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European Journal of Pharmacology 579 (2008) 418–425
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Lithospermic acid B ameliorates the development of diabetic nephropathy in OLETF rats☆

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Received 20 August 2007; received in revised form 17 October 2007; accepted 22 October 2007
Available online 1 November 2007

Abstract

Lithospermic acid B (LAB), an active component isolated from *Salvia miltiorrhizae*, has been reported to have renoprotective effects in type 1 diabetic animal models. In the present study we investigated the effects of LAB on the prevention of diabetic nephropathy in type 2 diabetic Otsuka Long-Evans-Tokushima Fatty (OLETF) rats. LAB (20 mg/kg) was given orally once daily to 10-week-old male OLETF rats for 28 weeks. Treatment of OLETF rats with LAB had little effects on body weight and blood glucose levels. Treatment with LAB resulted in significant reduction in blood pressure. LAB markedly attenuated albuminuria and significantly lowered levels of lipid peroxidation, monocyte chemoattractant protein-1 (MCP-1), and transforming growth factor-β (TGF-β1) expression in renal tissues of OLETF rats. In addition, LAB inhibited the progression of glomerular hypertrophy, mesangial expansion, and expansion of the extracellular matrix in the renal cortex. Collectively, these results suggest that LAB has beneficial effects on the diabetic nephropathy in OLETF rats by decreasing blood pressure, oxidative stress, and MCP-1 expression. Our results suggest that LAB might be a new therapeutic agent for the prevention of nephropathy in type 2 diabetes.

Keywords: Lithospermate B; OLETF rats; Diabetic nephropathy; Oxidative stress; MCP-1; Type 2 diabetes

1. Introduction

Diabetic nephropathy is one of the major complications associated with type 2 diabetes and is a leading cause of end-stage renal disease. Diabetic nephropathy is characterized by a progressive accumulation of extracellular matrix components in the glomerular mesangium and tubular interstitium, eventually leading to proteinuria and renal failure. The underlying mechanisms of the evolution of diabetic nephropathy are extremely complex, and several mediators have been implicated. Several growth factors or metabolic products, including transforming growth factor-β (TGF-β), insulin-like growth factor-I, platelet-derived growth factor, angiotensin II, and advanced glycation end products, have been identified as contributing factors involved in the progression of diabetic glomerulopathy (Brosius, 2003; Ziyadeh, 2004). These factors can promote mesangial cell proliferation, glomerular hypertrophy, and extracellular matrix overproduction, or alter glomer-

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doi:10.1016/j.ejphar.2007.10.070

ular hemodynamics. Among these factors, reactive oxygen species are thought to play a central role in development of diabetic nephropathy. High glucose induces reactive oxygen species (Ha and Lee, 2000; Ha et al., 2002) and upregulates TGF-β1 and extracellular matrix (Oh et al., 1998; Ziyadeh et al., 1994) expression in the glomerular mesangial cells. Antioxidant treatments effectively inhibit high glucose-induced TGF-β1 and fibronectin upregulation (Ha and Lee, 2000; Ha et al., 1997), thus providing evidence that reactive oxygen species play an important role in high glucose-induced renal injury. Despite implementation of intensive glycemic and antihypertensive control, diabetic nephropathy remains an important clinical problem. Therefore, new therapeutic agents are needed for the treatment of diabetic nephropathy.

Salvia miltiorrhizae radix is a traditional Chinese herbal medicine that has been used for many years in China, Japan, and Korea for the treatment of diabetic complications. In addition, lithospermic acid B (LAB), a recently isolated component of *S. miltiorrhizae*, is known to have multiple pharmacological activities such as antihypertensive effect (Kamata et al., 1993; Kamata et al., 1994), beneficial effects on renal injury (Yokozawa et al., 1989), protective effect on hepatitis (Hase et al., 1997), and antioxidant effect (Soung et al., 2003; Yokozawa et al., 1997).

The Otsuka Long-Evans-Tokushima Fatty (OLETF) rat is an inbred strain that spontaneously develops type 2 diabetes and subsequently progresses to diabetic glomerulosclerosis. The animal is characterized by mild obesity and late-onset hyperglycemia (after 18 weeks of age) with complications related to chronic diabetes similar to those of human type 2 diabetes (Koga et al., 2002; Uehara et al., 1998).

We previously showed renoprotective effects of LAB on streptozotocin-induced diabetic rats (Jung et al., 2002; Lee et al., 2003). In this study, we investigated the effect of LAB treatment on renal function, oxidative stress, morphometric changes, and renal monocyte chemoattractant protein-1 (MCP-1) expression in OLETF rats.

2. Materials and methods

2.1. Animals

Ten-week-old male OLETF and age-matched control Long-Evans Tokushima Otsuka (LETO) rats were kindly supplied by the Tokushima research institute (Otsuka Pharmaceutical, Tokushima, Japan). All animals were individually housed and maintained on a 12:12-h light-dark cycle in a temperature-controlled (23±2 °C), humidity-controlled (55±5%), AAA-LAC (Association for Assessment and Accreditation of Laboratory Animal Care)-accredited vivarium. All rats were fed standard rat chow (Samyang, Seoul, Korea) and allowed free access to tap water. LETO and OLETF rats were randomly treated with either LAB (20 mg/kg, $n=10$) or phosphate buffered saline (PBS) ($n=10$). All agents were given orally by gavages (2 ml) once daily to 10-week-old male OLETF rats and LETO rats ($n=40$) for 28 weeks. LAB was isolated from *S. miltiorrhizae* as previously described (Jung et al., 2002; Lee et al., 2003). The effects of different concentrations of LAB (1–20 mg/kg/day) on albuminuria in OLETF rats were compared during our preliminary study (data not shown). Body weights were measured monthly and the amounts of 24-h urinary albumin excretion were measured 12 and 26 weeks after LAB treatment. Systolic blood pressure was measured monthly by tail-cuff photoplethysmography (MK-1100, Muromachi Kikai, Tokyo, Japan) in conscious pre-warmed rats. The average of five pressure readings was recorded for each measurement. At 38 weeks of age, animals were sacrificed and half of one kidney was fixed in paraformaldehyde for histologic examination; the remaining kidney was frozen in liquid nitrogen. All experiments in this study conformed to the guidelines for the care and use of laboratory animals of the Yonsei University College of Medicine.

2.2. Glucose tolerance test

At 38 weeks of age, animals were fasted overnight. At 8:00 a.m. of the following morning animals were given an intraperitoneal injection of glucose (2 g/kg). Blood samples were obtained from the tail vein, and blood glucose levels were determined using a glucometer (Roche-Diagnostic, Pleasanton, CA).

2.3. Histologic examination

Kidneys were fixed with 10% formalin, embedded in paraffin, sectioned into 3-um-thick slices at the level of the hilus, and stained with hematoxylin-eosin and Masson's trichrome. From the collected sections, 30 different superficial glomeruli were randomly sampled for morphometric analysis as follows. The microscopic slide was scanned clockwise along the superficial cortex, and every third glomerulus was analyzed. An automated image analysis system was used to measure the total glomerular profile area, the glomerular tuft area, and the mesangial matrix area. Collagen-stained areas were measured using MetaMorph 4.6 image analysis computer program (Universal Imaging, West Chester, PA) as described previously (Elliot et al., 2003; Kosugi et al., 2007; Kosugi et al., 2006).

2.4. Biochemical analysis

Blood samples were obtained from the aorta at the time of sacrifice and were immediately centrifuged at 5000 g for 5 min. Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and serum creatinine were determined with a Hitachi 911 automatic analyzer (Boehringer Mannheim, Indianapolis, IN). Total cholesterol, triglycerides, and free fatty acid concentrations were quantified at the end of the study using the Vitros 250 Chemistry Analyzer (Johnson & Johnson, Rochester, NY). Urine was collected for 24 h and samples were centrifuged at 3000 *g* for 10 min. Urinary albumin present in the supernatant was determined by ELISA using the Nephrat microalbumin assay kit (Exocell, Philadelphia, PA). All samples were assayed in triplicate and the mean value from a given rat was calculated.

2.5. Measurement of malondialdehyde (MDA) in the renal cortex

The degree of lipid peroxidation was measured using a modified form of the thiobarbituric acid reactive substances method as described by Ohkawa et al. (Ohkawa et al., 1979).

2.6. Measurement of mRNA expression

Total RNA was extracted from kidney cortex with TRIZOL reagent (Invitrogen, Carlsbad, CA). RNA extracts were kept at −80 °C until reverse transcription polymerase chain reaction (RT-PCR) analyses were performed. A RT-PCR kit (Qiagen, Valencia, CA) was used for semi-quantitative analyses of monocyte chemoattractant protein-1 (MCP-1) and β-actin mRNA expression. Following the reverse transcription reaction at 50 °C for 30 min, PCR reactions were done. The primer sequences used and PCR conditions are shown in Table 1. Relative RT-PCR was performed according to the manufacturer's recommendations. To measure quantitative MCP-1 mRNA expression in kidney, real-time PCR was performed using a 7300 real-time PCR system (Applied Biosystems, Foster City, CA). The reaction mixtures were prepared in a total volume of 20 μl containing 4 ug of cDNA, 10 μl of SYBR Green Master Mix (ABI), 300 nM of primers. The primer sequences used and PCR conditions are shown in Table 1.

2.7. Assay for renal cortical TGF-β1 protein

TGF-β1 in the renal cortex homogenate was converted into its active form by treatment with HCl (final concentration, 0.2 M) for 30 min at room temperature followed by neutralization with equimolar NaOH. The amount of TGF-β1 was determined by a quantitative sandwich enzyme immunoassay using the TGF-β1 E_{max}™ ImmunoAssay System (Promega, Madison, WI) according to the manufacturer's description. The E_{max}™ ImmunoAssay System has a detection limit of 32 pg/ml for TGF-β1. The sensitivity of the test was 94% and had a specificity with <5% cross-reactivity with TGF-β2 and TGF-β3 at 10 ng/ml.

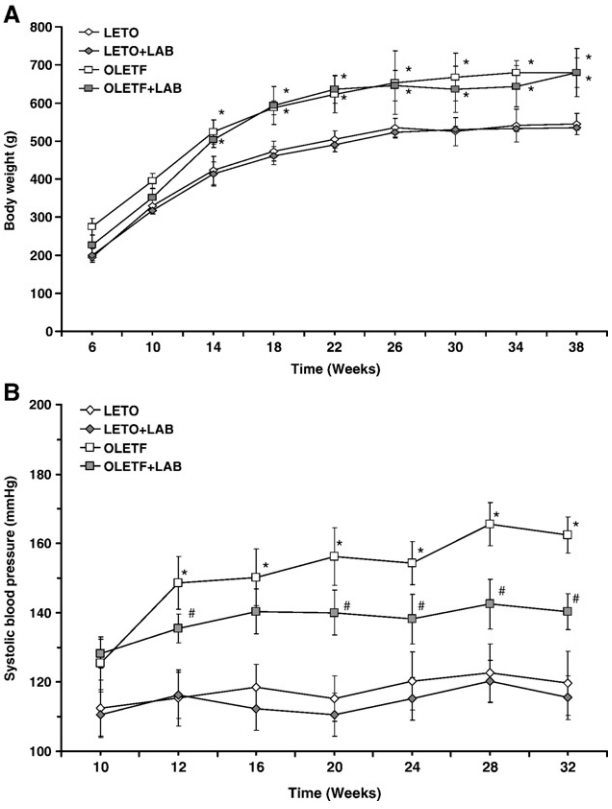

Fig. 1. Profiles of body weight change (A) and systolic blood pressure change (B). A: treatment with LAB had no effect on the body weight gain compared with OLETF control rats. B: OLETF rats progressively developed hypertension, whereas treatment with LAB reduced systolic blood pressure significantly. LETO; control rats treated with PBS, LETO+LAB; control rats treated with LAB, OLETF; OLETF rat control treated with PBS, and LAB; OLETF rats treated with LAB. *P<0.05 compared with LETO, #P<0.05 compared with OLETF.

2.8. Statistical analysis

All data are expressed as mean±S.E.M. unless otherwise stated. A Kruskall–Wallis test was used for comparison among groups, followed by a Mann–Whitney U test for comparison. Statistical analyses were performed using the SPSS for Windows software (version 12.0; SPSS, Chicago,

Table 1
PCR primer sequences and PCR conditions used

PCR condition								
Primers			Number of cycles	Initial denaturation	Denaturation	Annealing	Elongation	Final extension
MCP-1	F	5′-CACCTGCTGCTACTCATTCACT-3′	30	95°C for 5 min	95°C for 30 s	60°C for 30 s	72°C for 30 s	72°C for 10 min
	R	5′-GTTCTCTGTCATACTGGTCACTTCT-3′						
Beta-actin	F	5′-GACCCAGATCATGTT TGAGACC-3′	25	95°C for 5 min	95°C for 30 s	60°C for 30 s	72°C for 30 s	72°C for 10 min
	R	5′-GGCCATCTCTTGCTCGAAGTC-3′						
MCP-1 (real-time)	F	5'-CACCTGC TGCTACTCATTCACT-3'	40	95°C for 10 min	95°C for 15 s	60°C for 30 s		
	R	5'-GTTCTCTGTCATACTGGTCACTTCT-3'						
Beta-actin (real-time)	F	5'-GACCCAGATCATGTTTGAGACC-3'	40	95°C for 10 min	95°C for 15 s	60°C for 30 s		
	R	5'-GGCCATCTCTTGCTCGAAGTC-3'						

F, forward primer; R, reverse primer.

Table 2
Metabolic and histologic characteristics at 38 weeks

	LETO	LETO+LAB	OLETF	OLETF+LAB
FPG (mmol/l)	4.41±0.49	4.95±0.52	6.27±0.39[a]	6.32±0.19[a]
2Hr PPG (mmol/l)	5.62±0.15	5.75±0.23	11.17±0.54[a]	10.03±0.52[a]
Aspartate aminotransferase (IU/l)	85.5±2.7	92.4±3.9	97.0±4.1[a]	113.7±12.9[a]
Alanine aminotransferase (IU/l)	72.5±1.9	68.5±1.3	63.0±2.6	64.7±6.8
Total cholesterol (mmol/l)	2.21±0.14	2.84±0.26	3.68±0.44[a]	3.67±0.27[a]
Triglyceride (mmol/l)	2.72±0.71	2.87±0.85	6.45±1.10[a]	6.82±0.70[a]
Free fatty acid (uEq/l)	544.5±46.7	620.2±58.2	1078.5±106.9[a]	1061.3±141.7[a]
Serum creatinine (mg/dl)	0.70±0.03	0.63±0.05	0.65±0.01	0.67±0.03
Kidney weight (g)	1.42±0.08	1.45±0.13	2.55±0.24[a]	1.93±0.20[a, b]
Kidney weight (g)/body weight (100 g)	0.26±0.02	0.29±0.03	0.39±0.04[a]	0.29±0.03[a, b]
Mesangial matrix fraction (%)	15.2±4.3	16.4±4.3	21.7±5.3[a]	18.4±4.2[b]
Glomerular volume ($10^6\mu^3$)	1.50±0.25	1.43±0.20	1.70±0.31[a]	1.56±0.28[b]
Glomerular collagen (% of area)	4.78±2.23	4.22±2.42	12.94±3.78[a]	4.91±2.75[b]
Tubulointerstitial collagen (% of area)	3.48±1.50	2.69±1.11	10.45±3.41[a]	3.60±1.77[b]

Values are expressed as mean±S.E.M. Statistical analysis was performed among groups at 38 weeks. LETO; control rats treated with PBS, LETO+LAB; control rats treated with LAB, OLETF; OLETF rat treated with PBS, and LAB; OLETF rats treated with LAB. [a]$P<0.05$ compared to LETO; [b]$P<0.05$ compared to OLETF.

IL). A P value of <0.05 was considered statistically significant.

3. Results

3.1. Body weight and blood pressure

Fig. 1A shows data for body weight changes in control LETO, LAB-treated LETO, control OLETF, and LAB-treated OLETF rats. Treatment with LAB had no effect on the body weight gain compared with control rats. Body weight was always greater in the OLETF than LETO rats. At the end of the 38-week period, mean body weight had increased from 274.5±8.0 to 659.2±20.9 gram in OLETF rats, whereas the mean body weight of LETO rats had increased from 194.0±4.1 to 535.8±5.3 gram (Table 2). OLETF rats progressively developed hypertension, whereas treatment with LAB reduced systolic blood pressure significantly (Fig. 1B). There was no significant difference in systolic blood pressure between control LETO and LAB-treated LETO rats (Fig. 1B).

3.2. Glucose tolerance test and lipid profiles

Blood glucose levels were the greatest 30 min after the intraperitoneal glucose injection in all animals, which was followed by a distinct disposal phase. There was a delay in blood glucose disposal in OLETF rats. Treatment with LAB did not significantly influence fasting and postprandial plasma glucose levels in LETO and OLETF rats (Fig. 2 and Table 2). In addition, there were no significant differences in lipid profiles among treatment groups (Table 2).

3.3. Albuminuria and renal hypertrophy

The amount of albuminuria was markedly suppressed by treatment with LAB (Fig. 3). Following a similar pattern, kidney weight and kidney weight corrected for body weight

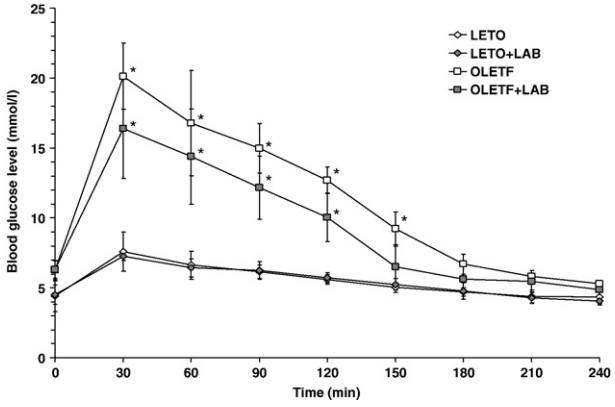

Fig. 2. Profiles of glucose tolerance test at 38 weeks. Blood glucose levels were the greatest 30 min after the intraperitoneal glucose injection in all animals, which was followed by a distinct disposal phase. There was a delay in blood glucose disposal in OLETF rats. Treatment with LAB did not significantly influence fasting and postprandial plasma glucose levels. Values are mean±SD LETO; control rats treated with PBS, LETO+LAB; control rats treated with LAB, OLETF; OLETF rat control treated with PBS, and LAB; OLETF rats treated with LAB. *$P<0.05$ compared with LETO.

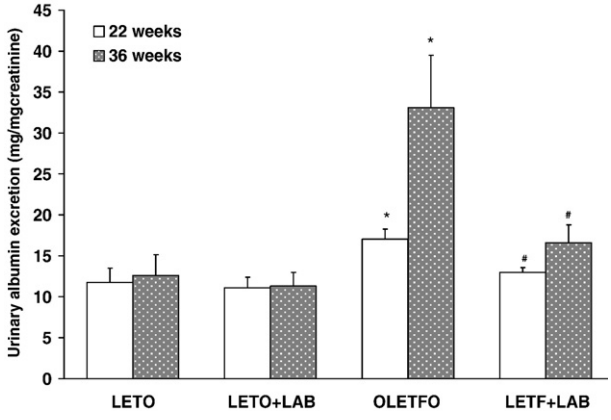

Fig. 3. Effects on albuminuria. The amount of albuminuria was markedly suppressed by treatment with LAB. LETO; control rats treated with PBS, LETO+LAB; control rats treated with LAB, OLETF; OLETF rat control treated with PBS, OLETF rats treated with LAB. Values are mean±S.E.M. *$P<0.05$ compared with LETO, #$P<0.05$ compared with OLETF.

were decreased significantly in rats treated with LAB (Table 2).

3.4. Glomerular histology and morphometry

Quantitative morphometry performed by automated image analysis showed diabetes-related increase in glomeruli volume and mesangial expansion (Fig. 4 and Table 2). The mesangial and glomerular volumes were 1.3-fold larger in OLETF rats than in LETO rats. Treatment with LAB significantly decreased glomerular hypertrophy and mesangial expansion (Table 2).

3.5. Accumulation of collagen protein in the renal cortex

Masson's trichrome staining for collagen demonstrated increased collagen deposition in both glomeruli and tubulointerstitium of the renal cortex in OLETF rats (Fig. 4 and Table 2). Semi-quantitative analyses for each parameter in the glomeruli and tubules of the different experimental groups are summarized in Table 2. Treatment with LAB effectively inhibited all diabetes-associated collagen up-regulation in the renal cortex (Table 2).

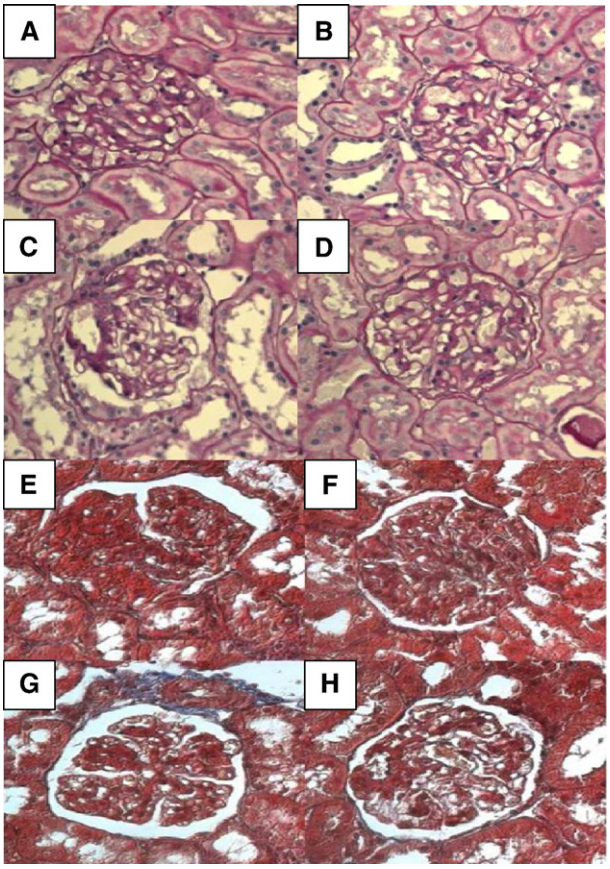

Fig. 4. Effects of LAB on renal histology (A–D) and renal collagen protein expression (E–H). PAS staining (A–D) and Masson's trichrome staining (E–H) for collagen were performed after 28 weeks of LAB treatment. LETO control rats were treated with PBS (A, E), LETO control rats treated with LAB (B, F), OLETF rats were treated with PBS and (C, G), and OLETF rats were treated with LAB (D, H). ×400 increased in glomeruli volume and mesangial expansion was observed in OLETF rat (C) compared with LETO (A) and LAB treated LETO (B). Treatment with LAB significantly decreased glomerular hypertrophy and mesangial expansion (D). Increased collagen deposition in both glomeruli and tubulointerstitium in OLETF rats was observed (G) which was reduced by LAB treatment (H).

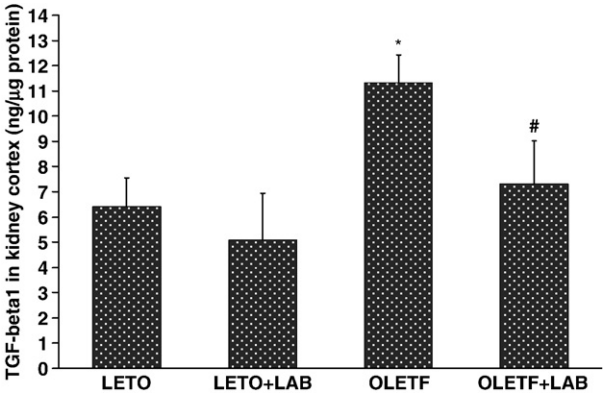

Fig. 5. ELISA results for TGF-β1 protein expression in the kidney cortex. Quantitative analyses of TGF-β1 showed that LAB inhibited TGF-β1 protein expression in the renal cortex of OLETF rats. LETO; control rats treated with PBS, LETO+LAB; control rats treated with LAB, OLETF; OLETF rat control treated with PBS, and LAB; OLETF rats treated with LAB. Values are mean±S.E.M. *$P<0.05$ compared with LETO, #$P<0.05$ compared with OLETF.

3.6. TGF-β1 protein expression in the renal cortex

Quantitative analyses of TGF-β1 showed that LAB inhibited TGF-β1 protein expression in the renal cortex of OLETF rats (Fig. 5).

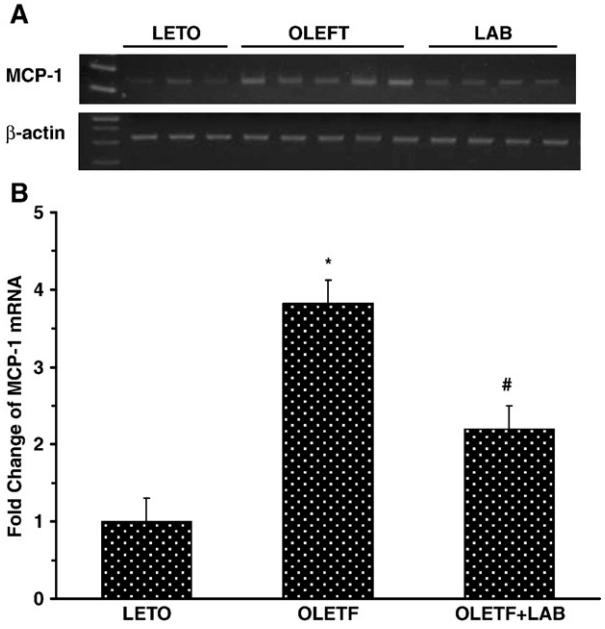

Fig. 6. A: profile of renal MCP-1 levels by relative PCR. B: quantitative level of renal MCP-1 mRNA expression by real time PCR. MCP-1 level in creased in OLETF rats compared with LETO rats. Treatment with LAB caused a decrease in renal cortical MCP-1 mRNA expression levels. LAB decreased amount of MCP-1 expression to the 57% level of OLETF control. LETO; control rats treated with PBS, OLETF; OLETF rat control treated with PBS, and LAB; OLETF rats treated with LAB. Values are mean±S.E.M. *$P<0.05$ compared with LETO, #$P<0.05$ compared with OLETF.

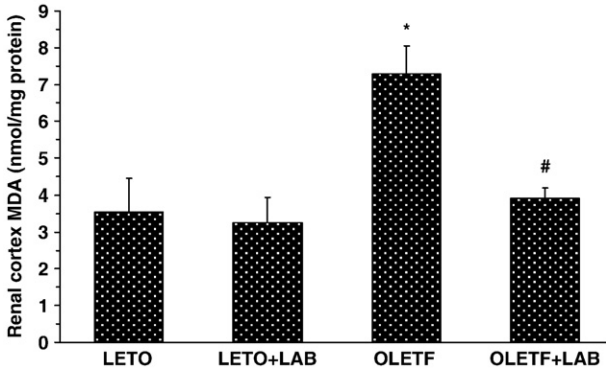

Fig. 7. Effect of treatments on renal cortex MDA levels. OLETF rats showed higher levels of renal cortical MDA contents compared with LETO rats. LETO; control rats treated with PBS, LETO+LAB; control rats treated with LAB, OLETF; OLETF rat control treated with PBS, and LAB; OLETF rats treated with LAB. Values are mean±S.E.M. *$P<0.05$ compared with LETO, #$P<0.05$ compared with OLETF.

3.7. Renal MCP-1 mRNA expression levels

MCP-1 mRNA expression in the kidney increased in OLETF rats compared with LETO rats (Fig. 6A). Treatment with LAB caused a decrease in renal cortical MCP-1 mRNA expression levels (Fig. 6A). LAB decreased amount of MCP-1 expression to the 57% level of OLETF control (Fig. 6B).

3.8. MDA concentration in the renal cortex

OLETF rats showed higher levels of renal cortical MDA contents compared with LETO rats. Treatment with LAB decreased the renal cortical MDA concentration (Fig. 7).

3.9. Cytotoxic effect of LAB

LAB treatment at a dose of 20 mg/kg/day did not cause any cytotoxicity, as assessed by AST and ALT. LAB slightly induced levels of AST or ALT levels, although the changes were not significant (Table 2).

4. Discussion

We have previously reported that LAB has positive effects on the intracellular signal pathway in the pathogenesis of nephropathy in a type 1 diabetic animal model by inhibiting the accumulation of extracellular matrix and protein kinase C (PKC) activity (Lee et al., 2003). These effects may be due to inhibition of reactive oxygen species production by LAB. In many previous *in vivo* studies, it has been revealed that oxidative stress induced by hyperglycemia precedes the clinically evident complications of diabetes.

In the present study, OLETF rats were used to investigate whether LAB has a favorable effect in a type 2 diabetic animal model. OLETF rats are characterized by late-onset hyperglycemia, a mild and chronic course of diabetes mellitus, and the association of diabetic complications. Diabetes develops in almost all cases of male OLETF rats by 25 weeks of age. As the disease progresses, renal complication becomes inevitable in this animal. Indeed, OLETF rats are widely used in many laboratories because they mimic type 2 diabetes in humans. Thus, we investigated the effect of LAB treatment on the development of diabetic nephropathy in OLETF rats.

Attenuated amount of albuminuria observed in LAB treated group was irrelevant to glucose metabolism in OLETF rats because LAB did not affect the changes in plasma glucose levels. Also, LAB treatment did not have any effect on the lipid profiles of OLETF rats.

Treatment with LAB exhibited a significant blood pressure lowering effect. LAB has been reported to have hypotensive effect, which may be due to the endothelium-dependent vasodilation of resistant arteries (Kamata et al., 1994) and modulation of angiotensin converting enzyme (ACE) activity (Kang et al., 2003).

Because oxidative stress has been widely reported to play an important role in the pathogenesis of diabetic nephropathy (Chander et al., 2004; Ha and Lee, 2001; Ha et al., 1994), we investigated oxidative stress by measuring lipid peroxide content in the renal cortex. Renal cortex MDA in OLETF rats was two-fold higher than in LETO rats and LAB treatment showed a significant antioxidant effect in kidney cortex.

Next we examined the effect of LAB on renal MCP-1 expression and macrophage infiltration. It has been reported that oxidative stress induces MCP-1 expression in various cell types (Ha et al., 2002; Takaishi et al., 2003; Tanifuji et al., 2005), and MCP-1 has been shown to play a role in the pathophysiology of inflammatory renal disease through selective monocyte attraction and activation (Chow et al., 2006; Tejera et al., 2004; Viedt

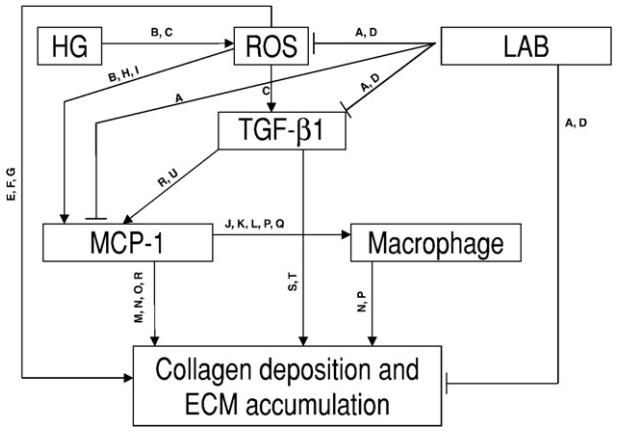

Fig. 8. Possible mechanism of renoprotective effect of LAB. LAB shows antioxidant effect, inhibits renal MCP-1 expression, suppresses renal TGF-β1 expression, and finally inhibits collagen deposition and extracellular matrix accumulation. Alphabet letters represent articles cited in this study. → ; up-regulation or activation, ⊢; inhibition, HG; high glucose, ROS; reactive oxygen species, MCP-1; monocyte chemoattractant protein-1, TGF-β1; transforming growth factor-β1, and ECM; extracellular matrix. A: represents this study, B: (Ha and Lee, 2000), C:(Ziyadeh et al., 1994), D:(Lee et al., 2003), E:(Chander et al., 2004), F:(Ha et al., 1994), G: (Ha and Lee, 2001), H: (Takaishi et al., 2003), I: (Tanifuji et al., 2005), J: (Tejera et al., 2004), K: (Viedt and Orth, 2002), L:(Chow et al., 2006), M:(Di Mario and Pugliese, 2001), N:(Ikezumi et al., 2003), O:(Ceol et al., 2003), P:(Tesch et al., 1999a), Q:(Tesch et al., 1999b), R: (Schneider et al., 1999), S:(Duncan et al., 1999), T:(Riser et al., 2000).

and Orth, 2002). MCP-1 is an important chemokine for macrophages and plays a crucial role in mesangial cell proliferation, glomerulosclerosis and renal fibrogenesis (Ceol et al., 2003; Ikezumi et al., 2003). Also, MCP-1 is known to be required for kidney macrophage accumulation (Tesch et al., 1999a; Tesch et al., 1999b). Treatment with LAB significantly decreased the levels of renal MCP-1. To measure quantitative MCP-1 mRNA level, real-time PCR was performed. We confirmed that LAB significantly decreased MCP-1 mRNA level in kidney.

Next we measured TGF-β1 in the renal cortex because TGF-β1 has been reported to stimulate MCP-1 expression in mesangial cells (Cheng et al., 2005), stimulates MCP-1 to promote collagen deposition (Schneider et al., 1999), and is believed to be a final mediator of extracellular matrix expansion, collagen deposition and fibrogenesis in diabetic nephropathy (Riser et al., 2000). Our data showed that LAB decreased levels of renal cortical TGF-β1. Together, our data suggest that LAB inhibits renal MCP-1 expression and renal TGF-β1 expression.

Finally, morphologic analysis showed that LAB inhibited the progression of glomerular hypertrophy and mesangial expansion. LAB effectively decreased extracellular matrix expansion and collagen deposition.

Together, these data suggest that renoprotective effects of LAB were due to antihypertensive effect, antioxidant effects and inhibition of MCP-1 and TGF-β1 (Fig. 8).

S. miltiorrhizae radix has long been used to treat both heart disease and cerebrovascular disease in China, Japan, and Korea. To date, no serious adverse effects of *S. miltiorrhizae* have been reported, and clinical usage data gathered from many years appears to suggest that *S. miltiorrhizae* products are generally safe (Zhou et al., 2005). Further, LAB did not cause a significant elevation in AST or ALT levels in this study.

In conclusion, we found that LAB attenuates the development of diabetic nephropathy in type 2 diabetic OLETF rats. These protective effects of LAB are thought to be due to antihypertensive effect, antioxidant effects, and inhibition of macrophage infiltration by decreasing MCP-1. LAB may be a new therapeutic strategy in the treatment of diabetic nephropathy in type 2 diabetes, as well as type 1 diabetes. The protective effects of LAB on experimental diabetic nephropathy provide scientific evidence to support the traditional use of *S. miltiorrhizae* radix for the treatment of diabetic complications.

Acknowledgements

This study was supported by a grant of the Oriental Medicine R&D Project, Ministry of Health & Welfare, Republic of Korea (0405-OD00-0815-0013) and a grant from Department of Internal Medicine, Yonsei University College of Medicine (2005).

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