International Immunopharmacology 55 (2018) 9–19

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Oridonin protects against the inflammatory response in diabetic nephropathy by inhibiting the TLR4/p38-MAPK and TLR4/NF-κB signaling pathways

Jushuang Li, Liping Bao, Dongqing Zha, Lian Zhang, Ping Gao, Juan Zhang, Xiaoyan Wu*

Department of Nephrology, Zhongnan Hospital of Wuhan University, Wuhan 430071, China

ARTICLE INFO

Keywords:
Oridonin
Diabetic nephropathy
Inflammatory response
TLR4
p38-MAPK
NF-κB

ABSTRACT

Inflammation plays a pivotal role in the development and progression of diabetic nephropathy (DN). Oridonin (Ori), a component isolated from Rabdosia rubescens, possesses remarkable anti-inflammatory, immunoregulatory and antitumor properties. However, the renoprotective effects of Ori and the underlying molecular mechanisms have not been explored in DN. In this study, we aimed to investigate the protective effects and potential mechanisms responsible for the anti-inflammatory effects of Ori in diabetes-induced renal injury in vivo and in vitro. Our results showed that Ori significantly attenuated diabetes-induced renal injury and markedly decreased urinary protein excretion levels, serum creatinine concentrations and blood urea nitrogen concentrations in rats. Ori also significantly alleviated infiltration of inflammatory cells (cluster of differentiation (CD)68) in kidney tissues and reduced the levels of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-1β and monocyte chemotactic protein 1 (MCP-1), both in vivo and in vitro. TLR4 is a principal mediator of innate immune and inflammatory responses and participates in the development of DN. Our molecular studies indicated that Ori administration significantly down-regulated TLR4 overexpression in DN. Additional studies were conducted to investigate the effect of Ori on the p38-mitogen-activated protein kinase (p38-MAPK) and nuclear factor (NF)-κB pathways. The results showed that Ori inhibited IκBα, p65, and p38 phosphorylation, as well as NF-κB DNA-binding activity. In conclusion, these results demonstrated that Ori exerts protective effects in diabetes-induced renal injury in vivo and in vitro. These effects may be ascribed to its anti-inflammatory and modulatory effects on the TLR4/p38-MAPK and TLR4/NF-κB signaling pathways.

1. Introduction

Diabetic nephropathy (DN) is one of the most common and serious microvascular complications of diabetes mellitus. DN has become the single largest cause of end-stage renal disease (ESRD), and its prevalence has been increasing worldwide [1–4]. However, the pathogenesis of DN is not completely understood. Consequently, there is a lack of specific therapies capable of preventing DN development and progression. Therefore, studies aiming to elucidate the pathogenesis of DN and search for new therapies for DN are urgently needed. Accumulating evidence collected in recent years indicates that inflammatory processes facilitated by the innate immune response are of paramount importance with respect to the pathogenesis of DN [5,6]. Toll-like receptors (TLRs) are a conserved family of pattern recognition receptors that play a fundamental role in the innate immune system by triggering pro-inflammatory signaling pathways in response to microbial pathogens. In addition, TLRs are activated by endogenous agonists of nonmicrobial origin and participate in noninfectious inflammatory processes [7]. TLR4, a component of the primary innate immune receptor-mediated inflammatory signaling pathway, is mainly distributed in the glomerular mesangial cells and renal tubular epithelial cells of kidney tissues [8]. Accumulating evidence shows that TLR4 expression in glomerular mesangial cells and renal tubular epithelial cells can clearly increase in response to high glucose (HG) and angiotensin II, which can activate the downstream p38-mitogen-activated protein kinase (p38-MAPK) and nuclear factor (NF)-κB pathways and accelerate the secretion of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-1β and monocyte chemotactic protein 1 (MCP-1), thereby aggravating kidney injury [9–11]. The p38-MAPK and NF-κB pathways are two important inflammatory signaling pathways that are closely associated with renal tissue damage. Of these pathways, the p38-MAPK signaling pathway is the “classical” pathway

* Corresponding author at: Department of Nephrology, Zhongnan Hospital of Wuhan University, No.169, Road Donghu, Wuhan 430071, Hubei, China.
E-mail address: ZN000081@whu.edu.cn (X. Wu).

https://doi.org/10.1016/j.intimp.2017.11.040
Received 24 September 2017; Received in revised form 22 November 2017; Accepted 28 November 2017
Available online 05 December 2017

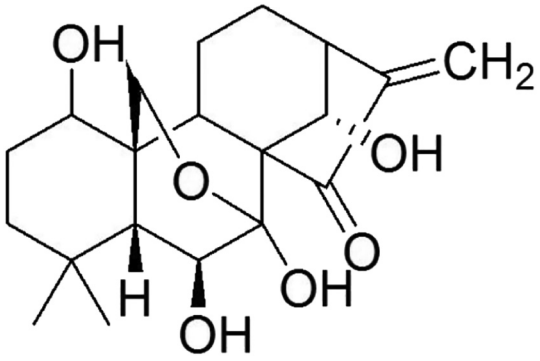

Fig. 1. The chemical structure of Oridonin.

through which anti-inflammatory drugs interfere with renal inflammatory tissue damage. Therefore, studying the natural immune inflammatory mechanisms underlying DN development and progression and searching for drugs that modulate immune-mediated inflammation may yield promising strategies for curing DN.

Oridonin (Ori, Fig. 1), a famous diterpenoid isolated from the Chinese medicinal herb Rabdosia rubescens, possesses a variety of biological properties, including antitumor [12], anti-inflammatory [13–15], immunoregulatory [13,16], antioxidant [17] and antibacterial properties [18]. Ori has been administered for the treatment of inflammatory diseases for hundreds of years in China and has become one of the most popular herbs used clinically. Zhou et al. [19] reported that Ori efficiently increases survival, alleviates proteinuria, attenuates renal damage and ameliorates the serological and clinical manifestations of systemic lupus erythematosus (SLE) in MRL-lpr/lpr mice. However, few studies have investigated the effects of Ori on kidney injury in diabetic rats and glomerular mesangial cells. Therefore, we aimed to investigate whether Ori protects against kidney injury in diabetic rats and rat glomerular mesangial cells and further elucidate the anti-inflammatory mechanism that involves the TLR4/p38-MAPK and TLR4/NF-κB signaling pathways. Our findings may support the clinical application of Ori as a treatment for DN.

2. Materials and methods

2.1. Materials

Oridonin was purchased from Shanghai Yuanye Biological Technology Co., Ltd. (Shanghai, China). The purity of Ori, which was measured by high-performance liquid chromatography (HPLC), was > 98%. Streptozotocin (STZ) was purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM/low glucose), DMEM/HG and fetal bovine serum (FBS) were purchased from HyClone (Logan, UT, USA). Commercial assay kits for urine protein, serum creatinine (Scr) and blood urea nitrogen (BUN) were purchased from the Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Laboratories. A TLR4 inhibitor (TAK242) was obtained from Life Technologies Corporation (Carlsbad, CA, USA). Antibodies for p-p65, p65, p-IκBα, IκBα, p-p38-MAPK, p38-MAPK, cluster of differentiation (CD)68, CD4 + and CD8 + were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibodies for TLR4 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were obtained from Novus Biologicals, LLC (Littleton, CO, USA) and Abcam (Cambridge, UK), respectively. qPCR was performed using SYBR® Premix Ex Taq™ (Takara, Japan). TNF-α, IL-6, IL-1β and MCP-1 enzyme-linked immunosorbent assay (ELISA) kits were purchased from R &D Systems (Minneapolis, MN, USA).

2.2. Animals and treatment

A total of 32 specific pathogen-free (SPF) grade male Sprague Dawley (SD) rats (aged 6–8 weeks and weighing 200 ± 20 g) were purchased from the Center for Disease Control and Prevention in Hubei province (Hubei, China). All of the rats were maintained under a standard temperature (21 ± 2 °C), humidity (55 ± 2%) and 12-h light/dark cycle. The rats had free access to a standard rodent diet and drinking water. All the animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the NIH.

After 1 week of adaptive feeding, all the rats were randomly assigned to the following four groups (8 rats per group): a normal control (NC) group, NC + Ori group, T2DM model (DM) group and DM + Ori group. The DM and DM + Ori groups were fed a high-fat diet (D12492) for 4 weeks to establish an insulin resistance model. All of the insulin resistance model rats were converted into DM rats by a single intraperitoneal injection of STZ (35 mg/kg dissolved in 0.1 mol/l citric acid buffer, pH 4.3). The NC and NC + Ori groups were fed normal chow (4% calories from fat) for 4 weeks and then intraperitoneally injected with the same dose of citric acid buffer (pH 4.3, 0.1 mol/l). At 72 h after STZ injection, we measured random blood glucose levels to confirm the successful establishment of the diabetes model. A random blood glucose level of > 16.7 mmol/l after STZ injection was indicative of the establishment of the model. All the rats in the model groups successfully developed T2DM. The rats in NC + Ori and DM + Ori groups were intraperitoneally injected with 10 mg/kg/day Ori, while the rats in the NC and DM groups received the equivalent dose of normal saline. The experiment lasted for 12 weeks. Body weights and random blood glucose levels were monitored weekly in all rats. All the rats were housed in metabolic cages for 24-h urine collection at the end of the 12th week and then sacrificed under chloral hydrate anesthesia. The data for the random blood glucose levels and body weights were compared among the groups. Blood samples were acquired by cardiac puncture at the time of sacrifice. Serum was then separated by centrifugation and stored at − 20 °C for subsequent experiments. Kidney tissues were also excised, weighed, and stored in liquid nitrogen or fixed in 4% paraformaldehyde. The kidney weight-to-body weight ratio was calculated for each rat.

2.3. Assessment of renal biochemical markers

Scr, BUN and 24-h urinary protein concentrations were determined using a creatinine assay kit, enzymatic assay kit and protein estimation kit, respectively, according to the protocols provided by the manufacturers.

2.4. Histopathological examination of kidney tissues

Portions of the renal cortex that were fixed in 4% paraformaldehyde were embedded in paraffin and cut into 4-μm-thick sections. The tissue sections were stained with hematoxylin-eosin (H&E) and periodic acid-Schiff (PAS) for assessment under a light microscope (Olympus, Japan).

2.5. Immunohistochemistry for TLR4 and CD68 expression in kidney tissue

Following deparaffinization and hydration, 4-μm-thick renal cortical tissue sections were treated with 3% H_2O_2 for 5–10 min to inactivate endogenous enzymes and then treated with 5% BSA in phosphate-buffered saline (PBS) blocking solution for 30 min at room temperature. After an incubation with the appropriate primary antibodies (1:100) overnight at 4 °C, the sections were incubated with a horseradish peroxidase-labeled goat anti-rabbit polyclonal antibody for 30 min at 37 °C. The sections were then counterstained with hematoxylin, dehydrated, cleared, and mounted with Permount mounting solution. Ten fields in each section were randomly chosen for