Life Sciences 137 (2015) 20–27

Contents lists available at ScienceDirect

Life Sciences

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

Branched-chain amino acids ameliorate heart failure with cardiac cachexia in rats

Yohei Tanada [a], Tetsuo Shioi [a,*], Takao Kato [b], Akira Kawamoto [a], Junji Okuda [a], Takeshi Kimura [a]

[a] Department of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan
[b] Cardiovascular Center, Tazuke Kofukai Medical Research Institute, Kitano Hospital, 2-4-20 Ohgimachi, Kita-ku, Osaka 530-8480, Japan

ARTICLE INFO

Article history:
Received 13 January 2015
Received in revised form 1 June 2015
Accepted 27 June 2015
Available online 2 July 2015

Keywords:
Heart failure
Cachexia
Branched-chain amino acids

ABSTRACT

Aims: Heart failure (HF) is associated with changes in energy metabolism of the heart, as well as in extra-cardiac organs such as the skeletal muscles. Cardiac cachexia is a common complication and is associated with poor prognosis. Branched-chain amino acids (BCAAs) reportedly improve sarcopenia and cancer cachexia. We tested the hypothesis that BCAA ameliorates HF with cardiac cachexia.

Main methods: We used Dahl salt-sensitive (DS) rats fed a high-salt diet as a model of HF. DS rats fed a low-salt diet were used as a control. BCAA were administered in drinking water from 11 weeks of age, when cardiac hypertrophy was established but the cardiac function was preserved. Survival and the cardiac function were monitored, and animals were sacrificed at 21 weeks of age and analyzed.

Key findings: In HF rats, BCAA treatment decreased the heart rate, preserved the cardiac function, and prolonged survival. BCAA also prevented body weight loss, associated with preservation of the skeletal muscle weight. Moreover, gene expression related to mitochondrial biogenesis and function was increased with BCAA in skeletal muscles.

Significance: BCAA preserved the body weight and cardiac function and prolonged survival in HF rats. The expression of genes involved in mitochondrial biogenesis and function in skeletal muscles was increased by BCAA.

1. Introduction

Although the prognosis of patients with heart failure (HF) has been improved, the mortality rate of such patients remains at almost 50% within 5 years of the diagnosis [1]. HF is associated with a significant change in energy metabolism of the heart. This change has been hypothesized to be important in the progression of HF [2].

HF is also associated with a change in systemic energy metabolism, such as insulin resistance [3] and cachexia [4]. Cardiac cachexia is a common complication in HF, and patients with cardiac cachexia show poor prognosis and disability. Several lines of evidence suggest that immune and neurohormonal abnormalities play a critical role in the wasting process and that the abnormal metabolic balance between catabolism and anabolism is associated with the development of cachexia [5]. Skeletal muscle is thought to be important in the development of cachexia [6]. A negative energy balance in skeletal muscle has been shown in an experimental model of HF [7,8]. In cardiac cachexia, wasting and weakness of skeletal muscle are observed and these changes are distinctly different from those of muscle atrophy due to reduced activity [9].

Recent studies showed that branched-chain amino acids (BCAAs) extend the chronological lifespan of *Saccharomyces cerevisiae* [10] and wild-type mice [11]. BCAAs consist of leucine, isoleucine, and valine. These amino acids are known to play a number of roles, such as those in muscle protein synthesis, insulin secretion, and energy production through their catabolism [12]. BCAA improves fiber atrophy of the skeletal muscle due to age-induced sarcopenia in rats [13], and prevent the loss of skeletal muscle weight associated with cancer cachexia in mice [14].

BCAAs improve the cardiac function in global ischemia of isolated rat hearts [15]. Patients with coronary artery disease have a negative protein balance and BCAAs exhibit an anabolic effect on myocardial protein metabolism [16]. Leucine attenuates myocardial infarction in mice [17]. These results show that BCAAs protect the heart from myocardial ischemic injury.

However, there has been no study evaluating the effect of BCAA on HF with cardiac cachexia. The Dahl rat model of HF shows progressive deterioration of the cardiac function [18]. We previously reported that there is a distinct change in the metabolic profile of the heart during the development of HF [19]. In addition, this model shows body weight loss associated with an increase in the level of proinflammatory cytokines, and can be deemed a model of cardiac cachexia [20]. Therefore,

* Corresponding author.
E-mail address: tshioi@kuhp.kyoto-u.ac.jp (T. Shioi).

http://dx.doi.org/10.1016/j.lfs.2015.06.021
0024-3205/

we tested the hypothesis that BCAAs ameliorate HF with cardiac cachexia using the Dahl rat model of HF.

2. Materials and methods

2.1. Animals

Inbred male Dahl salt-sensitive (DS) rats (Japan SLC, Hamamatsu, Shizuoka, Japan) were fed a 0.3% NaCl (low salt: LS) diet until the age of 6 weeks, after which they were fed an 8% NaCl diet (high salt: HS) [18]. Animal care and experiments were approved by the Institutional Animal Care and Use Committee of Kyoto University and conducted following the Guide for Care and Use of Laboratory Animals published by the United States National Institutes of Health.

2.2. Protocols

2.2.1. Experiment 1

At 11 weeks of age, the rats fed the LS or HS diet were randomly sorted into four groups to receive tap water or a branched-chain amino acid (BCAA) mixture at a dose of 1.5 mg/g body weight/day in drinking water. The rats were divided into four groups: the rats fed the LS diet and tap water (LS-C, n = 6), LS diet and BCAA (LS-BCAA, n = 6), HS diet and tap water (HS-C, n = 10), and HS diet and BCAA (HS-BCAA, n = 10). Serial measurements of the heart rate and blood pressure were performed before, 24 h after, and 48 h after BCAA supplementation. The heart rate and blood pressure are determined by the tail-cuff method using a noninvasive automated blood pressure apparatus (Softron BP-98A, Softron Co. Ltd. Tokyo, Japan) without anesthesia.

2.2.2. Experiment 2

At 11 weeks of age, the rats fed the LS or HS diet were randomly sorted into four groups to receive tap water or a branched-chain amino acid (BCAA) mixture at a dose of 1.5 mg/g body weight/day in drinking water. The survival of animals was compared among the rats fed the LS diet and tap water (LS-C, n = 8), LS diet and BCAA (LS-BCAA, n = 8), HS diet and tap water (HS-C, n = 30), and HS diet and BCAA (HS-BCAA, n = 30). Serial measurements of food intake, water intake, body weight, heart rate, and blood pressure were performed every 2 weeks from the age of 11 weeks until they were sacrificed at 21 weeks of age. The heart rate and blood pressure were determined by the tail-cuff method using a noninvasive automated blood pressure apparatus (Softron BP-98A, Softron Co. Ltd. Tokyo, Japan) without anesthesia.

The amino acid contents of the LS and HS diets are listed in Supplementary Table 1, and the content of BCAA mixture in drinking water was determined in a previous report [11] and is listed in Supplementary Table 2.

2.3. Cardiac echocardiography

Transthoracic echocardiography was performed as previously reported [18]. Rats were anesthetized briefly with inhaled diethyl ether (Wako Pure Chemical Industries, Osaka, Japan), and transthoracic echocardiography was performed using a Sonos-5500 echocardiograph (Agilent Technologies, Santa Clara, CA, USA) with a 15-MHz linear transducer every 2 weeks from the age of 11 weeks until being sacrificed. The heart rate (HR), intraventricular septal thickness (IVSd), left ventricular dimension in the diastolic phase (LVDd), and left ventricular dimension in the systolic phase (LVDs) were measured with M-mode echocardiography, and fractional shortening (FS) and the ejection fraction (EF) were calculated with Teichholz formula.

2.4. Tissue sampling

The 21-week-old LS-C group (n = 8), 21-week-old LS-BCAA group (n = 8), 21-week-old HS-C group (n = 11), and 21-week-old HS-BCAA group (n = 17) were sacrificed by decapitation without fasting. The heart, left gastrocnemius muscle, both kidneys, and both lungs were rapidly removed, and their weights were measured. The heart and gastrocnemius muscle were snap frozen in liquid nitrogen and stored at −80 °C, or fixed with 4% paraformaldehyde (PFA).

2.5. Quantitative reverse transcription-polymerase chain reaction

Total RNA was isolated from the heart tissue in each group by the acid guanidinium thiocyanate–phenol–chloroform method. Quantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed as in previous reports [21]. The oligonucleotide primers are listed in Supplementary Table 3. The mRNA level of each gene was standardized with the expression level of 18S ribosomal RNA as a control and calculated with the 2-DeltaCt method.

2.6. Fibrosis of myocardium

Hearts were fixed in 4% PFA, embedded in paraffin, and sectioned for histological evaluation. The fibrotic area was quantified in tissue sections with Sirius Red staining, as previously described [22].

2.7. Apoptosis of myocardium

Apoptosis was assessed with the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) method (Tanaka Bio Inc., Shiga, Japan), as previously reported [19].

2.8. Western blotting

The lysate of heart tissue and gastrocnemius muscle was extracted by homogenization in an ice-cold buffer (10% glycerol, 137 mM NaCl, 20 mM Tris–HCl pH 7.4, 4 g/mL leupeptin, 1 mM phenylmethylsulfonyl fluoride (PMSF), 4 g/mL pepstatin, 20 mM NaF, 1 mM sodium pyrophosphate, and 1 mM orthovanadate). The lysate was put on ice for 15 min and centrifuged at 15,000 *g* for 15 min at 4 °C. Then, 80 μg of the lysate was electrophoresed in the gel (Hybrid Gel, Kishida, Osaka, Japan) by the Laemmli method. The primary antibodies used for Western blotting were as follows: mTOR (1:1000, Cell Signaling, Danvers, MA, USA), phospho-mTOR (1:1000, Cell Signaling), p70S6K (1:200, Santa Cruz, Dallas, TX, USA), phospho-p70S6K (1:1000, Cell Signaling), and GAPDH (1:1000, Cell Signaling).

2.9. Measuring thiobarbituric acid reactive substances (TBARS)

TBARS levels in left ventricular tissue were measured according to the manufacturer's instructions (Alexis Biochemicals, Lausen, Switzerland).

2.10. Statistical analysis

Values are expressed as mean ± SEM. The survival of animals was analyzed using the Kaplan–Meier method with a Wilcoxon test. ANOVA was used for comparisons between multiple groups. In all tests, a value of $p < 0.05$ was considered significant.

3. Results

3.1. Short-term effect of BCAA on hemodynamic parameters in DS rats

In experiment 1, after 48 h of BCAA supplementation, the HS-BCAA group showed a significantly lower heart rate compared to that of the HS-C group (Fig. 1). No significant change in the heart rate or systolic blood pressure was noted between the LS-C and LS-BCAA groups (Fig. 1).