Ginseng Inhibits Cardiomyocyte Hypertrophy and Heart Failure via NHE-1 Inhibition and Attenuation of Calcineurin Activation

Juan Guo, PhD; Xiaohong Tracey Gan, MSc; James V Haist, BSc; Venkatesh Rajapurohitam, PhD; Asad Zeidan, PhD; Nazo Said Faruq; Morris Karmazyn, PhD

Background—Ginseng is a medicinal plant used widely in Asia that has gained popularity in the West during the past decade. Increasing evidence suggests a therapeutic role for ginseng in the cardiovascular system. The pharmacological properties of ginseng are mainly attributed to ginsenosides, the principal bioactive constituents in ginseng. The present study was carried out to determine whether ginseng exerts a direct antihypertrophic effect in cultured cardiomyocytes and whether it modifies the heart failure process in vivo. Moreover, we determined the potential underlying mechanisms for these actions.

Methods and Results—Experiments were performed on cultured neonatal rat ventricular myocytes as well as adult rats subjected to coronary artery ligation (CAL). Treatment of cardiomyocytes with the \(\alpha_1\) adrenoceptor agonist phenylephrine (PE) for 24 hours produced a marked hypertrophic effect as evidenced by significantly increased cell surface area and ANP gene expression. These effects were attenuated by ginseng in a concentration-dependent manner with a complete inhibition of hypertrophy at a concentration of 10 \(\mu g/mL\). Phenylephrine-induced hypertrophy was associated with increased gene and protein expression of the \(Na^+/H^+\) exchanger 1 (NHE-1), increased NHE-1 activity, increased intracellular concentrations of \(Na^+\) and \(Ca^{2+}\), enhanced calcineurin activity, increased translocation of NFAT3 into nuclei, and GATA-4 activation, all of which were significantly inhibited by ginseng. Upregulation of these systems was also evident in rats subjected to 4 weeks of CAL. However, animals treated with ginseng demonstrated markedly reduced hemodynamic and hypertrophic responses, which were accompanied by attenuation of upregulation of NHE-1 and calcineurin activity.

Conclusions—Taken together, our results demonstrate a robust antihypertrophic and antiremodeling effect of ginseng, which is mediated by inhibition of NHE-1-dependent calcineurin activation. (Circ Heart Fail. 2011;4:79-88.)

Key Words: ginsenosides ■ phenylephrine ■ NHE-1 ■ calcineurin ■ heart failure

Ginseng is a popular herbal medicine that has been used in Asia for centuries, although, in recent years, its potential therapeutic effects have become more widely recognized. Ginsenosides are the principal bioactive constituents of ginseng, and >40 ginsenosides have been isolated to date.\(^1\) Ginseng exerts numerous pharmacological properties in multiple species, including humans.\(^2\) The cardiovascular beneficial effect of ginseng has also been demonstrated for the treatment of angina pectoris\(^3\) and for the reduction of adriamycin-induced heart failure in rats.\(^4\) Moreover, ginseng has been demonstrated to attenuate right and left ventricular hypertrophy in a number of experimental models.\(^5-7\) Because the underlying basis for the antihypertrophic effect of ginseng is poorly understood, we studied the mechanisms for the antihypertrophic effect of ginseng using cultured ventricular myocytes and an in vivo model of heart failure secondary to chronic ischemia. The study centered primarily on the \(Na^+/H^+\) exchanger-1 isoform (NHE-1), which has been extensively shown to contribute to hypertrophy and heart failure.\(^8,9\) Accordingly, we determined the effect of ginseng on NHE-1 activity and its expression and subsequent effects on key signaling mechanisms underlying the hypertrophic program. For example, NHE-1 has been shown to contribute to intracellular \(Ca^{2+}\) overloading, resulting in the activation of \(Ca^{2+}\)-dependent prohypertrophic processes mediated by the protein phosphatase calcineurin and subsequent activation of prohypertrophic transcriptional factors.\(^10,11\) Here, we determined the role of this pathway in mediating the antihypertrophic effect of ginseng in cultured ventricular myocytes and an in vivo model of heart failure secondary to chronic ischemia. The study centered primarily on the \(Na^+/H^+\) exchanger-1 isoform (NHE-1), which has been extensively shown to contribute to hypertrophy and heart failure.\(^8,9\) Accordingly, we determined the effect of ginseng on NHE-1 activity and its expression and subsequent effects on key signaling mechanisms underlying the hypertrophic program. For example, NHE-1 has been shown to contribute to intracellular \(Ca^{2+}\) overloading, resulting in the activation of \(Ca^{2+}\)-dependent prohypertrophic processes mediated by the protein phosphatase calcineurin and subsequent activation of prohypertrophic transcriptional factors.\(^10,11\) Here, we determined the role of this pathway in mediating the antihypertrophic effect of ginseng in cultured ventricular myocytes and an in vivo model of heart failure secondary to chronic ischemia. The study centered primarily on the \(Na^+/H^+\) exchanger-1 isoform (NHE-1), which has been extensively shown to contribute to hypertrophy and heart failure.\(^8,9\) Accordingly, we determined the effect of ginseng on NHE-1 activity and its expression and subsequent effects on key signaling mechanisms underlying the hypertrophic program. For example, NHE-1 has been shown to contribute to intracellular \(Ca^{2+}\) overloading, resulting in the activation of \(Ca^{2+}\)-dependent prohypertrophic processes mediated by the protein phosphatase calcineurin and subsequent activation of prohypertrophic transcriptional factors.\(^10,11\) Here, we determined the role of this pathway in mediating the antihypertrophic effect of ginseng in cultured ventricular...
myocytes subjected to hypertrophic stimuli and applied these findings to an in vivo model of heart failure in rats.

Methods

Neonatal Cardiac Myocytes Culture and Treatment Protocol

The studies have been approved by the Animal Use Subcommittee of the University of Western Ontario and procedures conform to the guidelines of the Canadian Council on Animal Care (Ottawa, Ontario, Canada). Myocytes were prepared from hearts of 1- to 4-day-old Sprague-Dawley rats as described previously\textsuperscript{12} and cultured for 24 hours in serum containing medium followed by 24 hours in serum-free medium. To initiate hypertrophy, myocytes were then treated with 10 \( \mu \text{m/L} \) \( \alpha_1 \) adrenoceptor agonist phenylephrine (PE) for 24 hours in the absence or presence of ginsenosides (0.1, 1, or 10 \( \mu \text{g/mL} \)). For some experiments (see Results), cells were subjected to PE treatment for shorter durations.

Ginsenoside Extraction Procedure

Four-year-old North American ginseng (\textit{Panax quinquefolius}) roots were collected from 5 farms in Ontario, Canada and shipped to Naturex (South Hackensack, NJ) for ginsenoside extraction with use of a hydroalcoholic process. In brief, ground ginseng roots were soaked 3 times over 5 hours in an ethanol/water (75:25 \text{v/v}) solution at 40°C. The extract was filtered and excess solvent removed under vacuum at 45°C. The extract was concentrated again until the total solids on a dry basis were \textasciitilde 60\%. These concentrates were then lyophilized at the Ontario Ginseng Innovation and Research Consortium central laboratory (University of Western Ontario) to produce a powdered alcoholic ginseng extract, which was then subjected to analysis by high-pressure liquid chromatography (HPLC) to determine the presence of major (Rb1 and Re) and minor (Rg1, Rb2, Rd, and Rc) ginsenosides.

Measurement of Cell Surface Area

Myocytes were visualized using a Leica DMIL inverted microscope (Leica, Wetzlar, Germany) equipped with an Infinity 1 camera. At least 10 random photographs were taken from each dish, and the cell surface area of a minimum of 30 cells from each treatment was measured using SigmaScan Software (Systat, Richmond, Calif).

Measurement of Intracellular Na\(^+\) and Ca\(^{2+}\) Concentrations

Myocytes were incubated with CoroNa Red (excitation: 554 nm; emission: 578 nm) or Furo-2 (excitation: 338 nm; emission: 510 nm) for 30 minutes at 37°C, to measure Na\(^+\) or Ca\(^{2+}\) concentrations, respectively. Myocytes were washed twice with phosphate-buffered saline (PBS), and fluorescence intensity was measured using a Spectra Max M5 plate reader. Fluorescence intensity was normalized against control (CoroNa-Red-loaded cells or Furo-2-loaded cells) and 440 nm for excitation and 528 nm for emission using a monochromatic Deltascan-4000 system (Photon Technology International, Birmingham, NJ). The NH\(_4\)Cl prepulse technique was used to determine the presence of NHE-1 and the effect of treatments on NHE-1 activity in cardiomyocytes\textsuperscript{13}.

Calcineurin Phosphatase Activity Assay

Calcineurin activity was determined using commercially available kits according to the manufacturer’s instructions (Enzo Life Sciences, Plymouth Meeting, Pa).

Determination of NFAT3 Translocation

Myocytes were fixed in an aceton and methanol (20:80) mixture as described previously\textsuperscript{14}.

After permeabilization and blocking, cells were incubated with NFAT3 antibody (1:100 dilutions) overnight at 4°C followed by incubation with Alexa Fluor 594 goat anti-rabbit IgG (1:250 dilutions) for 1 hour at room temperature in darkness. The cells were mounted on the glass slide using DakoCytomation fluorescent mounting medium and visualized using a Zeiss Axio Observer D1 fluorescence microscope (Zeiss, Gottingen, Germany).

Electrophoretic-Mobility Shift Assay (EMSA)

EMSA\textsubscript{\textregistered}s were performed using the Panomics EMSA Gel-Shift Kit (Panomics, Inc., Fremont, Calif) according to the manufacturer’s protocol.

RNA Isolation, Reverse Transcription and Real-Time PCR Analysis

RNA was extracted using Trizol (Invitrogen, Carlsbad, Calif) according to the manufacturer’s instructions. RNA (2 \( \mu \text{g} \)) was used to synthesize the first strand of cDNA using M-MLV reverse transcriptase according to the manufacturer’s protocol and was used as a template in the following PCR reactions. The expression of ANP,
NHE-1, MCIP1, and 18S rRNA (loading control) genes was determined in 10-H9262 L reaction volumes using SYBR green Jumpstart Tag ReadyMix DNA polymerase and fluorescence was measured and quantified using DNA Engine Opticon 2 System. The following primer sequences were used: 5'-CTGCTAGACCACCTGAGGA-3' (forward) and 5'-AAGCTGTTGCAGCCTAGTCC-3' (backward) for ANP; 5'-ATGTGGCTGGGAAACAAGAC-3 (forward) and 5'-GACAGTCCCTCCCGTGTAAA-3 (backward) for NHE-1; 5'-GCC-CAATCCAGACAAACAGT-3 (forward) and 5'-TGATTTTTGGCTTGGGTCTC-3 (backward) for MCIP1; and 5'-GTAACC-CTTGAACCCCATT-3 (forward) and 5'-CCATCCAATCGGTAGTAGCG-3 (backward) for 18S rRNA. PCR conditions and cell cycle number were optimized for each set of primers. Melting curve analysis showed a single PCR product for each gene amplification. PCR conditions to amplify all 3 genes were 30 seconds at 94°C followed by annealing at 60°C for 25 seconds for ANP, MCIP1, and NHE1 and 54°C for 20 seconds for 18S rRNA followed by elongation at 72°C for 30 s. All genes were amplified for 40 cycles except 18S rRNA, which was amplified for 35 cycles.

Western Blotting for GATA-4 and NHE-1
After appropriate treatments, myocytes were washed with PBS and lysed with 150 μL of lysis buffer. Cell lysates were transferred to 1.5-mL Eppendorf tubes, homogenized, and centrifuged at 10 000 g for 5 minutes at 4°C. The supernatant was transferred to a fresh tube and the protein concentration determined by the Bradford protein assay method (Bio-Rad, Hercules, Calif). Thirty micrograms of protein were resolved on a 10% SDS-polyacrylamide gel and transferred to nitrocellulose membranes. The membranes were blocked in 5% milk for 1 hour and incubated with primary antibody for GATA-4 or NHE-1 for 1 hour, followed by a secondary antibody for 1 hour, then detected by enhanced chemiluminescence reagent (Amersham Biosciences Inc., Piscataway, NJ). The blots were stripped and reprobed with actin antibodies.

Coronary Artery Ligation
Male Sprague-Dawley rats (275 to 300 g) were randomly assigned to the following 4 treatment groups: sham and coronary artery ligation (CAL) with or without ginsenosides (100 mg/kg) treatment started 24 hours after surgery. Surgery was performed as previously described.15 All animals received 0.03 mg/kg buprenorphine immediately after completion of surgery for pain management.

Echocardiography
Four weeks after CAL, rats were anesthetized with 2% isoflurane and placed in a supine position on a heated platform. The chest and abdomen were shaved and the extremities were fixed to electrodes on the platform surface by use of tape and a highly conductive electrode gel. Echocardiography evaluations were performed using a Vevo 770 high-resolution in vivo microimaging system equipped with a real-time microvisualization scan head of 17.5 MHz (VisualSonics, Toronto, Ontario, Canada). M-mode 2-dimensional echocardiography images were obtained from the parasternal short axis. Images were analyzed using the Vevo 770 Protocol-Based Measurements software and calculations for the dimensions of the left ventricle (LV) diameter. Doppler measurements were taken to determine peak early diastolic filling velocity (E wave), peak late diastolic filling velocity (A wave), and E/A ratios.

Hemodynamic Measurements
The rats were anesthetized with pentobarbital sodium (50 mg/kg, ip). An anterior thoracotomy was performed, and the LV was catheter-
ized retrogradely via the right carotid artery using a 2.0F P-V Mikro-Tip catheter (Millar Instruments, Houston, Tex) as previously described.15 Data were recorded and analyzed by hemodynamic data analysis software (Notocord, Croissy-sur-Seine, France), digitized with a sampling rate of 1000 Hz, and recorded on a personal computer using Notocord-hem 4.2 software.

Statistical Analysis
Results are presented as means±SEM. The data were analyzed with 1-way ANOVA and group differences were detected using a Student-Newman-Keuls post hoc test when initial ANOVA analysis revealed statistically significant differences. P values of <0.05 were considered significant.

Results
Effect of Ginseng on PE-Induced Cardiomyocyte Hypertrophy
Myocytes treated with PE for 24 hours demonstrated a significant increase in cell surface area from 850±14 μm² to 1090±20 μm² (Figure 1A and 1B; P<0.05); whereas in the presence of 10 μg/mL ginsenosides cell surface area in the presence of PE was reduced to 942±44 μm² (Figure 1B; P<0.05 versus PE alone). PE increased gene expression of ANP by 2-fold (Figure 1B; P<0.05) and increased GATA-4–DNA binding (Figure 5B). Moreover, stimulation of NHE-1 activity was reduced to values not significantly different from control by 10 μg/mL ginsenosides (Figure 3B).

Effect of Ginseng on PE-Induced Changes in NHE-1 Protein, Gene Expression and Activity
Figure 3 shows that PE induced a 1.56±0.09-fold upregulation of NHE-1 gene (Figure 3A) and a 1.38±0.09-fold increase in protein abundance (Figure 3B) after 24 hours of treatment, which was associated with increased NHE-1 activity (Figure 3C). The upregulation of NHE-1 gene and protein expression was abrogated by the 2 highest concentrations of ginsenosides with increases for gene and protein levels in the presence of 10 μg/mL ginsenosides reduced to 1.18±0.04-fold and 1.07±0.10-fold, respectively (Figure 3A and 3B). Moreover, stimulation of NHE-1 activity was reduced to values not significantly different from control by 10 μg/mL ginsenosides (Figure 3C).

Effect of Ginseng on PE-Induced Changes in Intracellular Na⁺ and Ca²⁺ Concentrations
PE induced a rapid elevation in intracellular concentrations of both Na⁺ and Ca²⁺ that was evident 15 minutes after PE addition (Figure 4). No effect of ginseng (10 μg/mL ginsenosides) was observed up to 6 hours after its addition, although a significant reduction in the intracellular concentrations of both Na⁺ and Ca²⁺ was evident 12 and 24 hours after administering ginsenosides with values not significantly different from control after 24 hours.

Effect of Ginseng on PE-Induced Changes in Calcineurin Activity
As shown in Figure 5A calcineurin activity was rapidly (within 15 minutes) increased after PE administration with activity steadily declining in the presence of ginsenosides after 6 hours, although still significantly greater than control. Ginsenosides alone had no effect on calcineurin activity (Figure 5A).

Effect of Ginseng on PE-Induced Changes in NFAT3 Nuclear Import and GATA-4 Activation
Localization of NFAT3 in control myocytes was primarily restricted to the cytosol (Figure 5B), although substantial translocation to nuclei was evident after 24 hours of PE treatment. Ginsenosides clearly reduced PE-induced translocation resulting in substantial cytosolic localization of NFAT3 similar to that seen under control conditions (Figure 5B). PE significantly increased GATA-4 phosphorylation by 1.3-fold (Figure 5C; P<0.05) and increased GATA-4–DNA bind-
ing activity as determined by EMSA (Figure 5D). Both responses were prevented by ginsenosides (Figure 5C and 5D).

Effect of Ginseng on CAL-Induced Left Ventricular Dysfunction

We next determined whether the direct antihypertrophic effect of ginsenosides seen in cultured myocytes can be translated to protection in vivo in rats subjected to 4 weeks of sustained CAL. As shown in the Table, CAL produced marked systolic and diastolic abnormalities, which were attenuated by ginsenoside treatment. Moreover, ginsenoside treatment significantly reduced the increase in left ventricular inner diameters in rats subjected to sustained CAL (Figure 6A and 6B). In addition, CAL increased the E/A ratio obtained from Doppler echocardiographic analysis, which was normalized in animals treated with ginseng indicative of improved diastolic function (Figure 6C and 6D).

Effect of Ginseng on CAL-Induced Cardiac Hypertrophy

Animals subjected to CAL had significantly reduced body weights at the end of the 4-week ligation period, although this was unaffected by ginsenosides (Figure 7A). Animals subjected to CAL exhibited significantly increased left ventricle weights as well as ANP expression (Figure 7D), indicating development of left ventricular hypertrophy (Figure 7B to 7D). These responses were completely prevented by ginsenosides.

Effect of Ginseng on CAL-Induced NHE-1 and Expression and Calcineurin Activation

As shown in Figure 8A, rats subjected to CAL had significantly increased NHE-1 expression (1.98±0.16-fold) although this was partially but significantly reduced by ginsenoside treatment (1.41±0.11-fold) (Figure 8A). Two indicators of calcineurin activity, namely modulatory calcineurin interacting protein 1 (MCIP1) expression (Figure 8B) and calcineurin phosphatase activity (Figure 8C) were significantly increased in hearts subjected to CAL, although these responses were completely abrogated by ginsenosides (Figure 8B and 8C).

Discussion

Although ginseng has been used as a pharmacotherapeutic agent in Asian society for centuries, its potential cardiac
therapeutic properties have not been extensively studied and are poorly understood. Ginseng is also among the most common of the alternate medicines used by the American population, although not necessarily for cardiovascular disorders. Whether the use of natural compounds such as ginseng holds promise for the treatment of cardiovascular disorders is not known, possibly because of a paucity of data demonstrating their effects in well-established experimental models of cardiovascular disease and the lack of information on the mechanism of action of these compounds. Here, we determined the potential antihypertrophic effect of the bio-logically active components of ginseng, the ginsenosides,

Table. Hemodynamic Data

<table>
<thead>
<tr>
<th></th>
<th>Sham (n=8)</th>
<th>Sham + Gins (n=6)</th>
<th>CAL (n=8)</th>
<th>CAL + Gins (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVESP (mm Hg)</td>
<td>120.81±5.9</td>
<td>127.81±4.3</td>
<td>96.92±2.2†</td>
<td>113.75±3.3†</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>3.64±0.30</td>
<td>4.01±0.53</td>
<td>11.73±0.46*</td>
<td>7.76±0.40†</td>
</tr>
<tr>
<td>+dP/dt (mm Hg/s)</td>
<td>9711±408</td>
<td>8519±342</td>
<td>4770±682*</td>
<td>6550±164.2†</td>
</tr>
<tr>
<td>-dP/dt (mm Hg/s)</td>
<td>−8247±413</td>
<td>−7933±421</td>
<td>3755±860*</td>
<td>−6568±195.8†</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>395.68±8.2</td>
<td>385.98±7.4</td>
<td>397.68±6.1</td>
<td>393.62±9.0</td>
</tr>
<tr>
<td>LVESV (µL)</td>
<td>85.46±5.3</td>
<td>89.17±2.6</td>
<td>181.47±10*</td>
<td>108.45±6.0†</td>
</tr>
<tr>
<td>LVEDV (µL)</td>
<td>207.53±5.8</td>
<td>209.87±7.1</td>
<td>279.60±10.3</td>
<td>215.27±8.6†</td>
</tr>
<tr>
<td>SV (µL)</td>
<td>124.70±4.6</td>
<td>127.56±3.8</td>
<td>98.17±4.7*</td>
<td>113.76±4.0†</td>
</tr>
<tr>
<td>EF (%)</td>
<td>59.64±2.3</td>
<td>60.29±1.1</td>
<td>38.43±2.3*</td>
<td>48.97±1.2†</td>
</tr>
<tr>
<td>CO (mL/min per kg)</td>
<td>122.95±4.2</td>
<td>119.79±3.6</td>
<td>100.26±3.3</td>
<td>113.68±3.4†</td>
</tr>
</tbody>
</table>

All results are shown as means±SEM. CAL, coronary artery ligation; Gins, ginsenosides (100 mg/kg); LVESP, left ventricular end-systolic pressure; LVEDP, left ventricular end-diastolic pressure; +dP/dt and -dP/dt, rate of left ventricular pressure development and relaxation, respectively; HR, heart rate; ESV, end-systolic volume; EDV, end-diastolic volume; SV, stroke volume; EF, ejection fraction; CO, cardiac output. *P<0.05 versus sham; †P<0.05 versus CAL.

Figure 6. Effect of ginsenosides on CAL-induced changes in left ventricular internal diameters and transmitral velocity. Animals were subjected to either CAL or sham operation, with or without daily ginsenoside treatment (100 mg/kg) for 4 weeks. A, Short-axis biventricular M-mode images. B, Quantification of end-diastolic and end-systolic LVID. C, representative images of long-axis transmitral velocity. D, Quantification the E/A ratio. Data are shown as mean±SEM. *P<0.05 versus respective sham; #P<0.05 versus CAL, n=6 to 10. CAL indicates coronary artery ligation; LVID, left ventricular internal diameter; Gins, ginsenosides; LVIDd, end-diastolic LVID; LVIDs, end-systolic LVID.
extracted from North American ginseng, on the hypertrophic response of myocytes exposed to the α1 adrenoceptor agonist PE. We show that ginsenosides provide a robust antihypertrophic influence in this model of hypertrophy, although our study also suggests that the antihypertrophic effect of ginseng is likely not restricted to PE particularly because it would be unlikely that the robust salutary effects seen in vivo were mediated solely by an effect restricted to inhibition of α1 adrenoceptor-mediated hypertrophy. Moreover, our study shows that ginsenosides also markedly attenuate the direct hypertrophic of both angiotensin II and endothelin-1 on myocytes.

Overall, our study strongly suggests that the ability of ginsenosides to attenuate hypertrophy is related to preventing the activation/upregulation of NHE-1, which has been extensively implicated in the hypertrophic and heart failure process.17,18 This probably occurs subsequent to receptor-dependent NHE-1 activation. NHE-1 activation results in a number of intracellular alterations that can contribute to the hypertrophic program,10,19,20 although a particularly important consequence of NHE-1 activation is the elevation in intracellular Na+/H+ concentrations, which is followed by increases in intracellular Ca2+ concentrations via reverse mode Na-Ca exchange activity.21 As discussed below, this in turn would induce hypertrophy by activating key factors in the hypertrophic program, especially the phosphatase calcineurin that results in transcriptional changes due to NFAT3 dephosphorylation and its translocation into nuclei. The ability of ginsenosides to attenuate the hypertrophic effects of both angiotensin II and endothelin-1, 2 NHE-1 activators,22 further supports NHE-1 as a target for their salutary effects. Nonetheless, the possibility that ginsenosides are acting through other or additional nonspecific mechanisms cannot be ruled out and requires further studies.

In the present study we used an alcoholic extract of ginsenosides to demonstrate a potent antihypertrophic effect in vitro, as well as a highly effective ability to reduce hypertrophy and heart failure in vivo, through what appears to be identical mechanisms. The ability of ginsenosides to block the hypertrophic response to the α1 adrenoceptor agonist PE at the highest concentration equaled the antihypertrophic effect observed with the NHE-1 inhibitor cariporide (data not shown). The antihypertrophic effect of ginseng in vitro is in partial agreement with a previous study demonstrating that ginsenosides inhibit prostaglandin F2α-induced hypertrophy through a mechanism involving attenuation in the increased expression levels of calcineurin and various transcriptional factors.23 We were unable to observe any changes in abundance (either gene or protein) of calcineurin (data not shown), but rather, activation of calcineurin was the primary response to hypertrophic stimuli. Ginseng prevented the upregulation of NHE-1 gene and protein abundance and depressed NHE-1 activity 24 hours after PE treatment. Addition of PE produced a rapid elevation of intracellular Na+ and Ca2+ concentrations and calcineurin activation. Interestingly, the early up-regulation of these factors was unaffected by ginseng up to 6 hours after PE administration, except for a significant inhibition in intracellular Na+ concentrations at 6 hours, whereas all factors were significantly inhibited 12 and 24 hours after PE treatment. Addition of PE produced a rapid elevation of intracellular Na+ and Ca2+ concentrations and calcineurin activation. Interestingly, the early up-regulation of these factors was unaffected by ginseng up to 6 hours after PE administration, except for a significant inhibition in intracellular Na+ concentrations at 6 hours, whereas all factors were significantly inhibited 12 and 24 hours after PE administration. Because NFAT3 translocation into nuclei and activation of the transcriptional factor GATA-4 24 hours after PE administration were markedly inhibited by ginseng, the results suggest that late inhibition of calcineurin activation via Na+- and Ca2+-dependent mechanisms is important in attenuating the hypertrophic response to PE, at least with respect to the antihypertrophic effect of ginsenosides.

Using a rat CAL model we also show that oral administration of ginseng reduces hypertrophy and hemodynam-
myocytes vis-à-vis NHE-1 and calcineurin in that the substantial concordance with the effect seen in cultured beneficial effect of ginseng in this model demonstrated more difficult to establish using in vivo approaches, the and molecular markers. Although mechanistic insights are improved hemodynamic function was associated with di-
malization of hemodynamic dysfunction as assessed both
spectrum in humans, which can range between 0.5 and 15
roughly approximate the mid high end of the dosing
produced an optimal salutary effect in this model. Com-
failure. We used a ginseng dose of 100 mg/kg, which

**Figure 8. Attenuation of CAL-induced elevation in NHE-1, MCIP1 gene expression and calcineurin activation by ginsen-
oses. Animals were subjected to either CAL or sham proce-
dure, with or without daily ginsenoside treatment (100 mg/kg)
for 4 weeks. A, Quantification of NHE-1 gene expression levels.
B, Quantification of MCIP1 gene expression levels. C, Cal-
cineurin phosphatase activity. Data are shown as mean±SEM,
n=6 to 10. *P<0.05 versus respective sham; #P<0.05 versus CAL. CAL indicates coronary artery ligation; Gins, ginsenosides.**

ic abnormalities in this well-established model of heart failure. We used a ginseng dose of 100 mg/kg, which produced an optimal salutary effect in this model. Comparison of this dose with the clinical use of ginseng is difficult because of the lack of standardization in terms of dosing or the paucity of well-controlled clinical trials. Moreover, different ginseng varieties exhibit varied biological profiles. Nonetheless, the dose in our study would roughly approximate the mid high end of the dosing spectrum in humans, which can range between 0.5 and 15 g/d. The salutary effect of ginseng was evident in terms of substantial attenuation and, in some cases, the near normalization of hemodynamic dysfunction as assessed both by invasive catheter-based determinations of hemodynamic parameters and by echocardiography. Moreover, the improved hemodynamic function was associated with diminished hypertrophy determined by gravimetric analysis and molecular markers. Although mechanistic insights are more difficult to establish using in vivo approaches, the beneficial effect of ginseng in this model demonstrated substantial concordance with the effect seen in cultured myocytes vis-à-vis NHE-1 and calcineurin in that the improved cardiovascular properties were associated with diminished expression of NHE-1 and prevention of calcineurin activation, the latter determined by measurement of phosphatase activity and expression levels of MCIP1, an index of calcineurin activation.24

Based on current knowledge, the ability of ginseng to inhibit NHE-1 activity may represent its pivotal effect in terms of attenuating the hypertrophic response. As previously noted, from a general perspective, there is substantial evi-
dence implicating NHE-1 as a key contributor to myocardial hypertrophy, remodeling, and heart failure, because the antiporter is upregulated in heart failure and its inhibition reduces the severity of the hypertrophic response and the development of cardiovascular dysfunction.10,15,25 The nature of NHE-1 involvement in the hypertrophic response is not completely understood, although NHE-1 activation could result in a number of intracellular changes resulting in stimulation of the hypertrophic program.10,19,20 Ca^{2+}-mediated signaling has gained substantial attention among many molecular mechanisms that are known to coordinate development of pathological hypertrophy.26 In particular, Ca^{2+}-dependent activation of calcineurin leads to dephosphorylation of NFAT3 which subsequently translocates to the nucleus where it acts with other transcription factors including GATA-4 to initiate gene transcription. The ability of ginseng to attenuate calcineurin activation, NFAT3 translocation as well as GATA-4 phosphorylation and DNA-binding activity suggest that this represents the target for its antihypertrophic effect. Among these responses is the elevation in intracellular Ca^{2+} concentrations, secondary to elevations in intracellular Na+ concentrations, resulting in reverse-mode Na+-Ca^{2+} exchange activation that results in the active import of Ca^{2+} into the cell.27–31 There is emerging strong evidence in the literature that Ca^{2+}-dependent initiation of the hypertrophic program is NHE-1 dependent. For example, the antihypertrophic effect of NHE-1 inhibition in spontaneously hypertensive rats has been shown to be associated with normalization of the calcineurin pathway in the hearts of these animals.32 Moreover, a recent study demonstrated that cardiac NHE-1 overexpression in transgenic mice activates the Ca^{2+}-dependent hypertrophic program as manifested by increased intracellular Ca^{2+} concentrations, calcineurin activ-
ation, and establishment of cardiac hypertrophy and heart failure, in the absence of insult.10 Importantly, the activation of the Ca^{2+}-dependent hypertrophic cell signaling process and the hypertrophy itself can be abrogated by the NHE-1 specific inhibitor cariporide.10,33

In conclusion, we have used an integrative approach to demonstrate a robust antihypertrophic effect of ginseng in cultured neonatal myocytes exposed to hypertrophic stimuli as well as an in vivo model of heart failure secondary to sustained CAL. (The effects of ginseng in vivo translate to improved hemodynamic status 4 weeks after CAL.) Our results further suggest a common underlying mechanism involving NHE-1 inhibition resulting in the attenuation of calcineurin activation. A present limitation of our study is our inability to identify the nature of the specific ginsenoside that may account for the salutary effects on hypertrophy and heart failure. As alluded to previously, >40
ginsenosides have thus far been identified. Using HPLC analysis, the primary ginsenosides identified in the extract used in the present study were Rb1 and Re (each ~45% of total ginsenosides) whereas the content of minor ginsenosides (Rg1, Rb2, Rd, and Re) was between 1% and 7% (Dr EMK Lui, University of Western Ontario and Dr JT Arnason, University of Ottawa, personal communication). From a quantitative perspective, it is attractive to speculate that Rb1 and Re represent the principal ginsenosides accounting for the antihypertrophic/antiremodeling effects, although this needs to be determined with further studies. It is also possible that the beneficial effects represent the combined actions of a number of these compounds present in the ginseng extract. This limitation notwithstanding, our study suggests that administration of ginseng may represent an effective adjunct therapy for the limitation of myocardial hypertrophic response and for the treatment of heart failure. Indeed, interaction between ginseng and therapeutic agents used for the treatment of heart failure would be valuable for future studies to explore.

Sources of Funding

This study was supported by the Institute of Circulatory and Respiratory Health of the Canadian Institutes of Health Research and the Ontario Ginseng Innovation and Research Consortium (OGIRC) (Ontario Ministry of Research and Innovation Grant RE02-049). Dr J. Guo was supported by OGIRC. Dr M. Karmazyn holds a Canada Research Chair in Experimental Cardiology.

Disclosures

None.

References

29. Baartscheer A, Schumacher CA, van Borren MM, Belteman CN, Coronel R, Fiolet JW. Increased Na+/H+ exchange activity is the cause
of increased [Na⁺], and underlies disturbed calcium handling in the rabbit pressure and volume overload heart failure model. Cardiovasc Res. 2003; 57:1015–1024.


**CLINICAL PERSPECTIVE**

Heart failure is a major cause of hospitalization and is associated with high financial costs and societal burden. Cardiac hypertrophy and myocardial infarction are major risk factors for the development of heart failure. In recent years, there has been growing interest in botanicals as alternative medicines for cardiovascular disease. Ginseng is a popular traditional herbal medicine and has been widely used to treat cardiovascular diseases for thousands of years in Asia. Despite increasing interest in ginseng in Western societies, its efficacy and mechanism of action are poorly understood. We sought to understand the effect of ginsenosides on cardiomyocyte hypertrophy and myocardial infarction-induced heart failure. In in vitro studies, we showed that ginsenosides were able to attenuate cardiomyocyte hypertrophy. Further studies revealed that the antihypertrophic effect of ginsenosides was mediated by inhibiting NHE-1 activity and blocking calcium-mediated signaling, which is evidenced by decreased intracellular calcium levels, calcineurin activity, and NFAT3 translocation. In support of in vitro findings, our in vivo studies demonstrated that oral administration of ginsenosides prevented the progression of heart failure by reducing postinfarction myocardial hypertrophy and improving hemodynamics. Although results from animal studies must be interpreted cautiously, our findings support the development of ginsenosides as potential therapeutics for the treatment of cardiac diseases and demonstrate the cellular bases for these actions.
Ginseng Inhibits Cardiomyocyte Hypertrophy and Heart Failure via NHE-1 Inhibition and Attenuation of Calcineurin Activation
Juan Guo, Xiaohong Tracey Gan, James V Haist, Venkatesh Rajapurohitam, Asad Zeidan, Nazo Said Faruq and Morris Karmazyn

_Circ Heart Fail_. 2011;4:79-88; originally published online October 22, 2010; doi: 10.1161/CIRCHEARTFAILURE.110.957969

_Circulation: Heart Failure_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3289. Online ISSN: 1941-3297

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circheartfailure.ahajournals.org/content/4/1/79

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation: Heart Failure_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation: Heart Failure_ is online at:
http://circheartfailure.ahajournals.org/subscriptions/