Journal of Pharmacological Sciences xxx (2018) 1–8

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

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

Full Paper

Mangiferin alleviates hypertension induced by hyperuricemia via increasing nitric oxide releases

Hua Yang [a], Wenwei Bai [a], Lihui Gao [b], Jun Jiang [c], Yingxi Tang [d], Yanfen Niu [b], Hua Lin [b], Ling Li [b,*]

[a] The Second Affiliated Hospital of Kunming Medical University, Kunming, China
[b] Biomedical Engineering Research Center, Kunming Medical University, Kunming, China
[c] The Third People's Hospital of Yunnan Province, Kunming, China
[d] Kunming Medical University, Kunming, China

ARTICLE INFO

Article history:
Received 21 November 2017
Received in revised form
6 May 2018
Accepted 22 May 2018
Available online xxx

Keywords:
Mangiferin
Hypertension
Hyperuricemia
Nitric oxide
Endothelial function

ABSTRACT

Mangiferin, a natural glucosyl xanthone, was confirmed to be an effective uric acid (UA)- lowering agent with dual action of inhibiting production and promoting excretion of UA. In this study, we aimed to evaluate the effect of mangiferin on alleviating hypertension induced by hyperuricemia. Mangiferin (30, 60, 120 mg/kg) was administered intragastrically to hyperuricemic rats induced by gavage with potassium oxonate (750 mg/kg). Systolic blood pressure (SBP), serum levels of UA, nitric oxide (NO), C-reactionprotein (CRP) and $ONOO^-$ were measured. The mRNA and protein levels of endothelial nitric oxide synthase (eNOS), intercellular adhesion molecule-1 (ICAM-1), CRP were also analyzed. Human umbilical vein endothelial cells (HUVECs) were used *in vitro* studies. Administration of mangiferin significantly decreased the serum urate level and SBP at 8 weeks and last to 12 weeks. Further more, mangiferin could increase the release of NO and decrease the level of CRP in blood. In addition, mangiferin reversed the protein expression of eNOS, CRP, ICAM-1 and $ONOO^-$ in aortic segments in hyperuricemic rats. The results *in vitro* were consistent with the observed results *in vivo*. Taken together, these data suggested that mangiferin has played an important part in alleviating hypertension induced by hyperuricemia via increasing NO secretion and improving endothelial function.

1. Introduction

Uric acid (UA) is the terminal product of purine nucleotide metabolism in humans, higher primates and a small number of other species. Hyperuricemia characterized by the high level of uric acid in blood is a pathological condition which may originate from the increase of uric acid production in purine metabolism and/or impairment of renal clearance of uric acid.[1] Studies have demonstrated that hyperuricemia is greatly associated with hypertension and other cardiovascular diseases.[2,3]

The underlying cause of hyperuricemia-induced hypertension may be related to impaired nitric oxide (NO) production and subsequent endothelial dysfunction.[4] It has reported that a higher level of uric acid was correlated with worse endothelial function which might contribute to hypertension and cardiovascular morbidity.[5] Studies have shown that uric acid may reduce NO synthesis through decreasing the activity of endothelial nitric oxide synthase (eNOS), which is a key enzyme in the production of NO.[6] Moreover, pro-inflammatory factors, such as C-reactive protein (CRP), could also decrease NO synthesis through inhibiting the expression of eNOS and facilitate the development of hypertension.[7,8] Besides, oxidative stress may also plays an important part in this process. In hyperuricemia, there is much O_2^- production in purine metabolism which could reactive with NO to producing $ONOO^-$, and this process could consume much NO.[9]

Intercellular cell adhesion molecule-1 (ICAM-1), which is involved in adhesion reaction, is one of the important members in immunoglobulin superfamily (IGSF). It was reported that high

* Corresponding author. Biomedical Engineering Research Center, Kunming Medical University, NO. 1168, Chunrong West Road, Yuhua Street, Chenggong New Town, Kunming, China.
E-mail address: kmli62@163.com (L. Li).
Peer review under responsibility of Japanese Pharmacological Society.

https://doi.org/10.1016/j.jphs.2018.05.008

Please cite this article in press as: Yang H, et al., Mangiferin alleviates hypertension induced by hyperuricemia via increasing nitric oxide releases, Journal of Pharmacological Sciences (2018), https://doi.org/10.1016/j.jphs.2018.05.008

concentration of uric acid could stimulate generation of chemokines and adhesion molecules such as IL-8 and ICAM-1 *in vivo* experiments.[10] ICAM-1 has played an important role in endothelial disfunction and blood pressure elevation.[11]

Mangiferin, a natural bioactive xanthone C-glycoside, widely distributed in medicinal plants including *Mangifera indica*, *Anemarrhena asphodeloides*, and *Mangifera persiciformis*. Numerous published results have proved that mangiferin has many beneficial biological activities, such as anti-inflammatory, anti-diabetic, and anti-oxidant effects.[12] Recently, our previous works have showed that oral administration of mangiferin was able to significantly decrease the serum urate levels in hyperuricemic mice and rats, in a dose- and time-dependent manner. In addition, it has some advantages such as high efficiency and low toxicity compared to allopurinol.[13–15] However, whether mangiferin could alleviate uric acid-induced hypertension remains unknown.

Based on the important role that uric acid has played in the pathogenesis of hypertension, we hypothesis that mangiferin could guard against hypertension induced by hyperuricemia through increasing NO production and improving endothelial function. In the present study, we verified this hypothesis both *in vivo* and *in vitro*. To evaluate the preventive effect of mangiferin on high blood pressure in hyperuricemia, we used hyperuricemic rats model induced by potassium oxonate and treated them with different doses of mangiferin *in vivo* and the human umbilical vein endothelial cells (HUVECs) were used for further verification *in vitro*.

2. Materials and methods

2.1. Reagents

Mangiferin (purity > 90% by HPLC) was isolated from the leaves of *M. indica* as described previously.[13] Uric acid and potassium oxonate were purchased from Sigma–Aldrich (St. Louis, MO). Allopurinol was bought from Chongqing qingyang pharmaceutical co., LTD.

2.2. Animals

Adult Sprague–Dawley rats weighing 180–200 g were obtained from Chengdu Dashuo Bioengineering Company, Sichuan, China (Certificate No. SCXK(chuan)2013–24). The animals were maintained in specific pathogen-free (SPF) conditions. Rats were acclimated for at least 1 week before being used for experiments. All procedures were carried out in accordance with the Institute Ethical Committee for Experimental Use of Animals.

The rats were randomly divided into six groups (8 rats/group): normal control group, hyperuricemic control group, allopurinol control group (20 mg/kg), and three mangiferin groups receiving low does (30 mg/kg), middle dose (60 mg/kg), or high dose (120 mg/kg). The hyperuricemic rats were established using the same method as before.[13] Briefly, the hyperuricemic rats were induced by intragastrical administration with uricase inhibitor potassium oxonate (750 mg/kg) at 08:00 in the morning for 12 weeks. In the meanwhile, the hyperuricemic rats were administrated with allopurinol or mangiferin dissolved in 0.5% CMC-Na, whereas the normal control rats and untreated hyperuricemic control rats were treated with 0.5% CMC-Na, respectively. All drugs were given intragastrically based on body weight measured immediately prior to each dose at 4:00 pm. The last dosage was administrated at 1 h after intragastrication with potassium oxonate.

All rats were anesthetized by intraperitoneal injection of pentobarbital (30 mg/kg) at the end of experiment. Blood samples were obtained via abdominal aortic puncture and centrifuged at 3000 rpm, 4 °C for 20 min. Then the serum was stored at −20 °C until being assayed. The descending thoracic aorta was isolated and placed in cold Krebse Henseleit buffer (KHB). The part of aorta was then prepared for Hematoxylin and Eosin (HE) and Immune Histochemical (IHC) staining.

2.3. Estimation of blood pressure

Blood pressure (BP) was measured indirectly by the tail cuff method (BP-300A, Chengdu Taimeng Science And Technology Co., Ltd) in conscious rats with slightly restrictions at 8 weeks and 12 weeks after drug treatment. Briefly, rats were settled in the warming chamber and adapted 15 min/day for 3 days before BP recording. After a stabilisation period of 5–10 min in the warming chamber, BP in rats were recorded for 5 readings with an interval of 3 min. A mean of 5 readings was assigned as the SBP.

2.4. Cell culture

Human umbilical vein endothelial cells (HUVECs) were obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences. HUVECs were cultured in endothelial growth medium-2 (EGM-2; Lonza) at 37 °C in a humidified CO_2-controlled (5%) incubator. The third generation of cell after revived the same batch of frozen was used. HUVECs were treated with or without uric acid at 8 mg/dL, or uric acid with mangiferin at the concentrations of 75, 150 and 300 μM, or allopurinol 10 μM for 48 h, respectively. The levels of NO, CRP in cell-culture supernatants were measured using commercially available kits. The level of $ONOO^-$ and mRNA expression of eNOS, CRP and ICAM-1 in HUVECs were measured by immunofluorescent staining and quantitative real-time PCR, respectively.

2.5. Cytotoxicity assay

The cytotoxicity of mangiferin on HUVECs was examined using a MTT assay. Briefly, cells were cultured in 96-well plates for 24 h, then exposed to various concentration of mangiferin (0, 75, 150, 300, 600 μM) for 12–48 h as specified, afterwards incubated for 3 h at 37 °C with 0.5% tetrazolium saltin cell culture medium, then analysed with acidified isopropanol. Absorbance was measured at 570 nm using a microplate reader.

2.6. Measurement of urate level, NO level, and CRP level

Serum urate levels were determined by the method of phosphotungstic acid using commercially available kits (Nanjing Jiancheng Bioengineering Institute, China).

NO levels in serum and cell-culture supernatants were measured with Griess reagent using a nitricoxide assay kit (Beyotime, Shanghai, China) according to the manufacturer's protocol. Absorbance was measured at 540 nm using a microplate reader.

CRP levels in serum and cell-culture supernatants were evaluated by ELISA (Diagnostic System Laboratories, Webster, TX) according to the manufacturer's protocol.

2.7. $ONOO^-$ generation examination

$ONOO^-$ level in HUVECs was detected by immunofluorescent staining. Briefly, cells were fixed with 4% paraformaldehyde for 20 min, then incubated at room temperature with 1% BSA and 0.3% Triton X-100 for one hour. Afterwards, incubated with anti-3-nitrotyrosine (3-NT) polyclonal antibody at 4 °C overnight. The primary antibody was detected using a FITC-labeled secondary antibody (Upstate, USA). Finally cells were visualized by fluorescence

Please cite this article in press as: Yang H, et al., Mangiferin alleviates hypertension induced by hyperuricemia via increasing nitric oxide releases, Journal of Pharmacological Sciences (2018), https://doi.org/10.1016/j.jphs.2018.05.008