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Effects of magnolol (5,5′-diallyl-2,2′-dihydroxybiphenyl) on diabetic nephropathy in type 2 diabetic Goto–Kakizaki rats

Eun Jin Sohn [1], Chan-Sik Kim [1], Young Sook Kim, Dong Ho Jung, Dae Sik Jang, Yun Mi Lee, Jin Sook Kim *

Department of Herbal Pharmaceutical Development, Korea Institute of Oriental Medicine, 461-24 Jeonmin-dong, Yuseong-gu, Daejeon 305-811, Republic of Korea

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Abstract

We investigated the effect of magnolol (5,5′-diallyl-2,2′-dihydroxybiphenyl), a marker compound isolated from the cortex of *Magnolia officinalis*, in non-obese type 2 diabetic Goto–Kakizaki (GK) rats. The rats were treated orally with magnolol (100 mg/kg body weight) once a day for 13 weeks. In magnolol-treated GK rats, fasting blood glucose and plasma insulin were significantly decreased, and the pancreatic islets also showed strong insulin antigen positivity. Urinary protein and creatinine clearance (Ccr) were significantly decreased. Pathological examination revealed the prevention of the glomeruli enlargement in magnolol-treated GK rats. The overproduction of renal sorbitol, advanced glycation endproducts (AGEs), type IV collagen, and TGF-β1 mRNA were significantly reduced in magnolol-treated GK rats. Thus based on our findings, the use of magnolol could result in good blood glucose control and prevent or retard development of diabetic complications such as diabetic nephropathy.

Keywords: Magnolol; Goto–Kakizaki rat; Diabetic nephropathy

Introduction

The Goto Kakizaki (GK) rat is a model of spontaneous and moderate non-insulin-dependent diabetes. Between 3 and 4 weeks of age, GK rats develop mild hyperglycemia and hyperinsulinemia. The pathogenesis of diabetes in the GK rat involves impairment of insulin secretion, insulin resistance, abnormal glucose metabolism, and impaired ontogenetic development of pancreatic islet cells (Portha et al., 1991; Giroix et al., 1993).

Magnolol, a marker substance of *Magnolia officinalis*, has various pharmacological effects, including an antidepressant effect (Nakazawa et al., 2003), an inhibitory effect on tumor metastases (Ikeda et al., 2003), a protective effect against cerebral ischaemic injury (Chang et al., 2003), an anti-platelet effect (Pyo et al., 2002) and an antioxidant effect (Lo et al., 1994). On the other hand, *M. officinalis* is widely used for the treatment of diabetes and diabetic complications in Korean traditional herbal medicines and prescriptions (Hur, 1999). We have previously reported that an ethanolic extract of Magnoliae cortex showed an *in vitro* inhibitory effect on the formation of advanced glycation endproducts (AGEs), which play an important role in the development of diabetic complications (Kim et al., 2002). Furthermore, magnolol inhibits AGEs formation and sorbitol accumulation in streptozotocine-induced diabetic rats (Lee et al., 2006). So, in the present study, we investigated the pharmacological effects of magnolol on the progression of diabetes and diabetic nephropathy in a type 2 diabetes animal model.

Materials and method

Preparation of magnolol

The root barks of *M. officinalis* Rehder (Magnoliaceae) was obtained from the Baekje herbal medicine store in Daejeon, South Korea in 2003 and identified by Prof. J.-H. Kim, Division of Life Science, Daejeon University. The voucher specimen was

* Corresponding author. Tel.: +82 42 868 9465; fax: +82 42 868 9471.
E-mail address: jskim@kiom.re.kr (J.S. Kim).
[1] These authors contributed equally to this work.

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deposited at the Herbarium of Department of Herbal Pharmaceutical Development, Korea Institute of Oriental Medicine (No. 7). For this study, we used a recrystallization method that did not require column chromatography for mass isolation of magnolol (5,5′-diallyl-2,2′-dihydroxybiphenyl) (Fig. 1) from the root barks of *M. officinalis*. Briefly, powdered the plant materials (5 kg) were extracted with 80% EtOH (30 L) for 1 week at room temperature, concentrated with a rotary evaporator, and lyophilized, and the entire procedure was repeated for 4 times. To the concentrate, distilled water and *n*-hexane were added and the *n*-hexane layer was separated. The *n*-hexane layer was concentrated and magnolol (60 g) was isolated from the *n*-hexane layer directly by recrystallization (*n*-hexane/$CHCl_3$). The isolated magnolol was identified by comparing the NMR data obtained with those of published values (Bang et al., 2000) and by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) analysis with the standard compound (Wako Pure Chemical Industries, Japan).

Chemicals

Aminoguanidine (AG), carboxymethylcellulose (CMC), nicotinamide adenine dinucleotide (NAD) and sorbitol dehydrogenase (SDH) were purchased from Sigma (St. Louis, MO, USA); Blood glucose and creatinine levels test kit from Wako Pure Chemical Industries (Osaka, Japan); plasma insulin test kit from Linco research Inc. (St. Louis, MO, USA); fluorescein-conjugated horse anti-guinea pig IgG from Chemicon (Temecula, CA, USA); Texas red-conjugated goat anti-rabbit IgG from Santa-Cruz biotechnology Inc. (Santa-Cruz, CA, USA); TRIzol from MCRC (Cincinnati, OH, USA) reagent; RT-primix from Bioneer (Daejeon, Korea). All reagents were of biochemical grade.

Animals and experimental design

Male GK rats and control, age-matched Wistar rats, were used (SLC, Japan). Animals were allocated 1 per polycarbonate cage in a temperature (20–25 °C) and humidity (30–35%) controlled room. Light–dark cycle was 12 h/12 h and feed and water were supplied free to access. At 6 weeks of age, fasting blood glucose was determined in each rat, and they were divided into 4 groups of 8–10 rats each: (1) Wistar rats, (2) GK rats, (3) GK rats treated with magnolol (100 mg/kg body weight) and (4) GK rats treated with AG (50 mg/kg body weight). Magnolol and AG were dissolved in 1% CMC and given orally. In Wistar rats, 1% CMC was given orally. Rats were allowed free access to water and food for 13 weeks and, at 3-week intervals, their intake of water and food for a 24-h period was measured. AG, which is a prototype therapeutic agent for the prevention of various diabetic complications, was chosen as a positive control. The experiments were performed according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. The study was approved by the Committee on Animal Care of our institute.

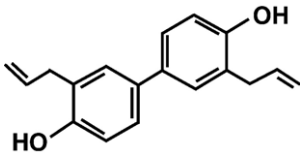

Fig. 1. Structure of magnolol.

Blood glucose levels and other parameters

Blood was obtained from the tail vein twice a month for fasting blood glucose determination (hexokinase method), using commercially available kits. Fasting plasma insulin concentrations at the end of experiment were determined by a two-antibody procedure using a radioimmunoassay kit. Plasma was used to measure creatinine levels.

The 19-week-old rats were kept in metabolic cages for 24 h, and their urine was collected in bottles. Urine was analyzed for total protein (Lowry method) and creatinine (Jaffe method), using a commercially available assay kit. The urinary protein content was calculated in terms of the creatinine concentration (urinary protein/urinary creatinine) (Velasquez et al., 2003). Creatinine clearance (Ccr) was calculated according to the following formula:

$$\frac{\text{urinary creatinine (mg/ml)} \times \text{urine volume (ml/kg)}}{\text{creatinine in plasma (mg/ml)}}$$

Determination of sorbitol level

The kidney medulla was homogenized in cold saline, and 6% perchloric acid was added to the supernatant. The protein was precipitated by centrifuging at 2500 g for 5 min; the supernatant was neutralized with 2.5 M K_2CO_3 and then centrifuged at 4 °C. Supernatant was reacted with the reaction mixture (glycine buffer pH 9.4, 0.2 mM NAD and 0.64 U SDH) for 30 min at room temperature, and the absorbance was measured by a fluorescence spectrophotometer (Bio-TEK® Synergy HT, USA; Ex/Em: 365/450 nm)(Clements et al., 1969).

Immunoflorescence staining of insulin and glucagons

For insulin and glucagon double-label experiments, the deparaffinized sections were hydrated with water and incubated with insulin antibody (1:100) for 2 h at room temperature. After washing with TTBS, the sections were incubated for 3 h with glucagon antibody (1:200). After washing, fluorescein-conjugated horse anti-guinea pig IgG (1:200) and Texas red-conjugated goat anti-rabbit IgG (1:200) were incubated for 1 h at room temperature. After washing, the sections were mounted and observed by Olympus fluorescence microscopy. The expression of type IV collagen in each glomerulus, and the expression of insulin and glucagon in the pancreas were observed by Olympus microscopy (Olympus BX51) equipped with an Olympus DP 70 camera (Olympus). For the morphometric analysis, NIH Image J software (National Institutes of Health, Bethesda, MD, USA) was used.

Immunohistochemical staining and histology of AGE and type IV collagen

At 13 weeks, after a 24-h fast, the kidney and pancreas were collected for histopathologic examination. In brief, the kidney and pancreas were preserved in 4% paraformaldehyde at room temperature for 24 h, embedded in paraffin, and sectioned (3 μm). Paraffin sections were deparaffinized, hydrated with water, and stained with periodic acid Schiff (PAS) reagent and haematoxylin as a counterstain.

For immunohistochemical AGE staining, formalin-fixed parafilm embedded section cuts (2 μm thick) were mounted on slide coated with 2-aminopropyltriethoxy silane, baked for 3 h at 58 °C, deparaffinized, rinsed with 3% hydrogen peroxide, and incubated with proteinase K (0.5 mg/ml) for 5 min at room temperature. These sections were washed with rinse buffer and blocked with protein blocking agent for 5 min and subsequently incubated with 6D12 anti-AGE mouse monoclonal antibody for 30 min at room temperature. After washing with rinse buffer, the sections were incubated with EnVision+labelled polymer peroxidase-conjugated mouse anti-IgG for 30 min at room temperature, followed by detection with 3,3′-diaminobenzidine tetrahydrochloride solution as chromogen and 50% haematoxylin as counterstain.

For the quantitative analysis, the average score of 80 randomly selected glomeruli was calculated using Image-pro-Plus (MediaCybernetics, MD, USA). The same set of experiments was repeated at least three times.

To determine type IV collagen deposition in the kidneys using immunohistochemistry, paraffin sections were deparaffinized and hydrated with water. Endogenous peroxidase was quenched by immersion in 3% hydrogen peroxide for 5 min. Sections were sequentially incubated with antibody to type IV collagen (1:200) for 1 h at room temperature, a secondary antibody for 15 min at room temperature (LSAB 2 kit, Dako, USA), and peroxidase-conjugated streptavidin for 15 min at room temperature. To evaluate the immunostaining for type IV collagen, the average score of 60 randomly chosen glomeruli was calculated using Image-pro-Plus (MediaCybernetics, MD, USA). The same set of experiments was repeated at least three times. The degree of AGEs and type IV collagen expression was performed using a semiquantitative scale by an observer blinded to the experimental design.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated using TRIzol reagent according to the manufacturer's instructions. cDNA was synthesized with 3 μg of RNA using RT-primix. The upstream and downstream primers for rat TGF-β1 mRNA were 5′-CGA GGT GAC CTG GGC ACC ATC CAT GAC-3′ and 5′-CTG CTC CAC CTT GGG CTT GCG ACC CAC-3′, yielding a 409-bp product. On the other hand, those for β-actin, used as an internal control, were 5′-CGT AAA GAC CTC TAT GCC AA-3′ and 5′-AGC CAT GCC AAA TGT GTC AT-3′, yielding a 350-bp product. RNA was similarly reverse transcribed. The RT-PCR products were separated by electrophoresis and DNA band intensities in agarose gels and quantitated with densitometry (Las-3000, Fuji photo, Tokyo, Japan).

Table 1
Changes of body weight

	Wistar	GK	GK+Mag	GK+AG
Begin (g) Aged 6 wk	215.9±11.2	158.5±15.3	149.1±11.8	164.5±11.2
End (g) Aged 19 wk	419.5±7.8	341.1±13.0	338.2±3.3	344.9±7.1
Gain (g)	203.6±6.8	182.6±8.3	174.7±4.3	180.3±5.7

Results are expressed as mean±S.E., n=8–10.

Table 2
Food and water intake for 24 h

	Wistar	GK	GK+Mag	GK+AG
Food (g/100 g body weight)				
3 wk	6.87±2.70	7.72±1.80	8.26±2.21	7.66±1.60
6 wk	5.89±1.90	6.48±1.80	6.53±1.90	6.02±1.92
9 wk	5.57±2.81	5.39±1.49	6.19±1.80	5.22±2.01
12 wk	5.94±2.51	4.73±2.54	5.26±2.84	5.06±6.47
Water (ml/100 g body weight)				
3 wk	18.40±10.25	15.91±4.75	16.93±6.90	15.01±3.81
6 wk	12.45±8.12	12.89±3.70	13.35±13.90	10.80±7.71
9 wk	11.68±7.89	9.95±5.32	11.53±5.98	10.43±4.87
12 wk	10.72±9.51	10.42±15.00	9.01±6.07	8.64±7.50

Results are expressed as mean±S.E., n=8–10.

Statistical analyses

Statistical evaluation of the results was performed using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using GraphPad *Prism 4.0* software (Graph pad, San Diego, CA, USA).

Results

Body weight and intakes of food and water

After 13 weeks, the body weight gains for the Wistar rats, untreated GK rats, GK rats treated with magnolol (100 mg/kg),

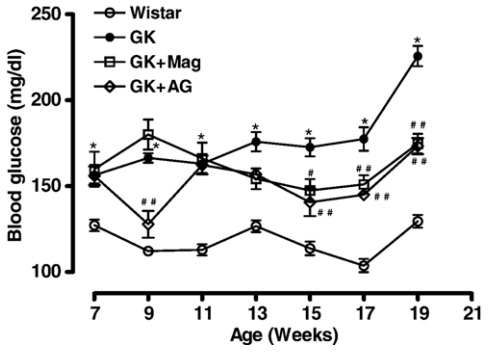

Fig. 2. Time course of blood glucose levels with once daily oral administration of magnolol (Mag, 100 mg/kg) or aminoguanidine (AG, 50 mg/kg) for 13 weeks: Data expressed as means±S.E., n=8–10. ★, p<0.01 vs. Wistar; #,##, p<0.05, p<0.01 vs. untreated GK rats.

and GK rats treated with AG (50 mg/kg) were 203.6±6.8 g, 182.6±8.3 g, 189±4.3 g, and 180.3±5.7 g, respectively. The weight gains of the four groups were not significantly different (Table 1). The food and water intakes of the four groups were also not significantly different (Table 2).

Blood glucose levels

During the experimental period, fasting blood glucose concentrations in untreated GK rats were higher than those in the Wistar rats ($p<0.01$). Fasting blood glucose levels were significantly reduced in the magnolol-treated GK rats ($p<0.01$, $p<0.05$) and in the AG-treated GK rats ($p<0.01$) compared to GK rats (Fig. 2).

Plasma insulin level and immunofluorescent staining for insulin and glucagon in pancreatic islets

The fasting insulin level in untreated GK rats (3.80±0.35 ng/ml, $p<0.01$) was higher than in Wistar rats (0.88±0.07 ng/ml).

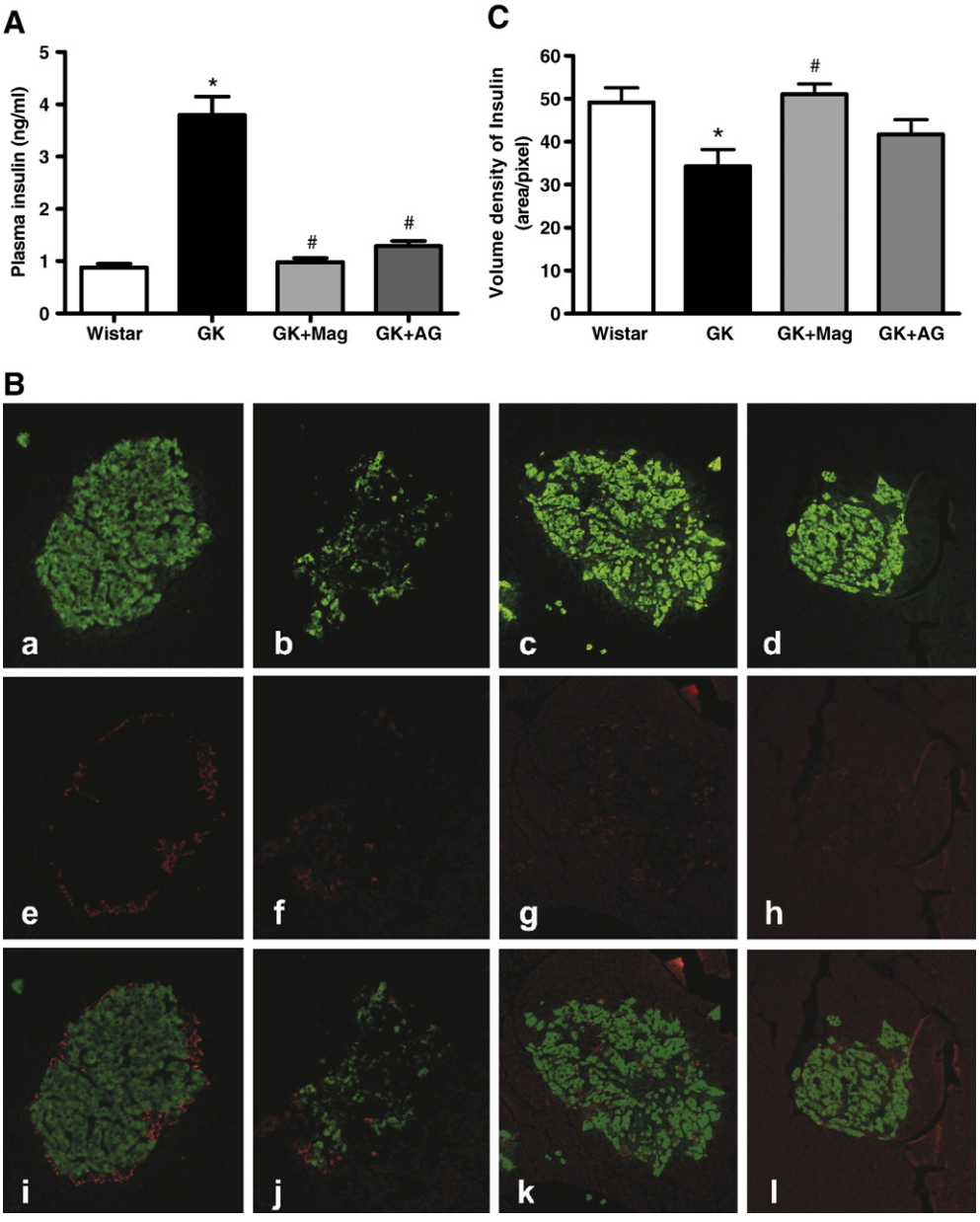

Fig. 3. Fasting plasma insulin levels and immunofluorescence staining of insulin and glucagon: (A) Fasting plasma insulin levels, data expressed as means±S.E., $n=8–10$. ★, $p<0.001$ vs. Wistar rats; #, $p<0.001$ vs. untreated GK rats. (B) Immunofluorescence staining of insulin and glucagon in the pancreas. Representative histological sections of pancreatic islets in Wistar rats (a, e, i), untreated GK rats (b, f, j), GK rats treated with magnolol (100 mg/kg) (c, g, k), and GK rats treated with aminoguanidine (50 mg/kg) (d, h, l) incubated with anti-insulin (a–d) and anti-glucagon (e–h) antibodies. Fig. 3B i–l are the result of merging 3B a–d and 3B e–h. Original magnification x100. (C) Quantitative analysis of insulin positive area. Data expressed as means±SEM, $n=8–10$. ★, $p<0.05$ vs. Wistar rats; #, $p<0.001$ vs. untreated GK rats.

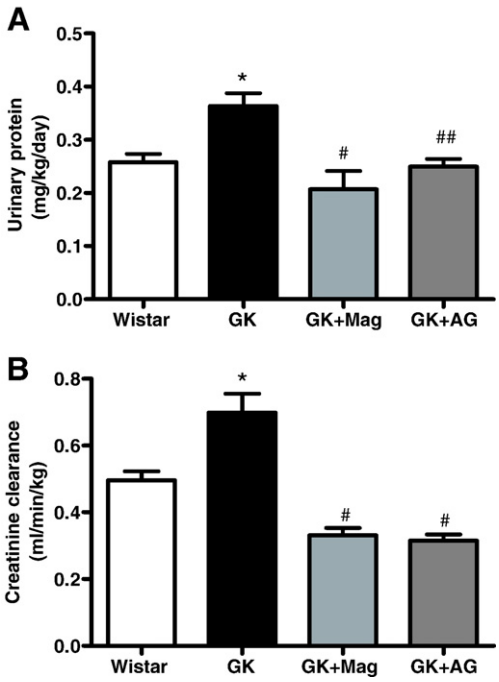

Fig. 4. Changes of urinary protein and creatinine clearance (Ccr) in 19-week-old experimental rats: (A) Changes of urinary protein. Data expressed as means±S.E., $n=7–10$. ★$p<0.01$ vs. Wistar rats; #,##, $p<0.001$, $p<0.01$ vs GK rats, respectively. (B) Changes of creatinine clearance (Ccr): Data expressed as means±S.E., $n=7–8$. ★, $p<0.01$ vs. Wistar rats; #, $p<0.001$ vs. untreated GK rats.

The insulin levels in magnolol-treated GK rats were significantly decreased (0.98±0.08 ng/ml, $p<0.01$ versus untreated GK rats) (Fig. 3A).

In untreated GK rats, immunofluorescent staining of pancreatic tissues showed weak insulin immunoreactivity in the islets of Langerhans (Fig. 3B-b). In magnolol-treated GK rats, there was strong insulin antigen positivity in the majority of the islets of Langerhans (Fig. 3B-c) and the density of insulin staining was also significantly increased ($p<0.001$ versus untreated GK rats; Fig. 3C). In Wistar rats, untreated GK rats, magnolol-treated GK rats and AG-treated GK rats, glucagon immunoreactive cells were located in their normal position at the periphery of the islets (Fig. 3B-e–h) and there were no statistically significant differences among these groups.

Determination of urinary protein and creatinine clearance (Ccr)

In diabetes, urinary protein excretion has been found to be responsible for glomerular dysfunction. Urinary protein levels in untreated GK rats gradually increased (0.33±0.03 mg/kg/day, $p<0.01$) compared to those in Wistar rats (0.20±0.02 mg/kg/day). However, urinary protein excretion was significantly decreased in the magnolol-treated group (by 0.20±0.04 mg/kg/day; $p<0.01$ versus untreated GK rats) and in the AG-treated group (by 0.21±0.02 mg/kg/day; $p<0.01$ versus untreated GK rats) (Fig. 4A). Creatinine clearance (Ccr) is one of the indicators of kidney function. As illustrated in Fig. 4B, Ccr was significantly increased in untreated GK rats (0.70±0.06 ml/min/kg, $p<0.01$) compared to Wistar rats (0.50±0.03 ml/min/kg). However, Ccr was significantly decreased in magnolol-treated GK rats (0.33±0.02 ml/min/kg, $p<0.001$ versus untreated GK rats) and in AG-treated GK rats (0.31±0.02 ml/min/kg, $p<0.001$ versus untreated GK rats).

Determination of sorbitol level

The sorbitol concentration in kidney medulla of untreated GK rats (1.16±0.07 μM/mg, $p<0.01$) was higher than in Wistar rats (0.73±0.07 μM/mg). The sorbitol levels in magnolol-treated GK rats were significantly decreased (0.82±0.07 μM/mg, $p<0.05$ versus untreated GK rats) (Fig. 5).

Immunohistological staining for AGEs and type IV collagen, and pathological changes in the kidney

In GK rats, an expansion of glomeruli was observed compared to age-matched Wistar rats. After the administration of magnolol for 13 weeks, the glomeruli enlargement was decreased (Fig. 6A, a–d). Immunohistochemical staining for AGEs showed that there was widespread staining for AGEs in the kidney glomeruli and tubles in untreated GK rats compared with Wistar rats (Fig. 6A-e,f). Treatment with magnolol visibly reduced the AGEs deposited in these regions (Fig. 6A-g). By quantitative scoring more AGEs were observed in glomeruli from untreated GK rats (13.06±0.96%, $p<0.001$) compared with Wistar rats (9.48±0.69%). Magnolol treatment significantly reduced the extent of AGEs staining in GK rats (2.83±0.17%, $p<0.001$; Fig. 6B).

In the GK kidney, it is known that type IV collagen is overexpressed in both the glomerular basement membrane and the mesangial matrix (Sato et al., 2003). Consistent with glomeruli enlargement, untreated GK rats demonstrated overexpression of type IV collagen as compared with Wistar rats (Fig. 6A-i,j). By quantitative scoring, an increased type IV collagen score was observed in the glomeruli of untreated GK rats (6.40±1.04%, $p<0.001$) compared with Wistar rats (1.28±0.27%). Treatment with magnolol significantly reduced the type IV collagen in the glomeruli of GK rats (1.23±0.13%, $p<0.001$) compared to the untreated GK rats (Fig. 6A-k, C).

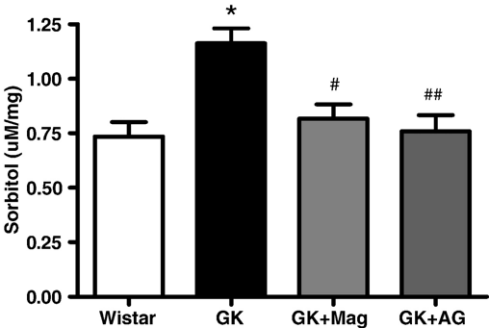

Fig. 5. Sorbitol levels in kidney medulla in 19-week-old experimental rats. Data expressed as means±S.E., $n=7–8$. ★, $p<0.01$ vs. Wistar rats; #,##, $p<0.05$, $p<0.01$ vs. untreated GK rats.

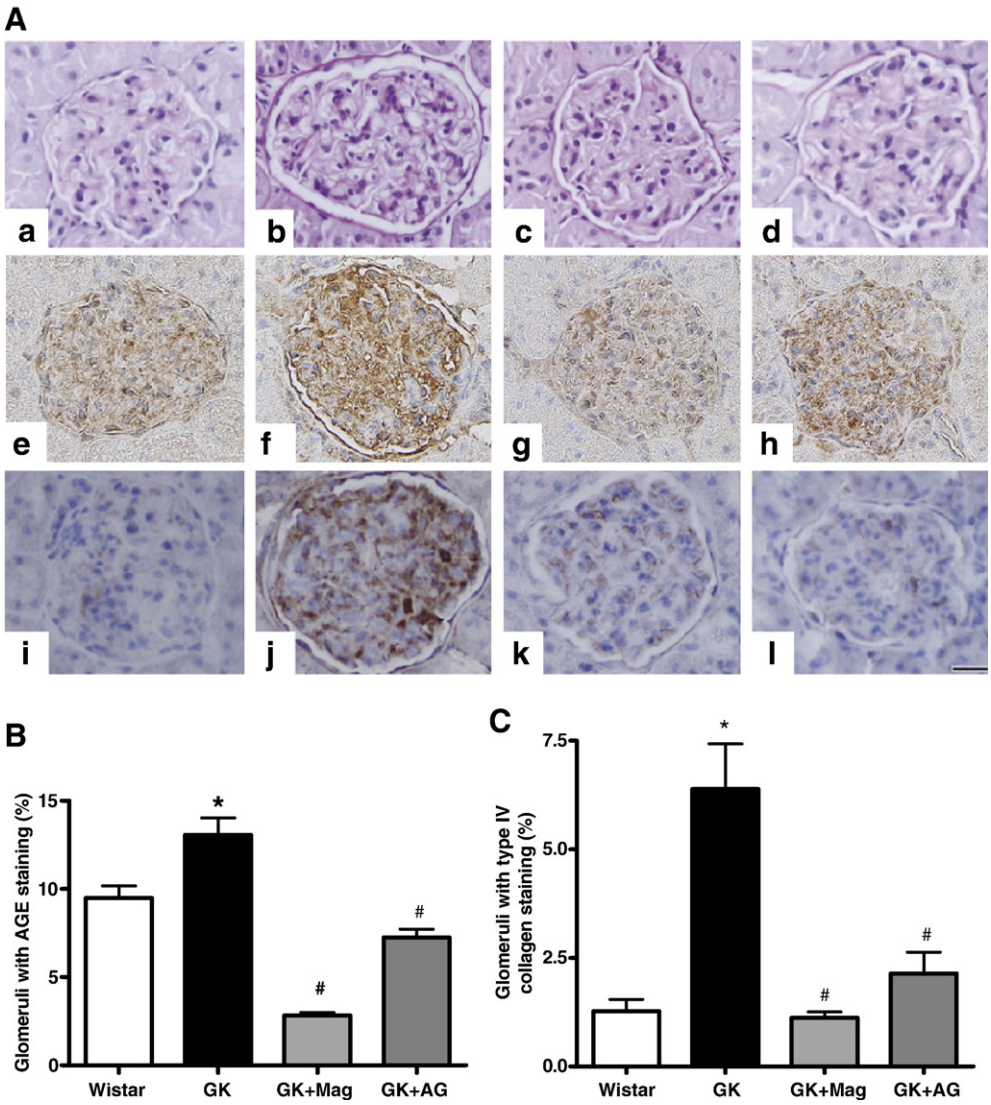

Fig. 6. Immunohistochemical staining of AGE and type IV collagen in kidney and pathological changes in the kidney: (A-a–d) Representative PAS staining in renal glomeruli of Wistar (a), untreated GK rats (b), GK rats treated with, magnolol (Mag, 500 mg/kg) (c) and GK rats treated with aminoguanidine (AG, 50 mg/kg) (d). Immunohistochemical staining of AGE (Fig. 6A-e–h) and type IV collagen (Fig. 6A-i–l) in Wistar (e, i), untreated GK rats (f, j), GK rats treated with magnolol (g, k) and GK rats treated with aminoguanidine (50 mg/kg) (h, l). Hematoxylin counterstain. Original magnification, 400x. (B) Quantitative analysis of AGE in glomeruli. The average score of 80 randomly selected glomeruli was calculated. Data expressed as means±S.E. ★, $p<0.001$ vs. Wistar rats; #, $p<0.001$ vs. untreated GK rats (C) Quantitative analysis of type IV collagen in glomeruli. The average score of 30 randomly selected glomeruli was calculated. Data expressed as means±S.E., $n=60$. ★, $p<0.001$ vs. Wistar rats; #, $p<0.001$ vs. untreated GK rats.

The expression of TGF-β1 mRNA in the renal cortex

The TGF-β1: β-actin ratio in untreated GK rats was increased 2.11 fold ($p<0.001$) compared with Wistar GK rats. Treatment with magnolol significantly suppressed the overexpression of renal TGF-β1 mRNA ($p<0.05$) (Fig. 7A, B).

Discussion

Diabetic nephropathy is one of the most common complications of diabetes and is characterized by increased urinary protein and loss of renal function. A number of studies have now definitely proven that improved metabolic control that achieves near-normoglycemia can significantly decrease the development and progression of diabetic nephropathy (Mogensen, 1984; Lee et al., 1995; Park et al., 1998). The metabolic factors such as AGEs, sorbitol, beyond blood glucose level are also implicated in the pathogenesis of diabetic nephropathy (Schrijvers et al., 2004). Magnolol (5,5′-diallyl-2,2′-dihydroxybiphenyl) produced a potent and strong antihyperglycemic effect, based on the fasting blood glucose levels (Fig. 2), and it also decreased insulin resistance in GK rats (Fig. 3A).

Previous studies have shown that high blood glucose causes the deterioration of pancreatic beta-cells due to oxidative stress (Marshak et al., 1999; Kaneto et al., 2001). Hyperglycemic stress is reflected structurally by degranulation of islet cells and reduced immunoreactivity to insulin in GK rats (Koyama et al., 1998). Magnolol, which has an antioxidant effect (Lo et al.,

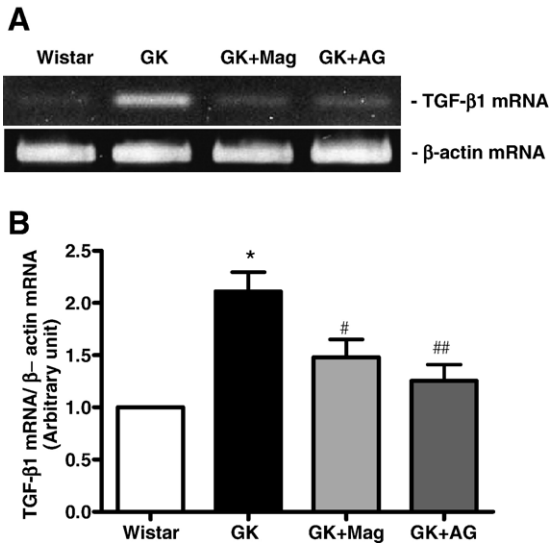

Fig. 7. The expression of TGF-β mRNA: (A) Expression of TGF-β1 mRNA by RT-PCR. (B) Intensity of TGF-β mRNA. Data expressed as means±S.E., $n=4$. ★, $p<0.01$ vs. Wistar rats; #,##, $p<0.05$, $p<0.01$ vs. untreated GK rats, respectively.

1994), can have beneficial effects on pancreatic beta cells by neutralizing oxidative stress. The present study showed that the decreased number of the pancreatic beta cells in untreated GK rats compared to Wistar rats. Furthermore, both the frequency and distribution of immunoreactive beta-cells were increased in magnolol-treated GK rats (Fig. 3B, C). However, glucagon immunoreactive alpha-cell expression was not significantly altered among the four groups tested. Therefore, the decrease in blood glucose levels in magnolol-treated GK rats might be attributed by the improvement of insulin resistance due to chronic glycemic control.

Over the last decade, reports have been accumulated that AGEs and TGF-β play a key role in the pathogenesis of diabetic nephropathy (Schrijvers et al., 2004; Yin et al., 2004). Hyperglycemia- or AGEs-increased TGF-β stimulates the synthesis of individual matrix components such as collagen, fibronectin, laminin, and proteoglycans in the diabetic kidney (Sharma and Ziyadeh, 1995; Ignotz and Massague, 1986; Cohen et al., 1999). In this study, the untreated 19-week-old GK rats showed early signs of diabetic nephropathy, including enlargement of the glomeruli, overexcretion of urinary protein and Ccr. AGEs, TGF-β1, and type IV collagen were also overproduced in untreated GK rats. Further, in the magnolol-treated GK rats, urinary protein excretion was decreased by 40% compared with untreated GK rats (Fig. 4A), Ccr levels also significantly decreased (Fig. 4B), and enlargement of the glomeruli reduced (Fig. 6A). Magnolol treatment significantly decreased type IV collagen and TGF-β1 mRNA expression in kidney of GK rats via inhibition of AGEs formation (Figs. 6, 7), suggesting that magnolol can prevent or retard the development of diabetic nephropathy through AGEs inhibition (Lee et al., 2006).

In diabetic patients, aldose reductase is greatly activated by high glucose and so sorbitol accumulated, which is associated with the depletion of myoinositol (Green et al., 1987). The importance of aldose reductase to diabetic nephropathy has been emphasized since the increase of aldose reductase activity is associated with enhanced TGF-β1 production (Ishii et al., 1998). In the current experiments, magnolol significantly inhibited sorbitol accumulation compared to untreated GK rats, indicating the reduction of aldose reductase activity (Fig. 5).

In conclusion, the present data clearly demonstrate that administration of magnolol reduces metabolic factors influencing diabetic nephropathy such as blood glucose, plasma insulin, sorbitol and AGEs in experimental type 2 diabetes. Furthermore, magnolol attenuates the up-regulation of renal TGF-β1 mRNA, reduces the expression of extracellular matrix protein as type IV collagen, and ameliorates renal dysfunction. Taking together these results, magnolol has therapeutic or preventive effects on several pharmacological targets in the complicated pathological mechanism of diabetic nephropathy. Thus it is worthwhile to be further investigated for its potential pharmacological effects in diabetic nephropathy.

Acknowledgments

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