Biomedicine & Pharmacotherapy 93 (2017) 130–145

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Fusaric acid (FA) protects heart failure induced by isoproterenol (ISP) in mice through fibrosis prevention via TGF-β1/SMADs and PI3K/AKT signaling pathways

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ARTICLE INFO

Article history:
Received 10 April 2017
Received in revised form 21 May 2017
Accepted 4 June 2017

Keywords:
Fusaric acid
Cardiac fibrosis
TGF-β1/SMADs
MAPKs
PI3K/AKT

ABSTRACT

Fusaric acid (FA) is a novel compound derived from a class of nicotinic acid derivatives, exhibiting activity against cancers. However, its role in regulating cardiac injury is limited. Our study was aimed to investigate the role and the underlying molecular mechanism of FA in heart fibrosis and hypertrophy. Isoproterenol (ISP) was used to induce cardiac fibrosis and hypertrophy in vitro and in vivo. FA administration ameliorated hypertrophy by reducing atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and β –myosin heavy chain (β-MHC) in vitro and in vivo. Additionally, FA reduced collagen accumulation and fibrosis-related signals, including α- smooth muscle actin (α-SMA), Collagen type I and Collagen type III. Transforming growth factor-β1 (TGF-β1)/SMADs and mitogen-activated protein kinases (MAPKs), including p38, extracellular signal regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK), signalling pathways were highly activated for ISP induction, which were prevented due to FA administration. Further, FA suppressed ISP-induced PI3K/AKT activity in a dose dependent manner. Of note, FA-reduced MAPKs phosphorylation was associated with phosphoinositide 3-Kinase (PI3K)/Protein kinase B (AKT) activity caused by ISP. However, PI3K/AKT activation showed no effects on TGF-β1/SMADs expression in FA-treated cells after ISP exposure. Together, FA might be an effective candidate agent for preventing cardiac fibrosis by modulating TGF-β1/SMADs and PI3K/AKT signalling pathways.

1. Introduction

Pathological cardiac hypertrophy develops in response to hemodynamic overload, a major predictor for the progression of coronary artery disease and heart failure [1]. Preventing and postponing the development of cardiac hypertrophy may serve as an effective therapeutic strategy for the prevention and treatment of heart failure for patients [2,3]. When the stress or injury is transient, cardiac hypertrophy is considered as a compensation molecular mechanism to overcome the accelerated workload [4]. Myocardial hypertrophy is distinguished by enhanced protein synthesis rate, sarcomeric reorganization, heart mass, and fetal genes activation, including brain natriuretic peptide (BNP), atrial natriuretic peptide (ANP), and β-myosin heavy chain (β-MHC) [5,6]. But, if cardiac stress lasts for a long time, then, the compensatory situation could result in a maladaptive condition, which is known as the cardiac hypertrophy, leading to arrhythmia, sudden death, and even heart failure ultimately [7]. Usually, the cardiac hypertrophy occurs with fibrosis, characterized by extracellular matrix proteins accumulation, such as collagen type I and III.

Studies before have indicated that transforming growth factor-beta 1 (TGF-β1) is involved in pathological remodel of the heart that occurs in response to pressure overload through the induction of interstitial fibrosis and cardiomyocyte hypertrophic growth, causing excessive collagen accumulation and extracellular matrix (ECM) deposition [8,9]. TGF-β1 over-expression has been identified in liver, lung and heart fibrosis in patients and animals [10]. α-smooth muscle actin (α-SMA) expression indicates the fibroblasts differentiation into myofibroblasts, which are sources of ECM signals, accelerating cardiac injury eventually [11]. Moreover, SMAD2 and SMAD3, as the significant down-streaming signals of TGF-β1, are essential for fibroblast-to-myofibroblast differentiation induced by TGF-β1 [12]. Thus, suppressing TGF-β1 expression is of potential value for preventing heart fibrosis.

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http://dx.doi.org/10.1016/j.biopha.2017.06.002
0753-3322/

MAPKs signalling pathways, such as extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal protein kinase (JNK) and p38 MAP kinase, are illustrated to be involved in hypertrophy [13], myocardial dysfunction [14], fibrosis and heart failure actively [15]. According to previous studies, MAPK pathways are up-regulated during cardiac hypertrophy and heart failure. These signals functions initiate a large number of studies for exploring the genetic blockers, pharmacological and activators as well as the related targets of MAPK signalling pathway in heart [16]. In addition, other protein kinases have been suggested to modulate MAPKs at up-streaming or down-streaming to regulate diverse physiology and pathophysiology [17]. AKT activation is showed to be essential for hypertrophy responses to physiological stimulation [18]. PI3K/AKT activity is reported to stimulate MAPKs phosphorylation to induce various genes transcription in cells under various stresses, such as cardiac muscle cells with inflammatory induction [19].

Fusaric acid (FA) (Fig. 1A), as a mycotoxin, is isolated from a novel class of nicotinic acid derivatives, displaying activity against cancer development [20,21]. Mycotoxins are compounds produced

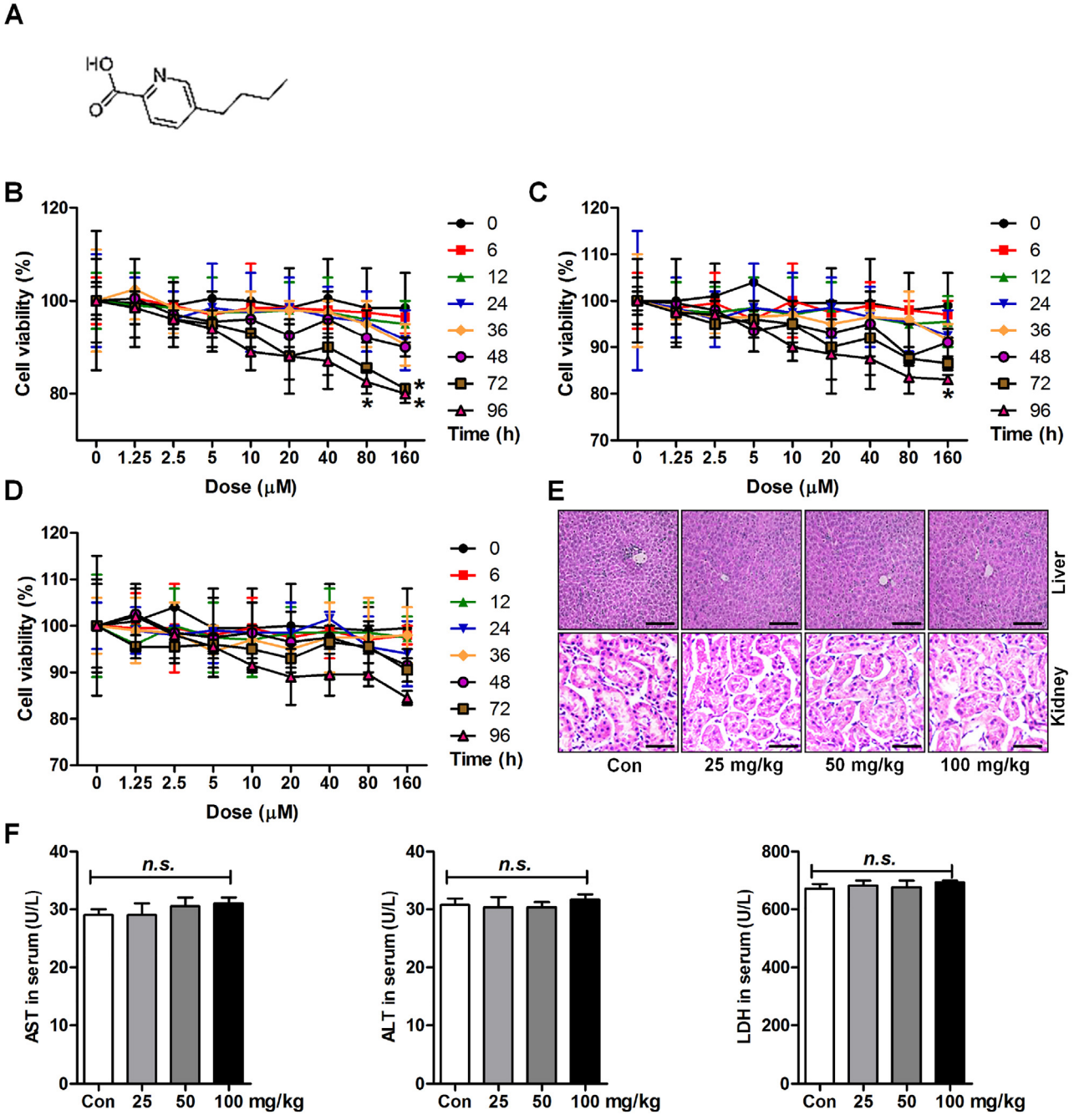

Fig. 1. Fusaric acid shows no significant toxicity in vitro and in vivo. (A) The chemical structure of fusaric acid. (B) The cardiomyocytes isolated from mice were treated with various concentrations (0 to 160 uM) of FA for different time ranging from 0 to 96 h, followed by cell viability analysis through MTT analysis. (C) The cardiomyocytes of H9C2 cells were treated with various concentrations of FA for different time as indicated. Then, the cell viability was analyzed by MTT analysis. (D) Human liver normal cell line, L02, was administered to FA at diverse concentrations for different time. Next, MTT assay was applied to calculate cell viability for cytotoxicity analysis. (E) H&E staining of liver and kidney isolated from mice treated with FA (25, 50 and 100 mg/kg) to explore the toxicity or safety of FA in vivo. (F) AST, ALT and LDH activities in the serum of mice treated under various conditions were evaluated. Data are presented as Mean ± SEM (n = 8). * indicates $P < 0.05$ (compared to the group without any treatments).