Ginsenoside Rb1 improves cardiac function and remodeling in heart failure

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Abstract: We investigated the effect of ginsenoside Rb1 on cardiac function and remodeling in heart failure (HF). Four weeks after HF induction, the rats were administrated with ginsenoside Rb1 (35 and 70 mg/kg) and losartan (4.5 mg/kg) for 8 weeks. Losartan was used as a positive control. Cardiac function was assessed by measuring hemodynamic parameters. Histological changes were analyzed by HE and Masson’s trichrome staining. Cardiac hypertrophy, fibrosis, mitochondrial membrane potential and glucose transporter type 4 (GLUT4) levels were evaluated. In the present study, high dose of (H−) ginsenoside Rb1 decreased heart rate, improved cardiac function and alleviated histological changes induced by HF. H-ginsenoside Rb1 attenuated cardiac hypertrophy and myocardial fibrosis by decreasing left ventricular (LV) weight/heart weight ratio and cardiomyocyte cross-sectional area and reducing the levels of atrial natriuretic factor (ANF), β-myosin heavy chain (β-MHC), periostin, collagen I, Angiotensin II (Ang II), Angiotensin converting enzyme (ACE) and Ang II type 1 (AT1) receptor. Moreover, H-ginsenoside Rb1 decreased mitochondrial membrane potential and enhanced the translocation of GLUT4 to plasma membrane. The TGF-β1/Smad and ERK signaling pathways were inhibited and the Akt pathway was activated. These findings suggest that ginsenoside Rb1 might restore cardiac/mitochondrial function, increase glucose uptake and protect against cardiac remodeling via the TGF-β1/Smad, ERK and Akt signaling pathways.

Key words: cardiac function, cardiac remodeling, ginsenoside Rb1, heart failure

Introduction

Heart failure (HF) is a disease that the heart cannot pump blood to organs to meet the needs of body. The symptoms of HF are edema, fatigue and shortness of breath [23]. HF is the end-stage of many heart diseases and it affects more and more people in the world [3]. According to the statistics, approximately 38 million people suffer from HF worldwide [2]. HF is a serious public health problem that has caused a huge economic loss [27]. Despite improvements in the treatment of HF, approximately 50% patients die within 5 years of diagnosis [1, 26].

Traditional Chinese Medicine (TCM) contains hundreds of commonly used herbs. In China and other Asian countries, TCM has been used in clinical treatment for thousands of years [39]. Ginseng is a perennial plant that belongs to Panax genus of Araliaceae family [12].
bioactive components of ginseng are ginsenosides [8]. Ginsenoside Rb1 is one of the most important members among the identified ginsenosides and has been reported to attenuate ischemia/reperfusion (I/R) injury in multiple organs [18, 20]. Li YH, et al. have demonstrated that ginsenoside Rb1 protected against I/R and hypoxia/reoxygenation (H/R) injury via Akt, GSK-3β and mitochondrial permeability transition pore (mPTP) [22]. Wang XF, et al. have found that ginsenoside Rb1 inhibited isoproterenol-induced apoptosis of rat cardiomyocytes and H9c2 cells [35]. Jiang QS, et al. have showed that ginsenoside Rb1 alleviated monocytes-induced cardiac hypertrophy in rats and protected cardiomyocytes from prostaglandin F2α (PGF2α)-induced cardiac hypertrophy [16, 17]. Based on the findings, we hypothesized that ginsenoside Rb1 might have therapeutic effect on HF.

In our study, the rats were underwent abdominal aortic coarctation to induce HF. We tested the protective effect of ginsenoside Rb1 on cardiac dysfunction and remodeling in a rat model of HF and further investigated the mechanisms involved.

Materials and Methods

Drugs
Ginsenoside Rb1 was purchased from Henan Lyle (Luoyang, China). Losartan was purchased from NOVARTIS (Beijing, China) and served as a positive control [32].

Animal model and groups
Male Sprague-Dawley rats weighting 200–250 g were obtained from Beijing Vital River Laboratory Animal (Beijing, China). The experiments were approved by Animal Care and Use Committee of Liaoning University of Traditional Chinese Medicine and performed according to the Guide for the Care and Use of Laboratory Animals. The animals were divided into 5 groups (n=6 in each group), including Sham group, heart failure (HF) group, low dose of ginsenoside Rb1 group (L-ginsenoside Rb1), high dose of ginsenoside Rb1 group (H-ginsenoside Rb1) and Losartan group. HF was induced by abdominal aortic coarctation as described previously by Lv SC, et al. with minor modifications [25]. The rats were anesthetized with 3.5 ml/kg chloral hydrate (3.5 ml/kg). Animal hair (3 × 5 cm) was shaved around the abdominal midline and the abdomen was opened. A number 7 needle (outer diameter, 0.7 mm) was placed on the site of abdominal aorta (0.5 cm above right renal artery branches) in parallel. Both abdominal aorta and the needle were tied and then the needle was removed. After surgery, penicillin (200,000 U for each day) was intraperitoneally injected for 3 days to prevent the rats from infection. Four weeks after induction of HF, the rats in the L-ginsenoside Rb1, H-ginsenoside Rb1 and Losartan groups were injected with 35 mg/kg ginsenoside Rb1 (Henan Lyle wormwood, Luoyang, China), 70 mg/kg ginsenoside Rb1, 4.5 mg/kg losartan (NOVARTIS, Beijing, China) for 8 weeks by gavage daily, respectively. The rats in the Sham group were underwent the same procedures without ligation of the aorta. The rats in the HF group were subjected to abdominal aortic coarctation to induce HF and treated with equal volume of distilled water by gavage.

Cardiac function assessment
After weighing the body weight, the rats were anesthetized with 3.5 ml/kg chloral hydrate and a BL-420 signal acquisition and process system (TECHMAN Software, Chengdu, China) was inserted into left ventricles via the common carotid artery. We then measured heart rate, left ventricular end diastolic pressure (LVeDP), left ventricular systolic pressure (LVSP), maximum rate of left ventricular pressure rise (+dP/dtmax) and the maximum rate of left ventricular pressure fall (−dP/dtmax).

LV weight/body weight ratio
After animal experiments, blood samples were collected. After sacrificing the rats, the heart was excised and LV was weighed. The ratio of LV weight to body weight was calculated.

Hematoxylin and eosin (HE) staining
Histological changes were analyzed by HE staining. The left ventricle of the heart was fixed in 4% paraformaldehyde, dehydrated in graded ethanol and embedded. Then, the paraffin-embedded tissues were cut into sections (5-µm thickness) and subjected to HE staining. The sections were photographed.

Masson’s trichrome staining
The myocardial fibrosis of left ventricle was examined by Masson’s trichrome staining. Briefly, the left ventricle of the heart was obtained, fixed, dehydrated and embedded in paraffin. After slicing into sections, the
sections were dewaxed, rehydrated and subjected to Mason’s trichrome staining. The sections were imaged under a Model DP73 Olympus Microscope (Tokyo, Japan).

**Wheat germ agglutinin staining**

Paraffin-embedded sections were dewaxed and rehydrated in graded ethanol. The sections were stained with wheat germ agglutinin lectin (FITC) (0.1 mg/ml) (GeneTex, Irvine, CA, USA) and the nuclei were counterstained with DAPI. Images were photographed under a BX53 fluorescence microscope (Olympus).

**RT-PCR**

Total RNAs were isolated using BioTeke RNA Extraction Kit (Beijing, China) and then reverse-transcribed into a volume of 20 µl cDNAs. Real-time PCR analysis was carried out on Exicycler™ 96 from BIONEER (Daejeon, Republic of Korea) according to the reaction conditions: 95°C for 10 min; 95°C for 10 s, 60°C for 20 s, 72°C for 30 s of 40 cycles. The primer sequences (5′-3′) were as shown in Table 1.

**Western blot**

Total proteins, membrane proteins and cytoplasmic proteins were extracted (Beyotime, Haimen, China) and equal amounts of proteins were run on SDS-PAGE. After electrophoresis, the proteins were transferred onto Millipore PVDF membranes (Billerica, Massachusetts, USA). The membranes were immersed in 5% nonfat milk and incubated with anti-Angiotensin converting enzyme (ACE) (1:200) (Santa Cruz, Dallas, Texas, USA), anti-Angiotensin II type 1 receptor (AT1 receptor) (1:1,000) (Abcam, Cambridge, MA, USA), anti-periostin (1:200) (Santa Cruz), anti-collagen I (1:400) (Wuhan Boster, Wuhan, China), anti-TGF-β1 (1:200) (Santa Cruz, USA), anti-Smad2/3 (1:200) (Santa Cruz), anti-p-ERK1/2 (1:500) (Bioss, Beijin, China), anti-ERK1/2 (1:500) (Bioss), anti-GLUT4 (1:200) (Santa Cruz), anti-p-Akt (1:200) (Santa Cruz) and anti-Akt (1:200) (Santa Cruz, USA) primary antibodies overnight. After washing with TBS-T buffer, the membranes were incubated for 45 min with horseradish peroxidase (HRP)-conjugated secondary antibody (1:5,000) (Beyotime) diluted in 5% nonfat milk. The proteins were developed using ECL reagent in the dark and quantified using Gel-Pro Analyzer (Media Cybernetics, Inc., Rockville, MD, USA).

**ELISA**

Peripheral blood was obtained and Angiotensin II (Ang II) was detected using ELISA Assay Kit purchased from Wuhan USCN Business Co., Ltd. (Wuhan, China) according to the manufacturer’s instructions.

**Measurement of mitochondrial membrane potential**

The left ventricle of the heart was washed with PBS and cut into 1–3 mm³ tissue mass. The tissue mass was digested with 0.125% trypsin at 37°C for 20 min and the cell suspension was centrifuged at 1,000 rpm. The supernatant was discarded. The cells were washed with PBS once again and resuspended in 500 µl JC-1 (KeyGEN, Nanjing, China). The suspension was incubated at 37°C for 20 min. The cells in the suspension were washed and resuspended in Incubation buffer. Mitochondrial membrane potential was examined by BD Biosciences model Accuri C6 flow cytometer (San Jose, CA, USA).

**Statistical analyses**

Values are expressed as mean ± standard deviation.

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**Table 1. Primers used for quantitative real-time PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequences (5′-3′ direction)</th>
<th>Tm (°C)</th>
<th>Gene ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE-forward</td>
<td>GGTGCCAATGACATAGAAAG</td>
<td>50.5</td>
<td>NM_012544.1</td>
</tr>
<tr>
<td>ACE-reverse</td>
<td>CACCACTCTGATTTGAGCG</td>
<td>54.1</td>
<td></td>
</tr>
<tr>
<td>ANF-forward</td>
<td>GGTGTTGAAATACCCCCCTG</td>
<td>54.5</td>
<td>NM_012612.2</td>
</tr>
<tr>
<td>ANF-reverse</td>
<td>CTGTTCCTGTTGCTGAA</td>
<td>55.3</td>
<td></td>
</tr>
<tr>
<td>periostin-forward</td>
<td>AGGAGGAGCGGTTTGGAG</td>
<td>57.3</td>
<td>_</td>
</tr>
<tr>
<td>periostin-reverse</td>
<td>GCTTACCTGCAACCGCTCA</td>
<td>56.2</td>
<td></td>
</tr>
<tr>
<td>β-MHC-forward</td>
<td>TCCATCTCTGACACCGCTGTA</td>
<td>56.2</td>
<td>NM_017240.2</td>
</tr>
<tr>
<td>β-MHC-reverse</td>
<td>GTCGCGACAATAACAGCA</td>
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<tr>
<td>AT1 receptor-forward</td>
<td>TAAACGGGCCCCACCTCGTG</td>
<td>60.6</td>
<td>NM_030985.4</td>
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<tr>
<td>AT1 receptor-reverse</td>
<td>CTGTGAACTGCTGCTACCCCTGC</td>
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<tr>
<td>β-actin-forward</td>
<td>GAGATTACTGCCCCTGGCTCTAGC</td>
<td>60.1</td>
<td>NM_031144.3</td>
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<tr>
<td>β-actin-reverse</td>
<td>GGCGGACTCATCGTACTCTGCTTT</td>
<td>62</td>
<td></td>
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Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software Inc., USA) by Student’s t-test or One-way ANOVA followed by Bonferroni post-hoc test. The difference was statistically significant when P value<0.05.

Results

Effect of ginsenoside Rb1 on cardiac function

Hemodynamic assessments showed that the HF group showed significant increases in heart rate (Fig. 1A) and LVEDP (Fig. 1B) and significant decreases in LVSP (Fig. 1C), \(+\text{dP/dt}_{\text{max}}\) (Fig. 1D) and \(-\text{dP/dt}_{\text{max}}\) (Fig. 1E) compared with the Sham group. L-ginsenoside Rb1 and H-ginsenoside Rb1 treatment decreased heart rate and LVEDP and increased LVSP, \(+\text{dP/dt}_{\text{max}}\) and \(-\text{dP/dt}_{\text{max}}\), but the difference was not statistically significant. Losartan injection improved HF-induced elevation of heart rate and LVEDP and HF-induced decreases of LVSP, \(+\text{dP/dt}_{\text{max}}\) and \(-\text{dP/dt}_{\text{max}}\) in model rats.

Effect of ginsenoside Rb1 on myocardial morphology and myocardial fibrosis in left ventricles

Myocardial tissues from the rats of HF group showed inflammatory cell infiltration, irregularly arranged cardiomyocytes and swelling of cardiomyocytes (Fig. 2) and myocardial fibrosis (Fig. 3) compared with those in the Sham group. However, all these histological changes were attenuated by injection of ginsenoside Rb1 or losartan.

Effect of ginsenoside Rb1 on cardiac hypertrophy

There was no significant difference in the body weight (Fig. 4A) between the drug-treated HF groups and non-treated HF group. The LV weight/body weight ratio (Fig. 4B) was significantly higher in the HF group than that in the Sham group. LV weight/body weight ratio was decreased after treatment with H-ginsenoside Rb1 as compared with the HF group, however the difference was not statistically significant. Losartan administration significantly decreased HF-induced increase of LV...
Fig. 2. HE staining. The HF model rats were administrated for 8 weeks with L-ginsenoside Rb1, H-ginsenoside Rb1 and losartan. The rats were sacrificed and LV tissues were excised. HE-stained sections of LV tissues were presented. Scale bars 100 µm.

Fig. 3. Masson’s trichrome staining. Masson’s trichrome-stained sections of LV tissues were shown. Scale bars 100 µm. **P<0.01 vs. the Sham group, ***P<0.01 vs. the HF group.
weight/body weight ratio. Cardiomyocyte cross-sectional area was measured by wheat germ agglutinin staining (Fig. 4C). We found that the cross-sectional area of cardiomyocytes in the HF group was significantly larger than that in the Sham group. However, HF-induced increase of cardiomyocyte cross-sectional area was markedly alleviated by L-ginsenoside Rb1, H-ginsenoside Rb1 or losartan administration. The markers of cardiac hypertrophy were examined by RT-PCR (Fig. 4D). We found that HF significantly increased the expression levels of ANF and β-MHC. Moreover, H-ginsenoside Rb1 or losartan treatment significantly reduced the upregulated gene levels of ANF and β-MHC.

**Effect of ginsenoside Rb1 on the expression of Ang II, ACE and AT1 receptor**

The level of Ang II in peripheral blood was higher in the HF group than that in the Sham group. L-ginsenoside Rb1, H-ginsenoside Rb1 or losartan treatment markedly decreased Ang II level (Fig. 5A) compared with the HF group. We then examined ACE and AT1 receptor using quantitative RT-PCR (Fig. 5B) and western blot (Fig. 5C). The results showed that H-ginsenoside Rb1 or losartan treatment decreased HF-induced upregulation of ACE and AT1 receptor in LV tissues.

**Effect of ginsenoside Rb1 on perioistin, collagen I, TGF-β1, Smad2/3 and ERK1/2**

We investigated the effect of ginsenoside Rb1 on perioistin expression. HF significantly upregulated perioistin expression at both gene (Fig. 6A) and protein levels (Fig. 6B), which were downregulated by H-ginsenoside Rb1 and losartan. The levels of collagen I in L-ginsenoside Rb1, H-ginsenoside Rb1 and losartan groups were lower than that in the HF group, but the differences were not statistically significant. We also found that L-ginsenoside Rb1, H-ginsenoside Rb1 or losartan abolished the stimulatory effect of HF on TGF-β1, nuclear translocation of Smad2/3 and ERK1/2 phosphorylation (Figs. 6B and C).

**Effect of ginsenoside Rb1 on mitochondrial membrane potential and metabolic pathways**

We assessed mitochondrial membrane potential using
GINSENOside RB1 ATTENUATES HEART FAILURE

**Fig. 5.** Effect of ginsenoside Rb1 and losartan on the components of the Ang II system. A. The level of Ang II was examined using ELISA. B. Gene expression of ACE and AT1 receptor. C. Protein expression of ACE and AT1 receptor. **P<0.01 vs. the Sham group, &P<0.05 and &&P<0.01 vs. the HF group.

JC-1 apoptosis detection kit (Fig. 7). HF significantly increased, whereas H-ginsenoside Rb1 and losartan notably decreased the apoptosis rates. Western blotting results showed that glucose transporter type 4 (GLUT4) (Fig. 8A) in the plasma membrane was significantly decreased in HF rats, while GLUT4 level in the cytoplasm was significantly increased. L-ginsenoside Rb1, H-ginsenoside Rb1 and losartan treatment upregulated GLUT4 levels in the plasma membrane and downregulated cytoplasmic GLUT4 levels. Additionally, western blot results showed that H-ginsenoside Rb1 and losartan treatment reversed HF-induced inactivation of the Akt pathway, as evaluated by p-Akt/Akt ratio (Fig. 8B).

**Discussion**

This study evaluated the effect of ginsenoside Rb1 on cardiac function, myocardial fibrosis, cardiac hypertrophy, mitochondrial membrane potential and GLUT4 translocation in a rat model of HF.

Hemodynamic parameters, including heart rate, LVSP, LVEDP, +dP/dt max and −dP/dt max were recorded at the end of the animal experiments. HF induced the changes of hemodynamic parameters, indicating the damage of cardiac function. We found that H-ginsenoside Rb1 or losartan administration restored the upregulation of LVEDP and the downregulation of LVSP, +dP/dt max and −dP/dt max induced by HF. The results suggest that H-ginsenoside Rb1 or losartan might improve heart function by altering hemodynamic parameters.

Myocardial fibrosis is one of the hallmarks of heart disease, which is characterized by ECM [19]. Collagen I is produced by myofibroblasts and it is the major component of ECM [38]. Ang II is an important member of renin-angiotensin system (RAS) [7]. RAS also includes ACE, ACE2 and Ang (1–7) [29]. ACE catalyzes Ang I into Ang II [7]. Ang II interacts with AT1 receptor, a receptor of Ang II, to induce fibrosis and inflammation [31]. Periostin (also called osteoblast-specific factor-2) is a secreted protein that firstly found in mouse osteoblast MC3T3-E1 cells [10]. Periostin is upregulated after heart failure and myocardial infarction [36]. Periostin is correlated with fibrosis, tissue injury and arthritis. Periostin-knockout mice showed significantly reduced levels of
liver fibrosis [15]. Xie XS, et al. have demonstrated that ginsenoside Rb1 alleviates renal interstitial fibrosis in unilateral ureteral obstruction (UOU) rat model [37]. Moreover, ginsenoside Rb1 has been demonstrated to be effective in attenuating liver fibrosis in vitro and in vivo [11, 24]. Our results showed that ginsenoside Rb1, especially H-ginsenoside Rb1, and losartan reduced the expression levels of Ang II, ACE, AT1 receptor, periostin and collagen I in a rat model of HF. The results suggest that H-ginsenoside Rb1 or losartan might protect rats against myocardial fibrosis by regulating fibrosis-related proteins, such as Ang II, ACE, AT1 receptor, periostin and collagen I.

TGF-β is an inducer of collagen production and fibroblast activation [4]. Smads are the downstream proteins of the TGF-β signaling. TGF-β1 binds its receptors in the cell surface and phosphorylates Smad2/Smad3, which form a protein complex with Smad4 to regulate the expression of target genes in the nucleus [42]. Accumulating evidences have shown that the TGF-β/Smad signaling pathway is involved in cardiac fibrosis [41]. Nowadays, inhibiting the TGF-β/Smad signaling pathway has been used to alleviate fibrosis [34]. We found that H-ginsenoside Rb1 or losartan significantly down-

**Fig. 6.** Effect of ginsenoside Rb1 and losartan on periostin, collagen I, TGF-β1, Smad2/3 and ERK1/2. A. Gene expression of periostin. B. Protein expression of periostin, collagen I and TGF-β1. C. The levels of cytoplasmic Smad2/3, nuclear Smad2/3, p-ERK1/2 and ERK1/2 were quantified by western blot. **P<0.01 vs. the Sham group, *P<0.05 and &P<0.01 vs. the HF group.**
regulated the levels of TGF-β1 and nuclear Smad2/3. The results suggest that H-ginsenoside Rb1 or losartan might attenuate myocardial fibrosis in HF rats by inhibiting the TGF-β1/Smad signaling pathway.

Abnormal enlargement of cardiomyocytes and extracellular interstitial fibrosis are the characteristics of cardiac hypertrophy [5, 28]. Cardiac hypertrophy can also induce the expression of fetal cardiac genes, such as β-MHC and ANF [33]. Recent evidences have shown that both ACE inhibitor and AT1 receptor antagonist improve LV remodeling and function in diabetic cardiomyopathy [9]. Zhao H, et al. have found that ginsenoside Rb1 inhibits cardiac hypertrophy and fibrosis in dilated cardiomyopathy [40]. Jiang QS, et al. have shown that ginsenoside Rb1 inhibits cardiac hypertrophy via the Ca²⁺-CaN signal transduction pathway [16, 17]. In the present study, we found that ginsenoside Rb1, especially H-ginsenoside Rb1, and losartan significantly decreased LV weight/body weight ratio and cardiomyocyte cross-sectional area and reducing the levels of ANF and β-MHC, suggesting that ginsenoside Rb1 may attenuate HF-induced cardiac hypertrophy in vivo.

It has been reported that the ERK signaling pathway is involved in cardiac hypertrophy and fibrosis [13, 21]. We found that L-ginsenoside Rb1, H-ginsenoside Rb1 and losartan notably inhibited the activation of the ERK signaling pathway induced by HF. The results suggest that ginsenoside Rb1 or losartan might alleviate cardiac hypertrophy and fibrosis by inhibiting the ERK signaling pathway.

Mitochondrial membrane potential is an important indicator of mitochondrial activity [30]. Our results showed that H-ginsenoside Rb1 and losartan significantly inhibited HF-induced mitochondrial depolarization and apoptosis, suggesting that ginsenoside Rb1 attenuated HF-induced mitochondrial dysfunction. GLUT4 is an important member of glucose transporter family and regulates blood glucose homeostasis and insulin
sensitivity. GLUT4 translocates from the cytoplasm to the plasma membrane upon insulin stimulation [14]. Akt has been reported to increase glucose uptake by promoting translocation of GLUT4 to plasma membrane [6]. Our present results showed that L-ginsenoside Rb1, H-ginsenoside Rb1 and losartan promoted the translocation of GLUT4 to the plasma membrane. The p-Akt/Akt ratios were significantly increased after H-ginsenoside Rb1 and losartan treatment. The results suggest that ginsenoside Rb1 might enhance glucose uptake through GLUT4 translocation via the Akt pathway.

In summary, ginsenoside Rb1 improved heart function, attenuated cardiac hypertrophy and fibrosis, restored mitochondrial function and enhanced GLUT4-mediated glucose uptake by inhibiting the TGF-β1/Smad and ERK pathways and activating the Akt pathway. Our study provides evidence of the protective effect of ginsenoside Rb1 on cardiac dysfunction and remodeling in HF. The limitation of our study was that we only examined the effects and mechanisms of ginsenoside Rb1 on HF in rats. Further studies in other mammals and human were required.

**Conflict of Interest**

The authors declare that they have no competing interests.

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