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Up-regulation of glyoxalase 1 by mangiferin prevents diabetic nephropathy progression in streptozotocin-induced diabetic rats

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ABSTRACT

Advanced glycation endproducts (AGEs) and its precursor methylglyoxal are associated with diabetic nephropathy (DN). Mangiferin has many beneficial biological activities, including anti-inflammatory, anti-oxidative and anti-diabetic effects. We investigated the effect of mangiferin on DN and its potential mechanism associated with glyoxalase 1 (Glo-1), a detoxifying enzyme of methylglyoxal, in streptozotocin-induced rat model of DN. Diabetic rats were treated orally with mangiferin (15, 30, and 60 mg/kg) or distilled water for 9 weeks. Kidney tissues were collected for morphologic observation and the determination of associated biochemical parameters. The cultured mesangial cells were used to measure the activity of Glo-1 in vitro. Chronic treatment with mangiferin significantly ameliorated renal dysfunction in diabetic rats, as evidenced by decreases in albuminuria, blood urea nitrogen, kidney weight index, periodic acid-schiff stain positive mesangial matrix area, glomerular extracellular matrix expansion and accumulation, and glomerular basement membrane thickness. Meanwhile, mangiferin treatment caused substantial increases in the enzymatic activity of Glo-1 in vivo and in vitro, and protein and mRNA expression of Glo-1, reduced levels of AGEs and the protein and mRNA expression of their receptor (RAGE) in the renal cortex of diabetic rats. Moreover, mangiferin significantly attenuated oxidative stress damage as reflected by the lowered malondialdehyde and the increased glutathione levels in the kidney of diabetic rats. However, mangiferin did not affect the blood glucose and body weight of diabetic rats. Therefore, mangiferin can remarkably ameliorate DN in rats through inhibiting the AGEs/RAGE aix and oxidative stress damage, and Glo-1 may be a target for mangiferin action.

1. Introduction

As a global disease, diabetes nephropathy (DN) is one of the most severe diabetic microangiopathies and accounts for approximately one third of end-stage renal disease (Rossing, 2006). Several mechanisms have been considered to be involved in the pathogenesis of DN and other diabetes-associated complications, such as the accumulation of advanced glycation endproducts (AGEs), and oxidative stress (Brownlee, 2001). AGEs are a major mediator of the untoward effects of hyperglycemia (Suzuki and Miyata, 1999). AGEs may generate from nonenzymatic reactions between proteins and carbonyl compounds, like methylglyoxal (MG), glyoxal, and 3-deoxyglucosone (Brownlee et al., 1988). Protein glycation caused by MG, a key precursor of AGEs formation, may be a central player in the complications of diabetes due to its ability to increase both inflammation and oxidative stress (Di Loreto et al., 2008; Rabbani and Thornalley, 2008; Sena et al., 2012; Yamawaki et al., 2008). MG can accumulate in body and accelerates the formation of AGEs. DN may be related to the accumulation of toxic alpha-oxoaldehydes such as MG. Therefore, therapeutic strategies aimed at reducing dicarbonyl compounds or enhancing their clearance and subsequently inhibiting AGEs formation would be effective to prevent the pathogenesis of DN.

The glyoxalase system is a major detoxication system for dicarbonyl compounds in human body, where glyoxalase 1 (Glo-1) is the rate-limiting enzyme. With reduced glutathione (GSH) as a cofactor, Glo-1 can promptly clear alpha-carbonyl aldehydes, e.g. MG, inhibiting AGEs formation. Glo-1 can also directly inhibit AGEs formation in bovine endothelial cells (Shinohara et al., 1998) and GM7373 endothelial cells (Thornalley, 2003). Moreover, Glo-1 over-expression has been shown to reduce indices of diabetic complications (Brouwers et al., 2011; Queisser et al., 2010). Hyperglycemia-induced reactive oxygen species have increased the expression of AGEs and RAGE (receptor for AGEs), which is mediated by MG, which can be normalized by Glo-1 over-expression (Brouwers et al., 2011; Yao and Brownlee, 2010). Glo-1 can

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attenuate damages in mitochondria from oxidative stress (Rabbani and Thornalley, 2008). Moreover, Glo-1 over-expression can reduce oxidative stress damage in diabetic rats (Brouwers et al., 2011). Thus, Glo-1 may become a preventative and therapeutic target of diabetic complications, including DN.

Mangiferin, also called chinonin, is a major glucoside of xanthone in *Rhizome Anemarrhena*. Mangiferin has many biological activities, including anti-inflammatory, anti-oxidative and anti-diabetic effects (Miura et al., 2001; Muruganandan et al., 2005; Prabhu et al., 2006). Mangiferin improves diabetic complications in heart and kidney (Li et al., 2010; Muruganandan et al., 2002). Several studies have showed that this compound inhibits AGEs formation and aldose reductase activity in in vitro study (Tang et al., 2004; Yoshikawa et al., 2001). Li et al. (2010) illustrate that mangifrein could prevent the progression of DN by suppressing renal fibrosis. All these reports indicate that mangiferin may have a beneficial effect on the progression of DN. However, the intimate mechanism of mangiferin on DN progression remains unknown. Therefore, our study is aimed to investigate the effects of mangiferin on the nephropathy in streptozotocin-induced diabetic rats, a rodent model of type 1 diabetes, and the pathological factors related to Glo-1 in diabetic condition.

2. Material and methods

2.1. Animals

Male Sprague Dawley rats (10 weeks) were bred in the Center of Experimental Animal, Xuzhou Medical College (Xuzhou, China). All animals were housed under a controlled room humidity (50% ± 10%), and maintained under a 12-h light/dark cycle with free access to water and food. All animal experiments were performed in accordance with the license by Jiangsu Province Science and Technology Office (Nanjing, China) and the approval from the Animal Ethics Committee of Xuzhou Medical College. All experiments were conformed to the Guidelines for Ethical Conduct in the Care and Use of Animals. Every effort was made to minimize stress to the animals.

2.2. Experimental design

The rats fasted for 12 h were subjected to a single intraperitoneal injection of 55 mg/kg streptozotocin (STZ) freshly dissolved in 0.1 mol/l sodium citrate buffer at pH 4.5. Age-matched normal rats were received sodium citrate buffer alone. The development of diabetes was accessed using the value of fasting blood glucose (FBG) with a reagent kit (Jiancheng Bioengineering Institute, Nanjing, China). The rats with the FBG level above 13.9 mmol/l were considered diabetic rats 72 h after STZ injection. Then, the diabetic rats were randomly divided into four groups with ten animals each, namely diabetic rats treated with three doses of mangiferin (15, 30 and 60 mg/kg) or distilled water through an oral gavage for 9 weeks. Mangiferin (> 97% purity, Fengshanjian Medicinal Research Co. Ltd., Kunming, China) was suspended in distilled water. Age-matched normal rats ($n=10$) were received distilled water. Blood glucose was examined for the fasted rats for 7–8 h.

After 9-week treatment, 24-h rat urine was collected by metabolic cages for albuminuria measurement. Then the animals were sacrificed under ethyl ether anesthesia, and blood was collected via femoral vein bleeding with serum separated. The rat kidneys were removed, with 1/4 of each kidney decapsulated and fixed in 10% formalin for 24 h before paraffin embedding. Renal cortex was cut into 1 mm^3 units and fixed in 2.5% glutaraldehyde for 2 h before transmission electron microscopic examination. The kidney left was rapidly removed with cortex isolated. The samples were stored at −80 °C before use.

2.3. Renal function assessment

Renal function was assessed by the measurement of kidney weight index, urinary protein and blood urea nitrogen (BUN). Decapsulated left and right kidneys were excised and weighted. Kidney weight index (mg/g) was the ratio of the two kidneys' weight and the body weight of a rat. The Coomassie brilliant blue method was used to quantify the urinary protein secretion by a urinary protein assay kit (Jiancheng Bioengineering Institute, Nanjing, China). BUN was measured using a diacetyl-latter colorimetric method by a BUN assay kit (Jiancheng Bioengineering Institute, Nanjing, China).

2.4. Periodic acid-Schiff (PAS) staining

Kidney tissues were fixed in a 10% buffered formalin solution and embedded in paraffin for histological analysis. The 3 μm thick paraffin sections were dewaxed and brought to water through graded ethanols. The sections were stained with PAS, cleared in xylene and mounted with neutral balsam before examined using an Olympus BX-50 microscope. The three most central sections of each defect were analyzed. Linear measurements were obtained with an image analysis system (Image-Pro Plus 4.0, Media Cybernetics, Silver Spring, MD).

2.5. Transmission electron microscopic examination

For transmission electron microscopic examination, small pieces of the kidney cortex (1 mm^3) were fixed in 2.5% glutaraldehyde in 200 mmol/l sodium phosphate buffer (pH 7.4) for 2 h. Then the tissue sections were washed with PBS for 3 h to remove glutaraldehyde, and fixed in osmic acid for 2 h before rinsed with water for 2 h. The sections were dehydrated in alcohol (from 50% to 90%) and dried using anhydrous acetone and Araldite® sequentially. Finally, the tissue sections were embedded in pure Araldite. Ultrathin (70 nm) sections were cut with a glass knife on a RMC HT-XL ultratome and mounted on a copper grid (200 mesh). The sections were stained with uranyl acetate and lead citrate. The TEM H-600A-2 (Hitachi, Japan) was used for viewing and photographing.

2.6. Fluorescent determination of AGEs levels in renal cortex

The weighed and frozen rat kidney cortex was homogenized in 10 volumes (w/v) of 100 mmol/l PBS extraction buffer (pH 7.4) with a motor-operated homogenizer (FLUKO Equipment Shanghai Co. Ltd., China) in an iced water bath. The homogenate was centrifuged at 4 °C at 10,000 g for 15 min, and the supernatant was collected for Glo-1 activity and other biochemical parameter assays. The pellets were washed three times with distilled water, to which 1.0 ml $CHCl_3$-MeOH (1:1) was added. The mixture was shaken overnight at room temperature. Next, 0.5 ml MeOH–H_2O (4:1) was added and centrifuged at 4 °C at 4000 g for 5 min. The pellets were washed sequentially with MeOH and distilled water for twice and then washed once with 0.02 mol/l Hepes buffer (pH 7.5) containing 0.1 mol/l $CaCl_2$. The resulting samples were suspended in 1.0 ml Hepes buffer overnight at 4 °C, and then in 1.0 ml Hepes buffer containing 290 U of type I collagenase (Sigma-Aldrich Co. LLC.) after removing the buffer by centrifugation. Antiseptics methylbenzene and chloroform (both 2.0 μl) were added and the mixture was shaken for 24 h at 37 °C for centrifugation. Next, the supernatant was collected for determination of fluorescence intensity using a fluorescent instrument (9203-941, Promega Biosystems Sunnyvale, Inc., CA, USA) with the Hepes buffer containing type I collagenase as a standard. The AGEs level in kidney cortex was expressed as the enzymatic activity of type I collagenase (U) per milligram of protein.