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Treatment with D-β-hydroxybutyrate protects heart from ischemia/reperfusion injury in mice

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ABSTRACT

The present study was designed to examine the protection of D-β-hydroxybutyrate (BHB) against ischemia/reperfusion (I/R) injury in heart and investigate its underlying mechanism. Male adult mice were exposed to 30 min of ischemia and 24 h of reperfusion. Osmotic pumps were implanted subcutaneously 5 min before reperfusion for continuous delivery of the exogenous BHB (1.6 mmol/kg/24 h). Treatment with BHB reduced infarct size and levels of cardiac troponin I, creatine kinase and lactate dehydrogenase in serum, attenuated apoptosis in myocardium, and preserved cardiac function of I/R mice. Importantly, treatment of I/R mice with BHB promoted autophagic flux, evidenced by reduced the ratio of LC3-II/LC3-I and protein expression of p62 and enhanced protein expression of lysosome associated membrane protein-2 (Lamp2) in myocardium. Treatment of I/R mice with BHB reduced mitochondrial formation of reactive oxygen species, enhanced adenosine triphosphate production, attenuated mitochondrial swelling, and partly restored mitochondrial membrane potential in myocardium. Furthermore, treatment of I/R mice with BHB abated oxidative stress and attenuated endoplasmic reticulum stress in myocardium. Our results indicated that treatment with exogenous BHB protected heart from I/R injury in mice.

1. Introduction

Ischemic heart disease, resulting from the shortage of nutrients and oxygen supply, is one of the leading causes of mortality and morbidity especially in the industrialized societies (Ferdinandy et al., 2014). It is reasonable to consider that the rapid and early reperfusion therapy, restoring blood flow to the ischemic regions, is a standard strategy to reduce infarct size and improve clinical outcome. Paradoxically, reperfusion leads to further irreversible myocardial cell death, which is known as lethal myocardial reperfusion injury (Yellon and Hausenloy, 2007), which accounts for up to 50% of the final infarct size in animal studies (Yellon and Hausenloy, 2007; Hausenloy and Yellon, 2013; Fröhlich et al., 2013). However, as yet no standard therapy exists, and it is of great clinical importance to develop new protective modalities to mitigate ischemia/reperfusion (I/R) injury in heart.

It was reported that short-term fasting (Snorek et al., 2012) or intermittent fasting (Godar et al., 2015) reduced the extent of myocardial infarction in animals exposed to I/R. Ketone body β-hydroxybutyrate, produced by degradation of fatty acids in liver and then transported to extrahepatic tissues (including the brain, heart and skeletal muscle), is one of the important metabolic substrates for energy production during fasting (Cotter et al., 2013). Recent findings suggest that β-hydroxybutyrate is not solely a metabolic intermediate, but also possesses a variety of signaling functions (Rojas-Morales et al., 2016). Treatment of ischemic brain with D-β-hydroxybutyrate (BHB) protected against cerebral hypoxia, anoxia and ischemia-induced metabolic change (Suzuki et al., 2001; Rahman et al., 2014). In addition, circulating ketone bodies have been previously reported to be increased in patients with congestive heart failure (Lommi et al., 1996). Pretreatment of BHB protected the heart from I/R injury (Zou et al., 2002). However it is unknown whether it has cardioprotection when given at the onset of reperfusion (postconditioning), a protocol with more clinical impact. In this work, we proposed that treatment with BHB during reperfusion protects heart from I/R injury and investigated the underlying mechanism.

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Table 1
Sequences of oligonucleotides used as primers.

Target gene		Sequence
GRP78	Sense	5′-TCATCGGACGCACTTGGAA-3′
	Antisense	5′-CAACCACCTTGAATGGCAAGA-3′
XBP- 1	Sense	5′-CCTGAGCCCGGAGGAGAA-3′
	Antisense	5′-CTCG AGCAGTCTGCGCTG-3′
CHOP	Sense	5′-GCATGAAGGAGAAGGAGCAG-3′
	Antisense	5′-CTTCCGGAGAGACAGACAGG-3′
GAPDH	Sense	5′-GCAAGGACACTGAGCAAGAG-3′
	Antisense	5′-GGGTCTGGGATGGAAATTGT-3′

CHOP, CCAAT enhancer binding protein homologous protein; GRP78, glucose regulated protein 78; XBP-1, X-box protein 1.

2. Materials and methods

2.1. Mouse model of I/R and treatment

All mice were maintained according to the Animals (Scientific Procedures) Act, 1986 of the UK Parliament, Directive 2010/63/EU of the European Parliament and 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). Animal studies are reported in compliance with the ARRIVE guidelines (Kilkenny et al., 2010; McGrath and Lilley, 2015).

10- to 12-week old C57BL/6J mice (male, Sino-British SIPPR/BK Lab Animal Ltd, Shanghai, China) were anesthetized with inhaled isoflurane, and positive pressure ventilation was provided with a constant-volume ventilator (mouse ventilator, Servo 900 C, Siemens, Germany). After a left lateral thoracotomy, an 8–0 prolene suture was passed under

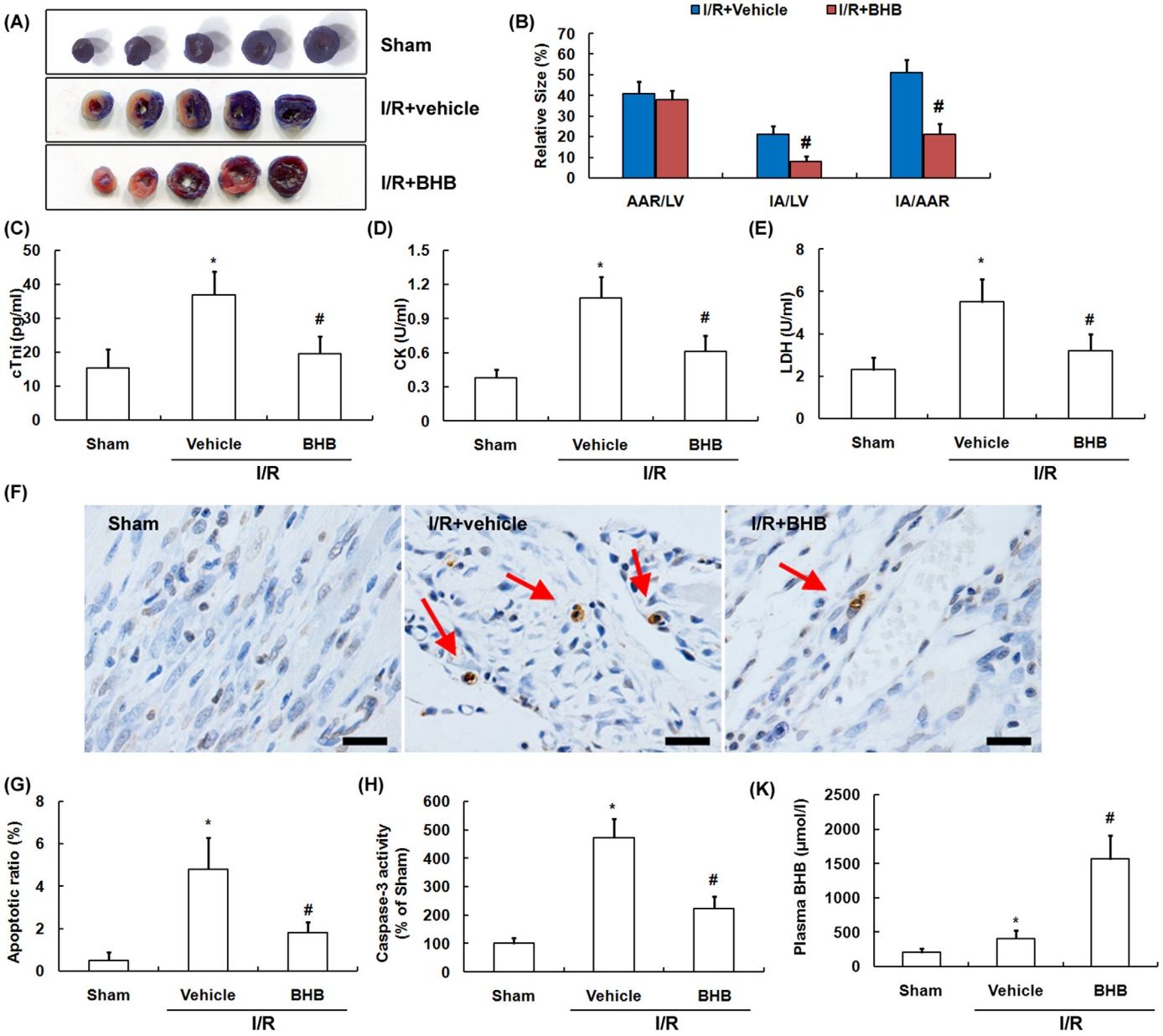

Fig. 1. BHB treatment attenuated myocardial I/R injury in mice. 24 h after reperfusion, infarct size (a, b), and serum levels of cTni (c), CK (d) and LDH (e) were measured. TUNEL (f, g, scale bar = 20 μm) staining was performed and activities of caspase-3 (h) in myocardium was measured to evaluated apoptosis. 24 h after reperfusion, plasma levels of BHB (k) were determined. BHB, D-β-hydroxybutyrate; I/R, ischemia/reperfusion; cTni, cardiac troponin I; CK, creatine kinase; LDH, lactate dehydrogenase; Values are means ± S.D.; n = 9 in each group; *P < 0.05 versus Sham-operated mice; # P < 0.05 versus I/R mice treated with vehicle.