Ginseng Reverses Established Cardiomyocyte Hypertrophy and Postmyocardial Infarction-Induced Hypertrophy and Heart Failure

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Background—A major challenge in the treatment of heart failure is the ability to reverse already-established myocardial remodeling and ventricular dysfunction, with few available pharmacological agents prescribed for the management of heart failure having demonstrated successful reversal of the remodeling and hypertrophic processes. North American ginseng (Panax quinquefolius) has previously been shown to effectively prevent cardiomyocyte hypertrophy and heart failure. Here, we determined whether North American ginseng can reverse established cardiomyocyte hypertrophy in cultured myocytes as well as hypertrophy and left ventricular dysfunction in experimental heart failure secondary to coronary artery occlusion.

Methods and Results—Ginseng was administered in drinking water (0.9 g/L) ad libitum to rats after 4 weeks of sustained coronary artery ligation when heart failure was established or to angiotensin II- (100 nmol/L), endothelin-1- (10 nmol/L), or phenylephrine- (10 µmol/L) induced hypertrophic cultured neonatal ventricular cardiomyocytes. Echocardiographic and catheter-based measurements of hemodynamic parameters 4 weeks after starting ginseng treatment (8 weeks postinfarction) revealed nearly complete reversibility of systolic and diastolic abnormalities. Similarly, ginseng administration to hypertrophic cardiomyocytes resulted in a complete reversal to a normal phenotype after 24 hours as determined by cell surface area and expression of molecular markers. The effects of ginseng both in vivo and in cultured cardiomyocytes were associated with reversal of calcineurin activation and reduced nuclear translocation of the transcription factor NFAT3 (nuclear factor of activated T cells 3) in cultured myocytes. Moreover, the beneficial effect of ginseng was associated with normalization in the gene expression of profibrotic markers, including collagen (I and III) and fibronectin.

Conclusions—This study demonstrates a marked ability of ginseng to reverse cardiac hypertrophy, myocardial remodeling, and heart failure, which was associated with and likely mediated by reversal of calcineurin activation. Ginseng may offer a potentially effective approach to reverse the myocardial remodeling and heart failure processes, particularly in combination with other treatment modalities. (Circ Heart Fail. 2012;5:504-514.)

Key Words: heart failure ■ cardiomegaly ■ Panax ■ remodeling ■ calcineurin ■ NFAT transcription factors

Cardiovascular disease remains a major cause of mortality in the world.1 Although survival in patients with ischemic heart disease has markedly improved over the past number of years, the incidence of heart failure continues to rise.2 Current pharmacotherapies for the treatment of heart failure have proven to be of substantial benefit in prolonging and improving the quality of life for patients with heart failure without markedly reducing mortality rates,3,4 which currently are >40% 5 years after diagnosis.5 The high mortality rate associated with heart failure likely reflects the complexity of the myocardial remodeling processes that contribute to heart failure and the difficulty in reversing these processes with current pharmacotherapy.6

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Improvement in the treatment of heart failure will likely result as a consequence of better understanding the underlying mechanisms for this complex syndrome, which should ultimately lead to the development of novel and effective pharmacological agents.7 Another possible and generally unexplored route for the identification of new heart failure therapeutic approaches may lie with alternative and less...
conventional medications, such as traditional Chinese medicines that have been used in Asian societies for treating a variety of disorders, including cardiovascular diseases, for thousands of years. Among these traditional Chinese medicines, North American ginseng (Panax quinquefolius) as well as other ginseng varieties have been garnering increasing interest in western societies for their salutary effects on the cardiovascular system. Recently, we have demonstrated a robust ability of North American ginseng to prevent cardiomyocyte hypertrophy and heart failure through a mechanism likely involving prevention of calcineurin activation, the latter representing a key factor for myocardial hypertrophy and remodeling. In view of a recent study indicating calcineurin-dependent cardiac hypertrophy as a reversible process and the potential importance of calcineurin as a target for the antihypertrophic effect of ginseng, we determined whether the latter can reverse already-established hypertrophy and heart failure using both in vitro and in vivo approaches.

**Methods**

The protocol for the use of animals was approved by The University of Western Ontario Animal Care and Use Committee and conformed to the guidelines in the Guide for the Care and Use of Laboratory Animals published by the Canadian Council of Animal Care (Ottawa, ON, Canada).

**In Vivo Studies**

The protocol for the in vivo study is summarized in Figure 1A. Forty male Sprague-Dawley rats (aged 53–55 days) with an average body weight of 228±8.3 g were randomly assigned to either a sham or coronary artery ligation (CAL) group without or with ginseng treatment (0.9 g/L in drinking water provided ad libitum) started 4 weeks after surgery and maintained for a further 4 weeks. CAL was performed as previously described under sodium pentobarbital (50 mg/kg body weight) anesthesia. Buprenorphine (0.03 mg/kg body weight) was immediately administered to all animals after completion of surgery for pain management. The studies were completed after a total of 8 weeks of sustained CAL or sham surgery at which time animals were subjected to final echocardiography and catheter-based hemodynamic assessment before euthanasia. Blood samples were also obtained at this time to determine plasma glucose and creatinine levels using commercially available kits (Cayman Chemical Co).

**Echocardiography**

Animals were subjected to echocardiographic analyses before surgery as well as 4 and 8 weeks after surgery (online-only Data Supplement).
Hemodynamic Measurements and Tissue Processing
Eight weeks after surgery, rats were anesthetized with pentobarbital sodium (50 mg/kg body weight), and an anterior thoracotomy was performed as previously described. A 2.0F P-V Mikro-Tip catheter (Millar Instruments) was retrogradely inserted into the left ventricle through the right carotid artery. Hemodynamic data were recorded and analyzed using Notocord-Hem 4.2 software (Notocord) digitized with a sampling rate of 1000 Hz. The hearts were then removed, blotted dry, and weighed. Left ventricular weights (free wall without the septum) were also obtained. For subsequent tissue biochemical or molecular assessment, the infarct area was removed, and viable tissue from the left ventricular free wall remote from the infarct area was processed as described later.

Cultured Cardiomyocyte Treatment and Experimental Groups
Neonatal ventricular cardiomyocytes from 1- to 3-day-old Sprague Dawley rats were isolated and cultured as previously described. Cells were grown in fetal bovine serum-containing medium for the first 24 hours after digestion, after which the serum-containing medium was replaced and the myocytes were washed and recultured in serum-free medium for 24 hours before agonist administration and throughout the treatment period. For reversal experiments, cardiomyocytes were pretreated with either angiotensin II 100 nmol/L, endothelin-1 (ET-1) 10 nmol/L, or the α-1 adrenoceptor agonist phenylephrine 10 μmol/L (all from Sigma-Aldrich) for up to 24 hours followed by the addition of ginseng extract 10 μg/mL for a further 24 hours in the presence of continued exposure to the respective hypertrophic stimulus.

Cell Surface Area Measurement
A Leica microscope equipped with an Infinity 1 camera (Lumenera Corp, Ottawa, Ontario, Canada) was used to obtain cardiomyocyte images using 10× magnification. The surface area of a minimum of 50 cells per treatment group was measured using SigmaScan Pro 5 software (Systat) and averaged to produce one N value.

Gene Expression Analyses
To determine gene expression, RNA was collected from either left ventricular tissue remote from the infarct area or cardiomyocytes and reverse transcribed to cDNA, and the gene product was quantified by real-time polymerase chain reaction as previously described. Primer gene sequences are shown in online-only Data Supplement Table I.

Calcineurin Activity Assay
Calcineurin activity from cellular and tissue extracts collected with a lysis buffer and protease inhibitor cocktail was measured using a colorimetric Calcineurin Cellular Activity Kit (Enzo Life Sciences) as per the manufacturer’s instructions. Calcineurin activity was calculated as the nanomol amount of phosphate released at 620 nm using a SpectraMax 5 (Molecular Devices) plate reader from tissue and cellular extracts.

Immunofluorescence
Nuclear factor of activated T cells (NFAT) translocation was also visualized using immunofluorescence as previously described (online-only Data Supplement).

Ginseng Extract
Ginseng extract was provided by the Ontario Ginseng Innovation and Research Consortium (The University of Western Ontario). Four-year-old North American ginseng roots collected in 2007 from 5 different farms in Ontario, Canada, were provided by the Ontario Ginseng Growers Association, and the 75% ethanolic extracts were prepared by Naturex (South Hackensack, NJ) and characterized as described previously.

Statistical Analysis
Data were analyzed by 1-way ANOVA followed by a post hoc Tukey test to determine the effect of CAL and potential influence of ginseng. Echocardiographic data were analyzed using 2-way ANOVA with repeated measures and a post hoc Tukey test. P<0.05 was considered significant.

Results
Ginseng Reverses CAL-Induced Cardiac Hypertrophy
We first determined whether ginseng administration 4 weeks after CAL reverses indices of myocardial remodeling and heart failure after a further 4 weeks of follow-up with continued ligation. Animals were administered North American ginseng at a dose of 0.9 g/L dissolved in the drinking water 4 weeks after initiating CAL (Figure 1A). Water consumption was monitored daily and found to be identical (=50 mL/day per animal) in all groups studied. Sustained CAL produced no mortality during the 8-week post-CAL period, although 20% of the animals died within 24 hours after CAL. These animals were replaced to ensure the maintenance of 10 animals per group at the end of the study. All animals exhibited identical growth patterns throughout the 8-week postsurgery period (Figure 1B). CAL produced a significant increase in cardiac hypertrophy as evidenced by increased expression of α-skeletal actin (Figure 1C), myosin heavy chain (Figure 1D), and both total (Figure 1D) and left ventricular (Figure 1E) heart weights, all of which were normalized by ginseng treatment. Plasma glucose or creatinine concentrations were unaffected by any treatment (online-only Data Supplement Table II).

Evidence of Reversibility of CAL-Induced Left Ventricular Dysfunction by Serial Echocardiography
Reversibility of cardiac dysfunction by ginseng was assessed by serial echocardiography in which animals were analyzed before surgery, 4 weeks after surgery but before starting ginseng treatment, and 8 weeks after surgery. As shown in Figure 2, animals subjected to 4 weeks of CAL had significantly depressed ejection fraction, fractional shortening, cardiac output, and stroke volume, with values progressively declining during the remaining 4-week follow-up period. However, administration of ginseng 4 weeks after CAL resulted in a dramatic reversal of left ventricular dysfunction as evidenced by restoration of all parameters to values not significantly different from baseline but significantly greater than those observed in animals not treated with ginseng.

Figure 3 shows echocardiograms and corresponding quantified data for cardiac parameters. As illustrated in Figure 3A through 3C, left ventricular internal diameters during systole and diastole were significantly increased by 4 weeks after CAL, although ginseng significantly reversed both parameters. Furthermore, E/A ratios determined by Doppler echocardiography analysis were increased in CAL rats, although ginseng restored values to control levels (Figure 3D and 3E).
As summarized in the Table, there were no statistically significant changes in echocardiographic parameters in rats subjected to sham surgery throughout the 8 week follow-up period. There also was no direct influence of ginseng on any parameter studied in sham-operated animals.

Hemodynamic Parameters in 8-Week Postinfarcted Animals
Catheter-based hemodynamic measurements were performed at the end of the study to further assess left ventricular systolic and diastolic function. Eight weeks of CAL resulted in significantly increased diastolic blood pressure and decreased systolic function, which was accompanied by markedly enhanced left ventricular end-diastolic pressures and volumes (Figure 4).

Heart Rates
Mean baseline heart rates for all experimental groups (pooled data) were 358 ± 16 beats/minute (N=40). In rats subjected to CAL without subsequent ginseng administration, heart rates were 318 ± 24 beats/minute (N=10) and 328 ± 17 beats/minute (N=10) 4 and 8 weeks after CAL,

Figure 2. Indices of left ventricular function obtained from serial echocardiography in rats subjected to 8 weeks of coronary artery ligation. For the treated group, ginseng was administered 4 weeks after induction of coronary artery ligation. Data are presented as mean±SE (N=10). *P<0.05 versus week 0. **P<0.01 versus week 0. †P<0.05 versus week 4. ††P<0.01 versus week 4. For all parameters, values between the 2 treatment groups were significantly different at 8 weeks (P<0.05).

Figure 3. Ginseng reverses coronary artery ligation-induced increases in LVIDs and diastolic LVIDd and changes in the E/A ratio. Echocardiographic recordings were measured before surgery (0 weeks), 4 weeks after surgery (4 weeks), and at the end of the study (8 weeks). A, M-mode images. B, LVIDs quantified data. C, LVIDd quantified data. D and E, Representative Doppler images for transmural velocity with corresponding quantified results, respectively. *P<0.05 versus week 0. †P<0.05 versus week 4. **P<0.01 versus week 0. ††P<0.01 vs week 4. For all parameters, values between the 2 treatment groups were significantly different at 8 weeks. LV indicates left ventricle; LVIDd, left ventricular internal diameter during diastole; LVIDs, left ventricular internal diameter during systole.

Figure 5 illustrates CAL-induced contractile and diastolic dysfunction as evidenced by a decreased slope in the end-systolic pressure-volume relationship and an increased slope in the end-diastolic pressure-volume relationship. However, as shown in Figures 4 and 5, animals treated with ginseng demonstrated almost complete normalization of hemodynamic parameters.
respectively. Corresponding heart rates in rats treated with ginseng 4 weeks after CAL were 342 ± 13 beats/minute (N=10) and 358 ± 20 beats/minute (N=10). There were no significant differences in heart rates between any treatment groups.

Expression of Profibrotic Genes

We examined changes in expression in myocardial profibrotic genes at the end of the 8-week postsurgery period. As shown in Figure 6, the increased expression levels of collagen I and III as well as fibronectin were normalized by ginseng.

Direct Reversal by Ginseng of Cardiomyocyte Hypertrophy in Culture

To further assess the ability of ginseng to reverse cardiomyocyte hypertrophy and obtain insights into mechanisms, we studied cultured ventricular myocytes exposed to either of 3 hypertrophic stimuli, including ET-1, angiotensin II, or phenylephrine. Ginseng was then added 24 hours after addition of the specific hypertrophic stimulus. Figure 7 illustrates individual hypertrophic responses to each stimulus after either 24 or 48 hours of treatment. As shown in the micrograph images (Figure 7A) and quantified data (Figure 7B), untreated myocytes significantly increased in size when culture duration increased from 24 to 48 hours in the absence of ginseng, although this was not accompanied by any changes in α-skeletal actin expression (Figure 7C). Each agonist significantly increased myocyte surface area by ≈37% compared with 24-hour untreated myocytes, whereas this was increased to ≈55% in 48-hour treated myocytes. These changes in cell surface area were accompanied by significant increases in myocardial profibrotic gene expression, as normalized by ginseng treatment.

Table. Echocardiographic Parameters in Sham-Operated Animals Without or With Ginseng Administered 4 Weeks Postsurgery

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Sham + Ginseng</th>
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<tbody>
<tr>
<td></td>
<td>Week 4</td>
<td>Week 8</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>79.5 ± 1.7</td>
<td>80.4 ± 2.1</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>49.8 ± 1.9</td>
<td>49.8 ± 2.1</td>
</tr>
<tr>
<td>Cardiac output, mL/min per kg</td>
<td>199 ± 21</td>
<td>167 ± 4.9</td>
</tr>
<tr>
<td>Stroke volume, μL</td>
<td>268 ± 27</td>
<td>248 ± 11</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>7.2 ± 0.3</td>
<td>7.6 ± 0.3</td>
</tr>
<tr>
<td>LVIDs, mm</td>
<td>3.9 ± 0.4</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.4 ± 0.1</td>
<td>1.3 ± 0.04</td>
</tr>
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Data are presented as mean ± SE (N=10). There were no significant differences among the 4 groups for any parameter. LVIDd indicates left ventricular internal diameter during diastole; LVIDs, left ventricular internal diameter during systole.

Figure 4. Normalization of hemodynamic parameters by ginseng. All values were obtained at the end of the study period (week 8) in sham-operated animals or animals subjected to CAL with a Millar P-V catheter as described in the Methods section. Data are presented as mean ± SE (N=10). *P < 0.05 versus sham group. **P < 0.01 versus sham group. †P < 0.05 versus CAL no treatment group. ††P < 0.01 versus CAL no treatment group. CAL indicates coronary artery ligation; DBP, diastolic blood pressure; dP/dt max, peak rate of left ventricular pressure development; dP/dt min, minimum rate of left ventricular pressure development; LVEDP, left ventricular end-diastolic pressure; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVESV; left ventricular end-systolic volume; SBP, systolic blood pressure.
by increased α-skeletal actin expression, although gene expression upregulation was relatively similar for 24- and 48-hour treatment values.

We next determined whether ginseng administration could reverse the hypertrophic response when added 24 hours after the addition of the respective agonist. Myocytes were then maintained for a further 24 hours in the presence of the hypertrophic agonist under study in the absence or presence of ginseng. As shown in Figure 7, cells treated with ginseng according to this protocol demonstrated no
evidence of agonist-induced hypertrophy when assessed by either surface area (Figure 7B) or gene expression levels of α-skeletal actin (Figure 7C) or myosin heavy chain (Figure 7D).

**Potential Role of Calcineurin as a Target for Ginseng-Induced Reversal of the Hypertrophic Response**

All 3 agonists significantly increased calcineurin activity at 24 hours, although significant activation of calcineurin was observed in ET-1-treated cells only at 48 hours (Figure 8A). Treatment with ginseng after 24 hours administration of hypertrophic agonists completely abolished the increases as observed at 24 and 48 hours. Calcineurin activation in angiotensin II-, ET-1-, or phenylephrine-treated myocytes at 24 hours was not significantly different from activation at 48 hours. Similarly, upregulation of myocyte-enriched calcineurin-interacting protein (MCIP-1) gene expression at 24 and 48 hours by any of the 3 hypertrophy inducers was reversed by treatment with ginseng (Figure 8B). Increased MCIP-1 gene expression at 24 hours induced by any of the agonists was not significant from expression at 48 hours.

NFAT3 nuclear translocation was visualized using immunofluorescence (Figure 8C) and quantified according to the nuclear:cytosolic fraction (Figure 8D) using ImageJ software as described previously.14 Significant NFAT3 nuclear translocation was observed in angiotensin II-, ET-1-, and phenylephrine-treated myocytes at 24 and 48 hours, whereas this effect was reversed by ginseng (Figure 8C and 8D).
Ginseng alone was without effect on calcineurin activity, MCIP-1 gene expression, or NFAT3 nuclear translocation.

We also determined potential similarities with respect to calcineurin activity in hearts subjected to CAL. As illustrated in Figure 8E, there was a strong trend for ginseng to inhibit CAL-induced increase in calcineurin activity (P=0.05) as well as MCIP-1 expression (P=0.06) (Figure 8F), whereas ginseng treatment alone was without effect.

**Discussion**

Although the use of ginseng as a therapeutic tool dates back >2000 years,\textsuperscript{16,17} its increasing use as well as that of other traditional Chinese medicines in western societies have resulted in a resurgence of investigating their possible therapeutic properties for the treatment of cardiovascular diseases.\textsuperscript{8} This has been primarily evident in the case of ginseng as a cardioprotective agent for mitigating ischemic and reperfusion injury, although emerging evidence suggests that ginseng may be effective in preventing cardiomyocyte hypertrophy and heart failure.\textsuperscript{8} Here, we show for the first time to our knowledge, that ginseng can reverse cardiomyocyte hypertrophy in cultured ventricular myocytes in response to 3 hypertrophic factors: angiotensin II, ET-1, and the $\alpha_1$-adrenoceptor agonist phenylephrine. More importantly, ginseng administration 4 weeks following induction of CAL, at which time marked left ventricular dysfunction and hypertrophy is clearly evident, produces marked reversal, resulting in the near normalization of all parameters studied. It should be emphasized that at the dose studied, ginseng produced no effect on blood pressure, and there were no effects on either plasma glucose or creatinine concentrations. These observations, coupled with direct effects seen in cardiomyocytes, suggest a direct reversal of the hypertrophic program by ginseng.

In vivo reversal of postinfarction ventricular remodeling and heart failure was demonstrated first by serial
pharmacotherapy independently of load reduction, such as supports the underlying hypothesis that myocardial remod-
graham hypertrophy and heart failure, although it is one of a number of key signaling pathways in the patho-
mal conditions can be much better controlled. Calcineurin
nate represents an important component of the therapeutic
ability to reverse cardiomyocyte hypertrophy, potentially
identical to sham animals, demonstrating potent reversibility
properties of ginseng.
A major contributing factor for the ability of ginseng to reverse remodeling and heart failure likely stems from its ability to reverse cardiomyocyte hypertrophy, potentially mediated by regression/reversal of calcineurin upregu-
lation (discussed later). Indeed, reduction in cardiac hypertro-
phy represents an important component of the therapeutic
strategy for treating heart failure.\(^\text{6,21,22}\) The present study
supports the underlying hypothesis that myocardial remodel-
ing is a bidirectional process that can be reversed by pharma-
cotherapy independently of load reduction, such as that observed with therapeutic devices.\(^\text{8}\) The key role for
cardiac hypertrophy as a target for ginseng-induced revers-
ibility is supported by a direct reversal of hypertrophy in
cultured myocytes exposed to 3 different prohypertrophic
agents. Moreover, cardiac dysfunction in control animals
subjected to CAL was accompanied by both gravimetric and
gene analyses data, indicating a hypertrophic phenotype.
Additionally, echocardiographic analysis demonstrated sig-
nificantly increased systolic and diastolic left ventricular
internal diameter values, which were reversed by ginseng
administration.
We assessed the possible underlying mechanisms for the
ability of ginseng to reverse myocardial remodeling in gen-
eral and hypertrophy in particular. Ginseng exerts a myriad
of cell-signaling effects, which could contribute to its anti-
hypertrophic properties.\(^\text{8}\) For example, we have recently
reported that ginseng can prevent hypertrophy produced by
the satiety-inducer adipokine leptin by inhibiting the activa-
tion of the RhoA-ROCK (Rho-associated kinase) pathway.\(^\text{13}\)
In the present study, we focused exclusively on the calcineurin
pathway both in vivo and in cultured myocytes, where experi-
mental conditions can be much better controlled. Calcineurin
is one of a number of key signaling pathways in the patho-
genesis of cardiac hypertrophy and heart failure, although it
likely plays a particularly important role in carrying out the
hypertrophic program.\(^\text{10,11}\) This reflects calcium-calmodulin-
dependent upregulation of calcineurin activity, which results
in dephosphorylation of the NFATC transcriptional factor and
its subsequent translocation into the nucleus.\(^\text{10}\) A role for cal-
cineurin in hypertrophy and heart failure is further supported
by studies showing that pharmacological inhibition of this
enzyme prevents cardiac hypertrophy and improves cardiac
function.\(^\text{23,24}\) We have previously shown that activation of cal-
cineurin in the 4-week postinfarcted myocardium as well as
in hypertrophied cultured ventricular myocytes is prevented
by ginseng.\(^\text{9}\) We have assessed calcineurin activation by deter-
mining phosphatase activity and expression levels of MCIP-1,
which has been shown to be related to the degree of calcineu-
rin activation.\(^\text{25}\) Robust calcineurin activation was clearly seen
in cultured myocytes 24 hours after initiation of hypertrophy,
which persisted at 48 hours, although in the presence of gin-
seng added at 24 hours, values returned to control levels. A
similar strong trend was observed in hearts subjected to CAL,
although the inhibition in calcineurin activity was not statisti-
cally significant. We believe that the latter reflects the tempo-
rals (peak calcineurin activation, which
clearly precedes the 8-week post-CAL time point. Indeed,
we have shown a 2-fold increase in calcineurin activity in the
4-week postinfarcted rat heart.\(^\text{3}\) Interestingly, the results bear
some conceptual similarity to a recent report demonstrating
reversibility of calcineurin-dependent cardiac hypertrophy.\(^\text{12}\)
That study showed that calcineurin-dependent hypertrophy
produced by overexpression of a mutant calcineurin transgene
precedes the development of heart failure, thus precluding the
necessity of sustained calcineurin activation for the produc-
tion of ventricular dysfunction associated with heart failure.
Moreover, turning off calcineurin activity reversed the hyper-
rophic phenotype.\(^\text{12}\)
In conclusion, the present study shows that North
American ginseng reverses cardiomyocyte hypertrophy in
vitro and myocardial hypertrophy, remodeling, and heart fail-
ure in rats subjected to sustained CAL. Reversal of cardiac
hypertrophy remains a major therapeutic challenge but one
that is critical for the development of improved therapeutic
strategies for treating heart failure.\(^\text{4}\) The results based on find-
ings in cultured myocytes and in vivo heart failure as well
as evidence in the literature\(^\text{12}\) suggest that reversal of calci-
neurin-dependent processes represents a viable mechanistic
basis for the reversal of hypertrophy. The results, therefore,
reinforce the concept that targeting the calcineurin pathway
may represent a key approach toward developing effective
therapeutic strategies, although it is recognized that other
potential intracellular targets for reversal of hypertrophy
likely exist and cannot be excluded. However, it should be
emphasized that adverse remodeling is mediated by a large
number of other factors, including apoptosis and changes in
the extracellular matrix associated with fibrosis. Although
it was beyond the scope of the present study to investigate
additional aspects in depth, the ability of ginseng to normal-
ize gene expression levels of collagen and fibronectin, which
reflect myocardial fibrosis,\(^\text{26,27}\) suggests that ginseng may
also be effective in reversing extracellular remodeling in
the postinfarcted myocardium, but this needs to be confirmed
with additional studies examining various aspects of the adverse remodeling process.

A limitation of the present study is our inability to identify and implicate the constituents responsible for the therapeutic properties of ginseng, a challenge rendered particularly difficult in view of the large number of bioactive compounds, including >100 ginsenosides alone (in addition to other bioactive compounds) that are present in ginseng.\(^{28,29}\) Interestingly, ginsenosides undergo metabolic biotransformation after oral administration, especially under the influence of intestinal microflora, and some of these metabolites are known to be pharmacologically active and have good systematic bioavailability.\(^{30,31}\) Thus, the ability of ginseng to reverse hypertrophy in cardiomyocytes implies that this property likely does not depend on its biotransformation. This observation may provide direction for future research to define ginseng bioactive compounds for treatment of heart failure, especially adjunctive therapy with existing medications such as p-adrenergic blockers, which, at least with respect to metoprolol, have some evidence of reverse remodeling in clinical trials\(^{32,33}\) or in combination with left ventricular assist device support to improve efficacy.\(^{34}\) Finally, the results may be interpreted to suggest that in addition to ginseng, other naturally occurring phytochemicals may be of value in the treatment of heart failure (and likely other cardiovascular diseases), thus deserving of further studies.

**Sources of Funding**

This study was funded by the Ontario Ginseng Innovation and Research Consortium (OGIRC), the Canadian Institutes of Health Research, and the Heart and Stroke Foundation of Ontario. Ms Moey was supported by OGIRC during the course of these studies. Dr Karmazyn holds a Tier 1 Canada Research Chair in Experimental Cardiology.

**Disclosures**

None.

**References**


**CLINICAL PERSPECTIVE**

A number of studies have now shown that alternative therapies, such as traditional Chinese medicines, may play a role in the treatment of cardiovascular disorders. Among these is ginseng, which has been used in Asian societies for thousands of years for treating a large number of maladies. Work from our laboratory as well as reports from others have shown that ginseng is a potent antihypertrophic and remodeling factor when administered before insult and, therefore, of possible benefit for treating heart failure. Here, we expand on previous studies and report that ginseng can also reverse already-established hypertrophy, remodeling, and heart failure in a rat model in which heart failure was induced by sustained coronary artery ligation. We have postulated that this benefit arises primarily from the ability of ginseng to suppress calcineurin activation. Importantly, the beneficial effect of ginseng occurred in the absence of any blood pressure reduction, thus precluding the potential contribution of unloading to its salutary effect. These results have a number of clinical implications. First, they demonstrate, conceptually, that myocardial remodeling is a reversible process that can occur independently of afterload reduction. Second, they implicate the likely importance of targeting the calcineurin system as a critical mechanism for reversal of hypertrophy and remodeling. Third, they offer the potential of combination therapy of ginseng with established medications for the treatment of heart failure. However, the feasibility of translating this approach to patients with heart failure awaits confirmation through rigorously controlled clinical trials.
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SUPPLEMENTAL MATERIAL

Ginseng Reverses Established Cardiomyocyte Hypertrophy and Post Myocardial Infarction-Induced Hypertrophy and Heart Failure

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Supplemental Methods

Echocardiography: Echocardiography measurements were taken at the following three different time points: baseline/before surgery (week 0), 4 weeks after surgery (week 4) and at the end of the study (week 8). Rats were prepared for echocardiography as previously described. Briefly, animals were anesthetized with 2% isoflurane and placed supine on a heated platform. Echocardiography measurements 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) to obtain M-Mode 2-dimensional and Doppler images. M-Mode was obtained from the parasternal short axis to analyze left ventricular dimensions during diastole and systole and Doppler mode was obtained from the parasternal long axis to determine the E/A ratio calculation. All images were analyzed using the Vevo 770 Protocol-Based Measurements software.

RNA Isolation, Reverse Transcription (RT) and Real-time Polymerase Chain Reaction (PCR)

RNA was collected from either left ventricular tissue or cardiomyocytes using QIAzol Reagent (Qiagen Canada, Toronto, Ontario, Canada) as per the manufacturer’s instructions and reverse transcribed to complementary DNA (cDNA) for real-time PCR analysis of α-skeletal actin and
modulatory calcineurin interacting protein-1 (MCIP-1) as previously described.\textsuperscript{2} cDNA was synthesized from 5 μg of total RNA using random primers (Invitrogen) and M-MLV Reverse Transcriptase (Invitrogen) as per the manufacturer’s protocol. The reaction was performed with a SYBR Green Master Mix (Applied Biosystems, Foster City, CA) and the gene products quantified with a DNA Engine Opticon 2 thermal cycler (MJ Research, Waltham, MA). Primer sequences are summarized in Supplemental Table 1 below. PCR cycle conditions involved 40 cycles of denaturation at 95°C for 30 seconds, followed by annealing at 50°C and 60°C for 30s for 18S and α-skeletal actin and MCIP-1, respectively, ending with elongation at 72°C for 45s. The housekeeping gene, 18S, was measured and quantified to normalize cDNA levels.

**Immunofluorescence.** Cardiomyocytes were prepared for immunofluorescence on collagen coated (3 μl of collagen/1 ml of PBS A) glass cover slips and incubated at 37°C for a minimum of 30 minutes. Cells were allowed to attach to prepared cover slips in serum-containing medium for 24 hours followed by serum starvation for an additional 24h before appropriate treatments. Cardiomyocytes were pre-treated with angiotensin II, ET-1, or phenylephrine for 24h without ginseng or 48h with or without treatment of ginseng for an additional 24h. Cells were fixed with 2:5 acetone-methanol for 1 hour at 4°C followed by permeabilization of cells for 15 minutes with 0.1% (v/v) Triton X-100 and blocking with blocking solution (1% BSA in PBS A). Cells were incubated with NFAT3 antibody (Santa Cruz Biotechnology, Santa Cruz, CA; 1:100 dilution) in 2% BSA in PBS A overnight at 4°C. Cells were subsequently probed with the secondary antibody IgG anti-rabbit AlexFluor-596 (Invitrogen) (1:250 dilution) in 2% BSA in PBS A for 1h at room temperature under light-free conditions. Cells were mounted with Vectashield® mounting medium containing 4’,6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA) for detection of nuclei onto microscope slides for image capture using a Zeiss (Oberkochen,
Germany) inverted fluorescence microscope at 630x magnification. Nuclear translocation was measured using ImageJ software as described previously.  

**References**


### Supplemental Tables

**Supplemental Table 1. Primer sequences of analyzed genes of interest**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer Sequence</th>
<th>Reverse Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-skeletal actin</td>
<td>5'-CACGGCATTATCACAACACTG-3'</td>
<td>5'-CCGGAGGCATAGAGAGACAG-3'</td>
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<tr>
<td>myosin heavy chain</td>
<td>5'-CATCACCAGGAGATCCGGAGC-3'</td>
<td>5'-CTATGGAGCCACAGTCGTC-3'</td>
</tr>
<tr>
<td>18S</td>
<td>5'-GTAACCGGTTGAAACCATT-3'</td>
<td>5'-CATCCAATCGGTAGTACG-3'</td>
</tr>
<tr>
<td>MCIP-1</td>
<td>5'-TCTCCAAGCTGAGGACAGAG-3'</td>
<td>5'-ATCAGAAGCGGCTGTCGT-3'</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>5'-GCGACTCTGACTGCGCTAC-3'</td>
<td>5'-CCGTGAAGGTCGAGCAAT-3'</td>
</tr>
<tr>
<td>Collagen I</td>
<td>5'-TGCTGCTTTTCTGTTTCTT-3'</td>
<td>5'-AAGGTCGGGTAAGGAAG-3'</td>
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<tr>
<td>Collagen III</td>
<td>5'-GTCCACAGGGTGAAGGAGT-3'</td>
<td>5'-CATCCTTTCCAGGAGGTCCA-3'</td>
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</table>

**Supplemental Table 2. Plasma glucose and creatinine concentrations**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Glucose</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Untreated (N=8)</td>
<td>70.5 ± 10.4</td>
<td>0.63 ± 0.04</td>
</tr>
<tr>
<td>Sham + Ginseng (N=6)</td>
<td>75.2 ± 16.3</td>
<td>0.65 ± 0.11</td>
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<tr>
<td>CAL Untreated (N=9)</td>
<td>79.8 ± 10.7</td>
<td>0.52 ± 0.07</td>
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<tr>
<td>CAL + Ginseng (N=6)</td>
<td>65.1 ± 8.5</td>
<td>0.50 ± 0.07</td>
</tr>
</tbody>
</table>

Values indicates means ± SE with number of animals in parentheses. All values are given in mg/dL and were determined from samples obtained at the end of the 8 wk post-surgery period.