ARTICLE IN PRESS

Biochemical and Biophysical Research Communications xxx (2017) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Resveratrol attenuates skeletal muscle atrophy induced by chronic kidney disease via MuRF1 signaling pathway

Li-Jing Sun, M.D. [a], Yan-Ni Sun, M.D. [b], Shun-Jie Chen, M.D. [a], Shuang Liu, M.S. [a], Geng-Ru Jiang, M.D. [a, *]

[a] Department of Nephrology, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai 200092, China
[b] Department of Emergency, Putuo Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200062, China

ARTICLE INFO

Article history:
Received 31 March 2017
Accepted 5 April 2017
Available online xxx

Keywords:
Resveratrol
Chronic kidney disease
Muscle atrophy
MuRF1
Signaling pathway

ABSTRACT

Skeletal muscle atrophy is an important clinical characteristic of chronic kidney disease (CKD); however, at present, the therapeutic approaches to muscle atrophy induced by CKD are still at an early stage of development. Resveratrol is used to attenuate muscle atrophy in other experimental models, but the effects on a CKD model are largely unknown. Here, we showed that resveratrol prevented an increase in MuRF1 expression and attenuated muscle atrophy in vivo model of CKD. We also found that phosphorylation of NF-κB was inhibited at the same time. Dexamethasone-induced MuRF1 upregulation was significantly attenuated in C2C12 myotubes by resveratrol in vitro, but this effect on C2C12 myotubes was abrogated by a knockdown of NF-κB, suggesting that the beneficial effect of resveratrol was NF-κB dependent. Our findings provide novel information about the ability of resveratrol to prevent or treat muscle atrophy induced by CKD.

© 2017 Elsevier Inc. All rights reserved.

1. Introduction

Muscle wasting is a common clinical characteristic of patients with chronic kidney disease (CKD) and increases morbidity and the risk of death [1]. Skeletal muscle atrophy is an effective indicator of muscle wasting, which is defined as a decrease in the mass of skeletal muscle. Molecular pathways underlying muscle atrophy are complicated, and many studies have shown that multiple pathways lead to skeletal muscle atrophy in patients with CKD [2]. Muscle ring-finger 1 (MuRF1) consists of 353 amino acid residues and contains a canonical N-terminal RING domain, which has been identified as a key muscle-specific E3 ubiquitin ligase that is highly expressed during muscle atrophy in CKD and increases muscle proteolysis by the ubiquitin-proteasome system (UPS) [3-4]. The expression of MuRF1 is regulated by NF-κB during muscle atrophy, as revealed in MuRF1$^{-/-}$ mice: a significant reduction in muscle loss in these mice revealed that transcriptional activation of MuRF1 by NF-κB is a key step in NF-κB-induced muscle atrophy [5].

Current strategies aimed at preventing muscle atrophy in clinical practice are focused on correction of acidosis plus promotion of physical exercise among patients with CKD, whereas in CKD mice with muscle atrophy, low-frequency electrical stimulation has been found to mimic acupuncture or exercise, both of which significantly improve weights of the soleus and extensor digitorum longus (EDL) muscles [6]. Currently, therapeutic approaches to muscle atrophy in CKD are still at early stages of development [7]. Specific therapies that inhibit a relevant signal transducer need to be developed to reduce the skeletal muscle atrophy induced by CKD.

Resveratrol (3,5,4′-trihydroxystilbene) is a natural polyphenol present in peanuts, pines, the skin of grapes, and red wine [8]. It has been shown to have many beneficial biological effects, including cardioprotection, antioxidant effects, inhibition of NF-κB activity, and activation of AMP-activated protein kinase (AMPK) [9-11]. In addition, resveratrol has been reported to stimulate activity of histone deacetylase SIRT1; this effect may represent a key mechanism of action of this drug [12]. Growing evidence indicates that resveratrol may have beneficial effects in various muscle atrophic conditions, such as diabetes, cancer cachexia, and Duchenne muscular dystrophy; it can attenuate skeletal muscle atrophy by multiple mechanisms [13-15].

* Corresponding author. Department of Nephrology, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, No. 1665 Kongjiang Road, Shanghai 200092, China.
E-mail address: xinhuakidney@163.com (G.-R. Jiang).

http://dx.doi.org/10.1016/j.bbrc.2017.04.022
0006-291X/© 2017 Elsevier Inc. All rights reserved.

Please cite this article in press as: L.-J. Sun, et al., Resveratrol attenuates skeletal muscle atrophy induced by chronic kidney disease via MuRF1 signaling pathway, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.04.022

A recent study showed that resveratrol may be a promising therapy for CKD patients [16], although studies evaluating its effects in CKD are scarce. Some clinical studies based on polyphenol-containing supplementation have shown anti-inflammatory effects and improvements in antioxidant activity in patients with CKD or end stage renal disease (ESRD) [17-18]. During basic research on CKD, Liang et al. found that resveratrol treatment can inhibit oxidative stress and renal interstitial fibrosis [19]. Until recently, however, no study has provided comprehensive insights into the effects of resveratrol on CKD-induced skeletal muscle atrophy although it is plausible that resveratrol can provide several benefits that may attenuate muscle atrophy. The protective effect of resveratrol against skeletal muscle atrophy during CKD and the effect on NF-κB activation are not well understood. In the present study, we tested the hypothesis that resveratrol prevents CKD-induced muscle atrophy by inhibiting the expression of MuRF1 and protein degradation, and we determined whether the protective effects of resveratrol are NF-κB dependent.

2. Materials and methods

2.1. Mouse strains and the CKD model

C57BL/6 mice aged 8 weeks were randomly subdivided into two groups (Sham group and CKD group); 5/6 nephrectomy (Nx) was utilized to create the CKD model and means resection of approximately 2/3 of the left kidney, followed by removal of the right kidney 1 week later. Sham-treated mice received sham operations; the appropriate kidney was exposed and mobilized but not treated in any other way.

After the CKD model was established, the mice were randomly redistributed into four groups, namely, Sham treatment + vehicle (n = 5), Sham treatment + gavage with 200 mg/(kg•day) resveratrol (n = 5), CKD + vehicle (n = 5), CKD + gavage with 200 mg/(kg•day) resveratrol (n = 5); the duration of resveratrol treatment was 21 days. Vehicle-treated groups received an equal volume of normal saline. Resveratrol was purchased from Copalyton Chemical Materials Co., Ltd., Shanghai, China. The use of animals in our studies was in compliance with protocols approved by the Institutional Animal Care and Use Committee of Xinhua Hospital.

The levels of blood urea nitrogen (BUN) and creatinine in mice were measured using Infinity™ Urea (Nitrogen) and Liquid Stable Reagent (Thermo Fisher Scientific).

2.2. Isolation of total RNA and quantitative real-time PCR (RT-qPCR)

TRIzol reagent (Sigma-Aldrich, St. Louis, MO) was used to extract total RNA from tissue samples. Reverse transcription reactions were performed using the iScript cDNA Synthesis Kit (Quanta, Gaithersburg, MD). SYBR Green Real-Time Quantitative PCR was performed on a Bio-Rad CFX96 System (Bio-Rad Laboratories). The amplification conditions were as follows: 3 min at 95 °C and 40 cycles of 15 s at 95 °C, 60 s at 60 °C, and 5 min at 95 °C. The expression levels of all mRNAs were normalized to GAPDH. The primer sequences for RT-qPCR were as follows: MuRF1 Forward: 5′-AGTGTCCATGTCTGGAGGTCGTTT-3′, Reverse: 5′-ACTGGAGCACTCCTGCTTGTAGAT-3′;GAPDH Forward: 5′-ACCACCATGG AGAAGGCCGG-3′, Reverse: 5′-CTCAGTGTAGCCCAAGATGC-3′; NF-κB Forward: 5′-AGTTTGACGGTGAGCTGGTA-3′, Reverse: 5′-GCCTCGGCCTGCC GCAAGCCT-3′.

2.3. Protein extraction, western blotting, and antibodies

Total protein extracts were prepared as follows; for western blotting, muscles were lysed in RIPA buffer (20 mM Tris-HCl pH 7.5, 5 mM EDTA, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.025% SDS, 1 mM sodium orthovanadate, 10 mM NaF, 25 mM β-glycerophosphate) containing protease and phosphatase inhibitors (Thermo Fisher Scientific). After centrifuging at 12,000 × *g* for 12 min at 4 °C, the supernatants were subjected to western blotting. Nuclear protein extracts were prepared using the NE-PER® Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific, Asheville, NC). The following antibodies were used: anti-phospho-NF-κB p65 (S536) antibody (1:500, Cell Signaling Technology), anti-NF-κB p65 (1:1000, Cell Signaling Technology), anti-IκB-α (1:1000, Cell Signaling Technology), anti-GAPDH (1:1000, Cell Signaling Technology), and an anti-MuRF1 antibody (1:1000, ECM Biosciences). The data on the target protein expression were normalized to GADPH.

2.4. Protein synthesis and degradation using an isotopic technique

To measure protein synthesis and degradation in the muscles of CKD mice, we incubated soleus and EDL muscles in 3 mL of Krebs–Henseleit bicarbonate buffer containing 0.5 mmol/L L-phenylalanine, 10 mmol/L glucose, and 0.05 μCi of L-^{14}C-phenylalanine (MP Biomedicals, Solon, OH) for 30 min. After gassing with 95% O_2/5% CO_2, the muscles were incubated for 30 min. We incubated the muscles in the fresh buffer gassed with 95% O_2/5% CO_2 for another 2 h. The rate of protein synthesis was measured as incorporation of L-^{14}C-phenylalanine into muscle protein. The rate of protein degradation was measured as the rate of release of tyrosine into the medium during the 2 h of incubation.

2.5. Immunostaining and measurement of the size of myofibers

The frozen TA muscles' slices (4 μm thick) was fixed with 4% formaldehyde for 5 min, then blocked with a protein blocking solution for 20 min. Dystrophin antibody (1:300, Abcam) was incubated with the slides at 4 °C overnight. The secondary Alexa Fluor-conjugated antibody (1:600, Life Technologies) was incubated with the muscles' slices for 30 min at room temperature. The areas of myofibers were measured using the NIS-Elements software (Nikon, USA), and at least 1000 myofibers per TA muscle were analyzed.

2.6. Cell culture and transfection with NF-κB small interfering RNA (siRNA)

C2C12 mouse myoblasts (ATCC; Manassas, VA) were routinely cultured at 37 °C and 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% of fetal bovine serum (FBS) and 1% of the penicillin/streptomycin solution (Haoran Biological Technological Co., Shanghai). At 90% confluence, FBS was replaced with 2% horse serum to induce C2C12 myoblast cells to differentiate into myotubes. The C2C12 myotubes were transfected with scramble control siRNA (siCTL) or NF-κB siRNA (GenePharma Co., Shanghai) for 48 h in DMEM containing 2% of horse serum in 6-well plates. After 48 h, the myotubes were treated with 1 μM dexamethasone (Dex), 100 μM resveratrol, co-administration of Dex and resveratrol for 24 h. We used Lipofectamine RNAiMAX Reagent (Invitrogen) to transfect the siRNA. The working concentrations of Dex and resveratrol were based on methods reported in other studies [20-21].

2.7. Statistical analyses

Data are presented as mean ± standard error of the mean (SEM) of three biological replicates. The body weight results were subjected to one-way ANOVA. Other results were statistically analyzed by two-tailed Student's *t*-test to determine p values. Differences

Please cite this article in press as: L.-J. Sun, et al., Resveratrol attenuates skeletal muscle atrophy induced by chronic kidney disease via MuRF1 signaling pathway, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.04.022

Download English Version:

https://daneshyari.com/en/article/5505748

Download Persian Version:

https://daneshyari.com/article/5505748

Daneshyari.com