STUDIES INTO THE MECHANISMS UNDERLYING THE CARDIAC ANTI-HYPERTROPHIC AND ANTI-REMODELLING EFFECTS OF GINSENG

(Spine Title: Ginseng as Pharmacotherapy for Heart Failure)

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by

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Abstract

Ginseng is a widely prescribed herbal drug that has been used for over 2000 years in Asia for the treatment of several different disorders of the body including those of the cardiovascular system. Advances in ginseng research have identified the bioactive constituents considered responsible for eliciting its pharmacological effects known as 'ginsenosides'. Several published reports have demonstrated the potential ability of ginseng and their isolated ginsenosides in the prevention and treatment of heart disease. In the study presented here the effects of ginseng on agonist-induced cardiac hypertrophy in isolated cardiomyocytes (**Chapter 2 – 4**) as well as an *in vivo* model of heart failure (**Chapter 4**) were investigated.

In our first study **(Chapter 2)**, the ability of ginseng to prevent leptin-induced ventricular cardiac hypertrophy by inhibiting p115RhoGEF-RhoA/ROCK-dependent MAPK activation was investigated. Leptin (50 ng/ml, which is a concentration representative of plasma levels found in obese individuals) produced a robust hypertrophic response that was associated with RhoA/ROCK activation resulting in a significant increase in cofilin-2 phosphorylation and actin polymerization, the latter evidenced by a reduction in the globular to filamentous actin ratio. These effects were prevented by North American ginseng (alcoholic extract; 10 μg/ml), hereon referred to as "ginseng". The stimulation of RhoA/ROCK by leptin was associated with significantly increased p115RhoGEF gene and protein expression and exchange activity, all of which were inhibited by ginseng. The attenuation of leptin-induced activation of RhoA/ROCK by ginseng was further associated with diminished p38 MAPK activation and nuclear translocation.

In a follow-up study **(Chapter 3)**, the ability of ginseng to reverse leptin-induced cardiac hypertrophy by enhancing Rnd3-p190RhoGAP-mediated downregulation of RhoA/ROCK

activation, was investigated. Cardiomyocytes incubated with leptin for 48 h displayed significantly increased cell surface area, which was accompanied by an increase in the expression of the fetal gene α-skeletal actin. A decrease in the G/F actin ratio, most likely as a result of RhoA/ROCK cofilin-2 phosphorylation was observed in hypertrophied cells treated with leptin. Treatment with ginseng however reversed these effects. In leptin-treated cells, Rnd3 gene and protein expression were decreased however treatment with ginseng reversed these effects by leptin. In the left ventricular tissues of rats subjected to four weeks of sustained myocardial infarction (MI), Rnd3 protein expression was markedly reduced while p63RhoGEF and ROCK expressions, which reflect upregulation of RhoA, were increased. These MI-induced effects however were restored by ginseng to expressions as observed in sham.

For our third study **(Chapter 4)**, we investigated the ability of ginseng to reverse already established cardiac dysfunction as well as hypertrophy both *in vitro* and *in vivo* by inhibition of calcineurin/NFAT3 activation. The ability of a pharmacological agent to *reverse* HF is of particular importance as the majority of current treatments are unable to reverse already established myocardial remodelling and ventricular dysfunction. Accordingly, ginseng was administered in drinking water *ad libitum* to rats after 4 weeks of sustained coronary artery ligation (CAL) when hypertrophy and HF were established or to hypertrophic neonatal ventricular myocytes treated with angiotensin II, endothelin-1 or phenylephrine. Echocardiographic and catheter-based measurements of hemodynamic parameters revealed complete reversibility of systolic and diastolic abnormalities as well as increased myocardial collagen gene expression in CAL-rats after treatment with ginseng. Similarly, ginseng administration to hypertrophic cardiomyocytes resulted in complete reversal to a normal phenotype after 24 h as determined by cell surface area and α-skeletal gene expression. The

effects of ginseng *in vivo* were associated with a tendency to attenuate calcineurin activation and MCIP-1 gene expression. In the cultured cardiomyocytes however, ginseng completely reversed agonist-induced calcineurin activation and NFAT3 nuclear translocation. Taken together, results from our studies demonstrate a marked anti-hypertrophic and anti-remodelling ability of ginseng in the prevention and treatment of cardiovascular disease.

Keywords: cardiac hypertrophy, leptin, p115RhoGEF, RhoA/ROCK, ginseng, ginsenosides, heart failure, reversal, calcineurin/NFAT3