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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 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.

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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.

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