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Metformin ameliorates cardiac conduction delay by regulating microRNA-1 in mice

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ABSTRACT

Cardiac conduction delay may occur as a common complication of several cardiac diseases. A few therapies and drugs have a good effect on cardiac conduction delay. Metformin (Met) has a protective effect on the heart. This study's aim was to investigate whether Met could ameliorate cardiac conduction delay and its potential mechanism. Cardiac-specific microRNA-1 (miR-1) transgenic (TG) and myocardial infarction (MI) mouse models were used. Mice were administered with Met in an intragastric manner. We found that the expression of miR-1 was significantly up-regulated in H_2O_2 treated cardiomyocytes as well as in TG and MI mice. The protein levels of inwardly rectifying potassium channel 2.1 (Kir2.1) and Connexin43 (CX43) were down-regulated both in cardiomyocytes treated with H_2O_2 as well as cardiac tissues of TG and MI mice, as compared to their controls. Furthermore, the PR and QT intervals were prolonged, action potential duration (APD) was delayed, and conduction velocity (CV) was reduced, with upregulation of miR-1 in the hearts. In the meanwhile, intercalated disc injuries were found in the hearts of MI mice. Interestingly, Met can noticeably inhibit miR-1 upregulation and attenuate the changes mentioned above. Taken together, this suggested that Met could play an important role in improving cardiac conduction delay through inhibition of miR-1 expression. Our study proposes that Met is a potential candidate for the treatment of cardiac conduction delay and provides a new idea of treating arrhythmia with a drug.

1. Introduction

The cardiac conduction system is a network responsible for initiation and propagation of normal rhythm, which plays a vital role in coordinating cardiac performance (Jongbloed et al., 2012; Munshi, 2012). The incidence and prevalence of cardiac conduction disease has kept growing worldwide in recent years (Vijayaraman et al., 2017). As a major cardiac conduction disease, cardiac conduction delay may occur as a common complication of several cardiac diseases such as autonomic dysfunction, infectious endocarditis, and myocardial infarction (MI) (Sidhu and Marine, 2019). Cardiac conduction delay indicates prolonged conduction time from the sinoatrial node to the ventricles (Freyermuth et al., 2016). Few therapies are available for the effective treatment of cardiac conduction delay, apart from cardiac pacing. Therefore, finding a drug that would have a good therapeutic effect for cardiac conduction delay, and understanding its mechanisms would be a beneficial therapy for some cardiac diseases.

MicroRNAs (miRNAs) are a class of endogenous small non-coding RNAs that play major roles in post-transcriptional regulation of gene expression by binding to the 3′-untranslated regions of target genes

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(Xiao and MacRae, 2019). MicroRNA-1 (miR-1) is muscle-specific and its expression is enriched in the heart. It has been discovered that its variation is a causative factor of many heart diseases (Chistiakov et al., 2016). Our previous studies demonstrated that the overexpression of miR-1 slowed cardiac conduction and depolarized the cytoplasmic membrane by post-transcriptionally Kir2.1 and CX43 (Yang et al., 2007). A subsequent study showed that transgenic (TG) mice with cardiac-specific overexpression of miR-1 were more susceptible to atrioventricular block (Zhang et al., 2013). These findings suggested that miR-1 upregulation may induce atrioventricular blocks and cardiac conduction delay.

Met is a mainstay drug that has been used to treat type 2 diabetes mellitus (T2DM) for more than 60 years (Sanchez-Rangel and Inzucchi, 2017). Recent studies suggest that Met can reduce the possibility of heart failure in diabetic patients (Fung et al., 2015) and mitigate cardiac remodeling and fibrosis without T2DM (Kanamori et al., 2019). Met has also been shown to improve cardiac functions and alleviate myocardial cell apoptosis post MI (Sun and Yang, 2017). However, it has not yet been reported whether Met could improve cardiac conduction delay.

Our team's previous study showed that as a direct allosteric AMPK activator, Met could activate AMPK and significantly reduce C/EBP β to decrease the expression of miR-1 (Zhang et al., 2018a). This study's main purpose was, therefore, to clarify the effect of Met on miR-1 upregulation induced cardiac conduction delay, and the potential molecular mechanism of its effect, which would provide a new therapeutic method for some cardiac diseases.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Institutional Animal Care and Use Committee of Harbin Medical University, P.R. China (No. HMUIRB-2008–06). All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

2.2. Generation of miR-1 TG mice

Eight-week-old male wild-type (WT) and cardiac-specific miR-1 TG mice were used for the experiments in a mixed C57BL/6 background. The MiR-1 TG mice were generated in our laboratory, as described previously (Zhang et al., 2013). Briefly, a fragment (264 bp) containing the precursor miR-1-2 (pre-miR-1-2) sequence was amplified and then carried by the vector (Promega, Madison, WI) with a cardiac-specific α-myosin heavy chain (α-MHC) promoter. Mice eggs were individually microinjected with the DNA fragment. The injected eggs were then implanted into the mice. The genomic DNA was extracted from the tail tissue of the TG mice and subjected to polymerase chain reaction (PCR) detection for the presence of the miR-1 transgene.

2.3. Mouse model of MI

Healthy male C57BL/6 mice (20–22 g) were purchased from the Experimental Animal Center of the Harbin Medical University; and housed with free access to food and water; in an environment with a maintained temperature of 22 °C, humidity of 55%–60%, and a 12 h light/dark cycle. Fifteen mice were randomly divided into sham (n = 5), MI or Met + MI (n = 5/group). MI surgery was performed as previously described (Yang et al., 2005). Briefly, the mice were anesthetized with avertin (240 mg/kg, *i.p.*) and left anterior descending coronary artery ligation was performed. Sham-operated mice were subjected to identical surgery, but without ligation. After 12 h of ligation, the mice were killed, and the infarcted border zone tissues were obtained for subsequent detection.

2.4. Administration of Met to mice

Met was delivered to miR-1 TG mice through intragastric administration at a dosage of 200 mg/kg/d for 4 weeks, or 2 weeks before the MI surgery. The same volume of 0.9% NaCl was used as the vehicle for intravenous administration. Met was administered once a day and its dosage was selected based on the preliminary experiment (Higgins et al., 2019).

2.5. Cell culture and treatment

The procedures used for neonatal mouse ventricular cardiomyocytes (NMVCs) culturing have been described previously (Zhang et al., 2018b). Briefly, hearts from C57BL/6 mice (1–3 days old) were minced and dissociated with 0.25% trypsin (Beyotime Institute of Biotechnology, Shanghai, China). Dispersed cells were seeded into Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Biological Industries, Shanghai, China), and then cultured in a 5% CO_2 incubator at 37 °C. Cultured primary NMVCs were exposed to 1 μM Met for 30 min and thereafter treated for 12 h with 100 μM H_2O_2 (Sigma-Aldrich, St. Louis, MO, USA).

2.6. Optical mapping

The optical mapping system MICAM05 (Brainvision, Tokyo, Japan) was used for CV and APD90 of the heart. Animals were anesthetized by injecting avertin (240 mg/kg, *i.p.*) and 0.1 ml heparin (50 mg/ml, *i.p.*). Their hearts were rapidly excised and perfused using the Langendorff technique at 25–30 ml/min with oxygenated tyrode solution (in mM: NaCl 125, KCl 4.5, $NaHCO_3$ 24, NaH_2PO_4 1.8, $CaCl_2$ 1.8, $MgCl_2$ 0.5, and glucose 5.5) with a pH of 7.40. Blebbistatin (10–20 μM, Tocris Bioscience, Ellisville, MO) was added to the perfusion to reduce motion artifacts during optical recordings in dark. After the contraction of the hearts had ceased, the hearts were stained with 30 μl of voltage-sensitive dye (RH 237) (Invitrogen, Carlsbad, CA, USA) for membrane potential mapping. An image capturing software (BV_MC05E; Brainvision, Tokyo, Japan) was adopted for optical recording of the heart at 5, 6.7, 8, and 10 Hz field stimulation and an image analysis software (BV-Analyze; Brainvision) was used for data analysis.

2.7. Electrocardiogram (ECG)

Cardiac electrical activity in mice was monitored using a standard lead II ECG for a continuous period of 15 min under anesthesia.

2.8. Echocardiographic measurements

The mice were anesthetized with avertin (240 mg/kg, *i.p.*). A Vevo 2100 high resolution imaging system (Visual Sonics, Toronto, Canada) was used for the echocardiography. The percentage rates for cardiac function parameters such as ejection fraction (EF) and short axis fractional shortening (FS) were obtained.

2.9. Transmission electron microscopic detection

The myocardium was fixed with 2.5% glutaraldehyde (pH 7.40) at 4 °C for 12 h, and subsequently fixed in 1.0% osmium tetroxide for 1 h. The monolayer was blocked in uranyl acetate and observed under an electron microscope, JEM-1220 (JEOL, Ltd., Tokyo, Japan).

2.10. Real-time PCR

Total RNA samples were extracted from NMVCs or the cardiac tissues of mice using TRIzol reagent (Invitrogen, Carlsbad, CA, USA)

according to the manufacturer's instructions. Total RNA (1 μg) was reverse-transcribed to cDNA miRNA cDNA Synthesis Kit (ABMGood, Vancouver, Canada). Real-time RT-PCR was performed using 100 ng cDNA via ABI 7500 fast real-time RT-PCR System (Applied Biosystems, Foster City CA, USA) with EvaGreen PCR Master Mix Kit (ABMGood, Vancouver, Canada) to detect the levels of miR-1. U6 was set as the internal control for miRNA quantification. The reactive procedure of SYBR Green Real-time PCR was 95 ˚C for 10 min, 95 ˚C for 15 s, 60 ˚C for 30 s, and 72 ˚C for 30s. Relative expression level of miR-1 was calculated using the Ct ($2^{-\Delta\Delta Ct}$) method. (The primer sequence: miR-1-F: CGGGCACATACTTCTTTATA, miR-1-R: CAGCCACAAAAGAGCACAAT. miR-RT: CCTGTTGTCTCCAGCCACAAAAGAGCACAATATTTCAGGAGACAACA

GGATGGGCA. U6–F: GCTTCGGCAGCACATATACTAAAAT. U6-R: CGCTTCACGAATTTGCGTGTCAT. U6-RT: CGCTTCACGAATTTGCGTGTCAT) The sequences of the primers were synthesized by Sangon Biotech - Shanghai, China.

2.11. Western blot

The total proteins from the NMVCs or cardiac tissues were extracted using a lysis buffer with protease inhibitors. The protein concentrations were measured via a BCA Protein Assay Kit (Beyotime Institute of Biotechnology, Shanghai, China). Equivalent level of proteins was denatured and resolved with 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis; transferred to nitrocellulose membranes (Millipore, Massachusetts, USA); incubated with 5% skimmed milk; and probed with primary antibodies overnight at 4 ˚C. Primary antibodies were diluted as follows: anti-Kir2.1 (1:200; Santa Cruz, CA, USA), anti-CX43 (1:5000; Millipore, Massachusetts, USA), and anti-β-actin (1:5000; Proteintech, Wuhan, China). The membranes were washed thrice with PBST (PBS containing 5% Tween-20), then incubated with fluorescence-conjugated secondary antibody diluted in PBS for 1 h at room temperature. The blots were detected using the Odyssey v1.2 Infrared Imaging System (Gene Company Ltd, Hong Kong, China), quantified by the intensity, and normalized by β-actin.

2.12. Statistical analysis

Prism software version 5.0 (GraphPad Software, La Jolla, CA, USA) was used for data analysis. In accordance with the journal's policy, a minimum of five independent samples were obtained. All the data were presented as mean ± S.E.M. Statistical analyses were assessed with one-way ANOVA using SPSS 20.0 (SPSS, IBM Corp). A value of $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Met ameliorates cardiac conduction delay in miR-1 TG mice through inhibition of miR-1 expression

We quantified miR-1 levels in the cardiac tissues of mice. The miR-1 expression level in TG mice was significantly up-regulated as compared with WT mice (Fig. 1A). After intragastric administration of Met for 4 weeks, the expression level of miR-1 in TG mice decreased noticeably. Through an ECG, we then detected the cardiac electrical activity condition in mice. The ECG recordings illustrated significantly prolonged PR and QT intervals in TG mice. However, these pathological characteristics improved after Met was administered (Fig. 1B–D). We tested the protein levels of Kir2.1 and CX43 in the cardiac tissues of mice, and found that both Kir2.1 and CX43 expressions were down-regulated in miR-1 TG mice (Fig. 1E and F). Administration of Met was able to reverse this change (Fig. 1E and F). In addition, echocardiographic measurements showed that Met restored the reduction of EF % and FS % in TG mice (Fig. 1G and H).

All the above results indicated that Met could improve miR-1 induced cardiac conduction protein restraint and atrioventricular block to restore cardiac functions. To further explore, we detected the APD90 and CV of the hearts in mice through optical mapping. As shown in Fig. 2A and Fig. 2B, APD90 was delayed in miR-1 TG mice. Interestingly, intragastric administration of Met reversed this effect. In accordance with the results of APD90, the CV was lowered in miR-1 TG mice. However, Met could effectively restore the CV decline (Fig. 2C and D). These results suggest that Met could inhibit high levels of miR-1 induced cardiac conduction delay.

3.2. Met rescues Kir2.1 and CX43 expressions in H_2O_2 treated neonatal mouse ventricular cardiomyocytes through reducing miR-1 expression

Some studies have shown that the expression of miR-1 was up-regulated in H_2O_2 treated NMVCs. Hence, the influence of Met on H_2O_2 treated NMVCs was observed. Compared with the control group, the expression of miR-1 was up-regulated in NMVCs treated with H_2O_2. Pre-administration of Metinhibited the upregulation of miR-1 induced by H_2O_2 (Fig. 3A). Correspondingly, the protein levels of Kir2.1 and CX43 were downregulated in NMVCs treated with H_2O_2. Pre-administration of Met successfully attenuated the reduction of Kir2.1 and CX43 induced by H_2O_2 (Fig. 3B–D).

3.3. Met ameliorates cardiac conduction delay in MI mice through inhibition of miR-1 expression

The above results indicate that Met had a therapeutic effect on cardiac conduction delay caused by the overexpression of miR-1. Some studies showed that cardiac conduction delay is induced by MI accompanied with up-regulation of miR-1 expression. Therefore, we hypothesized that Met could also improve cardiac conduction delay post MI through the inhibition of miR-1 expression. To test our hypothesis in vivo, we carried out the following experiments. Mice received Met for two weeks prior to MI through intragastric administration. Then, we quantified miR-1 levels in cardiac tissues obtained from the different groups. ECG recordings illustrated significantly prolonged PR and QT intervals in MI mice (Fig. 4A–C). The expression level of miR-1 was up-regulated in MI mice when compared with Sham mice (Fig. 4D). However, administration of Met for 2 weeks prior to MI surgery could significantly decrease the expression level of miR-1 when compared with that in MI mice. This result suggested that pre-administration of Met successfully attenuated the upregulation of miR-1 induced by MI. Meanwhile, the protein levels of Kir2.1 and CX43 were down-regulated in cardiac tissues of mice that underwent MI surgery. Interestingly, pre-administration of Met successfully attenuated the reduction of Kir2.1 and CX43 in MI mice (Fig. 4E and F).

Further, echocardiographic results showed that the EF % and FS % in MI mice reduced significantly, indicating that their cardiac functions were severely damaged. However, Met restored the cardiac functions (Fig. 4G and H). Besides, transmission electron microscopic detection revealed that the intercalated discs were dissolved markedly with vacuolar degeneration of gap junctions and decreased density of the macula adherents in the hearts of MI mice, but such damaging changes improved noticeably in MI mice treated with Met (Fig. 4I).

To further confirm our speculation, we also detected the APD90 and CV through optical mapping. We found a significant delay in APD90 and reduction of CV in the hearts of MI mice. Similarly, pre-administration of Met for 2 weeks was able to attenuate the conduction delay (Fig. 5A–D).

Although these results indicate that MI can lead to cardiac conduction damage in mice; yet, Met is able to alleviate this damage through down-regulation of the cardiac miR-1 expression, and then rescuing Kir2.1 and CX43.

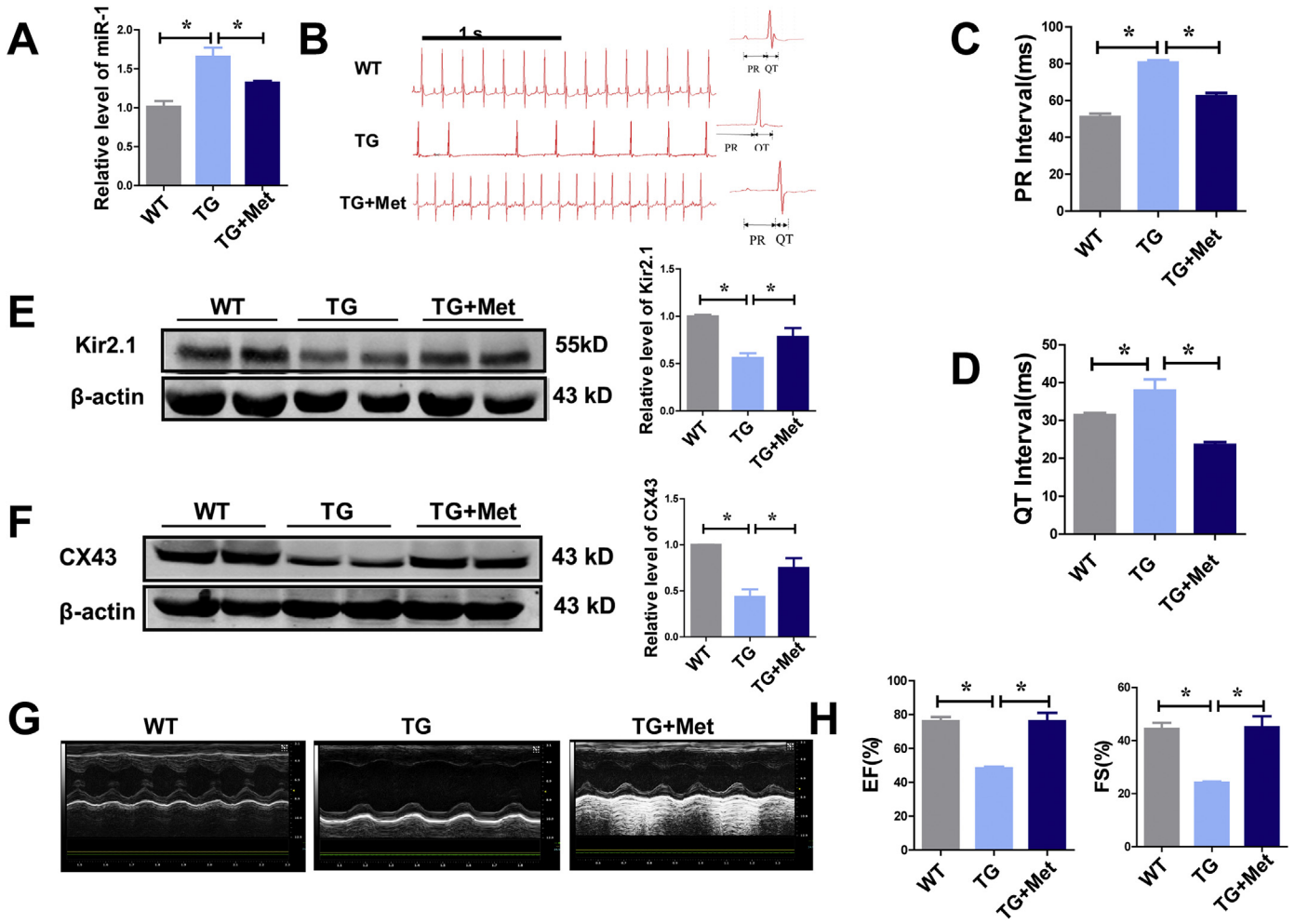

Fig. 1. Metformin resisted cardiac conduction injury in miR-1 TG mice. (A) The expression level of miR-1 in the heart was detected by real-time RT-PCR. $^*P < 0.05$, n = 5. **(B)** Representative ECG recordings of the three groups. **(C)** Quantitative comparison of PR interval in all groups. $^*P < 0.05$, n = 5. **(D)** Quantitative comparison of QT intervals in all groups. $^*P < 0.05$, n = 5. **(E)** Expression levels of Kir2.1 in heart was detected by Western blot. $^*P < 0.05$, n = 5. **(F)** Expression levels of CX43 in heart was detected by Western blot. $^*P < 0.05$, n = 5. **(G, H)** Representative echocardiographic images and parameters of cardiac functions in the three groups. $^*P < 0.05$, n = 5. WT: wild type, TG: miR-1 transgenic mice, Met: metformin. All data are presented as the mean ± S.E.M.

4. Discussion

Severe cardiac conduction delay increases the risk of death in patients with cardiovascular disease. MiR-1, a cardiac enriched miRNA, has been identified as a causative factor of cardiac conduction delay in a previous study (Yang et al., 2007; Zhang et al., 2013). In the meanwhile, Met, which is a drug for the treatment of T2DM, was found to be beneficial for a lot of heart diseases. This study's purpose was to survey whether Met had an effect on cardiac conduction delay through regulation of miR-1 expression. This study, therefore, has great significance for the treatment of cardiac conduction delay.

In conclusion, Met was able to reduce the cardiac miR-1 expression, and then rescue the expression level of miR-1's downstream targets CX43 and Kir2.1, which are essential for cardiac conduction. Furthermore, Met was able to improve the decreased density of the gap junctions in the hearts of MI mice. This effect of Met could attenuate cardiac conduction delay and injuries both in MI and miR-1 TG mice (Fig. 6).

MiR-1 was conserved from Drosophila to humans and was subsequently found to be a potential anti-arrhythmic target (Liao et al., 2016). MicroRNA expression disorders often occur after acute MI, such as the increase of miR-1 expressions, which would always lead to arrhythmias (Boon and Dimmeler, 2015; Goretti et al., 2014; Liu et al., 2014; Pinchi et al., 2019). Bao feng Yang et al. showed that miR-1 overexpression slowed cardiac conduction (Yang et al., 2007). A subsequent study showed that cardiac specific miR-1 TG mice were more susceptible to atrioventricular blocks (Zhang et al., 2013). This indicates that miR-1 overexpression induces atrioventricular blocks and conduction delay in the heart. A study showed that miR-1 is up-regulated in ischemic cardiac tissues, which contribute to cardiac conduction disorders (Liu et al., 2014). Based on these findings, we use miR-1 TG mouse and MI model to induce miR-1 mediated cardiac conduction disorders.

Met is a mainstay drug for T2DM therapy which could augment or mimic the function of insulin. The molecular mechanism mediating this effect is that Met inhibits mitochondrial complex I and increases the ratio of AMP/ATP, which activates the AMPK, leading to a decrease of the hepatic glucose output (Prattichizzo et al., 2018). However, another study showed that Met could restrain gluconeogenesis in hepatic cells, and mice not expressing AMPK (Miller and Birnbaum, 2010), which indicates that Met could play an independent role in AMPK. It was simultaneously reported that Met protects the heart against remodeling (Loi et al., 2019), autophagy (Kanamori et al., 2019), oxidative damage (Zhou et al., 2018), apoptosis (Cittadini et al., 2012) and fibrosis (Kanamori et al., 2019). Similarly, it was reported that the cardioprotective effect of Met is independent of the hypoglycemic effect. Thus, AMPK inhibitors such as compound C may not affect all the function of Met on the heart.

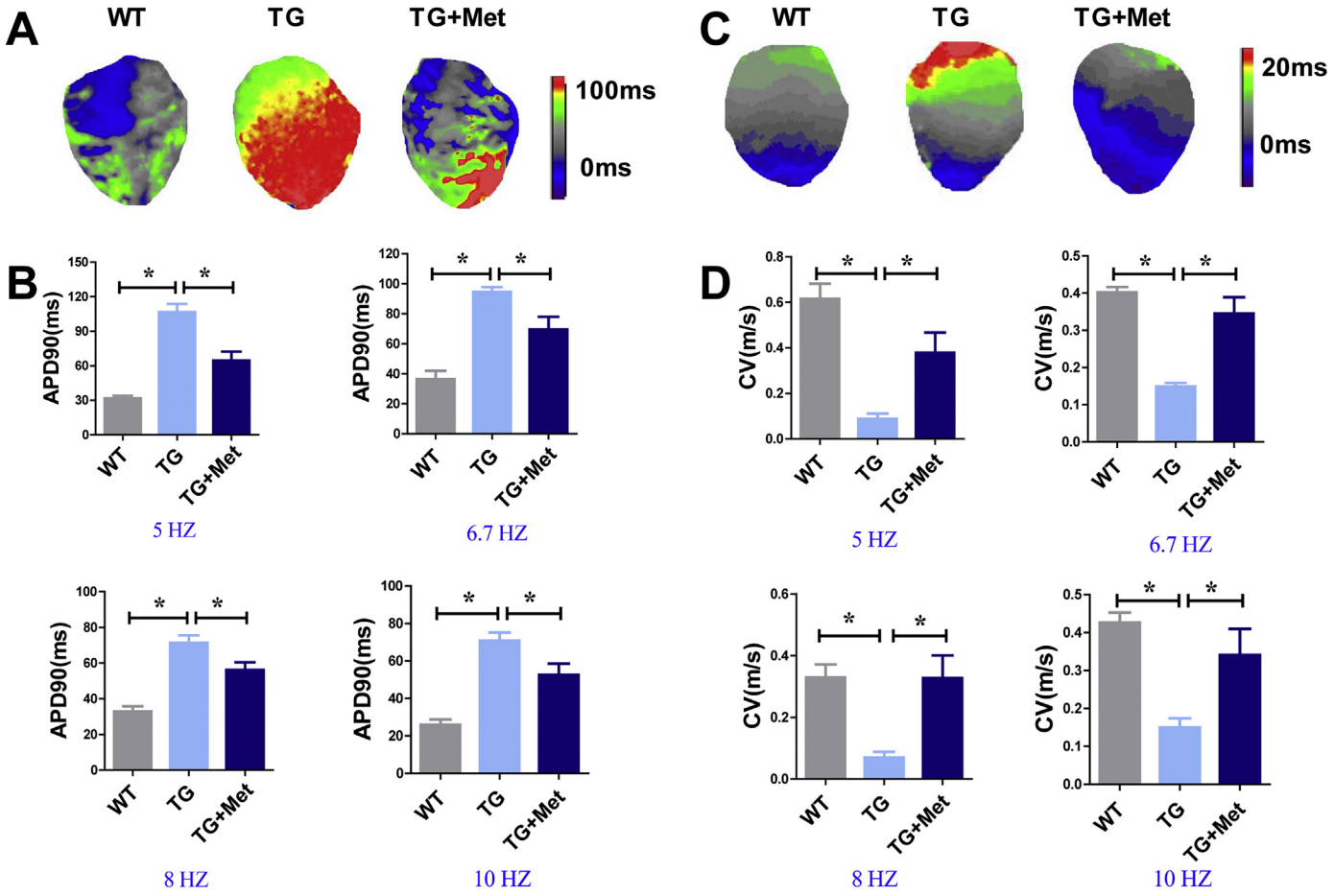

Fig. 2. Metformin attenuated the conduction delay in miR-1 TG mice. (A) Action potential duration (APD) in mice was detected by optical mapping in all groups. **(B)** Quantitative comparison of APD90 in all groups. *$P < 0.05$, n = 5. **(C)** Conduction velocity (CV) in mice was detected by optical mapping in all groups. **(D)** Quantitative comparison of CV in all groups. *$P < 0.05$, n = 5. WT: wild type, TG: miR-1 transgenic mice, Met: metformin. All data are presented as the mean ± S.E.M.

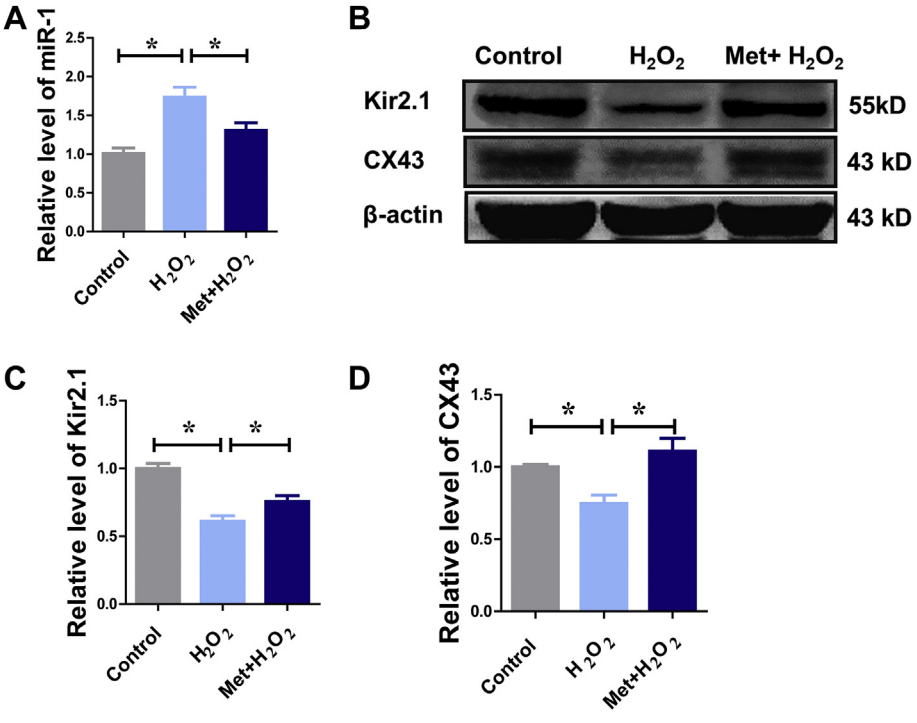

Fig. 3. Metformin mitigated H_2O_2 induced Kir2.1 and CX43 reduction. (A) The expression level of miR-1 in cardiomyocytes was detected by real-time RT-PCR. *$P < 0.05$, n = 5. **(B–D)** Expression levels of Kir2.1 and CX43 in cardiomyocytes were detected by Western blot. *$P < 0.05$, n = 5. Met: metformin. All data are presented as the mean ± SEM.

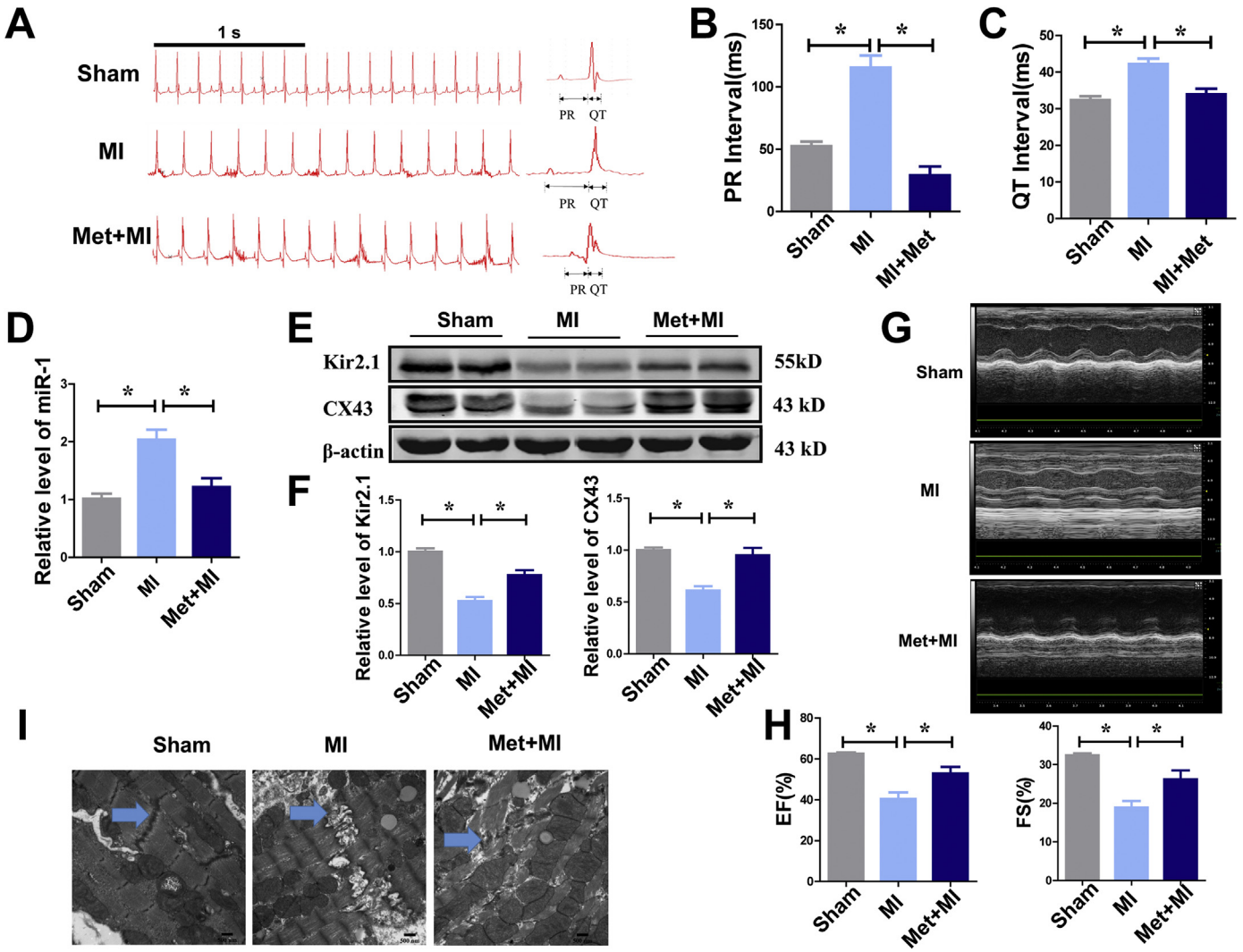

Fig. 4. Metformin inhibited cardiac injury in MI mice. (A) Representative ECG recordings of the three groups. **(B)** Quantitative comparison of PR intervals in all the groups. *$P < 0.05$, n = 5. **(C)** Quantitative comparison of QT intervals in all the groups. *$P < 0.05$, n = 5. **(D)** The expression level of miR-1 in the heart was detected by real time RT-PCR. *$P < 0.05$, n = 5. **(E, F)** Expression levels of Kir2.1 and CX43 in heart were detected by Western blot. *$P < 0.05$, n = 5. **(G, H)** Representative echocardiographic images and parameters of cardiac functions in three groups. *$P < 0.05$, n = 5. **(I)** Representative intercalated disc organization showing the gap junctions and macula adherents was detected by transmission electron (20,000× magnification). Met: metformin, MI: myocardial infarction. All the data are presented as the mean ± S.E.M.

The results of our study showed that Met was able to improve cardiac functions and protect the heart against cardiac conduction delays in both miR-1 TG and MI mice. It successfully enhanced the APD and increased CV in the heart. Met's protective function mechanism was mainly due to its ability to reduce miR-1 expression. As a direct allosteric AMPK activator, Met was able to activate AMPK which regulates the cardiac function (Gundewar et al., 2009). Another report demonstrated that C/EBP β is the molecular target of AMPK: C/EBP β, but not p-CREB β, whose level was shown to have decreased with the AMPK activator AICAR or constitutively activated AMPK, while the dominant-negative inhibitor of AMPK led to an increase in CEBP β expression (Choudhury et al., 2011). After that, a study of Ying Zhang et al. from our team confirmed that Met could activate AMPK and then suppress the C/EBP β expression significantly. They also experimentally showed that C/EBP β promoted miR-1 expression directly: C/EBP β knockdown led to a decrease in miR-1 expression, but overexpression was shown to lead to increased miR-1 expression (Zhang et al., 2018a). On the basis of these results, we confirm that Met can activate AMPK and then significantly reduce C/EBP β to decrease the expression of miR-1.

CX43 is the main cardiac gap junction channel, which is responsible for intercellular conductance in cardiac ventricles. Kir2.1 is the main K^+ channel subunit that mediates IK_1 current, which is responsible for forming and maintaining the cardiac resting membrane potential, indicating that CX43 and Kir2.1 are essential for cardiac conduction.

A previous study from our laboratory had shown that Kir2.1 (KCNJ2) and CX43 are the molecular targets of miR-1, whose overexpression results in the decrease of Kir2.1 and CX43 protein levels, which slowed cardiac conduction (Yang et al., 2007). In addition, the interplay between miR-1 and the CX43 gene in arrhythmic rodent hearts treated with valsartan had previously been reported (Curcio et al., 2013) and two potential binding sites were found in mice miR-1 and Cx43 3′ - untranslated regions (Xu et al., 2012) which could further prove that CX43 is a direct target of miR-1. Other studies have also confirmed that the 3′- untranslated region of Kir2.1 had miR-1 potential binding sites, and that miR-1 could regulate the expression of Kir2.1 (Liu et al., 2014; Shan et al., 2013). These studies provided an authentic analysis of the relationship between miR-1 and its direct targets Kir2.1 and CX43.

Based on the above studies and our present experiment's results, we come to the solid conclusion that Met accounts for cardiac protection

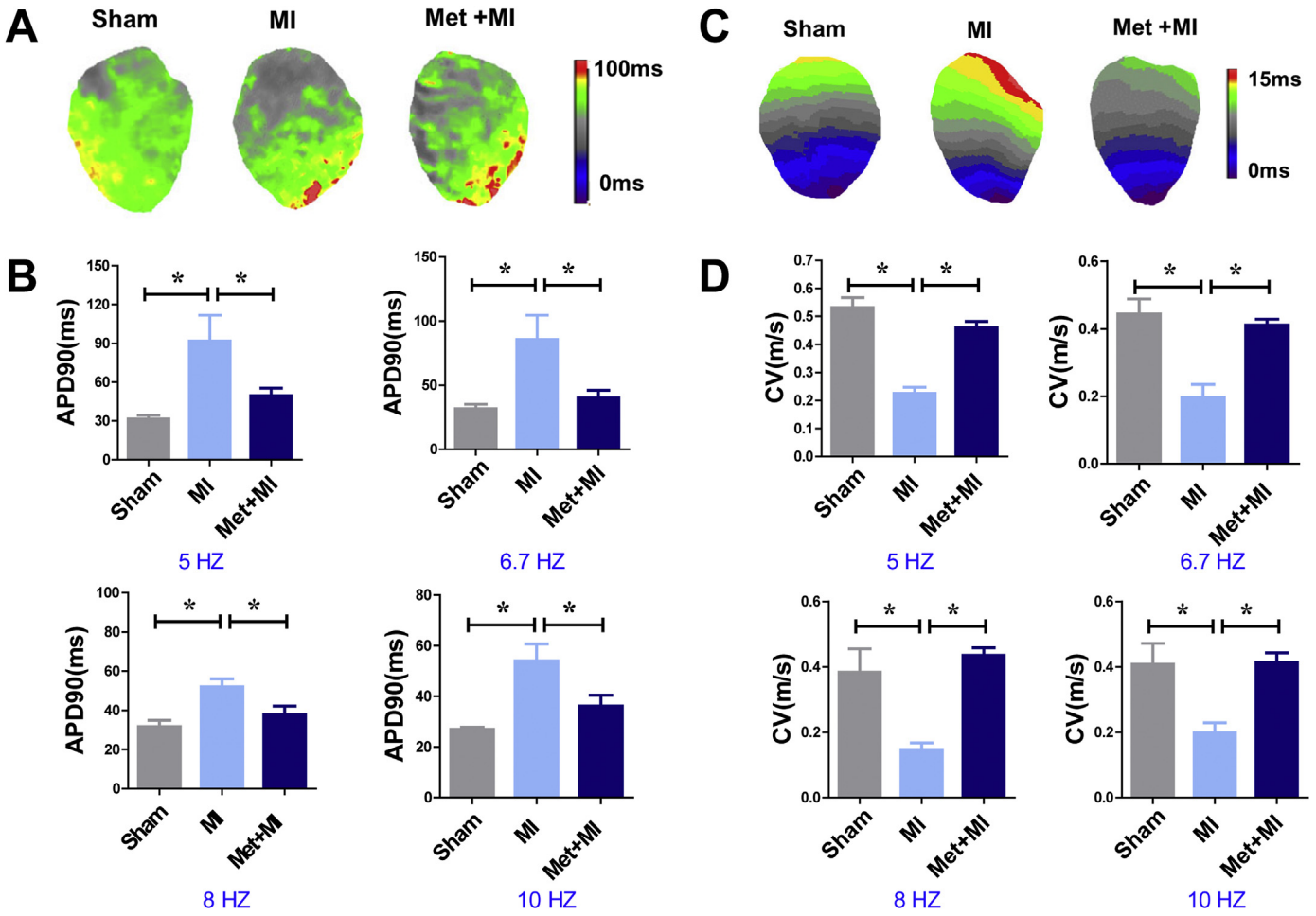

Fig. 5. Metformin attenuated cardiac conduction delay in MI mice. (A) Action potential duration (APD) in mice was detected by optical mapping in all groups. **(B)** Quantitative comparison of APD90 in all groups. *$P < 0.05$, n = 4. **(C)** Conduction velocity (CV) in mice by optical mapping in all groups. **(D)** Quantitative comparison of CV in all groups * $P < 0.05$, n = 4. All data are presented as the mean ± S.E.M.

potential, at least in part, through its anti-conduction delay effect by AMPK-C/EBP β-miR-1- CX43/Kir2.1 pathway.

However, miR-1 may not be the only upstream of CX43, and a study showed that Met restored CX43 protein repression by attenuating the autophagy pathway in hyperglycemic conditions (in vitro study only) (Wang et al., 2017). However, our study is consistent with previous analysis that miR-1 is a crucial regulator of CX43. Beyond that, we still need to explore if Met, exerts its effect on cardiac conduction delay, through other molecular targets.

Met's main and effective molecular targets for protective functions against cardiovascular injury still need to be explored. In a human Kir2.1 channel disease, Andersen-Tawil syndrome (ATS1), the cytoplasmic C terminus of the Kir2.1 mutation causes potassium channel down-regulation and accumulation in the Golgi apparatus (Ma et al., 2011). However, 40% of patients with ATS1 have no mutations in Kir2.1, and the genetic basis of the disease in these cases is completely unknown (Donaldson et al., 2004). Therefore, miR-1 up-regulation induced Kir2.1 decrease could be related to this phenomenon and Met

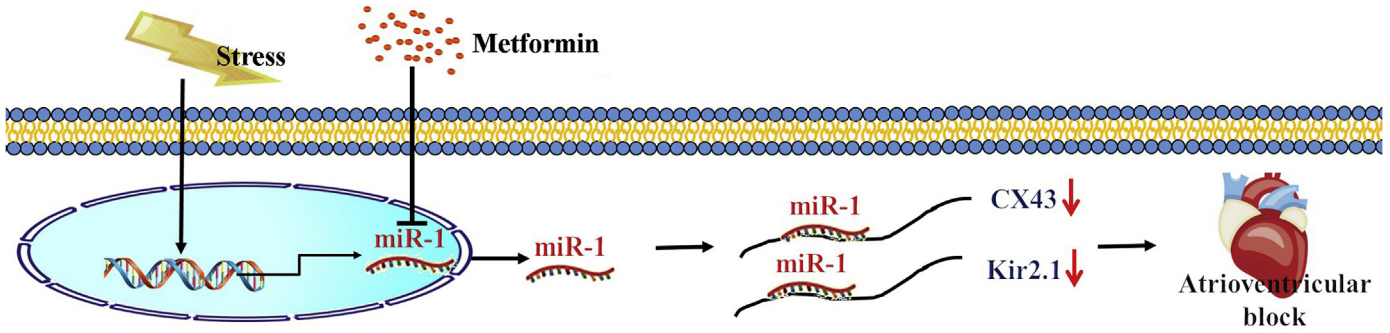

Fig. 6. Metformin inhibits miR-1 upregulation induced cardiac conduction delay. Cardiac-specific overexpression of miR-1 and its upregulation induced by MI lead to cardiac conduction delay in mice. Metformin was able to inhibit the upregulation of miR-1 and then rescue the expression of miR-1's downstream targets CX43 and Kir2.1, which are essential for cardiac conduction. By the miR-1- CX43/Kir2.1 axis, metformin attenuates cardiac conduction delay and injury in both MI and miR-1 TG mice.

could be a promising therapeutic drug.

We have accumulated scientific evidence on Met's protective role in cardiac conduction and hope that these novel findings will develop its potential as an evidence-based medicine and expand its application to cardiac conduction diseases.

Credit Author Statement

Lifang Lv: Writing - original draft. **Nan Zheng:** Formal analysis. **Lijia Zhang:** Formal analysis. **Azaliia Shabanova:** Writing - review & editing. **Haihai Liang:** Formal analysis. **Yuhong Zhou:** Supervision. **Hongli Shan:** Supervision.

Declaration of competing interest

All authors have no competing interests to declare, financial, or otherwise.

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