Alone=1/4, 32-Amio=0/8, three 32-Alone experiment did not undergo PES). Figure 6A shows an arrhythmia induced by PES at 36°C during ischemia with amiodarone. Top panel shows the ECG and action potentials from 3 sites, bottom panel shows activation time (upper) and corresponding repolarization time (lower) maps for each beat during initiation of VF. Extra endocardial beats were given with subsequent conduction block (site C, upper panel, red block symbol and S3 activation map, red bar) and reentrant excitation occurs originating from site C (arrows and first beat of VF). Block and reentry occur at the site of maximal RG on the previous beat (S2 repolarization time map, site C). VF initiation (upper panels and VF map) occurs by a reentrant mechanism.

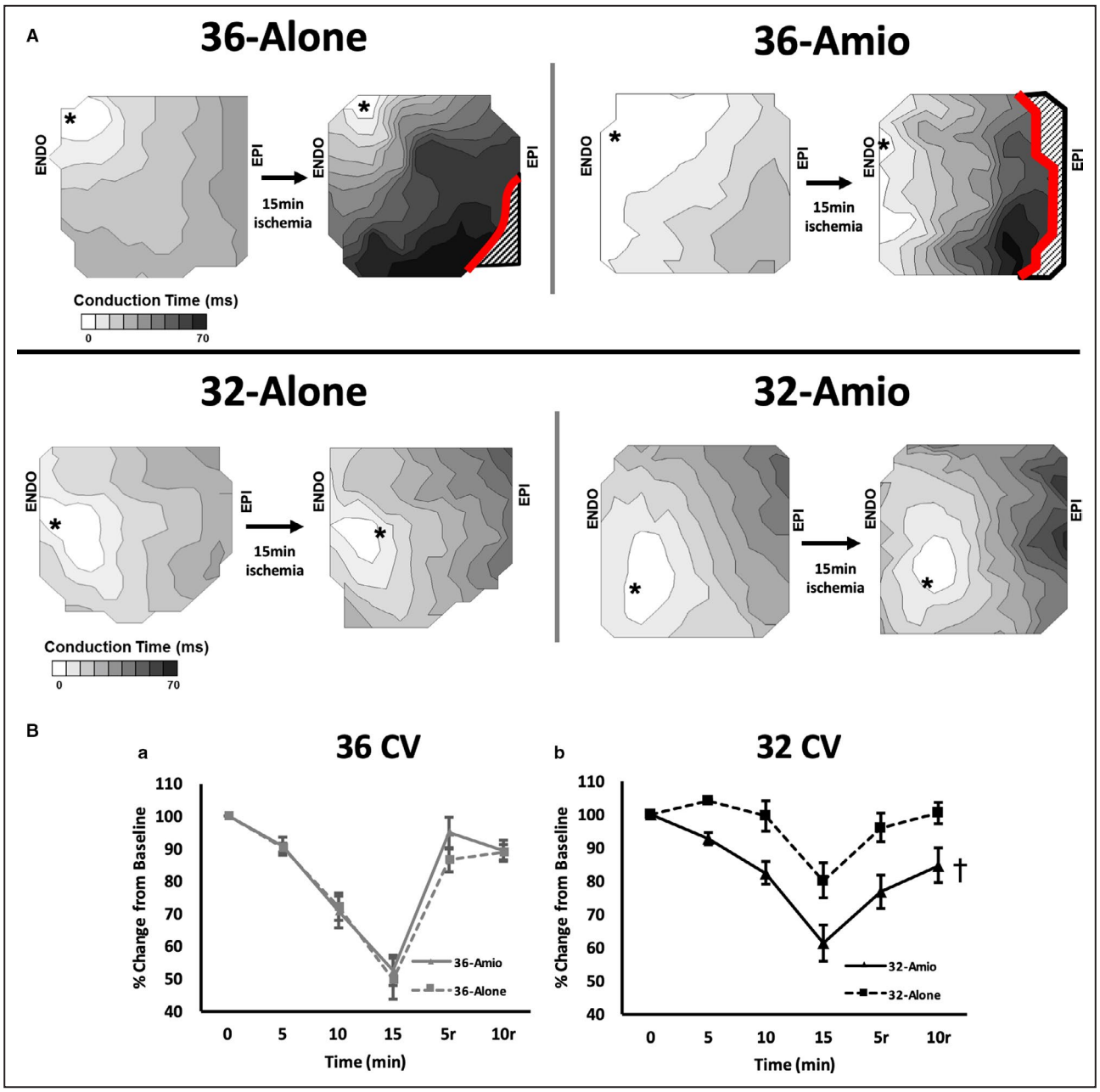

Figure 4. Effect of amiodarone on transmural conduction velocity.
A, *Left upper panel 36-Alone*. Transmural conduction slows during ischemia with subsequent subepicardial conduction block observed. *Right upper panel, 36-Amio*. The addition of amiodarone has no effect on either conduction slowing or conduction block. *Left lower panel, 32-Alone*. At 32°C, conduction is preserved and no conduction block is seen. *Right lower panel, 32-Amio*. With the addition of amiodarone, conduction is slowed but no conduction block is seen. **B**, (**a**) Summary data are shown. At 36°C, no difference is seen between the 36-Alone and 36-Amio groups during ischemia or reperfusion is observed. (**b**) At 32°C, amiodarone causes significant conduction slowing during ischemia †$P<0.05$ by repeated measures. Amio indicates amiodarone; CV, conduction velocity; ENDO, endocardium; and EPI, epicardium.

Figure 6B shows significant conduction slowing with amiodarone at 32°C (isochrones crowding on all activation maps); however, no arrhythmia was induced. This is attributable to the amelioration of RG, preventing conduction block in the presence of TH, so CV slowing did not increase susceptibility to arrhythmias during ischemia. Moreover, not only were RGs unchanged between groups, but also the location of the maximal RG was closer to the epicardium with amiodarone (Figure 6B, site B), where in control maximal gradients were observed in the midmyocardium (Figure 6A, site B). This observation was observed consistently over all experiments, where the maximal RG in the 36 Alone and 36 Amio where much closer to the endocardial

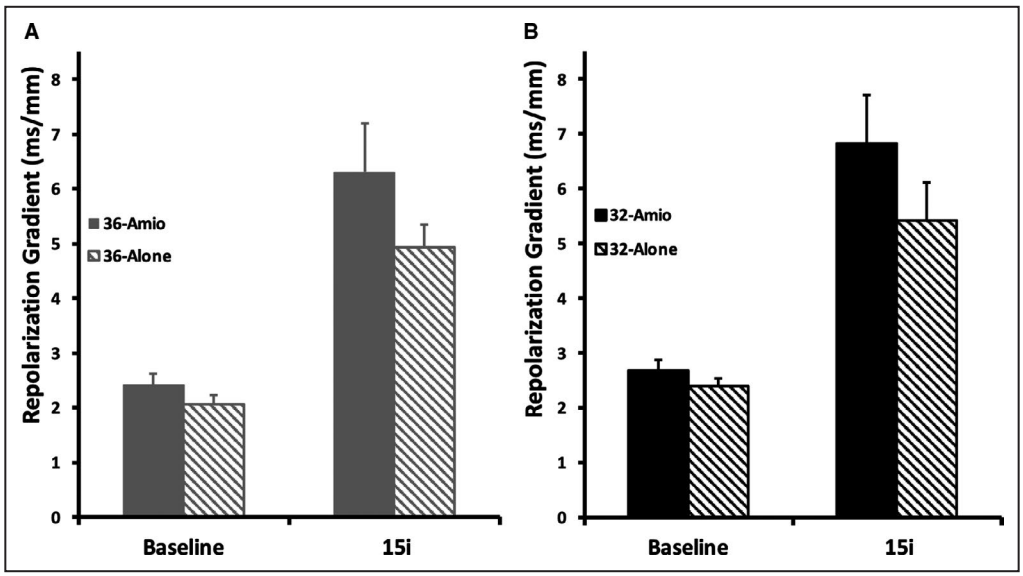

Figure 5. Effect of amiodarone on repolarization gradients.
A, At 36°C, repolarization gradients increased but no additional differences were seen with amiodarone (Amio). **B**, Similar increases in repolarization gradients were seen at 32°C, again with no change with amiodarone.

site of stimulation (4.9 and 4.4 mm, respectively) versus the 32 Amio group (8 mm, $P<0.03$). Therefore, also involved in the mechanism for prevention of CV block and arrhythmias despite increasing DOR in the 32-Amio group was that the RGs were more downstream of endocardial activation, and CV slowing allowed more time for these downstream sites to repolarize, making them no longer refractory during impulse propagation, preventing conduction block and arrhythmias.

In summary, the addition of TH to amiodarone produces conduction slowing and introduces APD heterogeneity increasing DOR but does not induce block or reentrant arrhythmias. Amiodarone without TH also did not significantly alter conduction block or reentrant arrhythmias. TH, with or without amiodarone, was protective for transmural conduction block and arrhythmia inducibility.

Effect of Amiodarone and TTM on In Vivo Electrophysiology During Resuscitation

We next evaluated the interaction of amiodarone and hypothermia on cardiac electrophysiology in our previously validated translational model of resuscitation from ischemia-induced myocardial infarction. We achieved temperatures consistent with clinical TTM at ≈16 minutes after left anterior descending coronary artery reperfusion in both hypothermic groups (Figure 7). Figure 8 shows representative examples of the ECG at baseline and at 240 minutes of experimental time, which was 180 minutes of reperfusion. Ischemia shortened the QTc interval at 180 minutes of reperfusion, which was attenuated by TTM. Although not statistically significant, the same trend of QTc shortening by ischemia and subsequent amelioration by amiodarone was seen (P=ns) (Figure 8). Also consistent with the known effect of TH on ventricular repolarization, when coldest temperature (240 minutes of the experiment, or 180 minutes of reperfusion) was achieved, action recovery intervals were prolonged in all regions (infarct, border, and normal zones), similarly in the presence or absence of amiodarone (data not shown, $P<0.05$). Tpeak-Tend is a validated measure of global DOR in the heart.[34] We observed no significant differences in Tpeak-Tend between any groups, but there was a trend in the presence of amiodarone, where TH attenuated Tpeak-Tend at 16 minutes of reperfusion (97±17 ms versus 60±13 ms, P=0.052 by repeated measures). When examining local DOR within any specific zone (infarct, border, and normal zones), there were no differences between control and amiodarone. Left ventricular activation time was similar in all groups.

Effect of Amiodarone and Temperature on Arrhythmic Rearrest After ROSC

We observed frequent rearrest because of VF and PEA in vivo. Incidence of VF and PEA after initial ROSC in each group is shown in Figure 9. There were no statistically significant differences between incidence of VF or PEA arrest among the 4 treatment groups. When comparing all subjects who received amiodarone, regardless of temperature, amiodarone suppressed rearrest because of VF (7/8 without amiodarone, 2/7 with amiodarone, P=0.041), but not PEA (2/8 without amiodarone, 5/7 with amiodarone, P=0.13).

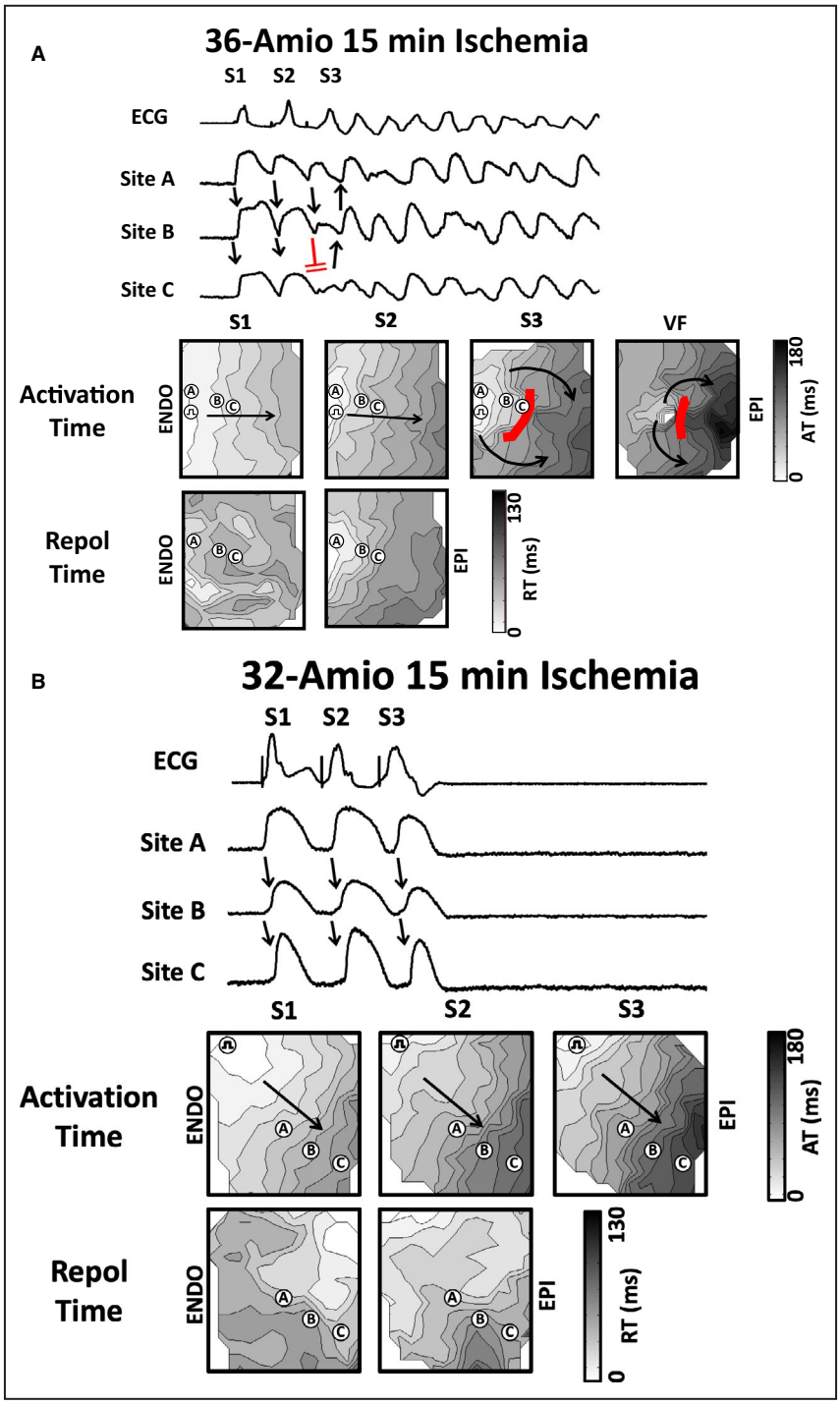

Figure 6. Effect of temperature on arrhythmia initiation with amiodarone.
A, Representative arrhythmia at 36°C and amiodarone. At 36°C with amiodarone (Amio), programmed electrical stimulation (PES) during ischemia induces ventricular fibrillation (VF). *Upper:* Representative recordings at 3 sites from endocardium (Site A) to midmyocardium (Site C). *Lower:* Activation time (AT) and repolarization (Repol) time (RT) maps for these PES trials are shown. Two premature beats (S2 and S3) are introduced during steady state pacing (S1). Conduction slowing and block (red line, upper and red bar, lower) is observed on the S3 beat, inducing reentrant VF (black curved arrows, lower). Steep repolarization gradients (crowding of isochrones) are observed in the subendocardium at the site of block (S2, Repolarization map). **B**, Amiodarone does not induce VF, despite worsening conduction velocity and dispersion of repolarization. No arrhythmia occurs under the same conditions at 32°C. *Upper:* Representative recordings at 3 sites in the midmyocardium (Site A) to epicardium (Site C). *Lower:* Activation time (AT) and repolarization time (RT) maps for these PES trials are shown. At 32°C, conduction is markedly slowed but premature beats do not block as repolarization has already occurred when the beats arrive. The repolarization slowing (crowding of isochrones) occurs further downstream towards the epicardium.

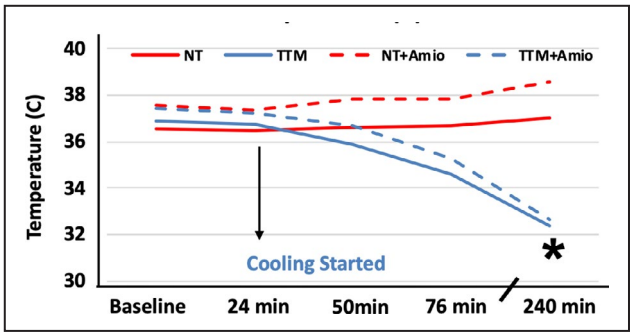

Figure 7. In vivo temperature during resuscitation.
Average temperature in all 4 groups during resuscitation. At baseline, no differences in temperature were noted. Cooling began at 30 minutes (arrow). At 76 minutes (16 minutes of reperfusion and 46 minutes after cooling was started) significant differences in temperature in the targeted temperature management (TTM) groups were noted (TTM, solid blue line and TTM+Amio, dashed blue line) compared to normal temperature (NT, solid red line) and NT with amiodarone (NT+Amio, dashed red line). By the end of the experiment, temperature remained significantly different in the TTM groups. *P<0.05. Amio indicates amiodarone; and NT, normal temperature.

DISCUSSION

Our data both ex vivo and in vivo suggest that, despite both promoting and ameliorating individual arrhythmia substrates, the overall effect of amiodarone is not proarrhythmic over the range of temperatures used in TTM.

Clinically and mechanistically, the effects of amiodarone are divided into chronic use and acute IV administration. Acute administration generally produces more conduction delay and less postrepolarization refractoriness than chronic administration.[25] During conduction, amiodarone affects I_{Na} and L-type calcium channels, causing conduction slowing. During repolarization, amiodarone has mixed effects on potassium currents, including I_{Kr}, I_{Ks}, I_{To}, and $I_{K,ATP}$, which explains the minimal effect on DOR under normal conditions.[23,25] Acute amiodarone administration also does not affect resting membrane potential.

The mechanism of amiodarone's antiarrhythmic effect during resuscitation is not entirely known. In animal models, IV amiodarone has been shown to improve VF waveform dynamics but not defibrillation thresholds as well as prevent VF spiral wave reentry.[29,35] One potential mechanism for the antiarrhythmic effect is the increase in postrepolarization refractoriness, thought to be caused by inactivation of sodium channels.[24] This is entirely consistent with our observations, where amiodarone did not increase ERP (a measure of refractoriness) during TH and ischemia. However, effects during resuscitation are likely very different than under other conditions.

Our data suggest that the addition of amiodarone to TH can worsen individual arrhythmia substrates (conduction slowing and increased transmural DOR), but not promote conduction block or arrhythmias. Our ex vivo data describe a possible mechanism for this observation during ischemia and TH, because although DOR increased, RG did not, in part because the transmural location of maximal RG (where block is likely to occur) occurs closer to the epicardium with amiodarone. Therefore, the slower endocardial-to-epicardial impulse propagation resulted in downstream repolarization before an impulse reaching subepicardial regions where RG had been greatest. This ensured uniform conduction across the transmural wall (Figure 6B). During normothermia with amiodarone, RGs remain unchanged and were located closer to the endocardial surface, facilitating subendocardial conduction slowing and block. Ex vivo, we did not identify a reduction in arrhythmia susceptibility with the addition of amiodarone either at 36°C or 32°C, although we only examined arrhythmias occurring transmurally during acute global I/R. Our rationale for using this model is that TH preferentially affects transmural conduction and repolarization during I/R. We first utilized the canine wedge model to identify any transmural effects. However, in vivo, pigs treated with amiodarone were less likely to have VF rearrest. Given the low numbers of our in vivo groups, we would likely be unable to detect small differences between groups. However, both our in and ex vivo data do not suggest an increased proarrhythmic effect of amiodarone during TH.

We chose to use a clinically relevant model of resuscitation and TH, with cooling initiated at ROSC, and as such, hypothermia was achieved only after ROSC and reperfusion (Figure 7). Although the model provides significant insight into this interaction during reperfusion after successful resuscitation, unlike our ex vivo model, the interaction between hypothermia and amiodarone was not evaluated during ischemia.

Our data on rearrest outcomes is consistent with frequent incidence of rearrest after initial ROSC observed clinically.[36–41] As expected, amiodarone did suppress recurrent ventricular tachycardia/VF after initial ROSC (Figure 9). We did observe more PEA rearrest in groups receiving amiodarone, although this was not statistically significant. Certainly, potential effects of amiodarone on promoting post ROSC PEA deserve further study. Most rearrest events we observed occurred during active cooling, but before reaching target temperature.

Limitations

Several limitations are noted for these experiments. First, to determine the effects of amiodarone and temperature on transmural arrhythmia substrates and obtain more detailed data on arrhythmia mechanisms, the canine ventricular wedge model was chosen. This model, utilizing optical mapping, although ideal for examining the

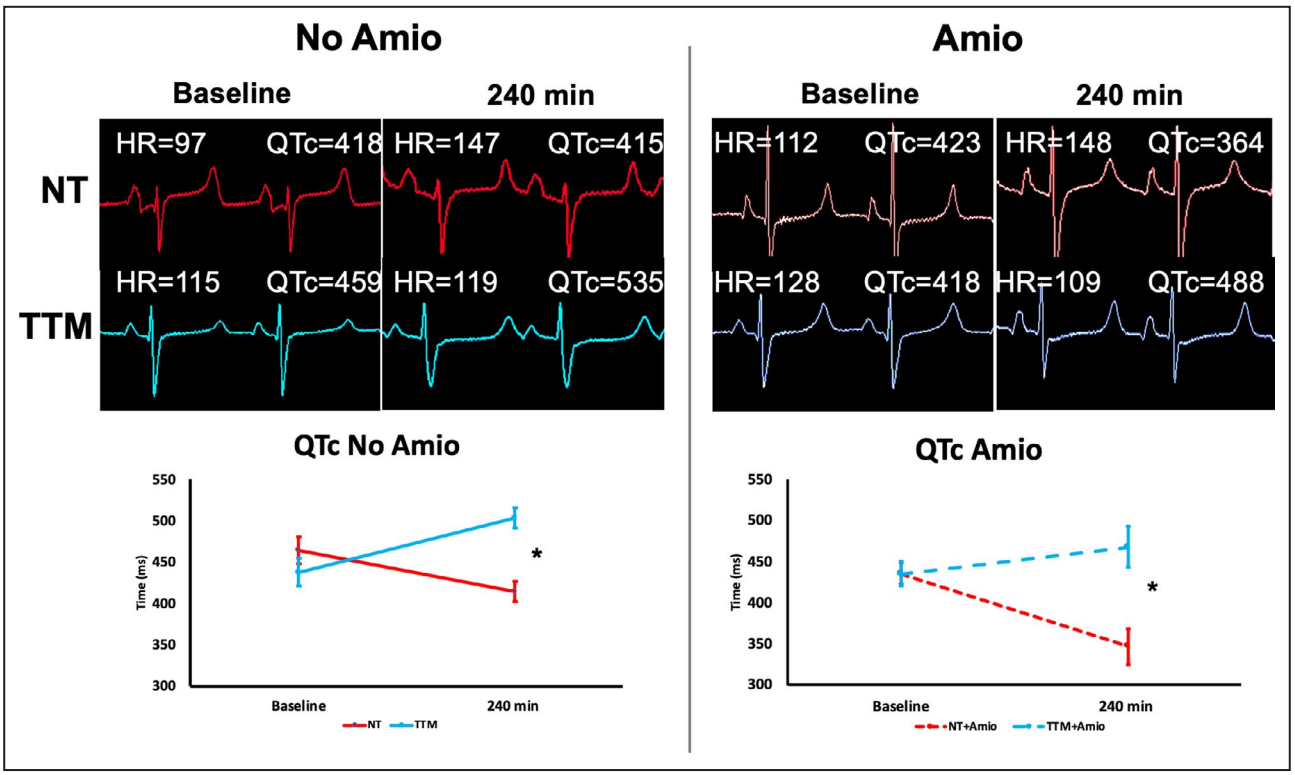

Figure 8. Effect of hypothermia and amiodarone on QTc.
Left upper. Representative ECGs at baseline and 240 minutes of experiment time. At 240 minutes, the QTc is longer in the targeted temperature management (TTM) group vs the normal temperature (NT) group. *Left lower.* Summary data showing the increase in QTc with temperature alone at 240 minutes between the NT and TTM group (*P=0.016). *Right upper.* With the addition of amiodarone, QTc shortened from baseline in the NT group, which was ameliorated in the TTM group. *Right lower.* Summary data showing the QTc nonsignificant shortening in the NT group compared with the TTM group (*P=0.126). Amio indicates amiodarone; HR, heart rate; and NT, normal temperature.

transmural heterogeneities important in mechanisms of arrhythmias during hypothermia, does not address arrhythmia substrates in the whole heart.[28] Furthermore, the temperature remained constant throughout the protocol and was stabilized before ischemia was induced. Also, amiodarone was given before ischemia was induced in order to determine the effects on acute ischemia. It is for these reasons we also examined the interaction of amiodarone and hypothermia in our in vivo translational model, where amiodarone was administered during resuscitation after VF arrest, and TH was initiated after ROSC. Importantly, our data in both models are consistent with the notion that amiodarone during TH is not proarrhythmic. We chose a lower end point of TTM with our target temperatures of 32°C (ex vivo) and 32°C to 34°C (in vivo) in order to determine the effect of the lowest recommended temperature on arrhythmia substrates. Although it is possible that a higher temperature range would show fewer potential effects, it was the goal of this study to investigate the potential detrimental effects of hypothermia and amiodarone in combination. We used pressure support after ROSC in order to maintain adequate BP. Although this was used to mimic the likely clinical scenario, adding pressure support may (1) increase arrhythmias or (2) limit our ability to identify PEA. However, this was closer to the clinical scenario of a resuscitation, and there was no difference in the amount of pressor support required between groups. Our in vivo experiments are underpowered to determine effects of "clinical outcomes." However, our aim was to investigate the interaction of amiodarone and hypothermia on arrhythmia substrates. Although our optical mapping experiments allow significant resolution to accurately determine CV and DOR, these measurements are somewhat limited in vivo given the electrophysiology data available. The studies were not blinded or randomized. Male dogs are used in canine wedge experiments to limit electrophysiologic variation.[42] Traditionally, female pigs have been used in our laboratory and recent evidence suggests a difference in cardiac arrest outcomes including VF.[43] A single sex is a limitation of this study.

CONCLUSIONS

The addition of amiodarone to hypothermia worsens ischemia-induced DOR and transmural CV slowing. Despite these apparent worsening of arrhythmia substrates ex

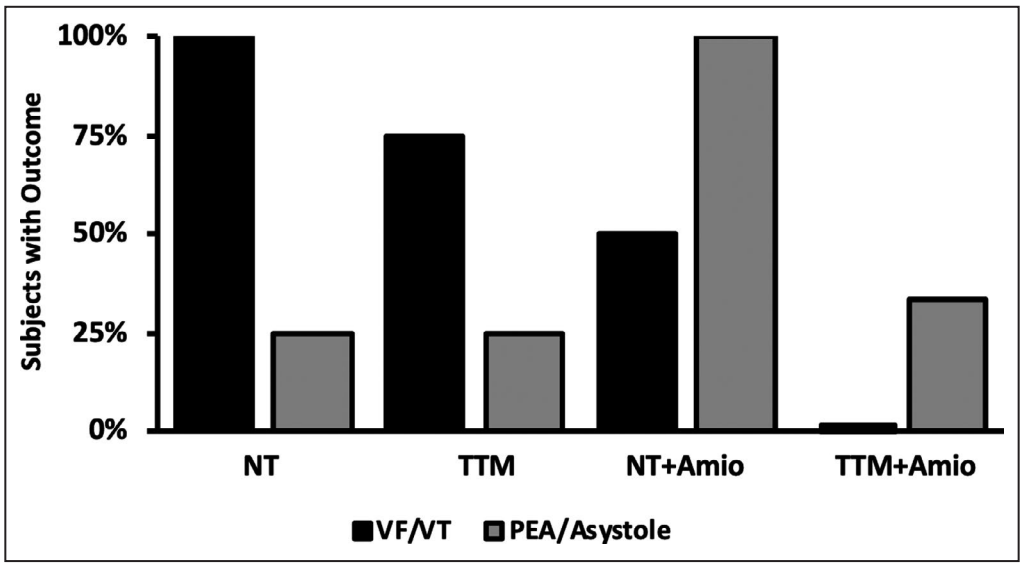

Figure 9. Incidence of ventricular fibrillation/tachycardia and PEA/Asystole after initial return of spontaneous circulation.
Percentage of subjects that had a rearrest in each group. No differences were seen between the 4 groups. However, when given amiodarone regardless of temperature, there was a decrease in VF/VT but an increase in PEA/Asystole. Amio indicates amiodarone; NT, normal temperature; PEA/Asystole, pulseless electrical activity/Asystole; VF/VT, ventricular fibrillation/tachycardia; and TTM, targeted temperature management.

vivo, there was no increase in conduction block or inducible arrhythmias in either model. These data suggest that amiodarone may not be proarrhythmic when used during TTM, particularly during ischemia/reperfusion as observed during resuscitation. A potential increase in incidence of PEA observed will be important to further evaluate.

ARTICLE INFORMATION

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Disclosures

None.

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