Pharmacological Research 111 (2016) 509–522

Contents lists available at ScienceDirect

Pharmacological Research

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

Dioscin alleviates lipopolysaccharide-induced inflammatory kidney injury *via* the microRNA let-7i/TLR4/MyD88 signaling pathway

Meng Qi, Lianhong Yin, Lina Xu, Xufeng Tao, Yan Qi, Xu Han, Changyuan Wang, Youwei Xu, Huijun Sun, Kexin Liu, Jinyong Peng*

College of Pharmacy, Dalian Medical University, Western 9 Lvshunnan Road, Dalian 116044, China

ARTICLE INFO

Article history:
Received 14 April 2016
Received in revised form 15 June 2016
Accepted 14 July 2016
Available online 16 July 2016

Keywords:
Lipopolysaccharide
Inflammatory acute kidney injury
Micro let7i
TLR4
Dioscin
MyD88

ABSTRACT

We previously reported the potent effect of dioscin against renal ischemia/reperfusion injury, but little is known about the role of dioscin in lipopolysaccharide (LPS)-induced inflammatory kidney injury. The present work aimed to investigate the effects and potential mechanisms of dioscin in preventing LPS-induced kidney injury. *In vivo* injury was induced in rats and mice with an intraperitoneal injection of LPS (10 mg/kg), and *in vitro* studies were performed on NRK-52E and HK-2 cells challenged with LPS (0.5 μg/ml). Our results indicated that dioscin significantly protected against renal damage by decreasing blood urea nitrogen and creatinine levels and reversing oxidative stress. Mechanistic studies demonstrated that dioscin markedly up- regulated the level of the microRNA let-7i, resulting in significant inhibition of TLR4 expression. Dioscin significantly down-regulated the levels of MyD88, NOX1 and cleaved caspase-8/3; inhibited the nuclear translocation of NF-κB; inhibited PI3K and Akt phosphorylation; increased the levels of SOD2; and decreased the mRNA levels of IL-1β, IL-6, MIP-1α, Fas and FasL. *In vitro*, transfection of microRNA let-7i inhibitor and TLR4 DNA were applied, and the results further confirmed the nephroprotective effect of dioscin in suppressing TLR4/MyD88 signaling and subsequently inhibiting inflammation, oxidative stress and apoptosis. Furthermore, the abrogation of cellular MyD88 expression by ST2825 eliminated the inhibitory effect of dioscin on the levels of nuclear NF-κB, cleaved caspase-3, SOD2 and ROS. These data indicated that dioscin exerted a nephroprotective effect against LPS-induced inflammatory renal injury by adjusting the microRNA let-7i/TLR4/MyD88 signaling pathway, which provided novel insights into the mechanisms of this therapeutic candidate for the treatment of inflammatory kidney injury.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Acute kidney injury (AKI), a critical care syndrome, is characterized by a rapid decrease in renal function, which is responsible for substantial resource utilization and mortality in intensive care unit (ICU) patients [1]. The clinical conditions leading to AKI are caused by various factors, including sepsis-induced infection [2], cardiac surgery [3], liver or kidney transplantation [4], and contrast media-induced nephropathy [5]. Endotoxic (lipopolysaccharide, LPS) shock, the secondary outcome of systemic infections, remains the most common trigger for AKI. Sepsis-induced AKI results in up to 50% of mortality in ICU patients [6]. Therefore, it is necessary to seek effective therapeutic methods or pharmaceuticals to reduce the mortality of AKI in the clinic.

Acute tubular necrosis (ATN) is usually caused by ischemia/reperfusion injury and LPS-mediated inflammatory responses [7]. LPS is derived from the cell wall of Gram-negative bacilli [8] and stimulates a renal inflammatory cascade resulting

Abbreviations: LPS, lipopolysaccharide; MDA, malondialdehyde; NO, nitric oxide; ROS, reactive oxygen species; GSH, Px glutathione peroxidase; CAT, catalase; TLR4, toll like receptor 4; MyD88, myeloid differentiation primary response gene; NOX1, NADPH oxidase 1; NF-κB, nuclear factor kappa B; PI3K, phosphatidylinositol 3-kinases; Akt, serine/threonine kinase; SOD2, superoxide dismutase; IL-1β, interleukin-1β; IL-6, interleukin-6; MIP-1α, macrophage inflammatory protein-1α; Fas, factor associated with suicide; FasL, factor associated with suicide ligand; AKI, acute kidney injury; ICU, intensive care unit; ATN, acute tubular necrosis; miRs, microRNAs; TLRs, toll-like receptors; TUNEL, Terminal-deoxynucleotidyl transferase mediated dUTP nick end labeling; FADD, factor associated with suicide-associated death domain-containing protein; DMSO, dissolving with dimethyl sulfoxide; CMC-Na, sodium carboxylmethyl-cellulose; DMEM, Dulbecco's minimum essential medium; FBS, Fetal bovine serum.

* Corresponding author.
E-mail address: jinyongpeng2008@126.com (J. Peng).

http://dx.doi.org/10.1016/j.phrs.2016.07.016
1043-6618/© 2016 Elsevier Ltd. All rights reserved.

from cytokine-chemokine responses that can result in kidney end-organ damage. The factors involved in LPS-induced AKI include renal hemodynamic changes, endothelial dysfunction, renal interstitial inflammatory infiltration, microthrombus in the glomeruli and renal tubular congestion, among others [9].

Some inflammatory cytokines that are released from renal cells play critical roles in the pathogenesis of AKI [10]. Although sepsis is the most important cause of AKI, the underlying mechanisms are not completely understood. Numerous basic and clinical experiments have demonstrated that inflammation plays a vital role in the primary and secondary injury phases of septic AKI [11,12]. LPS activates a large number of pro-inflammatory cytokines, which can exacerbate the production of reactive oxygen species (ROS) and trigger tubular cell death. Therefore, the development of a novel and effective anti-inflammatory drug may be an efficient method to reverse LPS-induced AKI [13].

MicroRNAs (miRs) are characterized by the inhibition of mRNA transcription or the promotion of mRNA degradation in the occurrence and development of various diseases [14]. Many studies have reported that miR-let-7i, a member of the let-7 family of miRs, targets the Toll-like receptor 4 (TLR4) gene and inhibits its expression [15,16]. Toll-like receptors (TLRs), a family of 13 identified members in mammals, are evolutionarily conserved, widely expressed and belong to type I transmembrane proteins [17]. TLR4, a leading receptor for LPS, can regulate innate and adaptive immune responses, and may have a pathophysiological role in inflammation [18]. Upon stimulation with LPS, TLR4 can activate two classic signaling pathways: the myeloid differentiation factor 88 (MyD88)-dependent and -independent pathways [19]. LPS induces the formation of a signaling complex between MyD88 and phosphatidylinositol 3-kinase (PI3K). Serine/threonine kinase (Akt), the downstream target of PI3K, triggers inflammatory cytokine release and stimulates nuclear factor kappa B (NF-κB) translocation [20]. NADPH oxidase 1 (NOX1) is adjusted by LPS through MyD88-dependent inflammatory signaling, which leads to ROS generation and a reduction in antioxidant enzymes [21]. In addition, MyD88 transforms pro- caspase-8 into cleaved caspase-8 through a series of downstream signals, followed by an increase in the levels of cleaved caspase-3 and the expression of several apoptosis-related genes [22]. Therefore, inhibiting the TLR4/MyD88 signaling pathway *via* up-regulating miR-let-7i to suppress inflammation-mediated oxidative stress and apoptosis represents a potential nephroprotective treatment strategy.

Traditional Chinese medicines have been used in China to prevent and treat diseases for thousands of years, and some active natural products, including alpinetin from *Alpinia katsumadai Hayata* [23], leonurine from *Leonurus cardiaca* [24] and an extract from *Cordyceps sobolifera* [25], have been applied to treat LPS-mediated AKI. Thus, natural products from medicinal herbs are promising for the treatment of LPS- induced AKI.

Dioscin (Dio, shown in Supplemental Fig. 1), a natural steroid saponin, is isolated from various herbs [26]. Pharmacological investigations have shown that dioscin has anti-tumor, anti-hyperlipidemic and anti-fungal activities [27–29]. Our previous studies have indicated that dioscin has potent effects against carbon tetrachloride (CCl_4)-and paracetamol-induced acute liver damage [30,31] and non-alcoholic fatty liver disease (NAFLD) [32] and anti-inflammatory activities against hepatic ischemia/reperfusion damage [33], cerebral injury [34], renal injury [35] and alcoholic liver fibrosis [36]. However, no studies have reported the effects and molecular mechanisms of dioscin in preventing LPS-induced AKI.

Therefore, we explored the effects and possible mechanisms of dioscin in preventing LPS-induced inflammatory kidney injury.

2. Materials and methods

2.1. Chemicals and reagents

Dioscin with the purity of over 98% was isolated from *Dioscorea nipponica* Makino in our laboratory [26,37,38]. Dioscin with a purity >99% was purchased from Shanghai Tauto Biochemical Technology Co., Ltd. (Shanghai, China). The dioscin was added to the serum-free medium after it was dissolved in a final concentration of dimethyl sulfoxide (DMSO) of less than 0.1% for the *in vitro* experiments or suspended in 0.5% sodium carboxylmethyl-cellulose (CMC-Na) for the *in vivo* experiments. The BUN, Cr, MDA, NO, GSH-Px, and CAT kits were obtained from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). The tissue protein extraction kit was obtained from Keygen Biotech. Co., Ltd. (Nanjing, China). The bicinchoninic acid (BCA) protein assay kit and Nuclear and Cytoplasmic Protein Extraction Kit were purchased from the Beyotime Institute of Biotechnology (Jiangsu, China). 4′,6′-Diamidino-2-phenylindole (DAPI), tris (hydroxymethyl) aminomethane (Tris), sodium dodecyl sulfate (SDS) and CMC-Na were purchased from Sigma-Aldrich (St. Louis, MO, USA). Penicillin and streptomycin were obtained from Hyclone Laboratories, Inc. (MA, USA). Dulbecco's minimum essential medium (DMEM), fetal bovine serum (FBS) and trypsin were purchased from Gibco (CA, USA). TRIZOL, a PrimeScript® RT Reagent Kit with gDNA Eraser (Perfect Real Time), and SYBR® Premix Ex Taq™ II (Tli RNase H Plus) were purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China). The SanPrep Column MicroRNA Mini-Prep Kit, MicroRNA First Strand cDNA Synthesis Kit and MicroRNA Quantitation PCR Kit were purchased from Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The miR-let-7i inhibitor and Lipofectamine 2000 were purchased from RiboBio Co., Ltd. (Guangzhou, China). LPS was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Cell culture

The NRK-52E normal rat kidney epithelial cell line and HK-2 human kidney tubular epithelial cell line were purchased from the Institute of Biochemistry Cell Biology (Shanghai, China) and maintained in DMEM supplemented with 10% FBS and antibiotics (100 IU/ml penicillin and 100 mg/ml streptomycin) in a humidified atmosphere of 5% CO_2 and 95% O_2 at 37 °C.

2.3. Toxicity assay

The NRK-52E and HK-2 cells were plated in 96-well plates at a density of 5×10^4 cells/ml per well for 24 h and treated with various concentrations of dioscin (0, 50, 100, 200, 400, 800 or 1000 ng/ml) for an additional 24 h. The cells were then analyzed according to the MTT method. The absorbance of the samples was quantified at 490 nm using a spectrophotometer (Thermo Fisher Scientific, MA, USA).

2.4. LPS-induced cell injury

LPS was prepared in ultrapure water and used to make a series of working dilutions of 0.0625, 0.125, 0.25, 0.5, 1.0 and 2.0 μg/ml in serum-free DMEM for direct application to the cell cultures [39]. The NRK-52E and HK-2 cells were plated in 96-well plates at a density of 5×10^4 cells/ml per well for 24 h before they were challenged with various concentrations of LPS and then incubated for 24 h. Cell viability was assessed with the MTT assay. Based on the MTT results, the concentration of LPS that was sufficient to induce the cell injury was optimized.

Download English Version:

https://daneshyari.com/en/article/5843513

Download Persian Version:

https://daneshyari.com/article/5843513

Daneshyari.com