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miR-29c is implicated in the cardioprotective activity of Panax notoginseng saponins against isoproterenol-induced myocardial fibrogenesis

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ABSTRACT

Ethnopharmacological relevance: Panax notoginseng (Burkill) F.H. Chen (Araliaceae) has a long history of clinical application in China for the treatment of cardiovascular diseases. Panax notoginseng saponins (PNS) have been proven to be the major cardioprotective substances of Panax notoginseng (Burkill) F.H. Chen (Araliaceae).

Aim of the study: The current study further investigated the molecular mechanisms associated with the cardioprotective effect of PNS.

Materials and methods: C57BL/6J mice were subject to isoproterenol (ISO)-induced myocardial injury in the absence or presence of PNS treatment. Histological, immunohistochemical and molecular biological approaches were taken to assess the effects of PNS treatment on ISO-induced myocardial injury and ensuing fibrogenesis.

Results: PNS treatment significantly attenuated ISO-induced myocardial injury and fibrosis. The expression of an anti-fibrotic microRNA, miR-29c, was significantly decreased in ISO-challenged mouse hearts. In contrast, PNS treatment resulted in increased cardiac expression of miR-29c. The expression of miR-29c target genes including Collagen (Col) 1a1, Col1a2, Col3a1 and Col5a1, fibrillin 1 (Fbn1) as well as TGFβ1 was significantly increased by ISO, which exhibited decreased expression by PNS intervention.

Conclusions: Our results demonstrate for the first time that the cardioprotective effects of PNS could in part implicate increased expression of miR-29c in the heart, which may help increase the understanding of the pharmacological activities of PNS in treating cardiovascular disorders.

1. Introduction

Myocardial fibrosis, characterized by excessive accumulation of extracellular matrix (ECM) in the myocardium, is one of the hallmark endpoint pathologies commonly shared by various cardiovascular disorders including hypertension, atherosclerosis and myocardial infarction. Myocardial fibrosis could lead to myocardial stiffness, cardiac dysfunction, arrhythmias and sudden death (Kong et al., 2014). Mechanism-guided therapeutics attenuating myocardial fibrosis would improve the structural and functional outcomes in patients, which remain to be developed.

Transforming growth factor β (TGFβ) signaling plays essential roles in the pathogenesis of fibrosis of different tissue origins including heart. Activation of TGFβ signaling induces the expression of genes encoding ECM, directly leading to tissue fibrosis (Lijnen et al., 2000a). MicroRNAs (miRNAs) have recently emerged as a novel class of gene expression regulators. MiRNAs are small non-coding RNAs in the approximate length of 22 nucleotides. MiRNAs regulate gene expression in a negative manner mainly by binding to the 3′-untranslated regions of the target gene, leading to inhibited mRNA translation or mRNA degradation (Rana, 2007). MiRNA-mediated gene expression regulation is uniquely featured by simultaneous regulation of multiple genes that are functionally associated. Change in one miRNA could well likely result in altered expression of many genes which collectively contribute to a given disease phenotype. MiRNAs mechanistically take important part in nearly all pathophysiological processes. MiRNAs modulate myocardial fibrogenesis through direct interaction with fibrogenic regulator such as TGFβ and genes encoding pro-fibrogenic molecules such as collagens and ECM (Ono et al., 2011; van Rooij and Olson, 2012). For instance, miR-29 plays important roles in suppressing myocardial fibrogenesis through downregulating the expression of multiple genes encoding collagens and ECM (van Rooij et al., 2008).

Panax notoginseng (Burkill) F.H. Chen (Araliaceae) has a long history of clinical application in China, which includes treatment of cardiovascular diseases. Its cardioprotective activities could largely be attributed to Panax notoginseng saponins (PNS) (Wang et al., 2016;

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Yang et al., 2014). Whether cardioprotective activities of PNS involve miRNA-mediated gene expression regulation remains to be investigated. Gaining novel understanding on the cardioprotective effects of PNS would benefit further optimization of therapeutic modalities centering around Panax notoginseng (Burkill) F.H. Chen (Araliaceae). In experimental models, β adrenergic receptor agonist isoproterenol (ISO) causes cardiac injury primarily characterized by cardiac hypertrophy, cardiomyocyte necrosis, as well as myocardial fibrosis. ISO-induced myocardial injury recapitulates the pathological features of various myocardial disorders in human and has been extensively employed for therapeutic screening tackling related diseases (Benjamin et al., 1989; Brooks and Conrad, 2009; Kahn et al., 1969). The current study thus explored the implication of miR-29c, an important player in myocardial fibrosis (van Rooij and Olson, 2012; van Rooij et al., 2008), in the cardioprotective effects of PNS in the model of isoproterenol (ISO)-induced myocardial injury.

2. Materials and methods

2.1. Reagent and animals

Supplemental materials and methods.

2.2. Immunohistochemistry (IHC)

Paraffin sections 4 μm thick were used to assess the expression of α-smooth muscle actin (α-SMA) and TGFβ1 using primary anti-α-SMA antibody (Sigma-Aldrich, USA) and anti-TGFβ1 antibody (Boster, Wuhan, China), respectively. Immunopositivity was observed using a light microscope (Leica, Germany) and analyzed by Image Pro Plus Software.

2.3. Real-time PCR analysis

Total RNA was isolated from the left ventricles using miRNeasy Mini kit (217004, Qiagen, USA). RNA (500 ng per sample) was reverse-transcribed using miScript reverse transcription kit (218061, Qiagen, USA). Primers sequences are listed in the Supplemental Table 1. Real-time PCR was carried out using SYBR Green PCR Master Mix (ABI, USA) on LightCycler 480 System (Roche, USA). Expression of GAPDH was examined as an internal control for gene expression analysis. The relative expression of miR-29c was normalized to that of RNU6B. The relative expression of a specific gene or miR-29c was calculated by $2^{-[Ct(gene)-Ct(GAPDH)]}$ and $2^{-[Ct(miR-29c)-Ct(RNU6B)]}$, respectively.

2.4. Statistical analysis

The data were expressed as means ± S.E.M. The statistical analysis was performed using SPSS 13.0 software. Data with normal distribution were analyzed with one-way ANOVA using LSD test or Games-Howell test depending upon the homogeneity of variances. Data without normal distribution or ranked data were analyzed by nonparametric test and difference between two groups was examined by Nemenyi test. p value less than 0.05 was considered statistically significant.

3. Theory/calculation

The implication of miRNA-mediated gene expression regulation in the cardioprotective activities of PNS has not been fully investigated. Therefore, the potential involvement of miR-29c, a miRNA with anti-fibrogenic functions, was investigated in ISO-challenged mice in the absence or presence of PNS treatment.

4. Results

4.1. PNS alleviated ISO-induced myocardial injury and fibrotic alterations

As shown in Supplemental Fig. S2A, compared to that from vehicle-treated normal controls, increased heart weight to body weight ratio was observed in ISO-challenged vehicle-treated mice, which was attenuated in ISO-challenged mice treated with PNS at 50 mg/kg bw and 150 mg/kg bw, respectively. Histopathological examination revealed increased size of surviving cardiomyocytes in ISO-challenged vehicle-treated mice compared to that from vehicle-treated normal controls, which was significantly decreased in ISO-challenged mice treated with PNS at 50 mg/kg bw and 150 mg/kg bw, respectively (Supplemental Fig. S2B). Moreover, as shown in Supplemental Fig. S3, contrary to well-aligned pattern of myocardial fibers and cardiomyocytes observed in vehicle-treated normal controls, loss of cardiomyocytes, inflammatory cell infiltration and granulation were readily observed in the left ventricles of ISO-challenged vehicle-treated mice, which was significantly attenuated by PNS treatment in a dose-dependent manner (Supplemental Table S2). Collagen deposition in myocardium was subsequently evaluated by Masson's trichrome staining. As shown in Fig. 1A and B, compared to that from vehicle-treated normal controls, ISO administration led to significantly increased Masson's trichrome positivity in the left ventricles of vehicle-treated mice, which was found to be remarkably decreased in ISO-challenged mice treated with PNS at 50 mg/kg bw and 150 mg/kg bw, respectively. Attenuated collagen accumulation in ISO-challenged PNS-treated mice was also witnessed by Picrosirius red staining (Supplemental Fig. S4). The immunoreactivity of α-SMA, a marker of myofibroblast, was further examined. As shown in Fig. 1C and D, compared to that from vehicle-treated normal controls, α-SMA immunopositivity indicative of myofibroblast activation was readily detected at the site of microscopic injury in ISO-challenged vehicle-treated hearts, which was remarkably attenuated in the hearts from ISO-challenged mice treated with PNS at 50 mg/kg bw and 150 mg/kg bw, respectively. Meanwhile, the level of pro-fibrogenic TGFβ1 was also shown to be decreased in the hearts by PNS treatment compared to that from ISO-challenged vehicle-treated mice (Supplemental Fig. S5). These results collectively indicate that PNS treatment protects against ISO-induced myocardial injury and fibrogenesis.

4.2. PNS treatment increased miR-29c expression and decreased the expression of miR-29c target genes in ISO-challenged mouse hearts

To further investigate the implication of miRNA in the cardioprotective activities of PNS, the expression of anti-fibrotic miR-29c was analyzed by real-time PCR. As shown in Fig. 2A, compared to that from vehicle-treated normal controls, significantly downregulated expression of miR-29c was observed in the left ventricles of ISO-challenged vehicle-treated mice, which was increased by PNS treatment. To further validate the implication of miR-29c in PNS-mediated cardioprotection in ISO-challenged mice, expression of miR-29c target genes encoding collagens and ECM including Col1a1, Col1a2, Col3a1, Col5a1 and Fbn1 was further analyzed. As shown in Fig. 2B–F, compared to that from vehicle-treated normal controls, ISO administration resulted in significantly increased expression of Col1a1, Col1a2, Col3a1, Col5a1 and Fbn1 in the left ventricles, whereas remarkably downregulated expression of Col1a1, Col1a2, Col3a1, Col5a1 and Fbn1 was observed as a result of PNS treatment in ISO-challenged mice. These results suggest that decreased miR-29c expression is associated with ISO-induced injury, which is in part counteracted by PNS treatment.

5. Discussion

Our current study showed that myocardial fibrogenesis induced by