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CYP2J2-derived EETs attenuated ethanol-induced myocardial dysfunction through inducing autophagy and reducing apoptosis

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ABSTRACT

Chronic excessive drinking leads to myocardial contractile dysfunction and dilated cardiomyopathy, where ethanol toxicity plays an essential role. Cytochrome P450 (CYP) epoxygenases metabolize arachidonic acids to form epoxyeicosatrienoic acids (EETs), which exert beneficial roles in the cardiovascular system, but their role in alcoholic cardiomyopathy is elusive. This study was designed to evaluate the effects and mechanisms of CYP2J2 gene delivery on ethanol-induced myocardial dysfunction with focus on autophagy and apoptosis. C57BL/6 J mice were challenged with a 4% Lieber-DeCarli ethanol liquid diet for 8 weeks, before which rAAV9-CYP2J2 was injected via the tail vein. Cardiac function was assessed using echocardiography, hemodynamic measurement, and cardiac histology. The results showed that chronic ethanol intake led to cardiac dilation, contractile dysfunction, cardiomyocyte hypertrophy, oxidative stress, and cardiomyocyte apoptosis, while CYP2J2 overexpression ameliorated these effects. Additionally, chronic ethanol consumption triggered myocardial autophagosome formation, but impaired autophagic flux via disrupting autophagosome-lysosome fusion, as evidenced by increased LC3 II/I, Beclin-1 and SQSTM1 levels, but reduced LAMP-2 expression. Interestingly, rAAV9-CYP2J2 treatment exerted cardioprotection via restoring autophagic flux in the alcoholic myocardium. Similarly, exogenous 11,12-EET addition significantly restored ethanol-induced neonatal rat cardiomyocyte autophagic flux impairment and inhibited apoptosis, both of which were mediated by AMPK/mTOR signaling pathway in vitro. In conclusion, our data suggest that CYP2J2-derived EETs attenuate ethanol-induced myocardial dysfunction through inducing autophagy and reducing apoptosis.

1. Introduction

Daily light to moderate consumption of alcohol (ethanol) exerts beneficial effects on the cardiovascular system [1]. In contrast, chronic binge alcohol intake (more than 80 g per day for over 5 years) leads to progressive cardiac dysfunction and heart failure, which is characterized by dilated left ventricle (LV), increased LV mass, normal or reduced LV wall thickness, and a reduced LV ejection fraction (< 40%) in the advanced stage [2,3]. Importantly, excessive ethanol consumption is considered to be the major cause of non-ischemic dilated cardiomyopathy (DCM) in western countries [4]. A Number of studies have declared the pathogenesis of alcoholic cardiomyopathy (ACM) to include the toxicity of ethanol and its metabolites, oxidative stress, mitochondrial dysfunction, autophagy, apoptosis, and fatty acid ethyl ester accumulation [3]. However, the precise underlying mechanisms of ACM remain to be elucidated. To date, none of studies have proposed a specific treatment for ACM other than that recommended for DCM in heart failure therapeutic strategies.

Cardiomyocytes respond to stress by activating various pathways enabling them to adapt to environmental changes. The fate of cells depends on the intensity of the external stimulus and activation of survival mechanisms. Extreme injury of cardiomyocytes results in oxidative stress, reactive oxygen species (ROS) production, mitochondrial dysfunction, and cytochrome c release, which finally lead to cell

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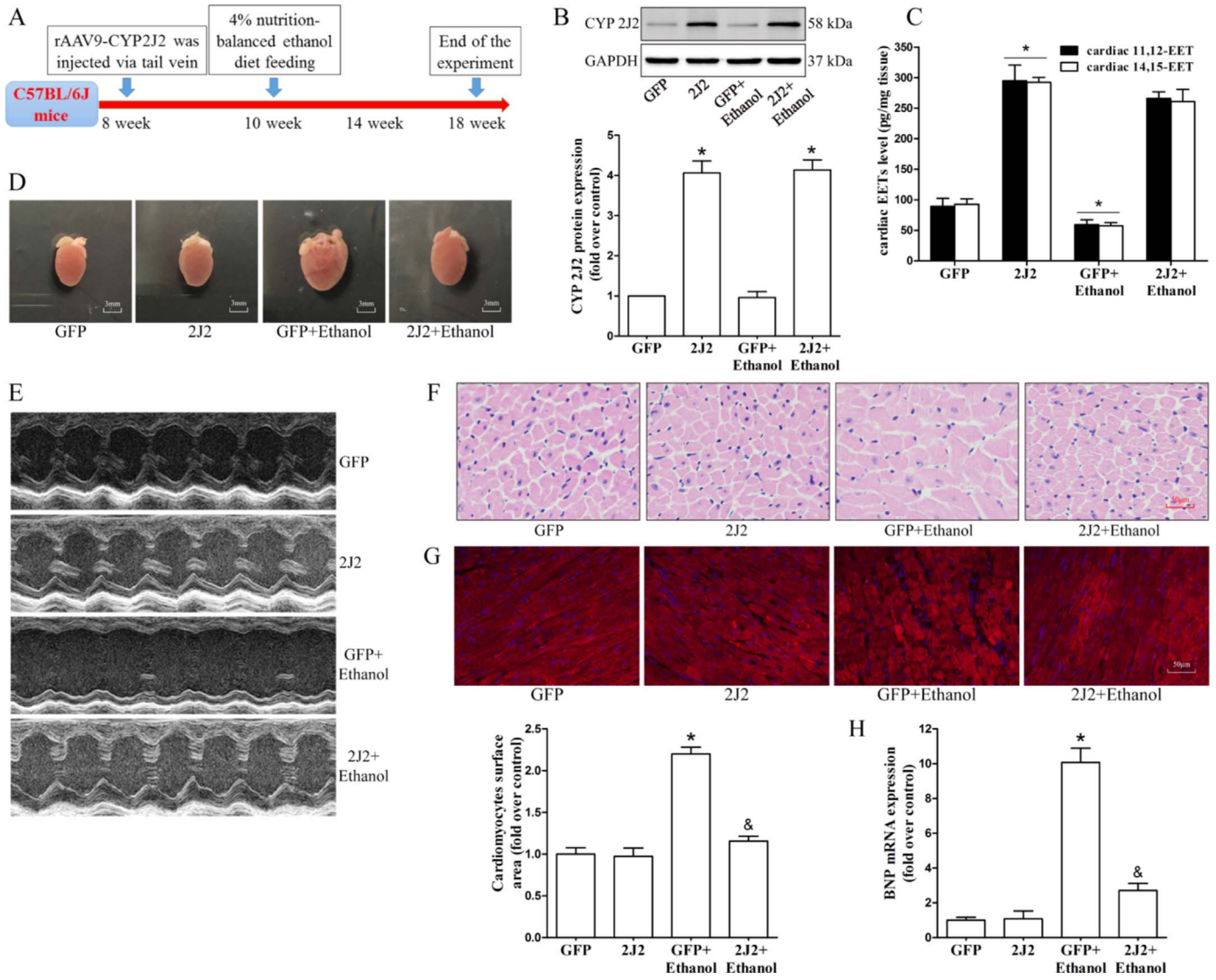

Fig. 1. CYP2J2 gene delivery prevented ethanol-induced myocardial dysfunction in C57BL/6 J mice. (A) Schematic of the animal model. (B) Representative immunoblots and quantitation of CYP2J2 protein expression in the myocardium after rAAV9-CYP2J2 gene delivery. (C) Cardiac 11,12-EET and 14,15-EET levels after rAAV9-CYP2J2 gene delivery measured by LC/MS. (D) Morphology and size of hearts from different groups. (E) Representative echocardiographic images of mice from different groups. (F) Histological analysis and quantitation of HE staining in myocardium from different groups. (G) Rhodamine-phalloidin staining for the cardiomyocyte cytoskeleton in myocardium from different groups. (H) BNP mRNA level in myocardium from different groups. Data were expressed as Mean ± SEM, n = 10 mice for each group. *p < 0.05 vs. GFP, &p < 0.05 vs. GFP + Ethanol.

apoptosis [5]. Interestingly, apoptotic cardiomyocytes have been found in the myocardium of long-term, high-dose alcohol consumers, and they contribute to the development of structural heart damage [6]. Autophagy is a highly conserved event of intracellular protein and organelle recycling, which regulates cell survival and functions [7]. Briefly, double-membrane-bound vesicles, called autophagosomes or autophagic vacuoles, form to swallow cargoes and sequester cytoplasm, then the vacuole membrane fuses with lysosomes, where they are degraded and the resulting macromolecules are recycled [8]. Autophagic flux refers to the entire process of autophagy. AMP-activated protein kinase (AMPK) is an intracellular sensor of energy status that promotes autophagy via inhibition of mammalian target of rapamycin (mTOR). It has been reported that autophagy is closely related to various cardiovascular diseases, including myocardial ischemia/reperfusion injury, diabetic cardiomyopathy, cardiac hypertrophy, and heart failure [9]. Importantly, a predomination of autophagy over apoptosis will result in cell survival over death. Recent evidence has described the contribution of autophagy and apoptosis in the pathogenesis of ACM. Studies from Jun Ren's team demonstrated that ethanol exposure triggers myocardial dysfunction through AMPK-mTORC1-ULK1-mediated autophagosome formation, along with impaired lysosomal degradation [10–12]. Additionally, high-dose ethanol induced oxidative stress and mitochondrial damage in cardiomyocytes, which activates caspases that initiate chromatin fragmentation and apoptosis [13]. Even though abundant studies have been reported, the precise mechanisms and therapeutic strategies of ACM remain to be elucidated.

Arachidonic acid (AA) is converted to eicosanoid mediators by three different enzyme pathways, namely, cyclooxygenase, lipoxygenase, and cytochrome P450 (CYP) epoxygenase pathways [14]. CYP2J2 is abundantly expressed in the human cardiovascular system, and metabolizes AA to four cis-epoxyeicosatrienoic acids (EETs): 5,6-, 8,9-, 11,12- and 14,15-EETs. Soluble epoxide hydrolase (sEH) hydrolyzes EETs to less biologically active dihydroxyeicosatrienoic acids. Accumulating evidence has indicated that CYP/EETs exert crucial and diverse biological activities in maintaining cardiovascular homeostasis. Our previous studies demonstrated that cardiomyocyte-specific CYP2J2 overexpression prevents angiotensin II-induced cardiac remodeling and heart failure by attenuating oxidative stress-mediated NF-κB nuclear translocation [15] and reducing endoplasmic reticulum stress [16]. Recently, CYP/EETs were found to be closely related to the process of