Role of Nitric Oxide in Ginsenoside Rg1-Induced Protection against Left Ventricular Hypertrophy Produced by Abdominal Aorta Coarctation in Rats

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Ginsenoside Rg1 (Rg1), one of the active components of Panax ginseng, has been reported to promote endogenous nitric oxide (NO) production in some tissues, and to inhibit left ventricular (LV) hypertrophy in rats. This study aimed to investigate whether Rg1-induced inhibition of rat LV hypertrophy is mediated by NO-production. Rat LV hypertrophy was induced by abdominal aorta coarctation. Rg1, 15 mg/kg/d, t-arginine 200 mg/kg/d, and the nitric oxide synthase (NOS) inhibitor Nω-nitro-L-arginine-methyl ester (L-NAME) 100 mg/kg/d used with the same dose of t-arginine or Rg1 were given starting from 1 d after surgery for 21 consecutive days. LV hypertrophy was evidenced by determining LV weight and mRNA expression of atrial natriuretic peptide, a marker of cardiac hypertrophic response, as well as by histopathology. Rg1 and t-arginine administration significantly reduced LV hypertrophy. These results demonstrate that Rg1-induced protection against LV hypertrophy elicited by abdominal aorta coarctation in rats is mediated, at least in part, via endogenous NO production and release.

Key words ginsenoside Rg1; left ventricular hypertrophy; endothelial nitric oxide synthase; nitric oxide

Ginsenosides are the most important active constituents identified in ginseng (Panax ginseng C. A. MEYER), a well-known and popular herbal medicine used in China. Based on the types of aglycone, ginsenosides (ginseng saponins) are mainly fractioned into two groups, namely the protopanaxadiol group and protopanaxatriol group.1 Ginsenoside Rg1 (Rg1) is a protopanaxatriol saponin and is abundant and highly active among different ginsenosides.2 We have previously reported that Rg1 can inhibit cardiac hypertrophy in vivo and in vitro,3,4 and the anti-hypertrophic mechanisms may involve calcineurin and mitogen-activated protein kinase (MAPK) signaling.3 However, the etiology of cardiac hypertrophy is very complicated; more studies should be done to elucidate the action means of Rg1-induced protection against cardiac hypertrophy.

It has been found that nitric oxide (NO) acts as an important transcellular signaling to exert many biological effects, such as anti-oxidative,5 anti-microbial,6 anti-inflammatory,7 anti-tumor,8 and immunosuppressive activity of macrophages.5,7 Notably, NO also plays an important role in cardiovascular protection,9 is produced in virtually every cell type in the heart, and exerts potent anti-hypertrophic effects.10 The observations indicate that in some instances, endogenous NO production should be considered in the anti-hypertrophic mechanism of drugs. Many studies have shown that Rg1,11 can cause endothelial-dependent relaxation in the rat aorta and enhance endogenous NO production in human umbilical vein endothelial cells,12 rat kidney,13 and in porcine coronary arteries.14 Although some protopanaxadiol saponins including ginsenoside Rb1 have been shown to promote NO release in some tissues,9 there seem some differences in NO-releasing effect between the protopanaxatriol and protopanaxadiol groups; for example, in rat aorta the protopanaxatriol saponins Rg1 and Re but not the protopanaxadiol saponins Rb1 and Rc enhance the release of NO from endothelial cells.15 In our previous studies on the relationship between the NO-releasing and anti-cardiac hypertrophic effects of ginsenosides, we found that Nω-nitro-L-arginine-methyl ester (L-NAME), a NOS inhibitor, had no influence on the anti-hypertrophic effect of Rb1,15 suggesting a limited role of NO in the effect of Rb1. However, little is known about whether there are any differences in the role of NO between the anti-hypertrophic effects of Rb1 and Rg1.

This study aimed to investigate whether Rg1-inhibition on rat left ventricular (LV) hypertrophy is mediated via NO production and release.

MATERIALS AND METHODS

Animal Model and Experimental Design Sprague-Dawley male rats (n = 60; 200 ± 20 g), purchased from Animal Center of Institute of Surgery Research of the Third Military Medical University (Chongqing, China), were maintained in animal facilities of Zunyi Medical College and allowed free access to rodent feed and tap water, under the Chinese Guidance of Humane Use of Laboratory Animals. For producing a LV hypertrophy model, the rats were anesthetized with sodium pentobarbital (40 mg/kg, intraperitoneally (i.p.)), and the abdomen was opened by surgical incision under sterile conditions. Abdominal aorta coarctation...
was produced by placing a blunt 7-gauge needle adjacent to the abdominal aorta proximal to the renal bifurcations junction, a ligature was made, then the blunt needle was withdrawn.16

Rats were randomly divided into 6 groups: abdominal aorta coarctation rats (i.e. model control) and normal rats (i.e. normal control) were given distilled water; other abdominal aorta coarctation animals were given Rg1 (Beijing Institute of Natural Medicine, China) 15 mg/kg/d, L-arginine (L-arg, ALEXIS BIOCHEMICALS, U.S.A.) 200 mg/kg/d, Rg2 15 mg/kg/d+i.‐NAME (ALEXIS BIOCHEMICALS, U.S.A.) 100 mg/kg/d (Rg2+i-NAME), and L-arg 200 mg/kg/d+i.‐NAME 100 mg/kg/d (L-arg+i-NAME). Rg1, and distilled water were given intraperitoneally, and L-NAME intragastric (i.g.) ally. All treatments were initiated 1 d after the operation, and continued once daily for 3 weeks.

**Hemodynamics Analysis and Assessment of LV Hypertrophy** Twenty-four hours after the last drug administration, the animal body weight was recorded and hemodynamics detected under anesthesia with sodium pentobarbital 40 mg/kg i.p. The systolic blood pressure of artery carotis communis was recorded, and then LV intubation, LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), and maximal rate of rise and decline of LV pressure (±dp/dt max) were detected by BL-420E system (Chengdu Taimeng Science and Technology Co., China). Then, the right ventricular (RV) free wall and LV (including interventricular septum) samples were separated and blotted dry. The weight of the right and left ventricle was recorded to calculate LV hypertrophic parameters17 including LV hypertrophy index (LVHI, i.e. the ratio of the left ventricle weight and body weight) and the LV/RV weight ratio (LVW/RVW).

**Histological Analysis** For light microscopic examination, the LV specimens were fixed with 10% paraformaldehyde in phosphate buffered solution (PBS), embedded in paraffin, and cut into 5-μm slices, which were stained with hematoxylin–eosin for morphological analysis.

**Expression Analysis for Atrial Natriuretic Peptide (ANP) and Endothelial Nitric Oxide Synthase (eNOS)** Total RNA was extracted from LV tissue using TRIzol™ (MRC Co., Cincinnati, U.S.A.), and purified by RNeasy mini kit (Qiagen Co., Valencia, U.S.A.). Total RNA was reverse transcribed with MuLV reverse transcriptase and OligodT primer. Real time polymerase chain reaction (RT-PCR) was carried out in iCycler iQ Real-Time PCR Detection System (BIO-RAD Co., CA, U.S.A.), with SYBR® Green PCR Master Mix (ABI Co., Foster, U.S.A.) and synthesized by TaKaRa Biological Engineering Com. (TaKRa, Dalian, China). The following primers were used:

- ANP (GenBank accession no. NM_012612) forward: 5’-TGA CAG GAT TGG AGC CCA GAG-3’; reverse: 5’-TGG AGC AGA TTT GGC TGT TAT CTT C-3’ (75 bp product);
- eNOS (GenBank accession no. NM_021838) forward: 5’-GTC ATC ACC AGG AAG AAG ACT T-3’; reverse: 5’-CAC AGC CTG CGC CAT CAC-3’ (97 bp product);
- β-actin (GenBank accession no. NM_031144) forward: 5’-TGA CAG GAT GCA GAA GGA GA-3’; reverse: 5’-TGG AGC CAC CAA TCC ACA CA-3’ (104 bp product).

The reaction conditions were: 95°C 10 min 1 cycle; 95°C 15 s, 60°C 1 min, 40 cycles. The threshold cycle (Ct) values of target genes were normalized with β-actin of the same sample, and expressed relative to controls.

**Statistical Analysis** All data are presented as mean± S.E.M. and were analyzed by one-way ANOVA followed by Student’s t-test (two-tailed) using SPSS 13.0 software (SPSS Inc., Chicago, IL, U.S.A.); significance was set at p<0.05.

**RESULTS**

**Rat Body Weight Change and Influence of L-NAME** As shown in Fig. 1, compared with normal control, body weight gain in model control animals was significantly decreased over the 3 week period after abdominal aorta coarctation operation (p<0.05). However, Rg1 15 mg/kg and L-arg 200 mg/kg administration significantly ameliorated the animal body weight loss induced by abdominal aorta coarctation, in comparison with model control rats (p<0.05). In addition, Rg2 also improved the general health conditions, while L-NAME abolished the beneficial effects induced by both Rg1 and L-arg.

**Beneficial Effects of Rg1 on LV Diastolic Function of Rats Following Abdominal Aorta Coarctation and Influence of L-NAME** After administration for 3 weeks, the systolic blood pressure of artery carotis communis and LVP were significantly increased in all the abdominal aorta coarctation operation groups compared with normal control (p<0.05), and no difference could be seen among these operation groups. On the other hand, although LVEDP was also higher and ±dp/dt max was lower in model control animals than in normal control (p<0.05), Rg1 and L-arg administration significantly reduced LVEDP and increased ±dp/dt max in the abdominal aorta coarctation operation rats (p<0.05), suggesting that Rg1 and L-arg could ameliorate the LV diastolic function of coarctation operation animals. When the administration was combined with L-NAME, however, the improving effects of Rg1 and L-arg on cardiac function were abolished (p>0.05; Table 1).

**Effects of Rg1 on Hypertrophic Parameters of Rats Following Abdominal Aorta Coarctation and Influence of L-**
Abdominal aorta coarctation dramatically increased systolic blood pressure (SBP), left ventricular systolic pressure (LVSP), and left ventricular end-diastolic pressure (LVEDP) and decreased maximal rate of rise and decline of left ventricular pressure (\(+dp/dt_{max}\) and \(-dp/dt_{max}\)) in rats. Rg1 treatment (15 mg/kg, i.p. for 21 d) ameliorated these changes in rats following abdominal aorta coarctation. Rg1, Rg1 15 mg/kg; Rg1 + L, Rg1 15 mg/kg (i.p.) and L-NAME (i.g.); normal, normal control; model, model control. Data are mean\(\pm\)S.E.M. of 6 rats. *Significantly different from normal control at \(p<0.05\); # significantly different from model control at \(p<0.05\); $ significantly different from Rg1 at \(p<0.05\); ** significantly different from L-arg at \(p<0.05\).

Abdominal aorta coarctation dramatically increased systolic blood pressure (SBP), left ventricular systolic pressure (LVSP), and left ventricular end-diastolic pressure (LVEDP) and decreased maximal rate of rise and decline of left ventricular pressure (+dp/dtmax, -dp/dtmax) in rats. Rg1 treatment (15 mg/kg, i.p. for 21 d) ameliorated these changes in rats following abdominal aorta coarctation. Rg1, Rg1 15 mg/kg; Rg1 + L, Rg1 15 mg/kg (i.p.) and L-NAME (i.g.); normal, normal control; model, model control. Data are mean\(\pm\)S.E.M. of 6 rats. *Significantly different from normal control at \(p<0.05\); # significantly different from model control at \(p<0.05\); $ significantly different from Rg1 at \(p<0.05\); ** significantly different from L-arg at \(p<0.05\).

**Table 1. Effects of Ginsenoside Rg1 on Hemodynamic Function in Abdominal Aorta Coarctation Rats and Influence of L-NAME**

<table>
<thead>
<tr>
<th>Group</th>
<th>BP (mmHg)</th>
<th>LVSP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>+dp/dtmax (mmHg/s)</th>
<th>-dp/dtmax (mmHg/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>118±4.7</td>
<td>137±3.8</td>
<td>7.9±2.20</td>
<td>4651±548</td>
<td>3433±391</td>
</tr>
<tr>
<td>Model</td>
<td>162±7.1*</td>
<td>165±9.6*</td>
<td>28.4±4.90*</td>
<td>1629±52*</td>
<td>1557±161*</td>
</tr>
<tr>
<td>Rg1</td>
<td>162±1.1*</td>
<td>167±6.5*</td>
<td>13.1±2.98*</td>
<td>3301±400*</td>
<td>3057±262*</td>
</tr>
<tr>
<td>Rg1 + L</td>
<td>163±6.5*</td>
<td>170±4.7*</td>
<td>22.6±2.45*</td>
<td>2373±173*</td>
<td>2312±209*</td>
</tr>
<tr>
<td>t-arg</td>
<td>153±5.9*</td>
<td>162±8.7*</td>
<td>13.1±3.14*</td>
<td>2915±201*</td>
<td>2851±176*</td>
</tr>
<tr>
<td>t-arg + L</td>
<td>163±7.4*</td>
<td>165±10.7*</td>
<td>23.1±1.38*</td>
<td>1920±170*</td>
<td>1903±504*</td>
</tr>
</tbody>
</table>

After 3 weeks of abdominal aorta coarctation, LV mass in model control rats was significantly increased, as evidenced by the elevated LVHI and LVW/RVW, in comparison with normal control animals (\(p<0.05\)). Administration of Rg1 and L-arg remarkably inhibited the elevated LVHI and LVW/RVW (\(p<0.05\)). L-NAME administration, however, abolished the protective effects by both Rg1 and L-arg (\(p<0.05\)), reversing the LV hypertrophic parameters to the levels of model control (Fig. 2).

**Effect of Rg1 on Histological Changes of Rats Following Abdominal Aorta Coarctation and Influence of L-NAME**

Figure 3 (normal) shows normal morphology of LV tissue in normal control rats. Abdominal aorta coarctation caused cardiomyocyte hypertrophy as evidenced by increased cell size and fibrosis in model control (Fig. 3; model). Rg1 and L-arg administration significantly ameliorated the LV morphological injuries induced by abdominal aorta coarctation, which was reversed by combining administration with L-NAME (Fig. 3; Rg1, L-arg, Rg1 + L-NAME, L-arg + L-NAME).

**Effects of Rg1 on ANP and eNOS mRNA Expression**

Expression of ANP and eNOS mRNA from LV tissue is shown in Fig. 4. It was found that basic expression of ANP mRNA was very low in normal control; however, this expression was significantly increased by abdominal aorta coarctation as shown in model control rats (\(p<0.05\)). Rg1 and L-arg administration downregulated this gene expression remarkably (\(p<0.05\)), and combining administration with L-NAME restored ANP mRNA expression to some extent (\(p<0.05\). In
of ANP mRNA was also taken as one of the parameters of LV hypertrophy in this study. Our results showed that Rg1 and the NO precursor L-arg could inhibit the elevated LVHI and LVW/RVW ratio, as well as the up-regulated ANP mRNA expression induced by abdominal aorta coarctation independent of the LVSP change, and these were abolished by L-NAME, a NOS inhibitor. The change of histology of LV myocardium was consistent with the above hypertrophic parameters. These findings primarily indicate that endogenous NO release may participate in the anti-cardiac hypertrophic effect of Rg1.

Our results also showed that Rg1 and L-arg could ameliorate body weight loss, and the LVEDP and ±dp/dt max changes induced by abdominal aorta coarctation, which were attributed to their improving effects on cardiac hypertrophy and function.3) Consistently with the findings in hypertrophic parameters, the beneficial effects of Rg1 and L-arg on body weight and cardiac diastolic function were also abolished or blunted by L-NAME. The results can be attributed to inhibition of L-NAME on the anti-hypertrophic effects of Rg1 and L-arg, through inhibiting endogenous NO production. Although we did not measure plasma NO concentration in the present study, we previously found that Rg1 at 15.6, 31.2, and 62.4 μmol/l could significantly increase NO metabolite level in culture medium of hypertrophic cardiac myocytes induced by prostaglandin F2α in a concentration-dependent manner.3)

It has been shown that cardiac myocytes express all isoforms of NOS, including neuronal (nNOS), inducible (iNOS), and eNOS,26) and both eNOS and nNOS-deficient mice have been shown spontaneously to develop cardiac hypertrophy.27) However, in the LV hypertrophic rats induced by abdominal aorta coarctation, eNOS, but not iNOS, plays a regulatory role in cardiac hypertrophy development.28) In transgenic mice with cardiac-specific overexpression of tumor necrosis factor-α, iNOS disruption did not alter myocardial inflammation and ventricular hypertrophy.29) Therefore only eNOS expression was detected in the present study. We found that eNOS mRNA expression in LV tissue was lowered by aorta stricture, and Rg1 administration could significantly upregulate the reduced eNOS mRNA expression. Rg1 has been reported to increase phosphorylation of glucocorticoid receptor, phosphatidylinositol-3 kinase, Akt/PKB, and eNOS leading to NO production in human umbilical vein endothelial cells.3) Whether the same mechanism exists in rat heart remains to be studied; however, the above data provide further evidence that Rg1 enhances endogenous NO production and release, which plays an important role in its protection against LV hypertrophy induced by abdominal aorta coarctation.

Taking the findings in the experiment using L-NAME together with the results in the on eNOS mRNA expression, we conclude that endogenous NO production is involved, at least in part, in Rg1-induced protection against LV hypertrophy produced by abdominal aorta coarctation in rats.

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REFERENCES