Inhibitory effect of ethyl acetate extract of *Aristolochia yunnanensis* on cardiac fibrosis through extracellular signal-regulated kinases 1/2 and transforming growth factor β/small mother against decapentaplegic signaling pathways

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***Aristolochia yunnanensis*, known as Nan Mu Xiang in traditional Chinese medicine, has long been used to treat hypertension and chest pain. In this study, the effect of ethyl acetate extract of Nan Mu Xiang (NMX) on cardiac fibrosis was assessed *in vitro* by cultured adult rat cardiac fibroblasts with angiotensin II (AngII) stimulation, and *in vivo* by rats with abdominal aorta constriction (AAC). In cultured adult rat cardiac fibroblasts stimulated by AngII, NMX inhibited cardiac fibroblast proliferation, reduced the expression of fibronectin, α-smooth muscle actin (α-SMA), and transforming growth factor β (TGF-β) in a dose-dependent manner; and suppressed AngII-induced phosphorylation of extracellular signal-regulated kinase (ERK)1/2, C- rapidly accelerated fibrosarcoma (C-Raf), and small mother against decapentaplegic (Smad) 2. Similar results were also observed in AAC rats with intraperitoneal injection of NMX, which not only ameliorated myocardial fibrosis, but also improved cardiac function. The therapeutic effect of NMX on myocardial fibrosis is attributed mainly to the inhibition of ERK and the TGF-β/Smad signaling pathways. NMX may be a promising potential drug candidate for myocardial fibrosis. (Translational Research 2014;163:160–170)**

Abbreviations: AAC = abdominal aortic constriction; AngII = angiotensin II; α-SNA = α-smooth muscle actin; C-Raf = C- rapidly accelerated fibrosarcoma; ECM = extracellular matrix; ERK = extracellular signal-regulated kinase; LDH = lactate dehydrogenase; MAPK = mitogen-activated protein kinase; NMX = ethyl acetate extract of Nan Mu Xiang; TGF-β = transforming growth factor β; Smad2 = small mother against decapentaplegic 2

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AT A GLANCE COMMENTARY

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Background

Aristolochia yunnanensis Franch (Aristolochiaceae) is known as Nan Mu Xiang in traditional Chinese medicine for its treatment of hypertension, chest pain, gastrointestinal diseases, and so forth. However, the pharmacologic effects and mechanisms of Nan Mu Xiang on cardiac fibrosis have not been clarified.

Translational Significance

The current study demonstrated that ethyl acetate extract of Nan Mu Xiang attenuated cardiac fibrosis significantly and ameliorated cardiac function induced by abdominal aorta constriction in rats. The antifibrotic effect was achieved by inhibiting the extracellular signal-regulated kinase (ERK) 1/2 and transforming growth factor β (TGF-β)/small mother against decapentaplegic (Smad) signaling pathways. The results provide evidence that ethyl acetate extract of Nan Mu Xiang (NMX) may be a promising potential drug candidate for myocardial fibrosis in clinics.

Hypertension is one of the major cardiovascular diseases worldwide. Patients with hypertension have cardiac fibrosis, which is characterized as fibroblast proliferation and excessive extracellular matrix deposition, leading to heart failure, sudden cardiac death, and other serious complications.[1] Cardiac fibroblasts are considered to be the major source of cardiac extracellular matrix (ECM), including structural and adhesive proteins such as collagen, fibronectin, and proteoglycans in myocardium.[2] Therefore, antifibrotic therapies are likely to be a crucial strategy in curbing this health problem.

In overloaded hearts with fibrosis, myocardial angiotensin II (AngII) levels are remarkably increased.[3] AngII stimulates cardiac fibroblasts to secrete growth-promoting substances, resulting in differentiation of cardiac fibroblasts into myofibroblasts.[4] Furthermore, AngII activates a variety of cell signaling pathways in rat cardiac fibroblasts, such as the mitogen-activated protein kinase (MAPK) pathway and transforming growth factor β (TGF-β)/small mother against decapentaplegic (Smad) pathway promoting myofibroblast differentiation.[5,6] However, in TGF-β1-deficient mice, AngII was not able to induce cardiac hypertrophy and fibrosis, indicating that TGF-β1 is essential for AngII-induced hypertrophic growth response and myofibroblast differentiation.[7] In addition to TGF-β, extracellular signal-regulated kinase (ERK) was also found to be an important modulator in AngII-induced collagen production in cardiac fibroblasts.[8,9] Activation of the ERK signaling pathway by various stimuli has been correlated to cell proliferation and remodeling of ECM.[10] Moreover, ERK inhibitor PD98059 exhibits a profound beneficial effect on myocardial fibrosis and heart function in mice.[11] Therefore, alleviation or prevention of the activation of the ERK and TGF-β/Smad signal pathways has been considered a promising therapeutic strategy for cardiac fibrosis.

Aristolochia yunnanensis Franch. (Aristolochiaceae), endemic to Yunnan Province, China, is known as Nan Mu Xiang in traditional Chinese medicine for its treatments of hypertension, chest pain, gastrointestinal diseases etc. Only a few experimental studies have attempted to clarify the pharmacological effects and mechanisms of Nan Mu Xiang.[12] Previously, we have demonstrated that several sesquiterpene lactones from ethyl acetate extract of *Aristolochia yunnanensis* (NMX) selectively inhibited ERK phosphorylation in HeLa cells.[13] In the current study, we attempted to evaluate the therapeutic effects of NMX on cardiac fibrosis and explore the potential mechanisms *in vitro* by cultured adult rat cardiac fibroblasts with AngII stimulation, and *in vivo* by rats with abdominal aorta constriction (AAC), a well-characterized animal model of pressure-overload hypertrophy.[14]

MATERIALS AND METHODS

Chemicals and reagents. The WST-8-based colorixmetric assay kit (Cell Counting Kit 8, CCK8) and lactate dehydrogenase (LDH) release assay kit was acquired from the Beyotime Institute of Biotechnology (Haimeng, China). Antibodies against phosphorylated ERK (p-ERK), phosphorylated JNK (p-JNK), phosphorylated p38, phosphorylated C-Raf, TGF-β, phosphorylated Smad2, total ERK, and total Smad2 were acquired from Cell Signaling Technology (Boston, Mass). Antibody against Glyceraldehyde-3-phosphate dehydrogenase was from Beyotime Institute of Biotechnology. Antibodies against fibronectin and α-SMA were from Santa Cruz (Dallas, Tex) and Thermo Scientific (Waltham, Mass), respectively. Antimouse and antirabbit secondary antibodies were from Promega (Madison, Wisc). AngII was from Millipore (Billerica, Mass).

Extract preparation. Stems of *A. yunnanensis* Franch. (synonym: *Aristolochia griffithii*) were collected in October 2010 from Yunnan Province, China, and were identified by Prof. You-Kai Xu of Xishuangbanna