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Mangiferin attenuates renal ischemia-reperfusion injury by inhibiting inflammation and inducing adenosine production

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ABSTRACT

Aim: Ischemia reperfusion injury (IRI) is a leading cause of acute kidney injury, which is associated with high morbidity. The aims of the present study were to examine whether mangiferin attenuates renal IRI in an animal model and to identify the underlying mechanism(s).
Methods: Male mice were subjected to right renal ischemia for 30 min followed by reperfusion for 24 h or to a sham operation during which the left kidney was removed. After the 24 h reperfusion, all mice were humanely euthanized and kidney tissues collected. Renal damage and apoptosis were investigated by examining hematoxylin and eosin-stained tissues, and by TUNEL assay and immunohistochemistry. Renal function was examined by measuring the concentrations of creatinine, blood urea nitrogen, and potassium (K^+) in the serum. MPO activity, the levels of NO, TNF-α, IL-1β, and adenosine, and CD73 expression in renal tissue were also examined.
Results: Mangiferin reduced ischemia reperfusion-induced injury, improved kidney function, and inhibited both proinflammatory responses and tubular apoptosis. In addition, treatment with mangiferin increased adenosine production and CD73 expression in kidney's suffering IRI.
Conclusion: Mangiferin appears to attenuate renal IRI by inhibiting proinflammatory responses and tubular apoptosis and by increasing adenosine production. These effects are associated with the adenosine-CD73 signaling pathway.

1. Introduction

Ischemia reperfusion injury (IRI) is a leading cause of acute kidney injury (AKI) in both native and transplanted kidneys [1]. IRI-induced AKI occurs in many clinical settings, including renal transplantation, shock, and vascular surgery [2]. However, therapeutic modalities that prevent or treat AKI are still extremely limited.

The xanthonoid, mangiferin, is a principal constituent of *Salacia* species. Previous studies show that mangiferin has a broad range of pharmacological effects, including antidiabetic, antioxidant, antitumor, antiviral, immunomodulatory, and antimicrobial [3–7]. Remarkably, it also displays potent antiapoptotic and anti-inflammatory activities [8–11]. The anti-inflammatory effect of mangiferin has been confirmed in various animal and cell-based models [8,9,11–13]. In fact, inflammation plays a key role in IR-induced AKI [14,15]. Furthermore, in rats, mangiferin protects renal tissues from streptozotocin-induced oxidative damage [3]. Thus, we hypothesized that mangiferin might be a possible candidate for treating acute renal IRI.

Recent studies suggest that adenosine, an endogenous signaling molecule, protects kidneys from ischemic injury [16]. One of the common causes of AKI is renal ischemia resulting from a reduced blood supply [17–19], and renal inflammation is the main mechanism by which tissues are damaged. Both the production of adenosine and signaling events mediated by adenosine receptors play a critical role in dampening hypoxia-driven inflammation and in preserving kidney function during episodes of renal ischemia [20–24].

Therefore, the aims of the present study were to examine whether mangiferin protects against acute renal IRI in an *in vivo* mouse model and, if so, to identify the underlying mechanisms. The results showed that mangiferin protected kidneys from IRI by inhibiting inflammation and inducing the endogenous production of adenosine.

2. Materials and methods

2.1. Reagents

Mangiferin ($C_{19}H_{18}O_{11}$; FW = 422.34, purity ≥95%) was purchased from Nanjing ZeLang Medical Technology Co. Ltd. (Nanjing, China). Mouse tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β) enzyme-linked immunosorbent assay (ELISA) kits were purchased from Bender MedSystems (Vienna, Austria). Blood urea nitrogen

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(BUN), creatinine, Potassium (K+), myeloperoxidase (MPO), and nitric oxide (NO) detection kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, PR China). The bicinchoninic acid (BCA) protein assay kit was purchased from Pierce (Rockford, IL, USA). Trizol reagent was purchased from Invitrogen (Grand Island, NY, USA). SYBR green PCR Master Mix was obtained from Promega (Madison, WI, USA). Anti-CD73 and -GAPDH antibodies were purchased from Abcam (Cambridge, MA, UK). The terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) assay kit and caspase-3 colorimetric assay kit were purchased from Abcam (Cambridge, MA, UK).

2.2. Animals

Male C57/BL6-mice (6–8 weeks old; weight, 20–22 g) were obtained from the Experimental Animal Center of Chongqing Medical University (Chongqing, China). All mice received humane care according to the guidelines set down by the Local Institutes of Health guide for the care and use of laboratory animals. Mice were housed in a specific pathogen-free (SPF) laboratory under optimum conditions (25 ± 2 °C, 55% humidity, and a 12 h light/dark cycle) and fed a standard laboratory diet and water. Mice were acclimatized for at least 1 week before use. All experimental procedures involving animals were approved by the Animal Care and Use Committee of Chongqing Medical University.

2.3. Experimental protocol

The mice were randomly divided into four groups: a sham-operated group (control); a sham-operated plus mangiferin-treated group (mangiferin); an ischemia-reperfusion (IR) group (IR); and an IR plus mangiferin-treated group (IR + mangiferin). Mangiferin (10, 30, 100 mg/kg, respectively) was dissolved in 0.5% carboxymethyl cellulose sodium/phosphate-buffered saline (PBS) and administered at 10 mg/kg/day, 30 mg/kg/day or 100 mg/kg/day by oral gavage, beginning 7 days before renal IR and ending at the time of sacrifice. Mice in the sham-operated and the IR groups received PBS alone as a control.

Renal IRI was induced by performing a left nephrectomy followed by ischemic treatment to the remaining right kidney. Briefly, the mice were anesthetized by intraperitoneal injection of a mixture of ketamine and xylazine (45 mg/kg and 8 mg/kg, respectively) and placed on a temperature-controlled heating table. A flank incision was performed using a coagulation electrode to prevent bleeding. The right renal pedicle was then clamped for 30 min, and the left kidney was removed without interfering with the adrenal vessels. For reperfusion, the clamp was released and the kidney was monitored for color changes to confirm blood reflow before the incision was closed. Sham control animals were subjected to the same procedure without clamping of the renal pedicle.

After surgery, the mice were kept on a warming blanket for 24 h and allowed food and water *ad libitum*. At the end of the 24 h reperfusion period, all animals were sacrificed by injecting a high dose of pentobarbital sodium. The blood and kidneys were then harvested.

2.4. Hematoxylin and eosin staining and TUNEL assay

Kidneys were harvested and fixed with 4% formaldehyde prior to paraffin embedding. Paraffin-embedded tissues were sectioned (5 μm thick) and stained with hematoxylin and eosin (H&E). Histological changes were evaluated by analyzing the percentage of renal tubules showing evidence of cell lysis and brush border loss.

The TUNEL assay was performed using the *in situ* Cell Death Detection kit according to the manufacturer's instructions. Positive staining of cell nuclei (indicative of DNA strand breaks) was identified under a fluorescence microscope.

2.5. Immunohistochemistry (IHC) detection of active caspase-3

IHC detection of apoptosis-related proteins was performed in 5 μm-thick deparaffinized sections. Before IHC, the sections were subjected to heat-induced epitope retrieval by incubation in a 0.01 M sodium citrate solution (pH 6) at 12 °C for 10 min, followed by a 2 h cool down. Active caspase-3 was detected with a mouse polyclonal antibody (diluted 1:100) that specifically recognizes the large fragment (17 kDa) of the active protein, but not full-length caspase-3. Primary antibodies were applied for 16 h at 4 °C. The sections were then washed in two changes of phosphate-buffered saline/Tween 20 (PBST; 0.1 M phosphate buffer, pH 7.4, 0.1% (v/v) Tween 20) for 10 min and then incubated with a biotinylated goat anti-mouse antibody (1:200; Vector Laboratories; Burlingame, USA) for 1 h at room temperature. After two washes in PBST (each for 10 min), endogenous peroxidase activity was blocked by a 10-min incubation in a 6% hydrogen peroxide solution in distilled water. The slides were washed twice in PBST (each for 5 min) and then incubated in streptavidin-peroxidase (diluted 1:150 in PBST) for 1 h at room temperature. After two further washes in PBST, bound peroxidase was identified using the AEC+ High sensitivity substrate chromogen (Dako, Denmark).

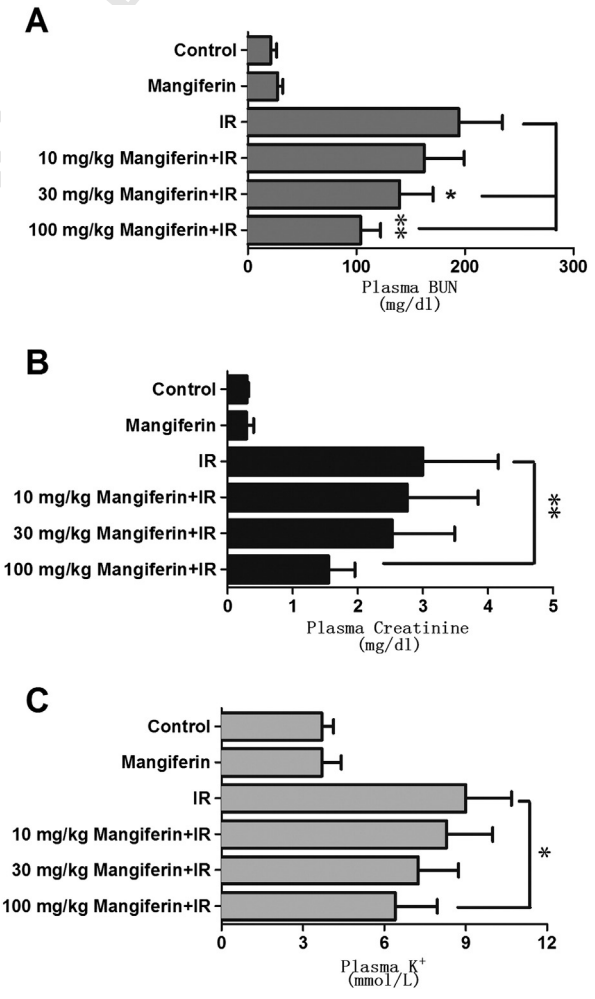

Fig. 1. Mangiferin reduces renal functional defects caused by ischemic reperfusion. C57/BL6 mice (n = 5–8 per group) were treated with vehicle or mangiferin (10 mg/kg/day, 30 mg/kg/day, 100 mg/kg/day) for 7 days before surgery. Twenty-four hours after the kidney was reperfused, blood was collected for renal function tests. Concentrations of (A) serum blood urea nitrogen, (B) serum creatinine, and (C) serum K^+ are shown. Data are expressed as the mean ± SD. $^*P < 0.05$; $^{**}P < 0.01$.

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