Published in final edited form as:

Heart Rhythm. 2009 April ; 6(4): 530–536. doi:10.1016/j.hrthm.2008.12.032.

Free Radical Scavenger Specifically Prevents Ischemic Focal Ventricular Tachycardia

Dezhi Xing, M.D., Ashok K. Chaudhary, Ph.D., Francis J. Miller Jr, M.D., and James B. Martins, M.D.

Department of Internal Medicine, University of Iowa College of Medicine and Veterans, Administration Medical Center, Iowa City, Iowa 52242

Abstract

Background—Focal ventricular tachycardia (VT) in acute myocardial ischemia is closely related to triggered activity (TA), which may be blocked by scavenging reactive oxygen species (ROS).

Objective—This study analyzed effects of acutely administered ROS scavenger-2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) on VT in vivo and TA in vitro.

Methods—Forty-three alpha chloralose anesthetized dogs with coronary artery occlusion were studied. 3-D activation mapping helped to locate the origin of focal or reentrant VT. TEMPO (30mg/kg, iv) or vehicle was given. Endocardium excised from the site of origin of VT was studied using standard microelectrode techniques and measures of ROS.

Results—Reentry and focal VT induction were both highly reproducible. TEMPO blocked focal VT in 6 of 11 dogs ($p < 0.05$), but 9 of 9 dogs with reentrant VT continued to have VT re-induced after TEMPO. TEMPO did not alter effective refractory period (168 ± 3 to 171 ± 3 ms), mean blood pressure (88 ± 3 to 81 ± 3 mmHg), and size of ischemia ($42 \pm 3\%$ vs $40 \pm 4\%$). In vitro, TEMPO (10^{-3} M, $n=14$) produced no change in action potentials. Nevertheless, TA was reversibly attenuated from 5.3 ± 1.1 to 0.4 ± 0.4 complexes with TEMPO ($n=15$, $p < 0.05$). Lucigenin-enhanced chemiluminescence and dihydroethidium staining showed increased ROS in ischemic endocardium; TEMPO dramatically reduced ROS in ischemic sites.

Conclusions—TEMPO, a scavenger of ROS, prevented triggered activity associated with focal VT during myocardial ischemia in areas of increased ROS. Antioxidant therapy may play an important role in blockade of focal VT under the conditions of myocardial ischemia.

Keywords

TEMPO; Scavenging of Reactive Oxygen Species; focal ventricular tachycardia; triggered activity

Correspondence to: James B. Martins, M.D., Department of Internal Medicine, Cardiology Division, E318GH, University of Iowa Hospitals and Clinics, 200 Hawkins Drive, Iowa City, IA 52242, Phone: 319-356-2740, Fax: 319-353-6343, E-mail: james-martins@uiowa.edu.

Conflict of interest: none

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

We previously reported that reproducible ischemic ventricular tachycardia (VT) or ventricular fibrillation (VF) has sites of origin on endocardium in up to 61%, which is closely correlated with triggered activity (TA) due to delayed afterdepolarizations (DADs) (1). VT/VF and DADs are further linked since lovastatin, an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, blocked both focal VT in vivo and TA in vitro possibly through free radical scavenging (2).

Considerable evidence suggests that free radicals are involved in myocardial ischemia/reperfusion injury, and could be important mediators of VT/VF (3,4). Increased post-ischemic susceptibility to oxygen radical damage results from build-up of a strongly reducing environment during ischemia, along with a decreased antioxidant defense capacity (3,5). Therapeutic strategies have attempted to reduce free radical damage either by intervening in their formation process or by scavenging free radicals already formed. Recently, in vivo study of rat hearts show that ischemia alone may generate reactive oxygen species (ROS) (6).

Prior studies suggest that there is a dose response of reperfusion VT/VF to antioxidants that must be given before reperfusion (7,8,9) even in patients. TEMPO (2,2,6,6-tetramethylpiperidine-N-oxyl) is a stable, low-molecular weight piperidine nitroxide, is highly permeable to the cell membranes, and has been demonstrated to be a superoxide dismutase mimic. Previous studies demonstrated that TEMPO prevents free radical damage in cell cultures (10), ischemia/reperfusion injury (11,12,13), and reduces myocardial infarct size in rats both in vivo and in vitro (14). We tested whether TEMPO blocked myocardial ischemic VT in vivo without reperfusion and TA in vitro.

Methods

Forty-three healthy dogs of either sex weighing 15–20 kg were used for the study. The Animal Care and Use Review Board at the University of Iowa approved the protocol, which conformed to the Guidelines of the American Physiological Society.

Surgical preparation

Dogs were pretreated with intra-muscular injections of 0.5 mg/kg acepromazine maleate mixed with 10 mg/kg of ketamine. Anesthesia was induced by intravenous administration of 10 mg/kg thiopental sodium followed by an α -chloralose bolus (100–200 mg/kg). Anesthesia was maintained by constant infusion of α -chloralose (8mg/kg/hr dissolved in saline and polyethylene glycol, molecular weight 200). The dogs were intubated and mechanically ventilated on a volume-cycled respirator (Harvard) to maintain a PO_2 of 80–110 mmHg, a PCO_2 of 35–45 mmHg, $NaHCO_3$ was infused as necessary to maintain the pH range of 7.35–7.45 (Radiometer, Copenhagen, Denmark). The femoral vein and artery were cannulated for administration of fluid and drugs and also for continuous measurement of mean arterial blood pressure (MAP).

The pericardium was incised through a midline sternotomy and sutured to the wound edges forming a pericardial support for the heart. A silk suture was passed under the left anterior descending coronary artery just distal to the first diagonal branch. The suture was threaded in tubing forming a snare for coronary artery occlusion. Epicardial temperature was maintained at $\sim 37^\circ\text{C}$ by an infrared heating lamp and a plastic sheet draped over the sternotomy. Warm saline was applied to the heart intermittently to prevent surface drying.

Electrophysiological preparation

Surface ECG leads (II and V₅R) were recorded continuously. The sinus node was permanently clamped so as to control the heart rate. The atria were stimulated at twice diastolic threshold with pulses of 2 ms duration with a bipolar electrode at a cycle length of 300 ms. To record transmural signals, twenty-three 16-pole plunge-needle electrodes (J. Kassell, Fayetteville, NC) were inserted into the myocardium in and surround the risk zone of LAD as previously reported(1). The inter-needle distance was about 1 cm depending on coronary artery anatomy.

Bipolar electrograms were recorded from up to 6 different sites on each 16-pole electrode. To maximize the capability of recording from Purkinje fibers, noise-free bipolar signals were chosen by sequential recording on a storage oscilloscope of each successive bipole. Purkinje, endocardial, midwall and epicardial electrograms were chosen as previously described (1). Electrograms were recorded simultaneously on two computers: one for the three endocardial-most bipoles, and the other for the three epicardial-most bipoles. Signals from the former were amplified by the gain of 100, band pass filtered between 3 and 1,300 Hz, and sampled at 3.2 kHz. The epicardial electrograms were sampled at frequency of 1 kHz per channel and band-pass filtered at 30–300 Hz. 3-D activation maps were developed from multiplexed signals. Data from both acquisition systems were incorporated for the construction of 3-D activation maps with a common surface electrocardiogram (II) recording pacing spikes, allowing for alignment of signals from both computers.

Each needle had 16 unipoles, which were used to select up to six optimal bipolar electrograms that were adjusted to maximize the capability to record Purkinje signals on the endocardial-most bipoles. The adjustment was performed by sequential recording on the digital oscilloscope for each single electrode. A switching box was utilized to connect the selected bipoles to each amplifier. Purkinje signal was identified at endocardial-most bipoles according to previous published criteria from this laboratory, including 0.5mV spikes lasting 1–2ms, preceding by 1–11 ms the larger and longer muscle spike and the surface QRS on the lead recording the earliest activity (1). Occasionally, the Purkinje activation was identified by the relationship to surrounding electrograms suggesting local Purkinje activation, particularly when the size of the Purkinje activation was substantially decreased when rapid rates occur. This phenomenon occurs in figure 1 for P-N during extra-stimuli and P-N, P-F and P-S activations at the origin of the VT complexes.

Experimental protocol

Blood gas and adequate anesthesia was confirmed before LAD ligation. The effective refractory period was determined by delivering extrastimuli after 8 paced complexes, with the ERP defined as the longest interval between the drive pacing (S₁) and the first extrastimulus (S₂) that failed to capture the ventricle at four times diastolic threshold. The drive cycle length was 300 ms. VT was induced by programmed electrical stimulation using up to four premature stimuli (S₂–S₅). Pacing protocols were started one hour after occlusion. Previous studies using this model have demonstrated that VT may be reproducibly induced over the next 1–3 hours. Sustained VTs were usually terminated by pacing or cardioverted with epicardial shocks of 10–20 joules. Repeat testing was done from apical, septal or lateral left ventricular pacing sites every 20 minutes and continued until the successful induction of two episodes of VT with similar surface lead morphology. If VT with similar morphology could not be induced twice within 3 hours after occlusion, the experiment was terminated. In dogs in which VT was reproducibly induced, TEMPO (30mg/kg, iv, Sigma-Aldrich) or saline was given randomly by IV infusion respectively. Five min into each infusion, extra-stimulus testing was performed from the same pacing site(s), which induced VT during the control period.

Intracellular Recording Techniques

After 3-D activation mapping, endocardial tissue from the focal origin of VT or ischemic zone (epicardial reentry) was excised and placed in Tyrode's solution with the following composition (mM): 125 NaCl, 24 NaHCO₃, 4.5 KCl, 1.8 CaCl₂, 0.5 MgCl₂, 0.25 NaHPO₄, and 5.5 dextrose (pH 7.4). The tissue was superfused with Tyrode's solution (37 °C) at 9 ml/min. Fibers were stimulated with a bipolar electrode at twice diastolic threshold. Action potential measurements were performed during pacing at 1.5 to 4 Hz. The most superficial cells were impaled with 3 M KCl filled glass capillary microelectrodes with tip resistances of 6 to 10 MΩ. Microelectrodes were connected to a high input impedance preamplifier (Axoclamp-2A, Axon Instruments, Foster City, CA). The bath was grounded with a Ag/AgCl pellet. Potentials were recorded and stored on a computer with the use of commercial software (Axon Instruments) with data filtered at 1 kHz and sampled at 2 kHz. Zero offsets were recorded to correct for drift. Purkinje cells were identified by spontaneous phase 4 depolarization or automaticity at cycle lengths more than 1 s. If there was no DADs or TA inducible, isoproterenol (0.5 μM) was added. Tissues were first assessed for presence or absence of DADs and TA prior to and during isoproterenol superfusion. To determine the effect on DADs and TA, TEMPO (10⁻⁴ to 10⁻²M) was added if they were induced. Washout of the drug and isoproterenol was also performed.

Chemiluminescence and dihydroethidium staining for reactive oxygen species (15)

Endocardial tissues were studied from electrode sites from two dogs with a total of 8 tissues (two normal and six ischemic sites with a focus of VT). Tissues were cut into 5- × 5-mm endocardial segments in cold oxygenated Tyrode's. Each were separated into halves for detection of ROS levels by two independent methods, lucigenin-enhanced chemiluminescence and dihydroethidium staining, as previously described (16). Briefly, endocardial segments were flash frozen, cut into 30-micron-thick sections and placed on a glass slide. Dihydroethidium (2×10⁻⁶ mol/L, Invitrogen-Molecular Probes) was topically applied to each tissue section and incubated at 37°C for 30 minutes in a light-protected humidified chamber. Images were obtained with a Zeiss LSM510 confocal microscope (excitation 514 nm at 4% laser power, emission 575-nm long-pass filter). All images were obtained at identical settings with 20x/0.8 Plan-Apochromat objective, pinhole size 100 μm, and mean line scan of four.

In separate experiments, ROS was detected by chemiluminescence. Endocardial segments were placed in PBS and lucigenin (5 μmol/L), and after 2 minutes of dark adaptation, relative light units (RLU) emitted were measured for 5 minutes in an FB12 luminometer (Zylux Corp). Mean RLU per second was normalized to the tissue weight.

Definitions and Analysis

Mapping of electrograms was done off line as previously described (1,2). VT was defined as at least five consecutive non-stimulated ventricular complexes and sustained VT was defined as VT that did not self-terminate within 10 sec; most episodes of VT were pace terminated. VF was defined as irregular ventricular rhythm that resulted in hemodynamic collapse and required a shock to terminate. The cycle length (CL) of each VT episode was measured by averaging the first, up to 10, CLs. VT mechanisms were defined as follows: Reentrant VT occurred when the electrode recording the earliest activity was immediately adjacent to the site of the latest activation from the previous complex and diastolic activity was recorded between complexes. Focal VT occurred when the electrode recording the site of origin was surrounded on 6 sides by other electrodes within 1–2 cm that recorded progressive and gradually later activity while moving away from the site of origin. There was no late (>50% cycle) electrical activity on adjacent sites. Ischemia was defined by voltage reduction as in our prior report (1,2).

Depolarization occurring after phase 3 of paced action potentials (APs) was defined as DADs. Non-paced APs occurring at the peak of a DADs showing overdrive stimulation was defined as TA.

Statistics

The effect of TEMPO on the incidence of VT, DADs and TA was analysed by a two-tailed Fisher's exact test at each dose level. The effect of TEMPO on cycle length of VT, effective refractory period and mean arterial pressure was analyzed by a two-way ANOVA with repeated measurements. $P < 0.05$ was considered statistically significant. All values are means $\pm$ SEM.

RESULTS

Of 43 dogs studied, 20 were treated with TEMPO and 23 were given saline, serving as a control group for inducibility over the 1–3 hour after coronary artery occlusion.

In the TEMPO treated group, 6 of 11 dogs with focal VT/VF origin had VT induction blocked. One third of dogs had inducible VF, of which only one (with focal origin) responded to TEMPO by becoming non-inducible. The overall response to TEMPO was significantly different than focal endocardial or Purkinje VT/VF in dogs with saline treatment of which 11 of 12 had continuously inducible VT/VF ($p < 0.05$).

Figure 1 shows induction of focal VT. Several sites including P-N, P-F and P-S recorded earliest activity prior to the surface QRS (with up-arrows), but only site P-F persisted as the site of origin for the subsequent VT. Note that all surrounding activity suggests focal activation with no late activation that would be expected with reentrant excitation. In general, individual episodes of VT and VF showed mechanisms that may change anatomical site as shown in figure 1. However, mechanisms did not change from reentry to focal or visa versa as observed by others in long-lasting VF (17).

Figure 2 shows 3-D map of the first stimulus of figure 1. Earliest activation is located in the PURK layer (13 ms after the QRS) with activation proceeding out to EPI with no significant conduction delay to suggest a reentry mechanism.

Figure 3 shows the 3-D map with endocardial and Purkinje foci separated by later activation times, especially in propagation to the epicardium. These figures demonstrate the typical focal Purkinje VT originating from Purkinje layer.

VT/VF with a reentrant mechanism was not affected by TEMPO; 9 of 9 dogs without block during TEMPO had reentry; this was no different in saline treated dogs with epicardial reentry VT/VF in which 10 of 11 were continuously inducible ($p = \text{NS}$). Thus TEMPO blocked focal VT but not reentry VT ($P < 0.05$). In addition, TEMPO did not change the mean cycle lengths of VT still inducible 138 ± 6 vs 140 ± 5 msec ($p = \text{NS}$). Also, cycle length was similar in two groups before treatment, averaging 138 ± 6 msec in TEMPO group and 134 ± 3 msec in saline group ($p = \text{NS}$). With TEMPO, mean arterial pressure decreased from 88 ± 3 mmHg to 81 ± 3 mmHg ($p = \text{NS}$); and refractory period was unaffected (168 ± 3 vs 171 ± 3 msec, $p = \text{NS}$). Similarly the size of the ischemic area was stable over time and unaltered by TEMPO ($42 \pm 3\%$ vs $40 \pm 4\%$, $p = \text{NS}$).

Of tissues excised from endocardium studied in vitro, TA was reversibly attenuated from 5.3 ± 1.1 to 0.4 ± 0.4 complexes with TEMPO ($n = 15$, $p < 0.05$), but no attenuation was seen in 6 tissues with vehicle alone ($p < 0.05$, vs TEMPO group). Two tissues had TA blocked at 10^{-4}M , while each of four tissues was blocked at 10^{-3}M and 10^{-2}M . In five tissues showing

no attenuation of TA with TEMPO all had 10^{-3} M only. Figure 4 showed DADs and TA in tissue excised from ischemic endocardium reversibly blocked by TEMPO. In tissues, which did not have TA, TEMPO did not facilitate the induction, nor was abnormal automaticity exposed. To examine possible direct electrophysiological effects on ion channels, we examined the effects of TEMPO on APs in vitro. The results suggested that TEMPO (10^{-3} M, n=15) produced no change in resting membrane potential (80 ± 3 vs. 78 ± 3 mV), action potential amplitude (94 ± 3 vs. 90 ± 3 mV), action potential duration at 90% depolarization (268 ± 10 vs. 270 ± 11 ms) and action potential duration at 50% depolarization (193 ± 8 vs. 191 ± 8 ms) respectively (P=NS).

Levels of ROS were found to be increased about three-fold in endocardium from ischemic zones, as compared to the endocardium from nonischemic regions, as detected by chemiluminescence. As expected, treatment with the antioxidant TEMPO markedly reduced endocardial levels of ROS in ischemic regions toward control levels (data not shown). Dihydroethidium staining confirmed dramatic increases in endocardial ROS levels in regions of ischemia in the untreated animal. In the second animal, TEMPO blocked focal VT originating from ischemic region A and markedly attenuated dihydroethidium staining in all four ischemic sites (figure 5). Of interest, the most intense staining was from region A.

DISCUSSION

We combined in vivo and in vitro studies on ischemic myocardium and confirmed our previous report that TA due to DADs plays a major role in generation of focal VT/VF (1); we showed that TEMPO, a free radical scavenger, could specifically block focal and not reentrant VT/VF correlated with inhibition of TA due to DADs. Additionally, we provide evidence of excess ROS documented in ischemic tissues that was markedly attenuated by TEMPO. These data provide the first mechanistic evidence that excess ROS causes ischemic focal VT/VF.

The role of oxidative stress in cardiovascular disease has been characterized (18). It has been suggested that ROS, including superoxide anions, hydroxyl radicals and hydrogen peroxide, contribute to the genesis of arrhythmias that are associated with ischemia/reperfusion. TEMPO is a membrane-permeable superoxide dismutase-mimic that has been demonstrated to scavenge superoxide anion and inhibits the formation of hydroxyl radicals (19), so as to protect tissues against a variety of ischemia/reperfusion arrhythmia (8,10,11,12,20). The ability of TEMPO to protect against arrhythmia was dramatic and consistent by all criteria used, such as duration, incidence, and the spontaneously developed final rhythm after 5 minutes of reperfusion. This protective effect was also dose dependent and not attributable to vehicle given with TEMPO (3). Guo, et al (8) found that high doses provided better protection. Our results also showed incremental doses of TEMPO were more effective in blocking TA in vitro, but raising the possibility that non-specific effects of such a high dose may play a role. When single doses of other agents have been used in a dog model of 25 minutes of coronary occlusion, partial effect on early but not late spontaneous VT occurred (21); higher doses were not investigated.

An effect of TEMPO administration was reported to slow down the heart rate, reaching 30% at 1 mM dose (3,8). However, in our model with fixed atrial pacing at CL 300 msec and sinus node permanently clamped, the elimination of this heart rate difference during equilibration and ischemia, still showed specifically blocking focal compared with reentry, indicating that the protection was not derived from the negative chronotropic effect. Intravenous administration of TEMPO also resulted in dropping in mean arterial pressure (8,22); it is suggested that both a direct vasodilation and an inhibitory action on sympathetic nervous system were involved. Kabell et al (23) reported that blood pressure may affect

focal arrhythmias arising in ischemically injured heart after 24 hours myocardial ischemia. However, in previous study of the same model (24), a decrease in MAP of up to 50 mmHg did not affect the inducibility of focal or reentrant VT/VF. In the present study, we found similar mean arterial pressure change after TEMPO administration with a fixed cardiac pacing rate, associated with block of only focal VT/VF. We believe these non-significant changes are not the cause of block of induction of focal VT/VF.

The protective effects of TEMPO on ischemia/reperfusion arrhythmias might be due to a direct antiarrhythmic activity. Gelvan and Guo et al (3,8) found that tempol, given at 1 min after reperfusion, at a time when arrhythmias had already occurred, did not reduce the incidence of VT/VF. Although their results were interpreted that nitroxides did not possess a direct antiarrhythmic property, ours showed that TEMPO blocked TA reversibly in vitro. This effect was not associated with changes in AP in vitro therefore consistent with effects on cellular effects involving calcium fluxes, and not typical ion channel targets (4,25). ROS (26) may increase $[Na^+]_i$, which when exchanged for extracellular Ca^{2+} via Na^+-Ca^{2+} exchanger causes cellular Ca^{2+} overload as evidenced by the DADs. Electrophysiological changes in the calcium channel function occur during exposure to free radicals (27).

Interventions that aimed at scavenging or inhibition their formation have been slow to effectively alleviate arrhythmias (8,9). In part, this may be related to the dosage required to scavenge ROS to produce an anti-arrhythmic action in an occluded vascular territory. TEMPO has better access to cells than many other scavenger. Our study with both methods showing evidence of intracellular free radical reduction in a large animal model suggests such doses may be enough to markedly reduce ROS enough to prevent TA. Our experiment showing the highest ROS levels and density of dihydroethidium staining at the site of focal VT further suggest ROS as a pathophysiological mechanism in TA.

Limitations

The model used for these studies involves open chest dogs with myocardial ischemia. Even with 23 plunge electrodes in the area of ischemic zone and surrounding recording up to 118 signals, we cannot exclude microentry. Our data show that TEMPO specifically blocked focal VT/VF compared with reentry, but some tissues may not respond owing to higher ROS or inability to achieve effective concentrations, or both. Our dose response studies support the former.

Conclusion

The results of our study suggest that TEMPO, a membrane permeable SOD mimic, selectively prevents induction of focal ventricular tachyarrhythmias due to delayed afterdepolarizations and triggered activity. This mechanism is based on reduction of excess ROS in ischemic sites of VT.

Acknowledgments

This work was supported by grants from the United States Department of Veterans Affairs to FJM and JBM and the National Institutes of Health (HL081750) to FJM

Abbreviations

VT	ventricular tachycardia
TA	triggered activity
ROS	reactive oxygen species

TEMPO	2,2,6,6-tetramethylpiperidine-N-oxyl
VF	ventricular fibrillation
DADs	delayed afterdepolarizations
RLU	relative light units
APs	action potentials

References

1. Xing D, Martins JB. Triggered activity due to delayed afterdepolarizations in sites of focal origin of ischemic ventricular tachycardia. *Am J Physiol Heart Circ Physiol*. 2004; 287:H2078–H2084. [PubMed: 15475531]
2. Xing D, Murry DJ, Schmidt MS, et al. Lovastatin specifically prevents focal ischemic ventricular tachycardia due to triggered activity. *Heart Rhythm*. 2007; 4(5):629–37. [PubMed: 17467633]
3. Gelvan D, Saltman P, Powell SR. Cardiac reperfusion damage prevented by a nitroxide free radical. *Proc Natl Acad Sci*. 1991; 88:4680–4684. [PubMed: 1647012]
4. Beresewicz A, Horackova M. Alterations in electrical contractile behavior of isolated cardiomyocytes by hydrogen peroxide: possible ionic mechanisms. *J Mol Cell Cardiol*. 1991; 23:899–918. [PubMed: 1942091]
5. Das, DK.; Engelman, RM. Oxygen Radicals: Systemic Events and Disease Processes. Das, DK.; Essman, WB., editors. Karger: Basel; 1990. p. 97-128.
6. Pasdois P, Beauvoit B, Tariosse L, et al. Effect of diazoxide on flavoprotein oxidation and reactive oxygen species generation during ischemia-reperfusion: a study on Langendorff perfused rat hearts using optic fibers. *Am J Physiol Heart Circ Physiol*. 2008; 294:H2088–H2097. [PubMed: 18296562]
7. Duilio C, Ambrosio G, Kuppusamy P, et al. Neutrophils are primary source of O₂ radical during reperfusion after prolonged myocardial ischemia. *Am J Physiol*. 2001; 280:H2649–H2657.
8. Guo R, Gao X, Wang W, et al. Tempol reduces reperfusion-induced arrhythmias in anesthetized rats. *Pharmacol Res*. 2005; 52:192–198. [PubMed: 15967386]
9. Hicks JJ, Montes-Cortes DH, Cruz-Dominguez MP, et al. Antioxidants decrease reperfusion induced arrhythmias in myocardial infarction with ST-elevation. *Front Bioscience*. 2007; 12:2029–2037.
10. Hahn SM, Mitchell SE. Tempol inhibits neutrophil and hydrogen peroxide-mediated DNA damage. *Free Radic Biol Med*. 1997; 23(6):879–884. [PubMed: 9378367]
11. Kato N, Yanaka K, Hyodo K, et al. Stable nitroxide tempol ameliorates brain injury by inhibiting lipid peroxidation in a rat model of transient focal cerebral ischemia. *Brain Res*. 2003; 979 (1–2): 188–193. [PubMed: 12850585]
12. Rak R, Chao DL, Pluta RM, et al. Neuroprotection by the stable nitroxide Tempol during reperfusion in a rat model of transient focal ischemia. *J Neurosurg*. 2000; 92(4):646–651. [PubMed: 10761655]
13. Sepodes B, Maio R, Pinto R, et al. Tempol, an intracellular free radical scavenger, reduces liver injury in hepatic ischemia-reperfusion in the rat. *Transplant Proc*. 2004; 36(4):849–853. [PubMed: 15194291]
14. McDonald MC, Zacharowski K, Bowes J, et al. Tempol reduces infarct size in rodent models of regional myocardial ischemia and reperfusion. *Free Radic Biol Med*. 1999; 27(5–6):493–503. [PubMed: 10490268]
15. Miller FJ Jr, Gutterman DD, Rios CD, et al. Superoxide production in vascular smooth muscle contributes to oxidative stress and impaired relaxation in atherosclerosis. *Circ Res*. 1998; 82:1298–1305. [PubMed: 9648726]
16. Miller FJ Jr, Sharp WJ, Fang X, et al. Oxidative stress in human abdominal aortic aneurysms: a potential mediator of aneurysmal remodeling. *Arterioscler Thromb Vasc Biol*. 2002; 22:560–565. [PubMed: 11950691]

17. Li L, Jin Q, Huang J, et al. Intramural foci during long duration fibrillation in the pig ventricle. *Circ Res*. 2008; 102:1256–1264. [PubMed: 18420942]
18. Zhang G, Kimura S, Nishiyama A, et al. Cardiac oxidative stress in acute and chronic isoproterenol-infused rats. *Cardiovasc Res*. 2005; 65:230–238. [PubMed: 15621051]
19. Mitchell JB, Samuni A, Krishna MC, et al. Biologically active metal-independent superoxide dismutase mimics. *Biochemistry*. 1990; 29(11):2802–2807. [PubMed: 2161256]
20. Hahn SM, DeLuca AM, Coffin D, et al. In vivo radioprotection and effects on blood pressure of the stable free radical nitroxides. *Int J Radiat Oncol Biol Phys*. 1998; 42(4):839–842. [PubMed: 9845107]
21. Agnes H, Nagy L, Parratt J, et al. N-2-mercaptopropionylglycine, a scavenger of reactive oxygen species, does not modify the early antiarrhythmic effect of ischaemic preconditioning in anaesthetised dogs. *Cardiovasc Drugs Ther*. 2004; 18:449–459. [PubMed: 15770432]
22. Xu H, Fink GD, Chen A, Watts S, et al. Nitro oxide-independent effects of tempol on sympathetic nerve activity and blood pressure in normotensive rats. *Am J Physiol*. 2001; 281:975–980.
23. Kabell G, Brachmann J, Scherlag BJ, et al. Mechanisms of ventricular arrhythmias follow one stage and two stage coronary reperfusion: evidence for both reentry and enhanced automaticity. *J Electrocardiol*. 1985; 18:87–96. [PubMed: 2579181]
24. Arnar DO, Xing DZ, Lee H, et al. Prevention of ischemic ventricular tachycardia of Purkinje origin: a role for α_2 adrenoceptors in Purkinje? *Am J Physiol*. 2001; 287:H1182–H1190.
25. Zima AV, Blatter LA. Redox regulation of cardiac calcium channels and transporters. *Cardiovasc Res*. 2006; 71:310–321. [PubMed: 16581043]
26. Song Y, Shryock JC, Wagner S, et al. Blocking late sodium current reduces hydrogen peroxide-induced arrhythmogenic activity and contractile dysfunction. *J Pharmacol Exp Ther*. 2006; 318(1): 214–222. [PubMed: 16565163]
27. Hool LC. Evidence for the regulation of L-type Ca^{2+} channels in the heart by reactive oxygen species: Mechanism for mediating pathology. *Clin Exp Pharmacol Physiol*. 2008; 35:229–234. [PubMed: 18197892]

Figure 1.

Ventricular pacing and three subsequent premature extrastimuli (down-arrows) induce ventricular tachycardia, last five complexes). From top to bottom are shown recording from ECG lead II, endocardial (E) and Purkinje (P) electrograms from sites at the focus (-F) and surrounding near the stimulating electrode (-ST) as well as northwest (-NW), southwest (-SW), north (-N), overlying (-O), east (-E), and south (-S) from the focus. The tracing labels are located above each tracing. Stimulated complexes give rise to VT complexes with P potentials (up-arrows) recorded earliest prior to surface QRS onset indicated by vertical lines in first, third and fifth complexes. Most Purkinje potentials loose voltage with rapid activation especially observed in P-N with each successive extra-stimuli as well as the first complexes of VT. Two P-N tracings are located in a line north of the focus with the lower being closer. Interpretation of very low amplitude Purkinje potentials is facilitated by observation of adjacent Purkinje activations in P-N, P-F and P-S. Tissue at site P-F was removed for *in vitro* study.

Figure 2.

Three-dimensional activation map of the first stimulus from the experiment shown in previous figure. Maps are shown in epicardial (EPI), subepicardial (SEPI), midwall, subendocardial (S-ENDO), endocardial (ENDO), and Purkinje (PURK) plans. Activation times are in milliseconds at each recording site. Maps are drawn in 20-ms isochrones (different color). Earliest activity is seen in the septal PURK layer (13 ms after the QRS) with activation proceeding out to EPI. The Purkinje layer has only 6 measurements owing to the fact that only six electrodes had Purkinje recordings

Figure 3.

Activation mapping of the third VT complex shown in previous figure. Map of the epicardial (EPI), subepicardial (S-EPI), midwall, subendocardial (S-ENDO), endocardial (ENDO) and Purkinje (PURK) planes are shown. Activation times are in ms, maps are drawn in 20ms isochrones. Earliest intracardiac activity is seen in PURK -64ms prior to the onset of surface ECG, with activation proceeding to surrounding Purkinje and to epicardium. There is not enough conduction delay shown to suggest reentry.

TEMPO reversibly blocked TA

20mV
2000ms

Figure 4.

Endocardial tissue was studied in vitro. Zero line, voltage and time calibration are indicated. Figure 4A: with isoproterenol (5×10^{-7} M) and rapid pacing produces a single TA complex (up arrow points to the TA) and Down arrow indicates DAD. Figure 4B: with isoproterenol and Tempo with the same pacing protocol, no TA was observed while DAD is still present. Figure 4C: with 15 minutes washout, 3 TAs were again induced.

Figure 5.

ROS levels are increased in ischemic endocardium. Segments of endocardium were sectioned, incubated with the oxidative fluorescent dye DHE, and examined by confocal microscopy. Cellular ROS levels are increased in multiple endocardial layers from **central** ischemic regions as compared with tissue from nonischemic regions. The TEMPO treated animal demonstrated reduced ROS levels in ischemic regions as compared to control. Region A was the origin of the focal VT, the induction of which TEMPO blocked. Region B was taken from an adjacent, central ischemic zone site. All images are obtained at the same laser settings and objective (see Methods). The scale bar represents 100 microns.