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Inhibition of high glucose-induced inflammation and fibrosis by a novel curcumin derivative prevents renal and heart injury in diabetic mice

Hongjin Chen[a,1], Xi Yang[b,e,1], Kongqin Lu[a], Chun Lu[c], Yunjie Zhao[a], Suqing Zheng[a], Jieli Li[a], Zhangjian Huang[a,d], Yi Huang[a,*], Yali Zhang[a,*], Guang Liang[a]

[a] Chemical Biology Research Center, School of Pharmaceutical Sciences, Wenzhou Medical University, Wenzhou, Zhejiang 325035, China
[b] The Eye Hospital of Wenzhou Medical University, Wenzhou, Zhejiang 325035, China
[c] The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, Zhejiang 325035, China
[d] College of Pharmacy, China Pharmaceutical University, Nanjing, Jiangsu 210038, China
[e] The First People's Hospital of Yichang, Yichang, Hubei 443000, China

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ABSTRACT

Hyperglycemia-induced inflammation and fibrosis have important roles in the pathogenesis of diabetic nephropathy and cardiomyopathy. With inflammatory cytokines and signaling pathways as important mediators, targeting inflammation may be an effective approach to new avenue for treating diabetic complications. **J17**, a molecule with structural similarities to curcumin, exhibited good anti-inflammatory activities by inhibiting LPS-induced inflammatory response in macrophages. However, its ability to alleviate hyperglycemia-induced injury via its anti-inflammatory actions remained unclear. Thus, we reported that **J17** exerts significant inhibitory effects on hyperglycemia-induced inflammation and fibrosis in NRK-52E cells, H9C2 cells and a streptozotocin-induced diabetic mouse model. We also found that the anti-inflammatory and anti-fibrosis activities of **J17** are associated with the inhibition of the P38 and AKT signal pathway, respectively. In vivo oral administration of **J17** suppressed hyperglycemia-induced inflammation, hypertrophy and fibrosis, thereby reducing key markers for renal and cardiac dysfunction and improving in fibrosis and pathological changes in both renal and cardiac tissues of diabetic mice. The results of this study indicated that **J17** can be potentially used as a cardio- and reno-protective agent and that targeting the P38 and AKT pathways may be an effective therapeutic strategy for diabetic complications.

1. Introduction

In recent decades, complications of diabetes have become increasingly prevalent health issues (Forbes and Cooper, 2013). Diabetic nephropathy (DN) is currently identified as the main cause of end stage renal disease; in addition, kidney disease plays an important role in the development of diabetic macrovascular complications, including diabetic cardiomyopathy (DCM). Meanwhile, DCM also can lead to increased disability, reduced life expectancy or quality of life, and considerably large health costs. Once DN is established, a series of pathophysiological changes will occur; thus, early-stage diabetic nephropathy and cardiomyopathy must be controlled (Israili, 2011). Current treatments have been mainly focused on managing blood glucose and insulin levels(Rotenstein and Close, 2010). Thus, a new and more effective drug category for the treatment of diabetic complications needs to be developed. Increasing evidences have recently suggested the involvement of inflammatory and adhesion molecules in the progression of diabetic complications (Bugger and Abel, 2014; Ferenbach et al., 2007; Goldberg 2009). Hyperglycemia, as a stimulus, contributes to macrophage infiltration and pro-inflammatory responses, as well as the over expression of numerous inflammatory cytokines (Goldberg, 2009; Snell-Bergeon et al., 2010), including tumor necrosis factor (TNF)-α, interleukin-6 (IL-6) and monocyte chemoattractant protein (MCP)-1 in the hearts and kidneys. Within a complex regulatory network, these cytokines are related to specific immunological processes that promote chronic inflammation and tissue destruction (Fioretto et al., 2008; Har et al., 2013). Therefore, investigating anti-inflammatory strategies using anti-inflammatory agents can provide new approaches for the treatment of DN and DCM.

Curcumin, a natural compound, has been extensively studied for its potential pharmacological regulatory effects on cancer, inflammation and inflammation related diseases (Prasad et al., 2014). Meanwhile, a

* Corresponding authors.
E-mail addresses: 112205596@qq.com (Y. Huang), ya-li000@163.com (Y. Zhang).
[1] These authors contribute equally to this work.

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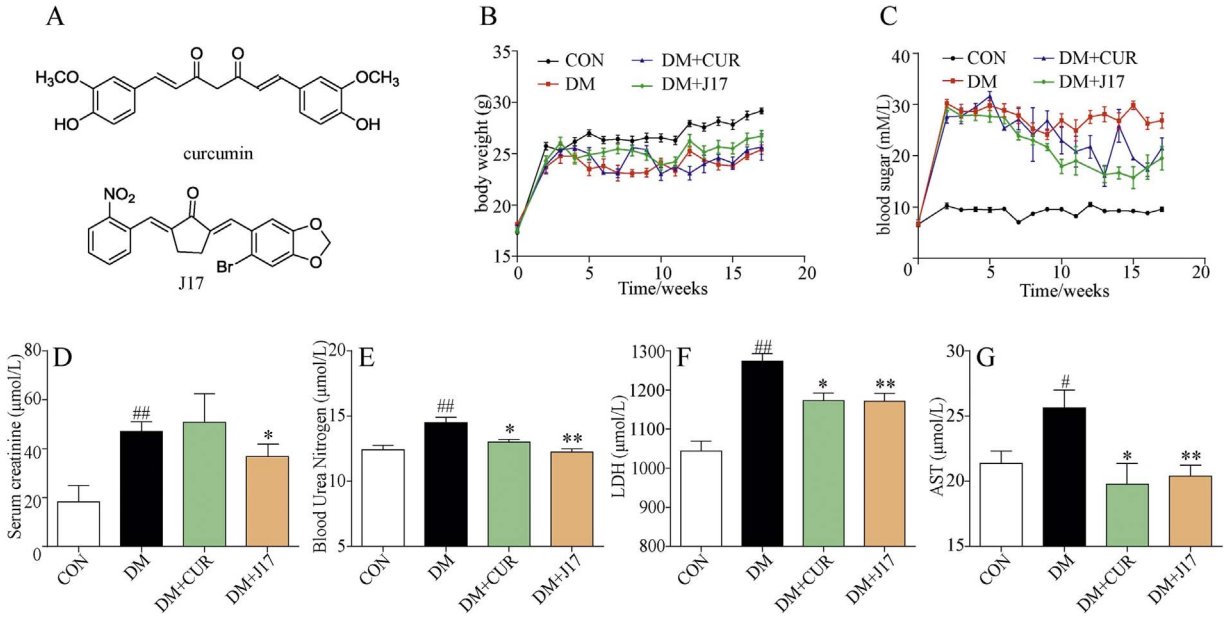

Fig. 1. J17 reduced hyperglycemia-induced biochemical indicators increase without significantly affecting the body weight of diabetic mice. (A) Chemical structure of J17. Diabetes mellitus was induced in male C57BL/6 mice by a single intraperitoneal injection of STZ and mice with fasting-blood glucose > 12 mM were considered diabetes and then diabetic mice were orally treated with curcumin (CUR, 50 mg/kg), J17 (10 mg/kg), or vehicle by gavage once every two days for 8 weeks (n = 7 in each group). In this process, the body weight and blood glucose were monitored once every week. At the end of experiment, the mice were sacrificed and the blood samples were collected and centrifuged for collecting serum for serum biochemistry analysis. (B) The body weight; (C) The blood glucose; (D) The level of serum creatinine; (E) The serum level of BUN; (F) The serum level of LDH; (G) The serum level of AST; data are presented as mean ± SEM, n = 7; *P < 0.05; **P < 0.01 v.s. DM group, # P < 0.05, # #P < 0.01 v.s. CON group.

number of molecular targets in various types of cells, such as NF-κB, P38, HIF-1α, and AKT, have reportedly been involved in the anti-inflammatory activity of curcumin. However, the development of curcumin as a potential therapeutic agent has been limited by its low bioavailability and instability after oral administration (Cheng et al., 2001; Dhillon et al., 2008; Pawar et al., 2012). We have synthesized and evaluated several series of curcumin derivatives to optimize the therapeutic effects of curcumin (Yu et al., 2015; Zhang et al., 2015, 2014). Some of these derivatives exhibit significant anti-inflammatory activities, including (2*E*,5*E*)-2-(3-Hydroxy-4-methoxybenzylidene)-5- (2-nitrobenzylidene)cyclopentanone (**J17**, Fig. 1A), which exerted a greater inhibitory effect on LPS-induced overexpression of TNF-α and IL-6 and lower toxicity in macrophages, compared with curcumin (Yu et al., 2015). However, the effect of **J17** on the treatment of diabetic complications is still unclear and the molecular mechanism underlying the action of **J17** remains undetermined. Thus, we explored whether **J17** could alleviate high glucose-induced inflammation and diabetic complications.

2. Material and methods

2.1. Chemicals

Glucose and curcumin (PubChem CID: 969516) were purchased from Sigma (St. Louis, MO). J17 was prepared with a purity of 99.2% as described in our previous study. Compounds J17 were dissolved in 0.5% sodium carboxyl methyl cellulose (CMC-Na) for in vivo experiments and were dissolved in DMSO for in vitro experiments.

2.2. Animals and treatment

Male C57BL/6 mice weighing 20–22 g were obtained from the Animal Center of Wenzhou Medical University (Wenzhou, China). The mice were housed at a constant room temperature with a 12:12 h light–dark cycle and fed with a standard rodent diet and water. All animal experimental procedures complied with the 'The Detailed Rules and Regulations of Medical Animal Experiments Administration and Implementation' (Document No. 1998-55, Ministry of Public Health, PR China.), and were approved by the Wenzhou Medical University Animal Policy and Welfare Committee (Approval Document No. wydw2014-0058).

A total of 28 mice were randomly divided into 4 wt-matched groups. 21 mice received streptozotocin (STZ, Sigma Chemicals, St. Louis, MO) via intraperitoneal injection at 100 mg/kg, dissolved in 100 mM citrate buffer (pH 4.5) to induce diabetes mellitus; control animals received buffered saline only; 7 mice used **J17** at 10 mg/kg; another set of 7 mice as positive controls received curcumin. Both J17, curcumin and placebo were given by gavage 8 days after the STZ injection. Blood glucose was measured at Day 7, 9, 16, 22, 40, and 49 after STZ induction using a glucometer. At Day 49 after STZ induction, the mice were killed under anesthesia, and then blood samples were drawn from the right ventricle by using heparinized syringe with a needle. At the time of death, the kidneys and the hearts were removed.

2.3. Measurement of serum creatinine, blood urea nitrogen (BUN), lactate dehydrogenase (LDH), and aspertate aminotransferase (AST) in serum

Serum creatinine, BUN, LDH and AST were determined by centrifuging blood for 10 min at 3000 rpm. They were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions and results are expressed as mg/dL. In brief, to test serum creatinine, the reaction mixture consisted of tungsten acid protein precipitant, picric acid solution and serum sample were mixed and heating at 37 °C water bath for 10 min and absorbance change was monitored at 510 nm. BUN sample was mixed with buffer enzyme liquid and tested at 640 nm after colored by phenolic agent and basic sodium hypochlorite. To test LDH and AST, we need to set the mixture of serum sample, matrix liquid, 2,4-dinitrophenylhydrazine and 0.4 M NaOH with water bath accordingly. LDH was monitor at 450 nm and AST was 510 nm.

2.4. Renal and heart histopathology

Kidneys and hearts were fixed in 4% paraformaldehyde and