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PRECLINICAL RESEARCH

Interleukin-1β, Oxidative Stress, and Abnormal Calcium Handling Mediate Diabetic Arrhythmic Risk

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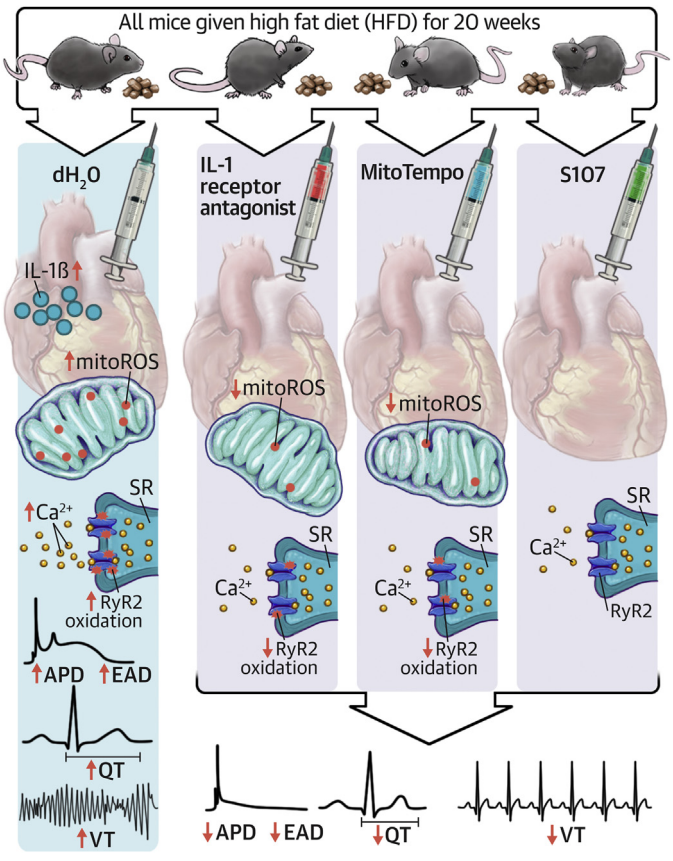
VISUAL ABSTRACT



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HIGHLIGHTS

- **Diabetes-induced arrhythmic risk involved activation of innate immunity, elevation of IL-1β, mitochondrial oxidative stress, SR calcium release channel oxidation, and QT prolongation.**
- **Diabetes-induced arrhythmic risk could be inhibited by IL-1β antagonism, mitoROS scavenging, and SR calcium release stabilization.**
- **The relationship of inflammation and arrhythmic risk may account for increased susceptibility of diabetic patients to the effects of COVID-19.**

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SUMMARY

Diabetes mellitus (DM) is associated with increased arrhythmia. Type 2 DM (T2DM) mice showed prolonged QT interval and increased ventricular arrhythmic inducibility, accompanied by elevated cardiac interleukin (IL)-1β, increased mitochondrial reactive oxygen species (mitoROS), and oxidation of the sarcoplasmic reticulum (SR) Ca^{2+} release channel (ryanodine receptor 2 [RyR2]). Inhibiting IL-1β and mitoROS reduced RyR2 oxidation and the ventricular arrhythmia in DM. Inhibiting SR $Ca2^{+}$ leak by stabilizing the oxidized RyR2 channel reversed the diabetic arrhythmic risk. In conclusion, cardiac IL-1β mediated the DM-associated arrhythmia through mitoROS generation that enhances SR Ca^{2+} leak. The mechanistic link between inflammation and arrhythmias provides new therapeutic options. (J Am Coll Cardiol Basic Trans Science 2021;6:42–52)

ABBREVIATIONS AND ACRONYMS

APD = action potential duration

DM = diabetes mellitus

EAD = early afterdepolarization

IL = interleukin

IL-1RA = interleukin-1 receptor antagonist

I_{to} = transient outward potassium current

mitoROS = mitochondrial reactive oxygen species

RyR2 = ryanodine receptor

SR = sarcoplasmic reticulum

T1DM = type 1 diabetes mellitus

T2DM = type 2 diabetes mellitus

VT = ventricular tachycardia

More than 400 million people worldwide have diabetes mellitus (DM). In 2016, an estimated 1.6 million deaths were caused directly by DM (1). Roughly 70% of patients with DM die from cardiovascular causes (2), of which around 50% are sudden cardiac deaths (3). The risk of sudden cardiac death in DM is double that of nondiabetic persons (4).

A prolonged QT interval on the surface electrocardiogram is a risk factor for sudden cardiac death (5). Clinical studies demonstrated a high prevalence of QT prolongation in DM patients (6). QT prolongation has been associated with sudden cardiac death in type 2 DM (T2DM), suggesting that electrical remodeling in DM may contribute to ventricular arrhythmias and sudden cardiac death (7–9). At a cellular level, an increase in the ventricular action potential duration (APD) with a subsequent increase in early afterdepolarizations (EADs) underlies long QT-associated torsades de pointes ventricular arrhythmias (10).

DM is associated with elevated inflammatory cytokines and mitochondrial oxidative stress. A recent publication implicates macrophage activation in the QT prolongation and increased arrhythmic risk in type 1 DM (T1DM) (11). We investigated how innate immune activation may contribute to arrhythmic risk in the more prevalent T2DM.

METHODS

Detailed methods are described in the Supplemental Appendix. Briefly, male C57BL/6 mice were fed with high fat diet for a minimal of 20 weeks to induce T2DM. Mice fed with normal chow were used as a control group. The cardiac electrical properties of diabetic mice, including cardiomyocyte APD, EADs, QT interval, and ventricular arrhythmic inducibility, were characterized by patch clamp, optical mapping, surface electrocardiogram, and in vivo electrophysiological test, respectively. The results were compared with control mice. Diabetic mice were then randomized into 3 treatment groups: interleukin (IL)-1 receptor antagonist (IL-1RA), mitochondrial antioxidant (MitoTEMPO), and ryanodine receptor 2 (RyR2) channel stabilizer (S107). At the end of each treatment, alterations on the cardiac electrical properties were evaluated. Mitochondrial reactive oxygen species (mitoROS) was were assessed using MitoSOX stain on isolated cardiomyocytes by flow cytometry. Heart tissue were collected for immunoblotting of IL-1β and oxidized RyR2 channels.

The animal experiments were carried in accordance with the National Institutes of Health Guide for the Care and Use of Experimental Animals and were approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

STATISTICAL ANALYSIS. Continuous data are presented as mean ± SEM when normally distributed. Data that were not normally distributed were logarithmically transformed prior to further statistical analysis. Data were then analyzed using a 2-tailed Student's *t*-test or 1-way analysis of variance with Bonferroni post hoc tests for multiple pairwise comparisons. Categorical data are expressed as counts

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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TABLE 1 Influence of MT, IL-1RA, and S107 on Action Potential and QT Interval

	Control Mice (n = 14)	DM Mice (n = 32)	DM + IL-1RA Mice (n = 20)	DM + MT Mice (n = 19)	DM + S107 Mice (n = 8)	p Value
APD90, ms	61 ± 14 (7)*	411 ± 42 (7)	105 ± 47 (5)†	188 ± 66 (9)‡	81 ± 42 (8)*	<0.001
QT interval, ms	53 ± 1†	59 ± 1	51 ± 1*	52 ± 1*	51 ± 2†	<0.001
QTc interval, ms	47 ± 1†	52 ± 1	46 ± 1*	47 ± 1*	47± 2‡	<0.001
HR, beats/min	495 ± 15	483 ± 8	498 ± 8	488 ± 10	525 ± 26	0.515

Values are mean ± SEM (n) or mean ± SEM. APD90 was calculated on action potentials without early afterdepolarizations. *p < 0.001 vs. DM. †p < 0.01. vs. DM. ‡p < 0.05 vs. DM.
APD90 = action potential duration at 90% repolarization; DM = diabetes mellitus; HR = heart rate; IL-1RA, interleukin-1 receptor antagonist; MT = mitoTEMPO.

with percentages and compared using a chi-square test. All statistical analyses were performed with GraphPad Prism 5.0 (GraphPad Software, San Diego, California). A p value < 0.05 was considered statistically significant.

RESULTS

T2DM CAUSES ABNORMAL ELECTROCARDIOGRAMS AND INCREASED ARRHYTHMIC RISK. A prolonged QT interval on the surface electrocardiogram is associated with increased arrhythmic risk (12). DM mice had QT intervals on the surface electrocardiogram that were significantly longer compared with the control mice (47 ± 1 ms in control mice vs. 52 ± 1 ms in DM mice; p < 0.001) (Table 1) under comparable heart rate. No other abnormalities on surface electrocardiogram were detected (Supplemental Table 1). Arrhythmia was also examined in conscious mice using telemetry (data not shown). No spontaneous ventricular tachycardia (VT) was observed in the DM mice. Testing for sudden death risk, programmed electrical stimulation revealed that none of the control mice were inducible for VT, whereas half the DM mice had inducible VT (0 of 7 in control mice vs. 7 of 14 in DM mice; p = 0.047) (Figures 1A and 1B). Differences in cardiac contractile function did not explain the increased arrhythmic risk in DM mice (ejection fractions of 54.7 ± 1.4% in control mice vs. 55.5 ± 0.9% in DM mice, n = 6 for each group; p = 0.651). Cardiac chamber size and wall thickness by echocardiography were comparable between the DM and control mice (Supplemental Table 2). Taken together, initial electrophysiological studies demonstrated that DM increased risk markers of sudden cardiac death.

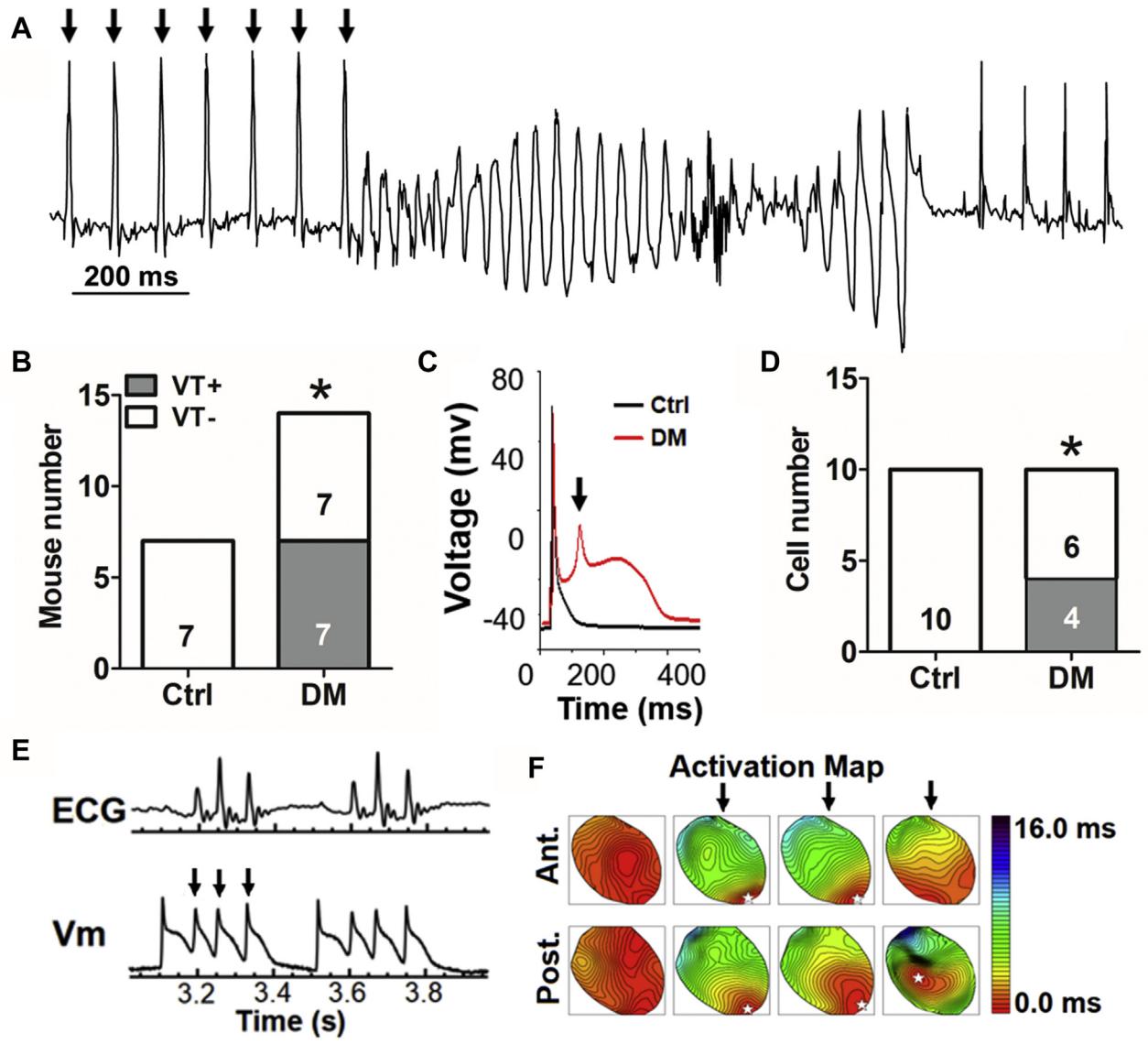
T2DM CAUSES APD PROLONGATION AND PRESENCE OF EADs. At the cellular level, cardiac ventricular APD prolongation is known to underlie surface QT interval lengthening and is associated with increased incidence of electrical oscillations before repolarization called EADs (10). EADs are a fundamental mechanism of ventricular arrhythmias and sudden cardiac death (13,14).

Using whole-cell current clamp of isolated cardiomyocytes, we demonstrated APD prolongation (61 ± 14 ms in control mice vs. 411 ± 42 ms in DM mice; p < 0.001) (Table 1) and increased presence of EADs (4 of 10 vs. 0 of 10; p = 0.025) (Figures 1C and 1D) in the DM myocytes compared with the control myocytes. These changes were confirmed by optical mapping on whole hearts. APD measured by optical mapping was 73 ± 3 ms (n = 6) in control and 85 ± 3 ms (n = 6) in DM (p < 0.001). EADs were detected in two-thirds of DM hearts at slow heart rates (Figure 1E and 1F).

DM UPREGULATED CARDIAC IL-1β AND mitoROS. In T2DM mice, cardiac IL-1β protein level was doubled as compared with control mice hearts (1.71 ± 0.18 normalized ratio in control mice vs. 3.45 ± 0.67 in DM mice; p = 0.037) (Figure 2A). IL-1β is known to promote oxidative stress and ROS production (15). We confirmed the increase of mitoROS (6,956 ± 220 arbitrary units electrocardiogram in control mice vs. 9,156 ± 468 arbitrary units in DM mice; p < 0.001) (Figure 2B), which was ameliorated by both IL-1RA (7,618 ± 120 arbitrary units; p = 0.010 vs. DM) and mitoTEMPO (7,298 ± 378 arbitrary units; p = 0.017 vs. DM), a mitochondrial-specific antioxidant. Moreover, adding IL-1β to the cultured cardiomyocytes increased the mitoROS in a dose-dependent manner (Figure 2C). These data implied that elevated IL-1β caused cardiac mitoROS production.

IL-1β INHIBITION AND MITOCHONDRIAL ANTIOXIDANT SUPPRESSED ARRHYTHMIC RISK. To test directly the roles of IL-1β or mitoROS in DM-associated arrhythmias, we treated DM mice with either IL-1RA (3 mg/kg) or mitoTEMPO (1 mg/kg) for 2 weeks. Both treatments caused substantial reductions in EAD occurrence (Figure 3A), APD (411 ± 42 ms in DM vs. 105 ± 47 ms in DM + IL-1RA; p < 0.01; vs. 188 ± 66 in DM + mitoTEMPO; p = 0.019) (Table 1), QT interval (52 ± 1 ms in DM vs. 46 ± 1 ms in DM + IL-1RA; p < 0.001;

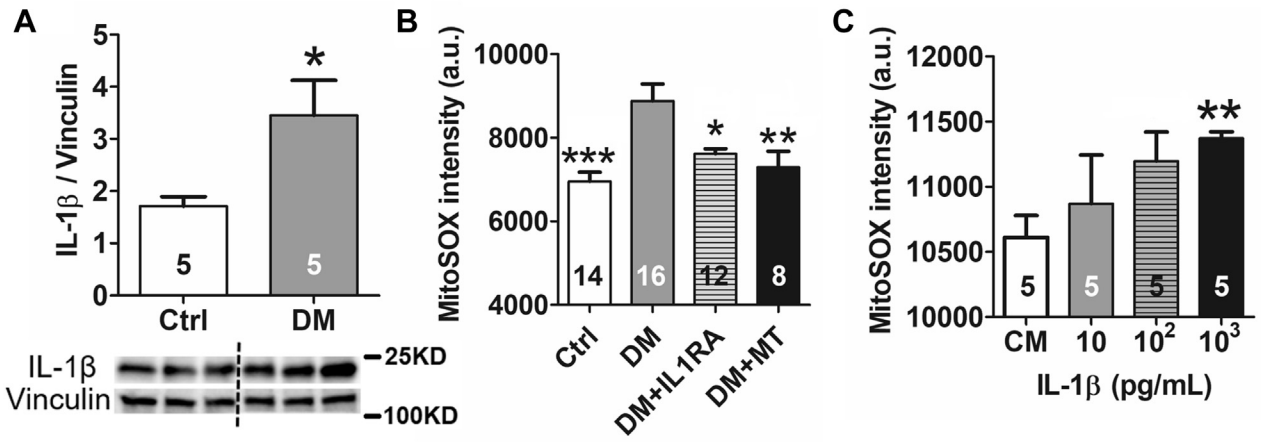
FIGURE 1 VT Induction and EADs in DM Mice



(A) Representative electrocardiogram trace showing induced ventricular tachycardia (VT). Arrows indicate the paced beats. **(B)** More diabetes mellitus (DM) mice had inducible VT. **(C)** Representative whole-cell patch-clamp recordings of ventricular action potentials from control (Ctrl) **(black)** and DM **(red)** cardiomyocytes showing an early afterdepolarization (EAD). The **arrow** indicates EAD. **(D)** More DM cardiomyocytes show EADs. **(E)** Optical mapping traces of triggered activities **(arrows)** under slow heart rate by atrioventricular node ablation (n = 2 of 3 DM hearts). **(F)** The representative activation maps show that EAD or polymorphic VT was caused by triggered activities **(arrows)**. **(B, D)** The n value is indicated within the **bars**. *p < 0.05. Ant. = anterior wall; Post. = posterior wall; Vm = membrane potential.

vs. 47 ± 1 ms in DM + mitoTEMPO; p < 0.001) (Table 1), and VT inducibility (Figure 3B). VT was induced in 7 of 14 DM mice, and the average VT duration was 1.3 ± 0.4 s. In contrast, only 1 of 11 IL-1RA-treated DM mice (p = 0.042) and 1 of 10 mitoTEMPO-treated DM mice (p = 0.040) had induced VT. The VT duration in these mice was 0.2 s and 0.3 s, respectively. Additionally, in optical mapping (Figure 3C), short-term in vitro perfusion with IL-1β (10 pg/ml for 20 min) prolonged APD and promoted EAD formation in DM mice (n = 3 of 4 hearts) compared with control mice (n = 0 of 4 hearts; p = 0.029), which was abolished by mitoTEMPO (n = 0 of 4 hearts; p = 0.029). Blood glucose level, insulin resistance, and body weight were not altered by mitoTEMPO or IL-1RA (Figures 3D to 3F), suggesting

FIGURE 2 Increased IL-1β Is Associated With MitoROS in DM



(A) Interleukin (IL)-1β was increased in DM hearts. **(B)** Mitochondrial reactive oxygen species (mitoROS) was increased in the DM cardiomyocytes. The mitoROS elevation was reversed by a 2-week treatment of IL-1 receptor antagonist (IL-1RA) (3 mg/kg) or mitoTEMPO (MT) (1 mg/kg). **(C)** IL-1β incubation for 4 h elevated mitoROS level in cultured cardiomyocytes in a dose-dependent manner. Values are mean ± SEM. The n value is indicated within the **bars**. *p < 0.05, **p < 0.01, ***p < 0.001. a.u. = arbitrary unit; CM = cardiomyocyte; other abbreviations as in Figure 1.

that their antiarrhythmic effect is independent of serum glucose, insulin, or adiposity.

ENHANCED SR Ca^{2+} RELEASE UNDERLIES THE EFFECT OF DM ON ARRHYTHMIC RISK MARKERS. Resting Ca^{2+} leak from the SR through the RyR has been implicated in slow repolarization, EADs and arrhythmias (16). We confirmed that the RyR was hyperactive using spontaneous Ca^{2+} sparks as a readout for RyR activity in saponin-permeabilized myocytes isolated from DM mouse hearts (5.6 ± 0.3 sparks $[100\ \mu M]^{-1} \cdot s^{-1}$ in control mice vs. 8.3 ± 0.3 sparks $[100\ \mu M]^{-1} \cdot s^{-1}$ in DM mice; $p < 0.001$) (Figures 4A and 4B). Parallel assessment of caffeine-induced Ca^{2+} transients amplitude in permeabilized myocytes revealed no difference in SR Ca^{2+} content between DM and control hearts ($p = 0.407$) (Figures 4C and 4D). Potassium current is another key contributor to cardiomyocyte repolarization. In rodents, transient outward potassium current (I_{to}) is a dominant repolarizing current (17). In our study, total potassium peak current density only slightly decreased in DM cardiomyocytes compared with that in control cardiomyocytes, while steady-state current density stayed the same (Figures 4F and 4G). There was almost no change in I_{to} peak density between these 2 groups (Figure 4E).

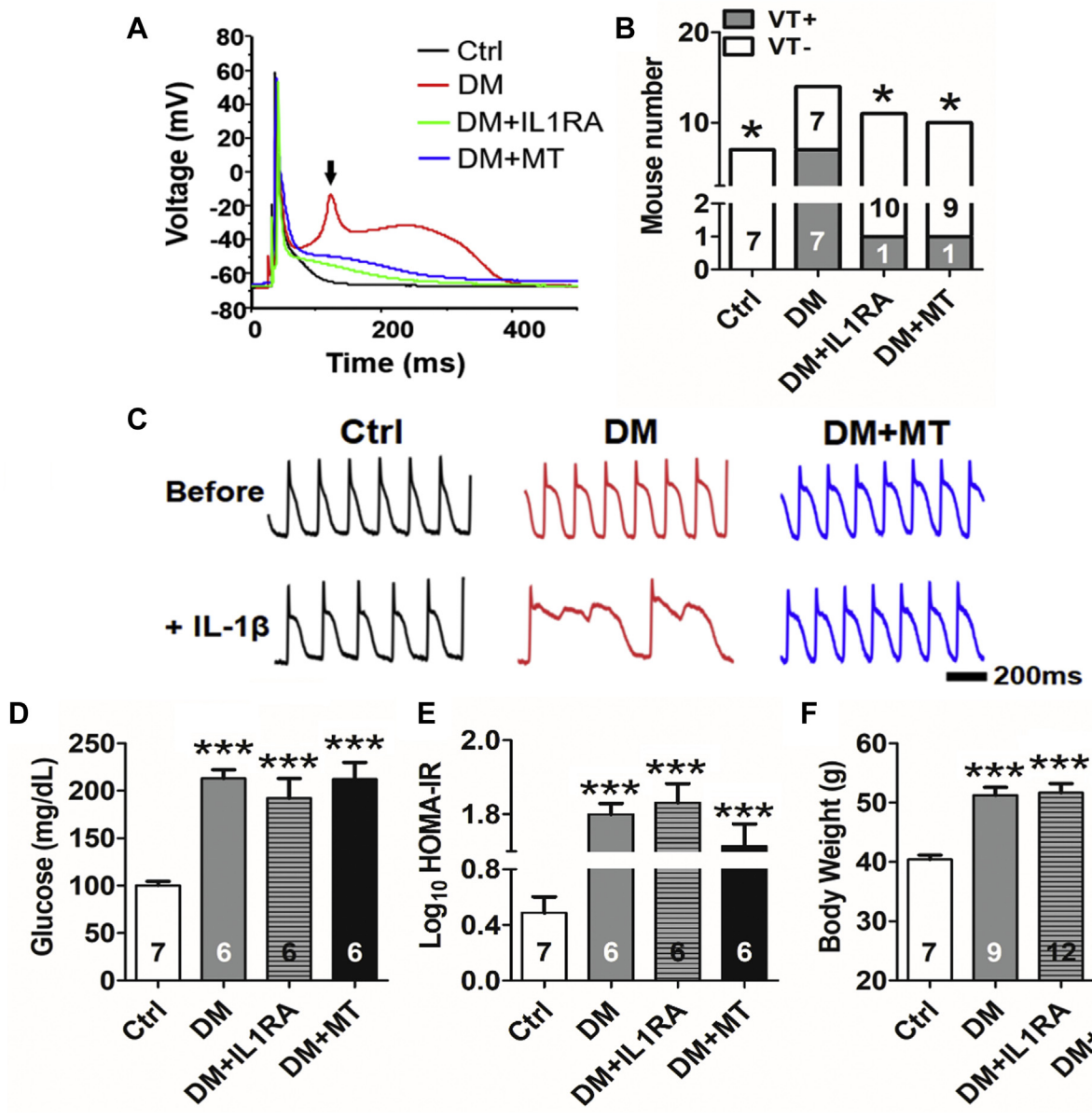
RyR activation by phosphorylation or oxidation leads to increased resting SR Ca^{2+} release (18–21). DM significantly enhanced RyR2 oxidation, while RyR2 phosphorylation was not altered (Figures 5A and 5B). Both mitoTEMPO and IL-1RA reduced the RyR2 oxidation (Figures 5A and 5B). RyR2 oxidation destabilizes an interaction with a channel-associated subunit, calstabin, to lead to Ca^{2+} leak (22). Recently, several drugs, such as S107, have been developed to stabilize the RyR-calstabin interaction and reduce resting SR Ca^{2+} leak (23–25). We treated cardiomyocytes immediately after they were isolated from diabetic hearts with the RyR stabilizer S107 (1.0 μM) for 2 h or injected S107 (30 mg/kg) in DM mice for 1 week. We found that S107 was able to ameliorate the APD prolongation in vitro (411 ± 42 ms in DM vs. 81 ± 42 ms in DM + S107; $p < 0.001$) (Table 1), shorten the QT interval (52 ± 1 ms in DM vs. 47 ± 2 ms in DM + S107; $p = 0.005$) (Table 1), and suppress EADs and arrhythmic inducibility in DM mice (5 of 8 in DM vs. 1 of 8 in DM + S107; $p = 0.039$) (Figures 5C and 5D).

These data indicated that DM-associated APD prolongation and arrhythmia was mediated by SR Ca^{2+} leak from oxidized RyR2 channels.

DISCUSSION

In the present study, we found that DM caused increased arrhythmic risk associated with a prolonged QT interval on the surface electrocardiogram and a corresponding increase in the cardiac APD and increased triggered activity at the cellular level. DM was accompanied by increased IL-1β and mitoROS. Inhibition of IL-1β signaling by IL-1RA or mitochondrial antioxidant ameliorated electrical remodeling

FIGURE 3 RyR2 Oxidation and SR Calcium Leak Mediated Arrhythmia in DM Mice

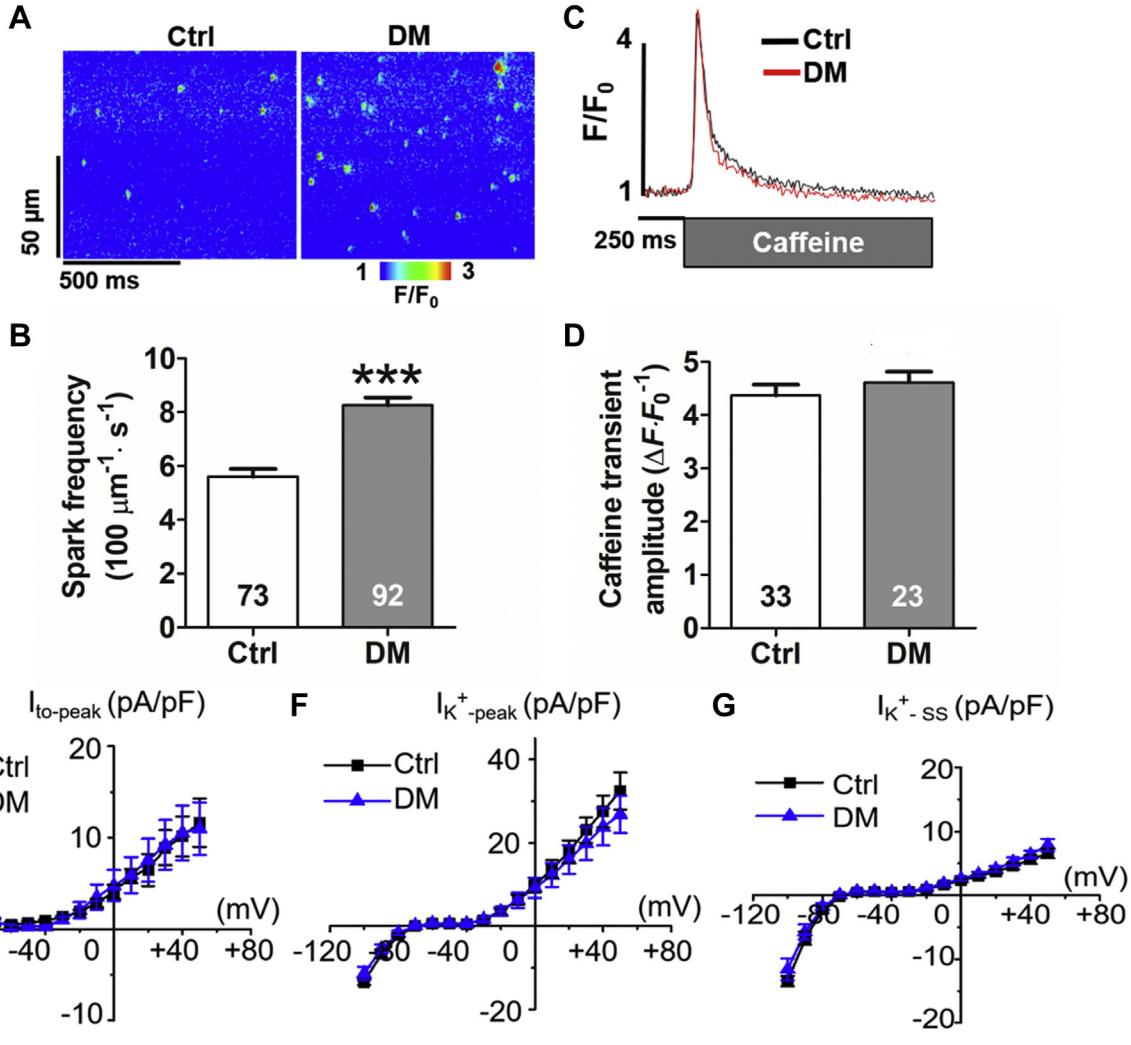


(A) Representative whole-cell patch-clamp recordings of action potentials showing 2-week treatment with IL-1RA (3.0 mg/kg) or MT (1.0 mg/kg) suppressed EAD formation. The **arrow** indicates EAD. **(B)** IL-1RA or MT reduced VT inducibility. **(C)** Representative tracing of action potential duration (APD) by optical mapping showing short-term in vitro perfusion with IL-1β (10 pg/ml for 20 min), prolonged APD, and promoted EAD formation in the DM hearts. MT treatment abolished the arrhythmic effect of IL-1β. **(D)** Hyperglycemia in DM mice was not affected by 2-week treatment with IL-1RA or MT. **(E)** HOMA-IR (Homeostatic Model Assessment of Insulin Resistance), an indicator of insulin resistance, and **(F)** body weight remained unchanged after either treatment. Values are mean ± SEM. **(B, D, F)** The n value is indicated within the **bars**. *p < 0.05, **p < 0.01, ***p < 0.001. Scale bar = 200 ms. Abbreviations as in Figures 1 and 2.

and arrhythmic risk. Underlying these electrical changes were alterations in Ca^{2+} release from oxidized RyR2 channels. Taken together, these findings indicate that DM activates innate immune mechanisms in the heart that link DM to increased arrhythmic risk by modulating mitoROS.

Growing evidence indicates that DM is a low-grade chronic inflammatory state characterized by oversecretion of proinflammatory cytokines in the circulation and in various tissues. The increased systemic and local inflammation are considered key mechanisms underlying the pathogenesis and the

FIGURE 4 Enhanced Ca^{2+} Sparks in DM Mice

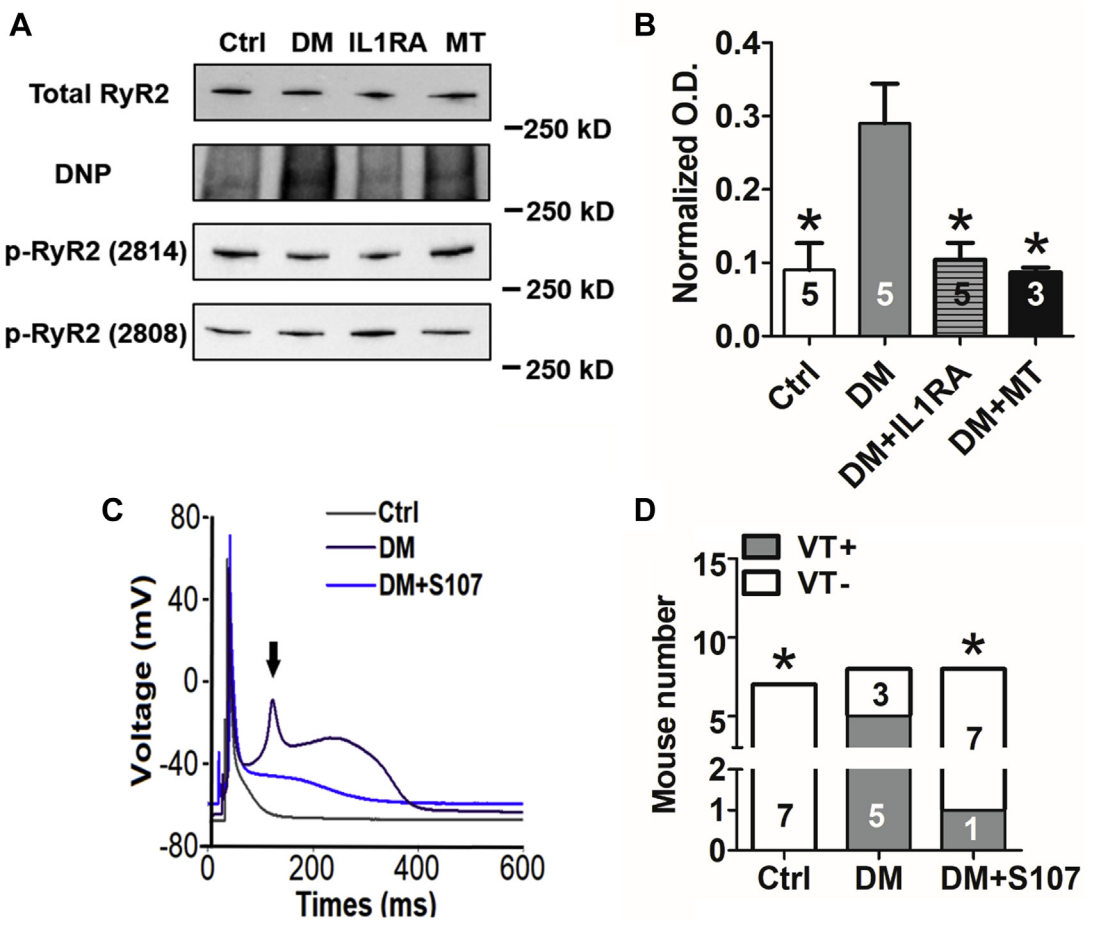


(A) Representative line-scan images of Ca^{2+} sparks. **(B)** Increased frequency of Ca^{2+} sparks in DM. **(C)** Representative trace of caffeine-induced Ca^{2+} transients. **(D)** Sarcoplasmic reticulum Ca^{2+} content is unchanged ($p = 0.407$). **(E)** Transient outward peak potassium current ($I_{to\text{-}peak}$), **(F)** total potassium current ($I_K{}^{+\text{-}peak}$), and **(G)** steady-state current ($I_K{}^{+}{}_{\text{-}SS}$), revealed similar current densities between Ctrl ($n = 5$) and DM ($n = 10$) mice. Values are mean ± SEM. **(B, D)** The n value within the **bars** indicates the total number of cells tested from >3 different mice. ***$p < 0.001$. Abbreviations as in Figures 1 and 2.

progression of DM and diabetic complications (19). Several proarrhythmic cytokines are elevated in DM, including IL-1β, tumor necrosis factor-α, IL-6, and IL-18 (26-28). Among those proinflammatory mediators, IL-1β lies upstream in the inflammation pathway (29). IL-1β augments tumor necrosis factor-α-mediated inflammation in lung epithelial cells by enhancing receptor expression (30). IL-1β modulates the production of IL-6 and IL-8 in human gingival epithelial cells (31). IL-1β inhibition leads to a significant reduction of C-reactive protein and IL-6 in the patients with myocardial infarction (29,32). A randomized double-blind placebo-controlled clinical trial (i.e., the CANTOS [Canakinumab Anti-Inflammatory Thrombosis Outcomes Study]) showed anti-inflammatory therapy with an IL-1β-specific antibody (canakinumab) significantly reduced cardiovascular events, independently of lipid-level lowering, supporting a role for IL-1β in cardiovascular disease complications. Therefore, we focused on IL-1β in this study (32).

Recently, Monnerat et al. (11) reported the role of innate immunity activation and IL-1β in cardiac electrical remodeling leading to ventricular

FIGURE 5 RyR2 Oxidation and SR Calcium Leak Mediated Arrhythmia in DM Mice



(A) Representative Western blots of total ryanodine receptor 2 (RyR2), oxidized (DNP), and phosphorylated RyR2 (s2814 and s2808). RyR2 oxidation was increased in DM and reversed by 2-weeks of IL-1RA (3.0 mg/kg) or MT (1.0 mg/kg) treatment. RyR2 phosphorylation was not changed in DM. **(B)** Statistical analysis of oxidized RyR2 normalized by total RyR2. **(C)** Representative whole-cell patch-clamp recordings showing immediate in vitro treatment with S107 (1.0 μM for 2 h) suppressed EADs. The **arrow** indicates EAD. **(D)** One-week of S107 (30 mg/kg, subcutaneous) treatment reduced VT inducibility. Values are mean ± SEM. **(B to D)** The n value is indicated within the **bars**. *$p < 0.05$. DNP = 2,4-dinitrophenylhydrazone; O.D. = optical density; other abbreviations as in Figures 1 and 2.

arrhythmia in T1DM. Our results show that IL-1β antagonism reduced the arrhythmic inducibility by over 80%, suggesting that IL-1β was the most prominent mediator of T2DM-associated arrhythmia. It is reasonable to postulate that IL-1β is the common arrhythmic mechanism shared between T1DM and T2DM. Monnerat et al. (11) observed a decrease in I_{to} and an increase in calcium sparks as the substrates for QT prolongation and arrhythmia, respectively. Concordantly, we observed increased SR Ca^{2+} sparks in the diabetic cardiomyocytes. Nevertheless, no alteration in potassium currents were noticed. Enhanced SR Ca^{2+} leak secondary to oxidative modification likely was sufficient to cause QT and APD prolongation by promoting Na^{+}/Ca^{2+} exchanger forward mode, thereby slowing repolarization and enabling L-type Ca^{2+} current reactivation (16). Contrasting data have been reported regarding how DM affects the properties of cardiac ion channels, depending on the DM models, channel activation status, or the parameters being reported (channel expression or function) (20,33). It is possible that the downstream effectors of IL-1β are different between T1DM and T2DM.

More importantly, our study provides a mechanistic linkage between IL-1β–mediated cardiac

inflammation and DM-induced arrhythmia. We demonstrated that inhibiting mitoROS had similar effects as IL-1 receptor antagonism and IL-1β-mediated electrophysiological effects could be masked by a mitochondrial targeted antioxidant, implying that mitoROS mediated the effects of IL-1β. It is well known that mitoROS plays a critical role in activating inflammasome and IL-1β (34-36). Nevertheless, debates exist on how IL-1β and inflammasome interplays with mitoROS (37). Consistent with previous reports (38,39), our results support that IL-1β fosters mitoROS production. This positive feedback between IL-1β and mitoROS would perpetuate the pathological process in DM. The exact mechanism whereby IL-1β regulates mitoROS in DM is yet to be determined.

MitoROS is known to be associated with arrhythmogenesis. For example, in nonischemic heart failure, mitoROS underlies QT prolongation and sudden death, a similar electrical phenotype as in diabetic patients and in our diabetic mice model (40). The putative mechanisms whereby mitoROS may cause arrhythmic risk are myriad and include facilitating focal activity and re-entry, altering cardiac ion currents, promoting cardiac fibrosis, and impairing gap junction function (41,42). RyR2 is the major SR Ca^{2+} release channel and an important oxidative target in cardiomyocytes (20,43). In aging hearts, mitoROS induces the redox modification of RyR2 causing aberrant Ca^{2+} handling (44). At the cellular and animal levels, we showed that, in diabetes, the arrhythmic effect of mitoROS was via oxidative modification of RyR2, leading to increased spontaneous SR Ca^{2+} leak, as S107 alone, a drug that inhibits RyR2 channel-mediated resting Ca^{2+} leak, reversed the diabetic arrhythmic phenotypes, implying that IL-1β and mitoROS acted predominantly on the RyR2 to mediate their effects. Excessive SR Ca^{2+} leak through oxidized RyR2 is known to increase free Ca^{2+} ion transportation into mitochondria causing mitochondrial Ca^{2+} overload, which subsequently results in more mitoROS production (45). This vicious cycle helps to sustain arrhythmia in DM.

It is possible that our findings may have implications for clinical trial results using sodium-glucose cotransporter-2 inhibitors and glucagon-like peptide-1 agonists to treat DM. Trials have found that these newer glucose-lowering drugs significantly reduce the incidence of major cardiovascular events in diabetic patients and that these cardiovascular benefits are unrelated to their glycemic control effects (46,47). Both treatments have been found to lower the expression and secretion of proinflammatory cytokines, such as IL-6, IL-1β, tumor necrosis factor-α, and C-reactive protein (48). This anti-inflammatory effect may contribute to reduced arrhythmic risk and improved outcomes. Consistent with this idea, blocking IL-1β signaling was antiarrhythmic and independent of the serum glucose level in our study.

STUDY LIMITATIONS. We examined neither ROS from other sources nor all ion current components of the cardiac action potential. Nevertheless, mito-TEMPO or S107 alone was sufficient to reverse cardiac electrical remodeling nearly to the control level, suggesting that the impacts from other ROS sources or ion currents are trivial. Second, we did not test the effects of S107 on other cardiac channels. Nevertheless, S107 is reported to bind specifically to RyR (49). Last, our study cannot rule out a potential effect of IL-1α. Nevertheless, a recent investigation on patients with T2DM revealed that the IL-1β concentration is 4 times as high as that of IL-1α (50). Thus, it is reasonable to conclude that IL-1β-mediated inflammation is the main driver in T2DM-associated arrhythmia. Further investigation using a specific IL-1β neutralizing antibody is needed to confirm our findings.

CONCLUSIONS

DM results in activation of a cardiac innate immune response associated with increased arrhythmic risk. This arrhythmogenic risk could be inhibited by antagonizing IL-1β, mitoROS, or SR Ca^{2+} leak. Each of these approaches represents a possible new therapy for inflammation-induced arrhythmic risk. Also, it is possible that our findings of increased cardiac inflammation in diabetes may explain the increased susceptibility of diabetic patients to the cardiac effects of COVID-19.

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AUTHOR DISCLOSURES

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: DM causes cardiac inflammation that contributes arrhythmic risk.

TRANSLATIONAL OUTLOOK 1: This diabetes-induced arrhythmic risk involves QT prolongation.

TRANSLATIONAL OUTLOOK 2: This diabetes-induced arrhythmic risk can be inhibited by IL-1β antagonism, mitoROS scavenging, and SR calcium release stabilization.

TRANSLATIONAL OUTLOOK 3: The relationship of inflammation and arrhythmic risk may account for increased susceptibility of diabetic patients to the effects of COVID-19.

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APPENDIX For expanded Methods and References sections as well as supplemental tables, please see the online version of this paper.