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The protective effect of trimetazidine on myocardial ischemia/reperfusion injury through activating AMPK and ERK signaling pathway

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Abstract

Introduction—Trimetazidine (TMZ) is an anti-anginal drug that has been widely used in Europe and Asia. The TMZ can optimize energy metabolism *via* inhibition of long-chain 3-ketoacyl CoA thiolase (3-KAT) in the heart, with subsequent decrease in fatty acid oxidation and stimulation of glucose oxidation. However, the mechanism by which TMZ aids in cardioprotection against ischemic injury has not been characterized. AMP-activated protein kinase (AMPK) is an energy sensor that controls ATP supply from substrate metabolism and protects heart from energy stress. TMZ changes the cardiac AMP/ATP ratio by modulating fatty acid oxidation, thereby triggering AMPK signaling cascade that contributes to the protection of the heart from ischemia/reperfusion (I/R) injury.

Methods—The mouse model of *in vivo* regional ischemia and reperfusion by the ligation of the left anterior descending coronary artery (LAD) was used for determination of myocardial infarction. The infarct size was compared between C57BL/6J WT mice and AMPK kinase dead (KD) transgenic mice with or without TMZ treatment. The *ex vivo* working heart perfusion system was used to monitor the effect of TMZ on glucose oxidation and fatty acid oxidation in the heart.

Results—TMZ treatment significantly stimulates cardiac AMPK and extracellular signal-regulated kinase (ERK) signaling pathways ($p < 0.05$ *vs.* vehicle group). The administration of

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Author Contributions

Design and conduct of the study: ZL, JMC, HH, MK, WS, NQ, LW, HY, JL, and PZ.
Data collection and analysis: ZL, HH, MK, SZ, WS, NQ, LW, HY, HMG, and JZ.
Data interpretation: JMC, SZ, HY, HMG, JL, JZ, and PZ. Manuscript writing: ZL, MK, JL, and PZ.

Conflict of Interest

None.

TMZ reduces myocardial infarction size in WT C57BL/6J hearts, the reduction of myocardial infarction size by TMZ in AMPK KD hearts was significantly impaired versus WT hearts ($p < 0.05$). Intriguingly, the administration of ERK inhibitor, PD98059, to AMPK KD mice abolished the cardioprotection of TMZ against I/R injury. The *ex vivo* working heart perfusion data demonstrated that TMZ treatment significantly activates AMPK signaling and modulating the substrate metabolism by shifting fatty acid oxidation to glucose oxidation during reperfusion, leading to reduction of oxidative stress in the I/R hearts. Therefore, both AMPK and ERK signaling pathways mediate the cardioprotection of TMZ against ischemic injury. The metabolic benefits of TMZ for angina patients could be due to the activation of energy sensor AMPK in the heart by TMZ administration.

Keywords

AMPK signaling; MAPK signaling; Trimetazidine; Cardioprotection; Ischemia/reperfusion

1. Introduction

Ischemic heart disease (IHD) is the leading cause of death as well as a major reason of disability due to nonfatal acute myocardial infarction (AMI), angina pectoris, or ischemic heart failure in the world [1,2]. In the United States alone, 15.4 million people were diagnosed with coronary heart disease, and 7.8 million had chronic angina and stable ischemic heart disease [3]. Current medical therapies for IHD involve anticoagulants, thrombolytic and percutaneous coronary intervention which aim to improve the blood supply of the heart [4]. However, these treatments will irreversibly cause myocardial ischemia/reperfusion injury. Over the last 30 years, researches demonstrate that partial inhibition of myocardial fatty acid oxidation, with mutual activation of carbohydrate oxidation, is an effective treatment for ischemia/reperfusion injury [5].

The greatest development in the application of metabolic therapy came in the last 15 years with the advent of compounds that partially inhibit myocardial fatty acid oxidation, specifically trimetazidine (1-[2,3,4-trimethoxibenzyl]-piperazine) and ranolazine. Trimetazidine selectively inhibits long-chain 3-ketoacyl-co-enzyme A (CoA) thiolase (3-KAT), which is the enzyme that catalyzes the terminal step of fatty acid β-oxidation, thereby shifting cardiac energy metabolism from fatty acid oxidation to glucose oxidation [6]. Fatty acid and pyruvate oxidation both occur in the mitochondrial matrix and share common substrates and products. Suppression of myocardial fatty acid oxidation lowers the mitochondrial ratios of $NADH/NAD^+$ and acetyl-CoA/free CoA, which relieves inhibition on pyruvate dehydrogenase (PDH) and increases glucose and lactate oxidation [7]. These results suggest that trimetazidine decreases the $NADH/NAD^+$ and acetyl-CoA/free CoA ratios in the mitochondrial matrix *via* inhibition of 3-KAT. As was demonstrated in the working rat heart, trimetazidine significantly increased the rate of glucose oxidation despite only modestly reducing the rate of fatty acid oxidation [6,8]. However, the detailed mechanism has not been illuminated.

We have demonstrated that the activation of AMP-activated protein kinase (AMPK) exerts a protective effect toward ischemia/reperfusion injury [9–15]. AMPK is a widely distributed

and highly conserved hetero-trimetric complex composed of a catalytic α (62KDa) subunit and the non-catalytic β and γ subunits which are responsible for the regulation of the kinase activity, enzyme stability, and localization [16]. Some of the catabolic, energy-producing pathways AMPK up regulates include glucose uptake, glycolysis, fatty acid uptake, fatty acid oxidation, and autophagy [17]. AMPK also functions directly as an important energy sensor for cells. It is activated in response to metabolic stress on the cell that lowers the energy state of the cell by either inhibiting ATP production (i.e. ischemia, hypoxia, glucose deprivation) or accelerating ATP consumption (i.e. muscle contraction) [18]. Does trimetazidine activate AMPK? Could trimetazidine shift metabolism through activating AMPK signaling pathway? Based on these questions, we hypothesized that trimetazidine exerts its protective effect through activating AMPK signaling pathway.

In this study, we investigated the cardioprotective effect of trimetazidine with wild-type mice and AMPK-kinase dead (KD) mice. In a mouse model of ischemia/reperfusion (I/R) injury in which the left anterior descending coronary artery (LAD) was occluded by suture and released, we demonstrated that trimetazidine can activate AMPK both at the basal level and the I/R level. In addition to that, p-ERK which is a component of mitogen-activated protein (MAP) kinases signaling pathway was also activated. Trimetazidine can significantly decrease the myocardial infarct size through AMPK and ERK signaling pathway. By measuring glucose oxidation and fatty acid oxidation with [U-^{14}C]glucose and [9,10-^{3}H]oleate, the results indicate that trimetazidine can shift metabolism from fatty acid oxidation to glucose oxidation through AMPK signaling pathway in the working heart system.

2. Materials and Methods

2.1. Experimental Animals

Male C57Bl/6J mice (12 weeks of age) and AMPK kinase-dead (KD, K45R mutation driven by muscle creatine kinase promoter) transgenic mice [19] were used in all experiments. All animal protocols in this study were approved by the Institutional Animal Care and Use Committee of the University at Buffalo–State University of New York.

2.2. Immunoblotting Analysis and Infarct Size Measurement

C57BL/6J and AMPK KD mice were anesthetized with pentobarbital (60 mg/kg, IP), disinfected, intubated and ventilated with a respirator (Harvard apparatus, Holliston, MA) as described previously [9,20]. After a left lateral thoracotomy, the left anterior descending coronary artery (LAD) was occluded for 20 min with an 8-0 nylon suture and polyethylene tubing to prevent arterial injury and reperfused for 15 minutes. Vehicle (saline) or trimetazidine (0.5 mg/kg) was administered *via* the tail vein injection 5 min before reperfusion. The ECGs confirmed the ischemic manifestations of ST-segment elevation during coronary occlusion and T-segment inversion during reperfusion (AD Instruments, Colorado Springs, CO). A cardiectomy was performed at the end of reperfusion. Left ventricular ischemic regions were isolated prior to freeze clamping in liquid nitrogen for further immunoblotting analysis. The tissue were lysed in a lysis buffer containing: 50 mmol/L β-glycerol phosphate, 2 mmol/L EGTA, 1 mmol/L DTT, 10 mmol/L NaF, 1 mmol/L

sodium orthovanadate, 20 mmol/L HEPES (pH 7.4), 1% Triton X-100, 10% glycerol, and a protease inhibitor cocktail tablet. Membranes were probed with anti-AMPK-α, p-AMPK, ACC, p-ACC, ERK1/2, p-ERK1/2, p-JNK, JNK, p-Akt, and Akt (Cell Signaling, Danvers, MA), followed by incubation with horseradish peroxidase (HRP)-coupled anti-rabbit secondary antibody (Cell Signaling Technology, Beverly, MA). Blue X-ray film (Phenix, Candler, NC) was used for photon detection and image development. Films were scanned with the Bio-Rad GS-700 scanner in the core facility of the School of Medicine and Biomedical Sciences and the relative density of the bands on the film was determined by Image J software [9,11,20].

For infarct size measurement, LAD was occluded for 30 minutes and reperfused for 24 h, compound C (0.1 μg/g) and/or PD98059 (10 μmol/L) were applied through IP injection an hour before ischemia to inhibit AMPK and/or ERK signaling pathway. After reperfusion, hearts were excised for dual staining. The non-necrotic tissue in the ischemic region was stained red with 2,3,5-triphenyltetrazolium (TTC) and non-ischemic regions were stained blue with Evans blue dye. The hearts were fixed with 10% formalin and sectioned into 1 mm slices, photographed utilizing a Lexica MZ95 microscope and analyzed by NIH Image analysis software [9,20]. The myocardial infarct size was calculated as the ratio of the percentage of myocardial necrosis to the ischemic area at risk (AAR).

2.3. Glucose Oxidation and Fatty Acid Oxidation Analysis

Cardiac substrate metabolism was determined in the working heart model as previously described [20]. The heart preload was set at 15 cm H_2O and afterload at 80 cm H_2O. The flow rate was maintained at 15 mL/min. Mouse hearts were cannulated through the aorta to initiate retrograde Langendorff perfusion followed by cannulation of the pulmonary vein to initiate anterograde perfusion in the working heart mode. Isolated mouse hearts were subjected to 20 minutes of baseline perfusion, followed by 10 minutes of global, no-flow ischemia and 20 minutes of reperfusion. Glucose oxidation was determined by the production of $^{14}CO_2$ from [U-^{14}C]glucose in the perfusate. Fatty acid oxidation was determined by the production of $^{3}H_2O$ from [9,10-^{3}H]oleate in the perfusate. The $^{14}CO_2$ in the coronary effluent was captured with hyamine hydroxide. The $^{14}CO_2$ and $^{3}H_2O$ were enumerated by scintillation counting.

2.4. Histological Evaluation

After subjected to 20 minutes of ischemia and 4 hours of reperfusion, hearts were perfused with relaxation buffer (25 mmol/L KCl and 5% dextrose in PBS) with heparin to wash out blood. The hearts were removed, fixed in 10% formalin and embedded in paraffin. Paraffin-embedded myocardial sections (5 μm), stained with hematoxylin and eosin, were examined by light microscopy.

2.5. Statistical Analysis

Data followed a normal distribution with constant variance and were expressed as means ± standard error of the mean [21]. Significance was tested by 2-tailed Student *t* tests or 2-way analysis of variance with post hoc analysis. A $p < 0.05$ was considered as significant.

3. Results

3.1. Trimetazidine Treatment Triggers Phosphorylation of AMPK and ERK

To determine whether trimetazidine (TMZ) protects against myocardial injury through AMPK signaling pathway, we first examined the effect of TMZ on cardiac AMPK signaling pathway. The C57BL/6J mice were subjected to tail vein injection of vehicle (saline) or TMZ (0.5 mg/kg) after intraperitoneal injection (IP) of pentobarbital (60 mg/kg), hearts were collected 10 minutes after injection. For ischemia and reperfusion (I/R) group, C57BL/6J mice were subjected to 20 minutes of ischemia followed by 15 minutes of reperfusion (Fig. 1A). Trimetazidine or vehicle (saline) was injected intravenously *via* the tail vein injection 5 minutes before reperfusion. The ECGs confirmed the ischemic manifestations of ST-segment elevation during coronary occlusion and T-segment inversion during reperfusion (Fig. 1B). Representative levels of p-AMPK, p-ACC, p-ERK and p-Akt are shown in Fig. 1C. Administration of trimetazidine at a 0.5 mg/kg dose significantly activated AMPK, and its downstream effector, ACC (Fig. 1C). The estrogen-regulated protein kinase (ERK) is identified as one of the major components of the RISK (reperfusion injury salvage kinase) pathway. Both p-ERK and p-Akt were augmented in both the basal and I/R groups. p-JNK, one of the kinase of MAP kinase signaling pathway did not change. p-AMPK, p-ACC, and p-Akt were not activated in the I/R vehicle group, however, p-ERK was stimulated in the I/R vehicle group.

3.2. Trimetazidine Reduces Myocardial Infarction Through AMPK and ERK Signaling Pathway

To determine whether trimetazidine can protect against myocardial injury through AMPK and ERK signaling pathway, we performed TTC staining to measure the infarction area. C57Bl/6J and AMPK KD mice were subjected to 30 min of ischemia followed by 24 h of reperfusion. At every group, trimetazidine (0.5 mg/kg) or vehicle (saline) was injected intravenously *via* the tail vein 5 minutes before starting reperfusion. Representative cardiac sections dually stained with TTC and Evans blue dye are shown in Fig. 2A. Ratios of the area at risk to the total myocardial area were similar among the ten groups, indicating that an equal extent of ischemic stress has been induced in all groups (Fig. 2B). Administration of trimetazidine at a dose of 0.5 mg/kg significantly decreased myocardial infarction in WT mice (Fig. 2B, 0.11 ± 0.02 *vs.* 0.23 ± 0.01, $p < 0.05$ *vs.* vehicle). With AMPK KD group, the infarction size of trimetazidine group increased compared with WT + trimetazidine group (Fig. 2B, 0.19 ± 0.01 *vs.* 0.11 ± 0.02, $p < 0.05$ *vs.* vehicle), indicating that trimetazidine reduces myocardial infarction injury partially through AMPK signaling pathway. However, the infarction size was smaller compared to corresponding vehicle group (0.34 ± 0.01 *vs.* 0.19 ± 0.02, $p < 0.05$ *vs.* corresponding vehicle), suggesting that there are other signal pathways that trimetazidine can interact with, so that it can relieve the injury caused by ischemia/reperfusion injury. Concerning immunoblotting results, we applied ERK inhibitor PD98059 to see the role of ERK signaling pathway in the ischemia/reperfusion injury. Injection of trimetazidine at a dose of 0.5 mg/kg significantly decreased myocardial infarction in WT + PD98059 mice (Fig. 2B, 0.21 ± 0.01 *vs.* 0.34 ± 0.02, $p < 0.05$ *vs.* vehicle). Between the two pathways, AMPK signaling pathway appears to be more

predominant than ERK signaling pathway. The histological results also demonstrated that trimetazidine can reduce myocardial infarction judging from morphological change (Fig. 3).

3.3. Trimetazidine Shifts Metabolism from Fatty Acid Oxidation to Glucose Oxidation

Decrease myocardial fatty acid oxidation and increase glucose oxidation will result in a greater ratio of ATP synthesis to O_2 consumption, and also reduce lactate production and the fall in pH, which is advantageous to the ischemia/reperfusion heart [22]. We examined the effect of trimetazidine in a well-established *ex vivo* model. In C57BL/6J + trimetazidine group, the glucose oxidation increased while fatty acid oxidation decreased compared to vehicle group in the reperfusion period (Fig. 4, $p < 0.05$ *vs.* vehicle). While in AMPK KD + trimetazidine group the glucose oxidation decreased while fatty acid oxidation increased compared to C57Bl/6J + trimetazidine group in the reperfusion period (Fig. 4). The metabolism between AMPK KD + trimetazidine group and AMPK KD vehicle group did not change. The results indicated that trimetazidine can shift metabolism from fatty acid oxidation to glucose oxidation during reperfusion period through modulating AMPK activation. It is interesting to note that at basal period in C57BL/6J + trimetazidine group, trimetazidine increases fatty acid oxidation and decreases glucose oxidation compared to vehicle group (Fig. 4A).

3.4. Trimetazidine Improves Contractile Functions of Cardiomyocytes during Hypoxia

The contractile function of cardiomyocytes isolated from mouse hearts was measured by IonOptix system [17]. Data shown in Fig. 5 indicate that TMZ significantly increased maximal velocity of relengthening (+dL/dt) and maximal velocity of shortening (−dL/dt), peak height and peak shortening (PS) amplitude compared with cardiomyocytes from respective vehicle groups, while TMZ treatment did not affect time-to-90% peak shortening (TPS90). These data indicate that TMZ facilitates the contractility of cardiomyocytes which is an ATP-consuming process.

To study how TMZ treatment induces the activation of AMPK, we measured intracellular ATP levels. Intracellular ATP levels of cardiomyocytes with or without TMZ (10 μmol/L) were measured using luminescence-based technique. The ATP levels were measured with or without exposure of cardiomyocytes to hypoxia/reoxygenation in the presence or absence of TMZ. The ATP levels without TMZ of basal and hypoxia/reoxygenation were 28 ± 2 and 27 ± 3.1 nmol/mg protein, respectively. The ATP levels with TMZ were 13.6 ± 2.1 and 14.6 ± 2.2 nmol/mg protein, respectively. Fig. 5G shows that TMZ treatment induced ATP depletion in cardiomyocytes in both the basal and hypoxia/reoxygenation groups, which indicates an increase of the AMP/ATP ratio.

4. Discussion

In this study for the first time we demonstrate that trimetazidine regulates metabolism during myocardial ischemia/reperfusion injury through regulating AMPK signaling pathway, and trimetazidine can reduce myocardial infarction size through AMPK and ERK signaling pathways. The result that AMPK KD mice or AMPK inhibitor compound C, ERK inhibitor PD98059 abolished the cardioprotective effect of trimetazidine further confirmed the

hypothesis that trimetazidine exerts its protective effect through activating AMPK signaling pathway as well as trimetazidine's protective effect through regulating ERK signaling pathway. Trimetazidine binds to mitochondria, with a high affinity/low density binding site on the outer mitochondrial membrane (Ka 0.96 μmol/L) and low affinity/high density site on the inner membrane (84 μmol/L) [23,24]. The long chain isoform of 3-KAT is also bound to the inner mitochondrial membrane [24,25], however it is still unknown if trimetazidine must bind tightly to 3-KAT in order to inhibit enzyme activity. AMPK switches on catabolic pathways, such as the uptake of glucose and fatty acids, and their metabolism by mitochondrial oxidation and glycolysis [26]. In physiological conditions, AMPK modulates lipid metabolism by phosphorylating and inactivating acetyl-CoA carboxylase (ACC) [27]. ACC is a biotin-dependent enzyme and catalyzes the synthesis of malonyl CoA, which is an essential substrate for fatty acid synthase and a potent inhibitor of carnitine palmitoyl-CoA transferase-I (CPT-I) [28,29]. In summary, AMPK acts as an important regulator of myocardial lipid oxidation by inactivating ACC and reducing malonyl CoA levels, which subsequently increase CPT-I activity and mitochondrial lipid oxidation. Our results indicates that trimetazidine activates p-AMPK and p-ACC at basal period. In the working heart system, at basal period of C57BL/6J + trimetazidine group, trimetazidine increases fatty acid oxidation and decreases glucose oxidation compared to vehicle group, because of the activation of p-ACC. In the ischemic heart, AMPK enhances glucose uptake by mediating the translocation of GLUT4 from storage vesicles to the cell surface [10,28]. AMPK also stimulates glycolysis by directly activating phosphofructo-kinase 2 (PFK-2) through phosphorylation at Ser^{466}, which further increases the production of fructose 2,6-bisphophate, an allosteric activator of PFK-1 in the glycolytic pathway [30]. AMPK activation maintains enhanced glucose uptake during the initial reperfusion stage to improve cardiac contractile functions, as shown by transgenic mice expressing a kinase dead mutation exhibiting impaired glucose uptake and post-ischemic contractile function [10]. After activating AMPK, trimetazidine increases glucose oxidation and decreases fatty acid oxidation probably through enhanced glucose uptake. These results, together with our previous findings, indicate that trimetazidine modulates cardiac glucose metabolism through activation of the AMPK signaling pathway.

Our results also indicate that trimetazidine reduces myocardial infarction size through stimulating p-ERK1/2, which is an important component of mitogen-activated protein kinases (MAPKs) signaling pathway. MAPKs are multifunctional regulators that play an indispensable role in a number of biological processes in heart including cell proliferation, survival, apoptosis, actin reorganization and cytokine production [31]. Primarily there are four MAP kinase subfamilies including extracellular signal-regulated kinases (ERK1/2), c-Jun NH2-terminal kinases (JNK1, JNK2 and JNK3), p38 kinase (α, β, γ, δ), and big MAPK (BMK or ERK5) [32,33]. Activation ERK1/2 signaling has been identified as one of the major components of the RISK (reperfusion injury salvage kinase) pathway [34]. Our results showed that during ischemia/reperfusion period, p42/p44 ERK increased, which is consistent to the research before [34–37]. During ischemia, reactive oxygen species (ROS) was produced in mitochondria and then an extra burst of ROS generation took place at reperfusion. The oxidative stress response occurred, and the imbalance of oxidation/antioxidant system was broken, resulting in myocardial tissue injury. In addition, ROS

activates a variety of inflammatory molecular cascades [38,39]. The activation of ERK1/2 is beneficial to cell survival and have a protective compensatory effect on antioxidant imbalance, whereas p38 MAPK and JNK can promote apoptosis [40,41]. Trimetazidine reduces myocardial infarction size through stimulating the activation of ERK1/2, so as to relieve the injury brought by ROS produced during reperfusion period. However, the detailed mechanism needs to be further explored.

Our results are not consistent with the study of Mahmood Khan et al. [42], which demonstrated that trimetazidine cannot activate ERK1/2 during ischemia/reperfusion period. This could be explained by the different experimental protocols employed. In the aforementioned research, the authors employed a protocol involving 30 min of ischemia and 48 h of reperfusion, and collected hearts after 48 h of reperfusion for immunoblotting analysis, which is drastically different from our protocol of 20 min of ischemia and 15 min of reperfusion. On the other hand, it is possible that the activations of ERK1/2 may have been missed. These differences in experimental design might explain the contrasting results.

Trimetazidine has been in clinical use for more than 20 years. It is an effective treatment for stable angina and a potential drug for treating systolic dysfunction in cardiac failure patients. Both *in vivo* and *in vitro* studies have exhibited that, during ischemia, trimetazidine relieves intracellular acidosis, inhibits sodium and calcium accumulation, maintains intracellular ATP levels, reduces creatine kinase (CK) release, and preserves mitochondrial function [6]. Furthermore, multiple studies reveal that patients treated with TMZ experience fewer episodes of angina and a longer interval before angina onset in exercise tests compared to patients given placebo or other anti-angina drugs [43]. In one particularly impressive study, 50 patients with stable angina were treated with the calcium blocker diltiazem with or without the addition of TMZ. A remarkable 68% of TMZ recipients experienced fewer angina attacks per week compared with baseline, while only 12% of the control group exhibited the same response [44]. This study revealed that trimetazidine, in addition to its above-mentioned therapeutic benefits, could shift fatty acid oxidation to glucose oxidation and attenuate reperfusion-induced injury by its activation of the energy modulator AMPK and the ERK1/2 which is an important component of MAPK signaling pathway. Our future studies will be focused on the more detailed mechanism, such as the relationship between AMPK and MAPK signaling pathway in trimetazidine's protective effect, and the upstream regulator of AMPK and MAPK, etc. In conclusion, the present study highlights the potential clinical significance of administering TMZ before the beginning of reperfusion, which may be valuable for patients undergoing coronary angioplasty or percutaneous coronary interventions.

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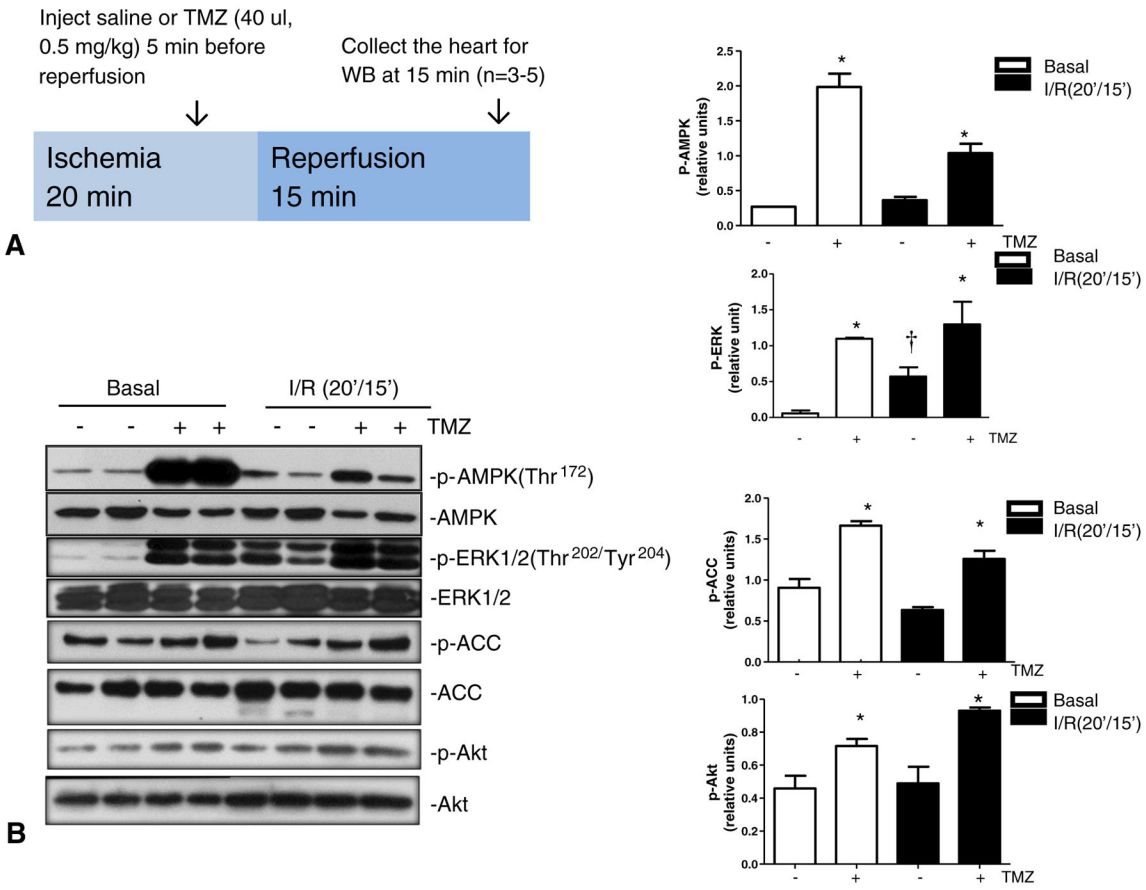

Fig. 1.

The levels of p-AMPK, p-ACC, p-ERK1/2 and p-AKT. The mice were subjected to 20 minutes of ischemia followed by 15 minutes of reperfusion. (A) Trimetazidine or vehicle (saline) was injected intravenously *via* the tail vein injection 5 minutes before reperfusion. (B) The effects of trimetazidine on p-AMPK, p-ACC, p-ERK1/2 and p-AKT at basal and ischemia/reperfusion levels in C57BL/6J mice. *$p < 0.05$ *vs.* corresponding vehicle group, respectively. †$p < 0.05$ *vs.* basal vehicle group.

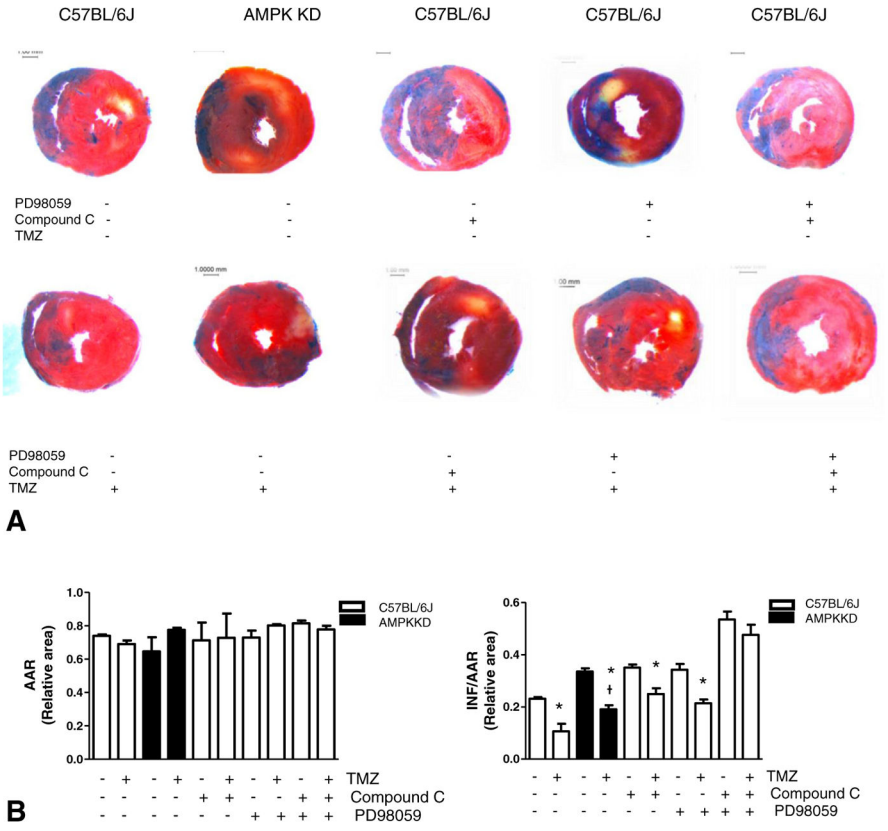

Fig. 2.

The mice were subjected to IP injection with compound C and/or PD98059 an hour before ischemia, followed by 30 min of ischemia and 24 h of reperfusion, trimetazidine (0.5 mg/kg) or vehicle (saline) was injected intravenously *via* the tail vein 5 minutes before reperfusion. (A) Representative sections of myocardial infarction. (B) The ratio of area at risk (AAR) to the myocardial area (left panel) and the ratio of infarct area to AAR (right panel). Values are means ± SEM. *p < 0.05 *vs.* corresponding vehicle group, respectively. †p < 0.05 *vs.* WT + trimetazidine group.

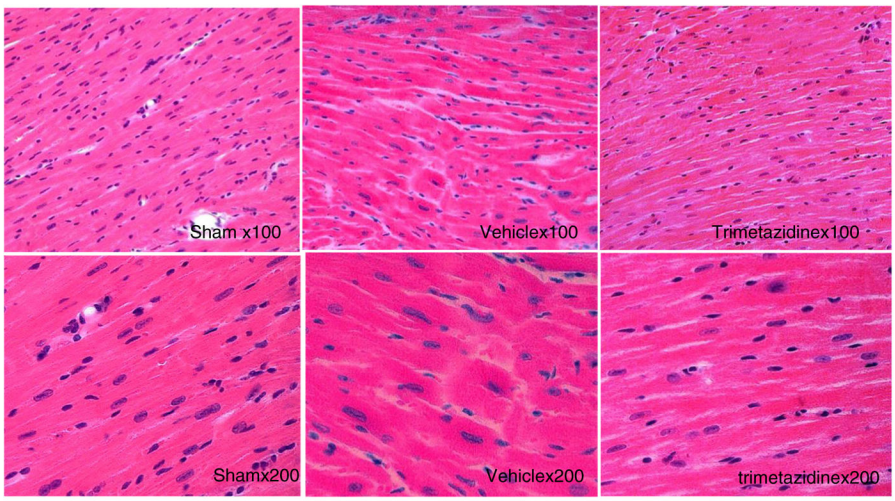

Fig. 3.

Histopathologic changes in ischemia/reperfusion (I/R)-injured hearts. Left ventricular sections from representative mice are shown. Vehicle refers to ischemia 20 min and reperfusion 4 h, trimetazidine refers to I/R (20 min/4 h) + trimetazidine (0.5 mg/kg) treatment. The sections are stained with hematoxylin and eosin, demonstrating interstitial hypercellularity and apoptosis.

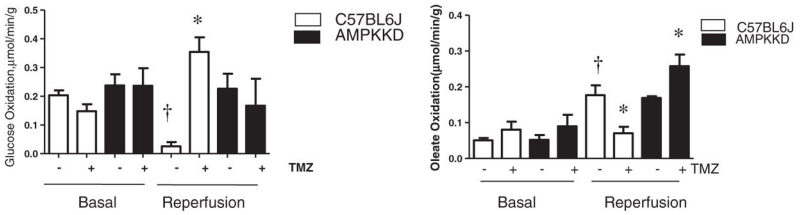

Fig. 4.

AMPK-dependent trimetazidine's shift metabolism from fatty acid oxidation to glucose oxidation. Values are means ± SEM for 4 dependent experiments. *$p < 0.05$ *vs.* corresponding vehicle group. †$p < 0.05$ *vs.* C57BL/6J + trimetazidine.

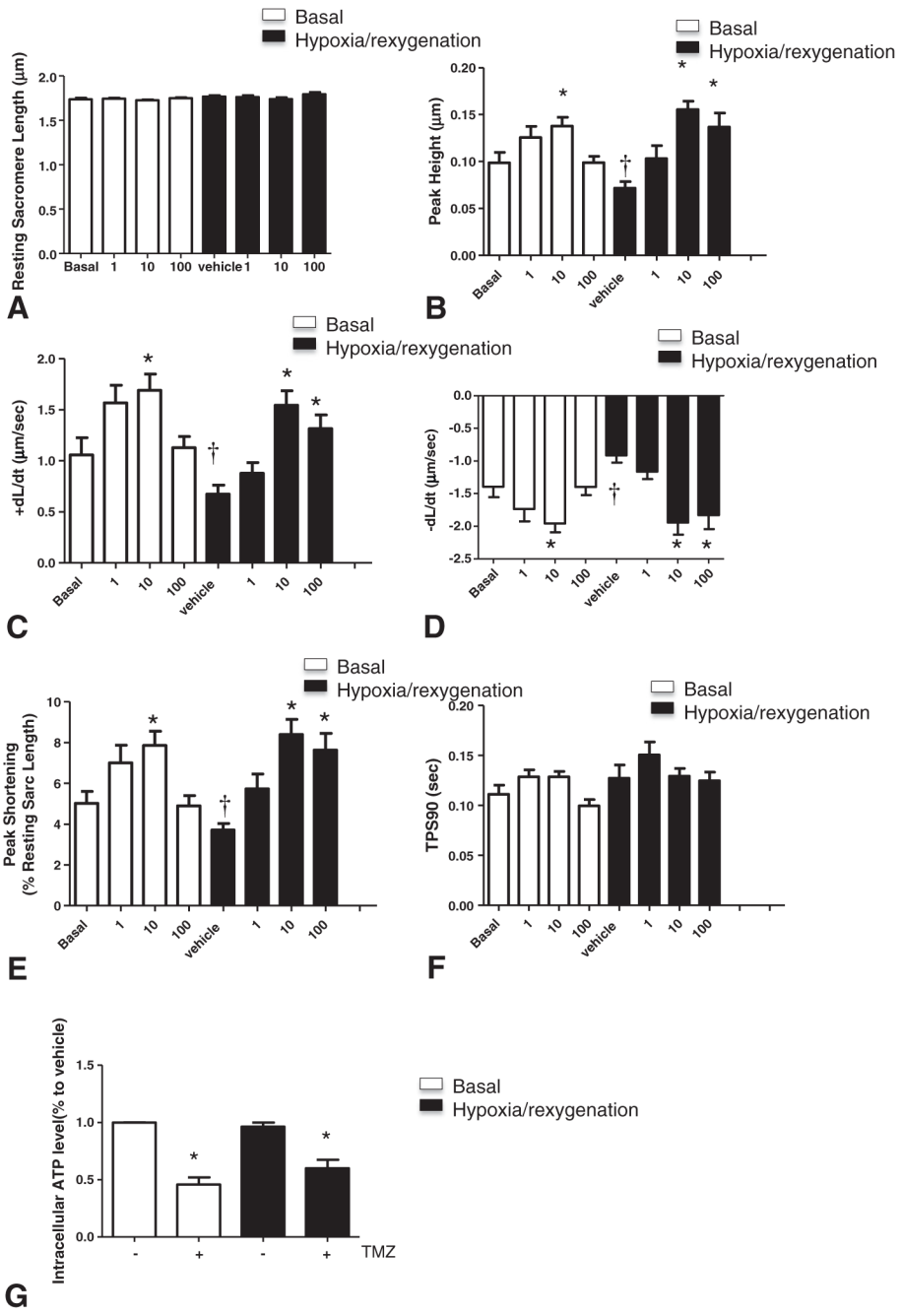

Fig. 5.

Contractile properties of cardiomyocytes with or without TMZ treatment. Contractile properties of cardiomyocytes isolated from WT mice with or without TMZ treatment. TMZ facilitates the contractile function of cardiomyocytes both at basal and hypoxia/reoxygenation levels. (A) Resting sarcomere length; (B) peak height; (C) maximal velocity of shortening (−dL/dt); (D) maximal velocity of re-lengthening (+dL/dt); (E) peak shortening (normalized to the resting sarcomere length); (F) time-to-90% peak shortening (TPS90) (G) the intracellular ATP change at basal at hypoxia/reoxygenation levels. After

TMZ treatment, intracellular ATP level is decreased both at basal and hypoxia/reoxygenation levels. Means ± SEM, n = 30 cells from 3 mice per group,*$p < 0.05$ *vs.* vehicle group, respectively. †$p < 0.05$ *vs.* basal group.