Journal of Molecular and Cellular Cardiology 101 (2016) 90–98

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Keto acid metabolites of branched-chain amino acids inhibit oxidative stress-induced necrosis and attenuate myocardial ischemia–reperfusion injury

Weibing Dong [a,1], Meiyi Zhou [a,1], Mei Dong [b,1], Bangfen Pan [a], Yunxia Liu [a], Jing Shao [a], Xiaoping Gu [a], Ying Huang [a], Guangping Li [b], Yibin Wang [a,c], Haipeng Sun [a,c,*]

[a] Key Laboratory of Cell Differentiation and Apoptosis of Ministry of Education, Department of Pathophysiology, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
[b] Tianjin Key Laboratory of Ionic-Molecular Function of Cardiovascular Disease, Department of Cardiology, Tianjin Institute of Cardiology, Second Hospital of Tianjin Medical University, Tianjin 300211, China
[c] Division of Molecular Medicine, Departments of Anesthesiology, Medicine and Physiology, Molecular Biology Institute, Cardiovascular Research Laboratories, David Geffen School of Medicine at University of California, Los Angeles, CA 90095, USA

ARTICLE INFO

Article history:
Received 21 October 2016
Received in revised form 1 November 2016
Accepted 3 November 2016
Available online 8 November 2016

Keywords:
Branched-chain amino acids
Branched-chain keto acids
Necrosis
Cell death
Mitochondria
Myocardial infarction

ABSTRACT

Branched chain α-keto acids (BCKAs) are endogenous metabolites of branched-chain amino acids (BCAAs). BCAA and BCKA are significantly elevated in pathologically stressed heart and contribute to chronic pathological remodeling and dysfunction. However, their direct impact on acute cardiac injury is unknown. Here, we demonstrated that elevated BCKAs significantly attenuated ischemia–reperfusion (I/R) injury and preserved post I/R function in isolated mouse hearts. BCKAs protected cardiomyocytes from oxidative stress-induced cell death in vitro. Mechanistically, BCKA protected oxidative stress induced cell death by inhibiting necrosis without affecting apoptosis or autophagy. Furthermore, BCKAs, but not BCAAs, protected mitochondria and energy production from oxidative injury. Finally, administration of BCKAs during reperfusion was sufficient to significantly attenuate cardiac I/R injury. These findings uncover an unexpected role of BCAA metabolites in cardioprotection against acute ischemia/reperfusion injury, and demonstrate the potential use of BCKA treatment to preserve ischemic tissue during reperfusion.

1. Introduction

Branched chain amino acids (BCAA), including leucine, isoleucine and valine, are essential amino acids which can be degraded via shared catabolic pathway. BCAA degradation yields branched chain α-keto acids (BCKAs), including α-ketoisocaproate (KIC), α-keto-β-methylvalerate (KMV), and α-ketoisovalerate (KIV) [1]. BCAA catabolic defects and elevated tissue levels of BCKA or BCAA have been reported in pathologically stressed mouse hearts or human cardiomyopathy [2–4]. Furthermore, abnormal plasma BCAA/BCKA is a common feature in diabetes and insulin resistance [1]. BCKAs inhibit mitochondrial respiration and energy metabolism in neuronal cells [5]. In heart muscle cells, BCKAs inhibit Complex I activity in mitochondria and induce superoxide production [2]. BCAA catabolic defects significantly contribute to heart failure and myocardial remodeling following chronic pressure-overload or myocardial infarction [2,3]. However, a direct effect of BCAA/BCKA on acute cardiac injury is unknown.

Prolonged ischemia followed by reperfusion (I/R) leads to loss of cardiac muscle cell viability and functional recovery [6,7]. Oxidative stress due to elevated reactive oxygen species (ROS) such as superoxide and hydrogen peroxide (H_2O_2) is a major contributor to I/R injury and myocyte death [8]. Apoptosis and necrosis are two fundamental types of cell death. Unlike apoptosis, necrosis was previously considered as an uncontrolled and energy independent cell death process characterized by early plasma membrane rupture and swelling of organelles [9]. However, recent studies have revealed that the necrotic process is also mediated through highly regulated process, referred to as programmed necrosis or necropotosis [10]. Pro-death stimuli trigger a series of cellular events involving protein kinases, ATP depletion, and proteolysis, to induced regulated necrosis [11]. In particular, tumor necrosis factor-α (TNF-α) induced Receptor-Interacting Serine/threonine Kinase (RIPK) pathway has been extensively studied and shown to be important for necrotic cell death [12]. It is well reported that high level of H_2O_2 induces necrosis [13–17]. Preventing or reducing myocyte

* Corresponding author at: Key Laboratory of Cell Differentiation and Apoptosis of Ministry of Education, Department of Pathophysiology, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China.
E-mail address: sun.haipeng@yahoo.com (H. Sun).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.yjmcc.2016.11.002
0022-2828/

necrosis death is a potentially effective therapeutic strategy to reduce heart attack injury as well as other ischemic/reperfusion induced organ injuries [18].

In the present study, we investigate the direct effect of BCKAs on oxidative stress induced myocyte death and acute I/R injury in heart. Opposite from our expectation based on previously observed detrimental effect of BCKA in cardiac remodeling and dysfunction, we observed a significant cardio-protective effect of BCKA administration against acute oxidative stress and I/R injury. Mechanistically, we demonstrate that BCKA treatment protected cells from H_2O_2 induced necrosis and mitochondrial damage. Our study uncovered an unexpected cardiac protective property of BCAA downstream metabolites and revealed a potential novel class of reagents as potential treatment for myocardial infarction.

2. Results

2.1. BCKAs protect hearts from I/R injury

To establish the direct effect of BCKAs on acute I/R injury, we performed studies in the Langendorff murine hearts following 35 min no-flow ischemia and 120 min reperfusion protocol. In addition to saline control, KIC, the branched-chain keto acid from leucine, was perfused throughout the experiment. Pretreatment of KIC significantly attenuated the infarct size after 120 min reperfusion (Fig. 1A and B). Meanwhile, comparing to the Control group of ~32% recovery of Left Ventricle Developed Pressure (LVDP) after 60 min reperfusion, KIC group showed a significant higher functional recovery of ~51% (Fig. 1C). The recovery of left ventricular systolic function (dP/dtmax) and diastolic function (dP/dtmin) during reperfusion was also substantially improved by KIC treatment (Fig. 1D and E). These data clearly demonstrate an unanticipated protective effect of BCKAs against myocardial I/R injury.

2.2. BCKAs protect cardiomyocytes against oxidative stress-induced death

Cardiomyocyte is the functional cell type in myocardium. Oxidative stress is one major contributor of cell death during I/R [8]. We found that H_2O_2 treatment at a dose of 20 μM induced rapid cell death in primary Neonatal Rat Ventricular Myocytes (NRVM), based on MTT activity, morphological alterations of rounding up and detachment. Treatment of BCKAs significantly reduced H_2O_2-induced NRVM death (Fig. 2A and B). Meanwhile, among the three BCKA species, KIC demonstrated the strongest protective activity against NRVM death (Fig. 2B). In addition, BCKA-dependent protection against H_2O_2-induced cell death was also dosage dependent (Fig. 2C). In addition to oxidative stress, calcium overload has also been demonstrated as one major contributor for cell death during I/R injury [19]. However, BCKAs failed to protect the calcium overloaded-induced cell death triggered by ionomycin (Fig. 2D). Thus BCKAs possess specific cyto-protective activity against oxidative stress- but not calcium overload-induced cell death of cardiomyocytes.

2.3. BCKAs protect different types of cells and inhibit mitochondrial respiration

We further tested if the protective effect of BCKAs is cardiomyocyte specific or applicable to other cell types. H_2O_2 treatment at a dose of 500 μM induced high level of cell death in mouse embryonic fibroblast (MEF) cells (Fig. 3A). As shown in Fig. 3B, either a 30-minute pre-treatment of BCKAs prior to H_2O_2 administration (0.5 h) or concurrent treatment of BCKA and H_2O_2 (0 h in Fig. 3B) showed significant protective effect in MEFs. Furthermore, adding BCKAs 30 min or 1 h after the initiation of H_2O_2 treatment still induced similar level of protective effects. In contrast, starting treatment of BCKAs 2 h after initial H_2O_2 treatment or later, the BCKA mediated protective effect diminished. BCKAs also protected Hela cells from oxidative stress induced cell death (Fig. S1). In addition, BCKAs failed to protect MEF against the Death Receptor-induced necrotic cell death (Fig. 3C). Similar to their inhibitory effect on isolated mitochondria [2], BCKAs suppressed the cellular respiration in cultured MEF (Fig. 3D). These data suggest that BCKA mediated cyto-protection against oxidative stress is a conserved effect across different cell lines and species associated with mitochondrial inhibition, although subtle differences do exist in terms of relative strengths of the protection by each of the BCKA species and in different cellular contents.

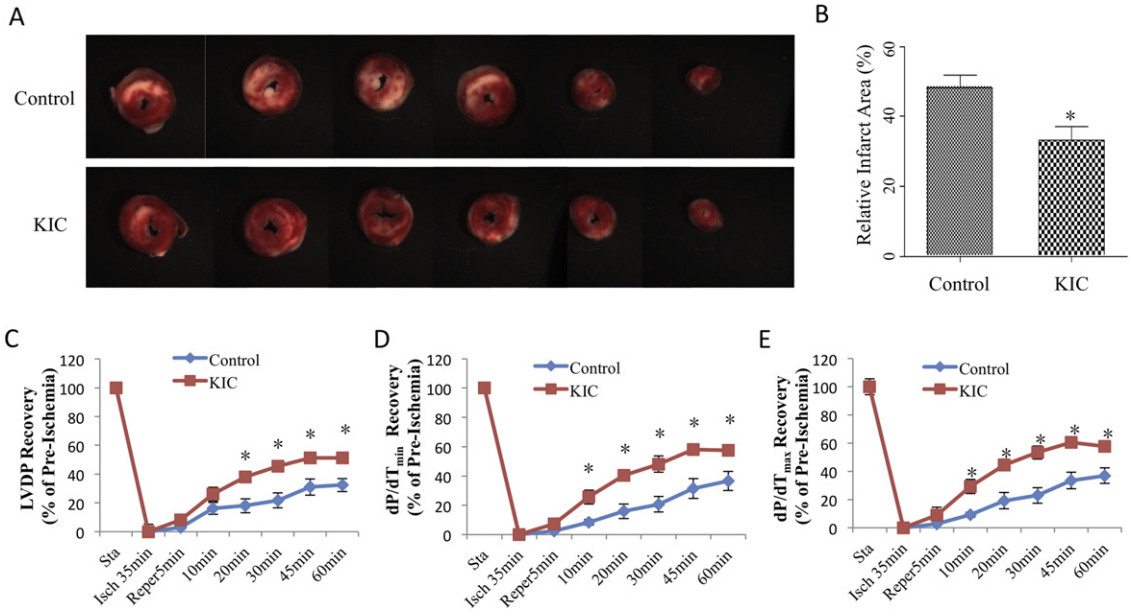

Fig. 1. BCKAs protected heart from ischemia/reperfusion injury. A. Representative images of cross-sections of TTC stained ischemic hearts with or without pretreatment of KIC. B, relative infarct size expressed as a percentage of the total ventricular area was calculated from control group (without KIC treatment) ($n = 11$) and KIC-pretreatment ($n = 15$) groups. C–E, time courses of functional recovery (percentage of baseline) of ischemic hearts with ($n = 14$) or without ($n = 11$) KIC pre-treatment. Pre-treatment was performed by including KIC in both perfusion and reperfusion buffer. LVDP, left ventricular developed pressure. *, $p < 0.05$.