European Journal of Pharmacology 747 (2015) 59–70

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Immunopharmacology and inflammation

Nuciferine restores potassium oxonate-induced hyperuricemia and kidney inflammation in mice

Ming-Xing Wang, Yang-Liu Liu, Ying Yang, Dong-Mei Zhang, Ling-Dong Kong *

State Key Laboratory of Pharmaceutical Biotechnology, School of Life Science, Nanjing University, Nanjing, People's Republic of China

ARTICLE INFO

Article history:
Received 8 July 2014
Received in revised form
27 November 2014
Accepted 28 November 2014
Available online 8 December 2014

Keywords:
Nuciferine
Hyperuricemia
Organic ion transporters
TLR signaling
NF-κB
NLRP3 inflammasome

ABSTRACT

Nuciferine, a major aporphine alkaloid of the leaves of *Nelumbo nucifera*, was found to decrease serum urate levels and improved kidney function, as well as inhibited system and renal interleukin-1β (IL-1β) secretion in potassium oxonate-induced hyperuricemic mice. Furthermore, nuciferine reversed expression alteration of renal urate transporter 1 (URAT1), glucose transporter 9 (GLUT9), ATP-binding cassette, subfamily G, membrane 2 (ABCG2), organic anion transporter 1 (OAT1), organic cation transporter 1 (OCT1), and organic cation/carnitine transporters 1/2 (OCTN1/2) in hyperuricemic mice. More importantly, nuciferine suppressed renal activation of Toll-like receptor 4/myeloid differentiation factor 88/NF-kappaB (TLR4/MyD88/NF-κB) signaling and NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome to reduce serum and renal IL-1β levels in hyperuricemic mice with renal inflammation reduction. The anti-inflammatroy effect of nuciferine was also confirmed in human proximal renal tubular epithelial cells (HK-2 cells) incubated with 4 mg/dl uric acid for 24 h. This study firstly reported the anti-hyperuricemic and anti-inflammatory effects of nuciferine by regulating renal organic ion transporters and inflammatory signaling in hyperuricemia. These results suggest that a dietary supplement of nuciferine rich in lotus leaf may be potential for the prevention and treatment of hyperuricemia with kidney inflammation.

1. Introduction

Hyperuricemia is known as an important risk factor for the development of gout, hypertension, diabetes, cardiovascular complications, metabolic syndrome and kidney disease (Johnson et al., 2013; Obermayr et al., 2008; Weiner et al., 2008). Renal urate transporter 1 (URAT1) (Enomoto et al., 2002), glucose transporter 9 (GLUT9) (Vitart et al., 2008), ATP-binding cassette, subfamily G, membrane 2 (ABCG2) (Nakayama et al., 2011) and organic anion transporter 1 (OAT1) (Hediger et al., 2005) contribute to urate homeostasis in kidney. Hyperuricemia is observed in the patients with kidney URAT1 defect (Enomoto et al., 2002). Variants of GLUT9 are related to low fractional excretion of uric acid (FEUA) and gout (Vitart et al., 2008). A genome-wide association study of serum urate levels demonstrates the close relationship between GLUT9 and ABCG2 in normal persons (Yang et al., 2014). Moreover, other kidney organic ion transporters participate in excretion of endogenous metabolites and exogenous drugs or toxins. The expression alteration of renal organic cation transporter 1 (OCT1), organic cation/carnitine transporter 1 (OCTN1) and OCTN2 is observed in hyperuricemic rodents with kidney dysfunction (Fan et al., 2014; Liu et al., 2014; Wang et al., 2010; Zhang et al., 2012).

Increasing evidence suggests that uric acid-induced inflammation is the central mechanism for kidney injury in hyperuricemic rodents (Fan et al., 2014; Wang et al., 2012) and diabetes patients (Sinha et al., 2014), in which NF-kappaB (NF-κB) pathway may play a central role. In fact, NF-κB is a key transcription factor to regulate the expression of pro-inflammatory cytokine interleukin-1β (IL-1β) mediated *via* Toll-like receptors (TLRs) signaling (Akira and Takeda, 2004), in which cytosolic TLR adapter protein myeloid differentiation factor 88 (MyD88) is involved. Induction of IL-1β by monosodium urate monohydrate crystals is suppressed in TLR-2$^{-/-}$ and TLR-4$^{-/-}$ mice and attenuated in MyD88$^{-/-}$ mice (Chen et al., 2014). Activation of the NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome triggers Caspase-1 activation to produce mature IL-1β. Thus, suppression of TLRs-MyD88-NF-κB-NLRP3 inflammasome activation-mediated inflammation may improve renal injury in hyperuricemia.

Nelumbo nucifera Gaertn. cv. Rosa-plena (commonly known as lotus, Nymphaeaceae) is a perennial aquatic plant grown in Asia. Its leaf extracts are widely applied in traditional herb or healthcare food to treat obesity (Guan et al., 2003a, 2003b), diabetes and hyperuricemia (Jin et al., 2011). Nuciferine, a major aporphine alkaloid found in the leaves, is found to prevent dyslipidemia, hepatic steatosis and oxidative stress in high fat-fed golden

* Corresponding author. Tel./fax: +86 25 8359 4691.
E-mail address: kongld@nju.edu.cn (L.-D. Kong).

http://dx.doi.org/10.1016/j.ejphar.2014.11.035
0014-2999/

hamsters (Guo et al., 2013; Lin et al., 2009) and stimulate insulin secretion in isolated islets (Nguyen et al., 2012). In this study, we firstly investigated the effects of nuciferine on serum uric acid levels, kidney function and inflammation response in potassium oxonate-induced hyperuricemic mice, as well as explored pharmacological mechanisms by detecting renal protein levels of organic ion transporters and inflammatory signaling pathway. Moreover, using human proximal renal tubular epithelial cells (HK-2 cells) as an *in vitro* model, the protective effect of nuciferine against hyperuricemia was confirmed by inhibiting inflammation responses in high uric acid stimulation.

2. Materials and methods

2.1. Reagents and chemicals

Nuciferine (purity > 90.0%) was obtained from Plant Bioengineering (Xi'an, P. R. China). Potassium oxonate (purity > 99.0%) and allopurinol (purity > 98.0%) were purchased from Sigma (St. Louis, USA). Assay kits of uric acid (UA), creatinine and blood urea nitrogen (BUN), and Hematoxylin–eosin (H&E) reagent were obtained from Jiancheng Biotech (Nanjing, P. R. China). An ELISA kit for IL-1β assay was purchased from IBL (Minneapolis, USA). The antibodies of URAT1 (001046-R), GLUT9 (001051-R), OAT1 (001019-R), and OCT1 (001017-R) were purchased from Cellchip Biotech (Beijing, P. R. China). Antibodies of OCTN1 (OCTN11-A) and OCTN2 (OCTN21-A) were purchased from Alpha Diagnostic International, Inc. (San Antonio, USA). Antibodies of ABCG2 (#4477S), MyD88 (#4283), inhibitor κB kinase (IKK) β (#2684), p-IKKα/β (Ser176/180, #2697), inhibitor of NF-κBa (IκBα) (#4814) and p-IκBα (Ser32, #2859), NF-κB p65 (#4764) and p-NF-κB p65 (Ser536, #3033) were purchased from Cell Signaling Technology (Boston, MA). Antibodies of TLR2 (sc-10739), TLR4 (sc-10741) and GAPDH (sc-25778) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, USA). Antibodies of NLRP3 (ab109314), Apoptosis-associated Speck-like protein containing a CARD (caspase recruitment domain) (ASC) (ab64808), Caspase-1 (ab17820) and IL-1β (ab9787) were purchased from Abcam (Cambridge, USA). HRP-conjugated anti-rabbit IgG antibody (074–1506) was purchased from KPL (Gaithersburg, USA), and HRP-conjugated anti-mouse IgG antibody (sc-2005) was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, USA). Antibodies of $CD3^+$(sc-20047) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, USA). The antibodies used in HK-2 cell experiments were the same as the *in vivo* experiments, except Caspase-1 (#2022) and IL-1β (#3866), which were purchased from Cell Signaling Technology (Boston, MA). 3,3′- diaminobenzidine (DAB) was purchased from Beyotime Institute of Biotechnology (Haimen, P. R. China). IumiGLO reagent was purchased from Cell Signaling Technology (Boston, MA). X-ray film was purchased from Kodak (New Haven, CT). Methanol, chloroform, dimethyl sulphoxide (DMSO), sodium chloride, formalin, ethanol, urethane, xylene, paraffin, neutral balsam and isopropyl alcohol were of analytical grade and purchased from Sinopharm Chemical Reagent (Shanghai, P. R. China).

2.2. Animals

Male Kun-Ming strain of mice (20 ± 2 g) were purchased from the animal center of Qing-Longshan (Nanjing, Jiangsu Province, P. R. China, Certificate no. SCXK 2009-0002). They were allowed at least 1 week to adapt to their environment before used for experiments. Animals were housed 5 per cage under a normal 12-h/12-h light/dark schedule with the lights on at 07:00 a.m. They were housed at room temperature (22 ± 2 °C) with relative humidity (55 ± 5%), and given a standard chow and water *ad libitum* for the duration of the study. All studies were carried out in accordance with the Institutional Animal Care Committee at the Nanjing University and the China Council on Animal Care at Nanjing University (The Ministry of Science and Technology of the People's Republic of China, 2006).

2.3. Hyperuricemic mice and drug administration

The uricase inhibitor potassium oxonate was used to induce hyperuricemia in mice according to previous reports (Wang et al., 2010). The hyperuricemic mice were randomly divided into 5 groups ($n = 10$ per group), receiving water (vehicle), nuciferine (10, 20 and 40 mg/kg) and allopurinol (5 mg/kg). Doses of allopurinol and nuciferine were determined based on the conversions from clinical adult dosages (China Pharmacopoeia Committee, 2010) and our preliminary studies. According to the State Pharmacopoeia of People's Republic of China, dosage of lotus leaves for adults is 3–10 g (the total raw materials)/day, which contains approximately 17.9–76.1 mg nuciferine. Equivalently, for mice, this dosage is 2.33–9.89 mg/kg/day calculated by the formula that converts body surface areas according to the Chinese Medicine Pharmacology Research Technology (China Pharmacopoeia Committee, 2010). Therefore we used 3 doses of nuciferine at 10, 20 and 40 mg/kg in this study. Food, but not water, was withdrawn from the animals 1 h prior to the administration. Potassium oxonate was administered by oral gavage once daily at 9:00 a.m. for 7 consecutive days. Nuciferine and allopurinol were orally initiated at 10:00 a.m. on the day when potassium oxonate was given.

2.4. Blood, urine and kidney tissue sample collection

During the 6 day of treatment, part of mice ($n = 10$ per group) were housed in metabolic cages with free access to standard chow and water. The 24-h urine was collected, recorded and centrifuged (2000*g*, 4 °C) for 10 min to remove particulate contaminants. Whole blood and urine samples were collected 1 h after final administration on the 7th day. The blood was allowed to clot for approximately 1 h at room temperature and centrifuged (10,000*g*, 4 °C) for 5 min to obtain serum. Serum and urine samples were then subjected to biochemical assays on the day of collection. Simultaneously, kidney cortex was rapidly and carefully separated on ice-plate and stored at −80 °C for assays.

2.5. Determination of uric acid and creatinine levels in serum and urine, as well as BUN levels in serum

Uric acid concentrations in serum (SUA) and urine (UUA) were determined by the phosphotungstic acid method (Hu et al., 2009). Creatinine levels in serum (SCr) and urine (UCr) were determined spectrophotometrically using a standard diagnostic kit. FEUA was calculated using the formula: $FEUA = (UUA \times SCr)/(SUA \times UCr) \times 100$, and expressed as a percentage. BUN levels in serum were determined using a urease ultraviolet kit.

2.6. Renal histological analysis

The removed mouse kidneys were fixed for 1 day at room temperature in formalin and preserved in 70% ethanol and embedded in paraffin. Each specimen was cut into 7 μm sections and mounted on APES-coated glass slides. Sections were deparaffinized in xylene, rehydrated in decreasing concentrations of alcohol in water, and then used for H&E staining, mounted with neutral balsam eventually. Sections were reacted with antibody against $CD3^+$ (1:100) at 4 °C overnight. After washing with PBS, the sections were incubated with HRP-labeled secondary antibody at 37 °C for 30 min. After washing with PBS, the slides were