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Research Article

Antiinflammatory and antifibrogenic effects of *s*-ethyl cysteine and *s*-methyl cysteine in the kidney of diabetic mice

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Protective effects of *s*-ethyl cysteine (SEC) and *s*-methyl cysteine (SMC) in kidney of diabetic mice were examined. SEC and SMC at 0.5, 1, 1.5, 2 g/L were added to the drinking water for 6 wk. Results showed that the intake of SEC or SMC alleviated body weight loss and urine output, as well as markedly decreased plasma blood urea nitrogen (BUN) and creatinine clearance (CCr) in diabetic mice ($P < 0.05$). The intake of SEC caused significantly dose-dependent increase in insulin and decrease in blood glucose, urinary albumin and type IV collagen ($P < 0.05$). SEC and SMC intake significantly and dose-dependently decreased malondialdehyde level and increased glutathione content in kidney ($P < 0.05$). The intake of these agents also increased renal GPx activity ($P < 0.05$), but there was no dose-dependent effect. SEC treatments dose-dependently decreased IL-6 and TNF-α levels, increased IL-4 and IL-10 levels, as well as upregulated IL-10 mRNA expression ($P < 0.05$). SMC treatments significantly suppressed renal IL-6 and TNF-α levels ($P < 0.05$), but did not affect IL-4 and IL-10 levels ($P < 0.05$). SEC or SMC intake significantly suppressed renal TGF-beta1 level and renal PKC activity ($P < 0.05$); however, only SEC treatments showed dose-dependent effect. SEC and SMC treatments significantly down-regulated mRNA expression of renal TGF-beta1 ($P < 0.05$), only SEC treatments had dose-dependent effects. Based on the observed antioxidative, antiinflammatory, and antifibrogenic effects, the supplement of SEC or SMC might be helpful for the prevention or treatment of diabetic kidney diseases.

Keywords: Diabetes / PKC activity / *s*-Ethyl cysteine / *s*-Methyl cysteine / TGF-beta1

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1 Introduction

Diabetic renal injury, or the so-called diabetic nephropathy, is one of the diabetic complications, which exacerbate the severity and the mortality of diabetes. It has been documented that oxidative stress, inflammation, and fibrosis contribute to the progress of diabetic associated renal injury [1–4]. Thus, any agent with antioxidative, antiinflammatory, or antifibrogenic effect may potentially prevent or delay the development of diabetic renal injury.

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Abbreviations: BUN, blood urea nitrogen; **CCr,** creatinine clearance; **ECM,** extracellular matrix; **GPx,** glutathione peroxidase; **GSH,** glutathione; **MDA,** malondialdehyde; **PKC,** protein kinase C; **SEC,** *s*-ethyl cysteine; **SMC,** *s*-methyl cysteine; **TGF-beta1,** tumor growth factor-beta1; **TNF-α,** tumor necrosis factor-α

s-Ethyl cysteine (SEC) and *s*-methyl cysteine (SMC) are two hydrophilic cysteine-containing compounds naturally formed in *Allium* plants such as garlic and onion [5, 6]. Our past study has indicated that the supplementation of these compounds at one dosage in drinking water for 4 wk could markedly alleviate hyperglycemia, hyperlipidemia, oxidative damage, and improve haemostatic balance in diabetic mice [7]. Thus, we hypothesized that these two compounds could protect kidney at least *via* decreasing hyperglycemic stress and oxidative stress. Therefore, a diabetic animal study regarding kidney protection from these two compounds at various doses was designed and conducted. On the other hand, the antiinflammatory effects of other hydrophilic cysteine-containing compounds such as *s*-acetyl cysteine against acetaminophen-induced liver injury in mice have been observed in our laboratory [8]. Thus, we also hypothesized that the dietary intake of SEC or SMC could protect kidney against diabetic associated inflammatory injury. In our present study, the influence of SEC and SMC upon cytokines such as IL-6 and IL-10 was examined.

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Under high glucose condition, like hyperglycemia in diabetes, the expression of transforming growth factor-beta1 (TGF-beta1) and the activity of protein kinase C (PKC) are increased in human and animal [9–12]. The upregulation of these parameters leads to massive production of extracellular matrix (ECM) such as fibronectin and type IV collagen, which further causes glomerular trophy, basement membrane thickening, mesangial expansion, and finally renal fibrosis. Consequently, renal dysfunction requiring dialysis and even renal replacement may be necessary in order to survive. Thus, any agent with TGF-beta1 and/or PKC inhibitory capability may be more efficacious in the prevention and/or therapy for diabetic associated renal fibrosis. In our present study, the influence of SEC and SMC upon TGF-beta1 production and PKC activity was examined in order to elucidate the possible action mode.

The major purpose of this study was to investigate the antiinflammatory and antifibrogenic effects, and possible action mode from SEC and SMC for renal protection in diabetic mice.

2 Materials and methods

2.1 Animals and diets

Male Balb/cA mice, 3–4 wk old, were obtained from National Laboratory Animal Center (National Science Council, Taipei City). Mice were housed on a 12-h light/dark schedule; water and the standard diet for rat and mouse were consumed *ad libitum*. The diet contained by weight (g/100 g): 64 starch, 23 protein, 3.5 fat, 5 fiber, 1 vitamin mixture, and 3 salt mixture (PMI Nutrition International). The use of mice was reviewed and approved by Chung Shan Medical University animal care committee. To induce diabetes, mice with body weights of 22.1 ± 0.5 g were treated with streptozotocin (STZ, 40 mg/kg body weight in 0.1 mol/L citrate buffer, pH 4.5) i.p. for five consecutive days. The blood glucose level was monitored on day 2, 5, and 10 from the tail vein using a one-touch blood glucose meter (Lifescan). Mice with fasting blood glucose levels ≥14.0 mmol/L were used for this study. After diabetes was induced, mice were divided into several groups (15 mice *per* group).

2.2 Experimental design

SMC (99%) and SEC (99.5%) were purchased from Sigma Chemical Each agent at 0.5, 1, 1.5, and 2 g/L was added to the drinking water. All mice had free access to food and water at all times. Consumed water volume and feed were recorded. Body weight, urine output, plasma level of glucose and insulin were measured weekly. After 6 wk supplementation, mice were killed with carbondioxide. Blood and kidney were collected. Plasma was separated from erythrocytes immediately. Kidney at 0.2 g was homogenized in ice in 2 mL phosphate buffer saline (pH 7.2) and the filtrate was collected. The protein concentrations of plasma or kidney filtrate were determined by the method of Lowry *et al.* [13] using BSA as a standard. In all experiments, the sample was diluted to a final concentration of 1 g protein/L using PBS, pH 7.2.

2.3 Measurement of plasma glucose, insulin, blood urea nitrogen (BUN) and creatinine clearance (CCr)

The plasma glucose level (mmol/L) was measured by a glucose HK kit (Sigma Chemical). Plasma insulin (nmol/L) was measured by RIA using a rat insulin RIA kit (SRI-13K; Linco Research). Plasma BUN, plasma creatinine (Cr) and urinary Cr concentrations were detected by a Beckman Autoanalyzer (Beckman Coulter, Fullerton, CA). CCr was calculated and expressed as mL/min *per* 100 g body weight.

2.4 Determination of glutathione (GSH) level and glutathione peroxidase (GPx) activity

GSH concentration (nmol/mg protein) in kidney filtrate was determined by a colorimetric GSH kit (Oxis Research). GPx activity (U/mg protein) in kidney filtrate was determined by GPx assay kit (Calbiochem, EMD Biosciences, San Diego, CA).

2.5 Lipid oxidation analysis

Lipid oxidation in the kidney filtrate was determined by measuring the level of malondialdehyde (MDA, μmol/L) using HPLC [14].

2.6 Renal cytokines determination

Perfused renal tissue was homogenized in 10 mM Tris-HCl buffered solution (pH 7.4) containing 2 M NaCl, 1 mM EDTA, 0.01% Tween 80, 1 mM PMSF, and centrifuged at 9000 × *g* for 30 min at 4°C [15]. The resultant supernatant was used for cytokine determination. The levels of proinflammatory cytokines, IL-6 and TNF-α, antiinflammatory cytokines, IL-4 and IL-10 were measured by ELISA using cytoscreen immunoassay kits (BioSource International Camarillo). Samples were assayed in duplicates according to manufacturer's instructions. The sensitivity of assay with the detection limit was at 5 pg/mg protein for IL-4, IL-6, and IL-10; and 10 pg/mg protein for TNF-α.

2.7 Measurement of urinary albumin, type IV collagen

Urinary albumin concentration was measured by an ELISA Albuwell M kit. Urinary type IV collagen concentration

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was measured by a Collagen IV M kit (Exocell, Philadelphia, PA), which measures both intact and fragments of type IV collagen at the same time.

2.8 Renal TGF-beta1 level analysis

Renal cortex was homogenized with ice-cold PBS containing 0.05% Tween 20. After centrifugating at 9000 × *g* for 15 min at 4°C, the supernatants were used for measuring renal TGF-beta1 level (ng/mg renal tissue). TGF-beta1 level was quantified by a commercial ELISA kit (R&D Systems, Minneapolis, MN).

2.9 Measurement of renal glomeruli PKC activity

The method described in Koya *et al.* [16] was used to measure glomeruli PKC activity. Briefly, bilateral kidneys were dissected, homogenized in ice-cold RPMI1640 media containing 20 mM HEPES. Glomeruli were isolated by removing the capsules, and passed through sieves of various sizes. After washing twice with RPMI1640 media containing 20 mM HEPES and once with a mixed salt solution, glomeruli were incubated with a salt solution for 15 min in the presence or absence of 100 μM PKC-specific substrate, RTLRRL, and followed by adding 5 mg/mL digitonin and 1 mM ATP mixed with γ-[^{32}P]ATP (< 1500 cpm/pmol). The reaction was stopped by 5% trichloroacetic acid, and then spotted onto P81 phosphocellulose paper and washed four times with 1% phosphoric acid and once with acetone. The amount of incorporated radioactivity into the substrate was determined by scintillation counting. Protein content of each sample was measured by the method of Lowry *et al.* [13]. Glomerular PKC activity was normalized by the corresponding protein content.

2.10 Quantitative RT-PCR for mRNA expression

Quantitative RT-PCR was used to examine the mRNA expression of IL-10 and TGF-beta1 in order to demonstrate the effect of SEC and SMC on the level of transcription. IL-10 was selected for examination because the inhibitory effect of IL-10 on nephrotoxic renal injury has been reported [17]. Renal cells were lysed, and RNA was extracted using TRIizol reagent, and was further digested with DNase. Total RNA was isolated from kidney tissue using the SV Total RNA Isolation kit (Promega, Madison, WI) according to the manufacturer's protocol. Two micrograms of total RNA was used to generate cDNA. Reverse transcription was performed in a one-step protocol using the iScript cDNA Synthesis Kit (BioRad) according to the manufacturer's protocols. Reactions were processed at 25°C for 5 min, 42°C for 30 min, 85°C for 5 min, and cooled at 4°C in a Thermocycler. The following primers were used. IL-10: forward, 5′-GGC CCT TTG CTA TGG TGT CC-3′, reverse, 5′- AAG CGG CTG GGG GAT GAC-3′; TGF-beta1: forward, 5′- CCG CAA CAA CGC CAT CTA TGA-3′, reverse, 5′-GGG GGT CAG CAG CCG GTT AC-3′. Quantitative analysis was performed with a BAS 2000 BIO-image analyzer (Fuji Photo Film, Tokyo, Japan). mRNA level was calculated as percentage value of the control group.

2.11 Statistical analysis

All data were expressed as mean ± SD. Statistical analysis was done using one-way analysis of variance (ANOVA), and post-hoc comparisons were carried out using Dunnet's *t*-test. *P* values < 0.05 were considered as significant.

3 Results

Feed intake and water intake at wk 1, 3, and 6 are presented in Table 1. There was no significant difference among groups ($P > 0.05$). Body weight and urine output at wk 1, 3, and 6 are shown in Table 2. At week 3, mice with SEC intake had significantly lower urine production than control and SMC intake groups ($P < 0.05$). At week 6, mice with SEC or SMC intake had significant higher body weight and lower urine output than control groups ($P < 0.05$).

Plasma level of glucose and insulin at wk 1, 3, 4, and 6 are presented in Fig. 1. When compared with control groups, SEC and SMC intake caused significantly lower blood glucose level and higher insulin level at wk 3, 4, and 6 ($P < 0.05$). SEC treatments at wk 4 and 6 caused dose-dependent decrease in glucose and increase in insulin ($P < 0.05$). Plasma BUN and CCr are presented in Fig. 2. SEC and SMC treatments significantly decreased BUN and CCr ($P < 0.05$), only SEC treatments had dose-dependent effect ($P < 0.05$).

Renal MDA, GSH levels, and GPx activity in mice consumed SEC or SMC are presented in Table 3. The intake of SEC and SMC caused dose-dependent MDA decrease and GSH increase ($P < 0.05$). The intake of these agents also significantly increased renal GPx activity, but there was no dose-dependent effect.

Renal levels of four cytokines are presented in Table 4. SEC treatments significantly and dose-dependently reduced renal IL-6 and TNF-α levels; as well as increased renal IL-4 and IL-10 levels ($P < 0.05$). SMC intake significantly suppressed IL-6 and TNF-α production ($P < 0.05$); but there was no dose-dependent effect. SMC treatments did not affect renal IL-4 or IL-10 level ($P > 0.05$). The effect of SEC and SMC on mRNA expression of renal IL-10 is shown in Fig. 3. SEC treatments caused dose-dependently upregulation in IL-10 mRNA expression when compared with control groups ($P < 0.05$). SMC treatments did not affect IL-10 mRNA expression.

As shown in Fig. 4, SEC treatments resulted in significantly dose-dependent decrease in urinary level of albumin

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Table 1. Feed intake (FI, g/mouse/d) and water intake (mL/mouse/d) of mice consumed 0, 0.5, 1, 1.5, 2 g/L SEC or SMC at 1, 3, and 6 wk. Data are mean ± SD, $n = 15$

Time (wk)	FI (g/mouse/d)			WI (mL/mouse/d)		
	1	3	6	1	3	6
SEC						
0	5.2 ± 0.4[a]	4.8 ± 0.3[a]	4.3 ± 0.6[a]	7.3 ± 0.9[a]	6.8 ± 0.7[a]	7.0 ± 0.6[a]
0.5	5.5 ± 0.6[a]	5.0 ± 0.4[a]	4.3 ± 0.5[a]	7.0 ± 1.0[a]	7.2 ± 0.9[a]	7.3 ± 0.8[a]
1	5.0 ± 0.5[a]	4.7 ± 0.5[a]	4.6 ± 0.7[a]	6.8 ± 0.8[a]	6.6 ± 1.1[a]	6.5 ± 1.0[a]
1.5	4.9 ± 0.6[a]	5.1 ± 0.2[a]	3.9 ± 0.5[a]	6.5 ± 1.1[a]	6.9 ± 0.6[a]	7.0 ± 0.5[a]
2	5.3 ± 0.3[a]	4.5 ± 0.6[a]	4.1 ± 0.3[a]	7.1 ± 0.7[a]	6.7 ± 0.8[a]	6.6 ± 0.7[a]
SMC						
0	5.4 ± 0.4[a]	5.0 ± 0.6[a]	4.7 ± 0.5[a]	6.9 ± 1.1[a]	7.1 ± 0.5[a]	6.6 ± 1.2[a]
0.5	5.2 ± 0.3[a]	4.6 ± 0.7[a]	4.4 ± 0.4[a]	6.4 ± 0.8[a]	6.5 ± 0.8[a]	7.0 ± 0.4[a]
1	5.6 ± 0.5[a]	4.4 ± 0.3[a]	4.5 ± 0.6[a]	6.5 ± 0.6[a]	6.8 ± 1.0[a]	7.1 ± 0.7[a]
1.5	5.7 ± 0.5[a]	5.1 ± 0.4[a]	4.8 ± 0.2[a]	7.1 ± 1.2[a]	7.2 ± 0.4[a]	6.6 ± 0.5[a]
2	5.3 ± 0.6[a]	4.9 ± 0.5[a]	4.3 ± 0.4[a]	6.8 ± 0.5[a]	6.7 ± 0.9[a]	6.8 ± 0.6[a]

a, b) Means in a column without a common letter differ, $P < 0.05$.

Table 2. Body weight (BW, g/mouse) and urine output (mL/mouse/d) of mice that consumed 0, 0.5, 1, 1.5, 2 g/L SEC or SMC at 1, 3 and 6 wk. Data are mean ± SD, $n = 15$

Time (wk)	BW (g/mouse)			Urine (mL/mouse/d)		
	1	3	6	1	3	6
SEC						
0	20.3 ± 1.4[a]	17.1 ± 0.8[a]	13.4 ± 0.5[a]	0.65 ± 0.10[a]	1.96 ± 0.21[a]	3.51 ± 0.29[a]
0.5	20.5 ± 1.6[a]	18.5 ± 0.8[a]	16.7 ± 0.8[b]	0.71 ± 0.11[a]	1.45 ± 0.18[b]	2.83 ± 0.17[c]
1	21.0 ± 0.9[a]	18.4 ± 1.0[a]	16.4 ± 0.4[b]	0.59 ± 0.08[a]	1.40 ± 0.14[b]	2.70 ± 0.20[c]
1.5	20.7 ± 1.0[a]	18.7 ± 0.5[a]	17.2 ± 0.9[b]	0.62 ± 0.06[a]	1.34 ± 0.10[b]	2.76 ± 0.18[c]
2	20.5 ± 1.3[a]	19.0 ± 1.1[a]	17.1 ± 0.6[b]	0.75 ± 0.12[a]	1.24 ± 0.17[b]	2.68 ± 0.21[c]
SMC						
0	20.9 ± 1.2[a]	17.3 ± 1.0[a]	13.7 ± 0.4[a]	0.72 ± 0.13[a]	2.14 ± 0.23[a]	3.67 ± 0.25[a]
0.5	21.1 ± 1.5[a]	18.5 ± 0.6[a]	16.2 ± 0.5[b]	0.63 ± 0.08[a]	1.85 ± 0.18[a]	3.22 ± 0.16[b]
1	20.6 ± 1.1[a]	18.8 ± 0.7[a]	15.9 ± 0.3[b]	0.68 ± 0.10[a]	1.78 ± 0.15[a]	3.18 ± 0.13[b]
1.5	20.2 ± 1.0[a]	17.9 ± 0.9[a]	16.1 ± 0.7[b]	0.70 ± 0.06[a]	1.70 ± 0.11[a]	3.20 ± 0.12[b]
2	20.7 ± 0.8[a]	18.7 ± 0.4[a]	16.0 ± 1.0[b]	0.73 ± 0.07[a]	1.81 ± 0.09[a]	3.12 ± 0.10[b]

a–c) Means in a column without a common letter differ, $P < 0.05$.

Table 3. Renal level of MDA (μmol/L), GSH (nmol/mg protein) and GPx activity (U/mg protein) in mice that consumed 0, 0.5, 1, 1.5, 2 g/L SEC (upper part) or SMC (lower part) for 6 wk. Data are mean ± SD, $n = 15$

	MDA (μmol/L)	GSH (nmol/mg protein)	GPx (U/mg protein)
SEC			
0	3.52 ± 0.45[e]	12.3 ± 0.9[a]	19.0 ± 1.5[a]
0.5	3.14 ± 0.26[d]	15.3 ± 1.1[b]	21.3 ± 1.8[b]
1	2.85 ± 0.17[c]	18.6 ± 1.4[c]	23.1 ± 2.0[b]
1.5	2.53 ± 0.21[b]	20.5 ± 1.2[d]	22.7 ± 2.2[b]
2	2.21 ± 0.15[a]	22.4 ± 1.5[e]	25.0 ± 2.1[c]
SMC			
0	3.62 ± 0.39[e]	13.2 ± 0.7[a]	19.2 ± 2.0[a]
0.5	3.21 ± 0.26[d]	15.8 ± 0.8[b]	21.8 ± 1.4[b]
1	2.90 ± 0.14[c]	17.7 ± 1.0[c]	22.0 ± 1.7[b]
1.5	2.67 ± 0.23[b]	19.8 ± 1.6[d]	22.7 ± 2.2[b]
2	2.42 ± 0.21[a]	21.6 ± 1.2[e]	21.5 ± 2.3[b]

a–e) Means in a column without a common letter differ, $P < 0.05$.

Table 4. Renal level (pg/mg) of proinflammatory cytokine (IL-6 and TNF-α) and antiinflammatory cytokine (IL-4 and IL-10) in mice that consumed 0, 0.5, 1, 1.5, 2 g/L SEC (upper part) or SMC (lower part) for 6 wk. Data are mean ± SD, $n = 15$.

	IL-6	TNF-α	IL-4	IL-10
SEC				
0	164 ± 15[e]	178 ± 19[e]	54.7 ± 2.5[a]	31.3 ± 2.0[a]
0.5	122 ± 10[d]	143 ± 14[d]	60.2 ± 2.8[b]	37.6 ± 2.7[b]
1	94 ± 7[c]	112 ± 10[c]	68.4 ± 3.0[c]	46.5 ± 3.1[c]
1.5	78 ± 5[b]	90 ± 8[b]	76.3 ± 2.4[d]	55.9 ± 3.7[d]
2	55 ± 7[a]	71 ± 6[a]	84.1 ± 2.3[e]	66.6 ± 3.4[e]
SMC				
0	168 ± 13[b]	174 ± 17[b]	55.3 ± 2.6[a]	32.0 ± 1.7[a]
0.5	126 ± 9[a]	138 ± 12[a]	57.6 ± 3.1[a]	34.4 ± 2.0[a]
1	130 ± 11[a]	131 ± 8[a]	58.2 ± 2.4[a]	35.3 ± 1.6[a]
1.5	123 ± 8[a]	125 ± 10[a]	60.7 ± 3.0[a]	33.5 ± 2.1[a]
2	119 ± 8[a]	134 ± 9[a]	59.5 ± 3.4[a]	35.6 ± 2.0[a]

a–e) Means in a column without a common letter differ, $P < 0.05$.

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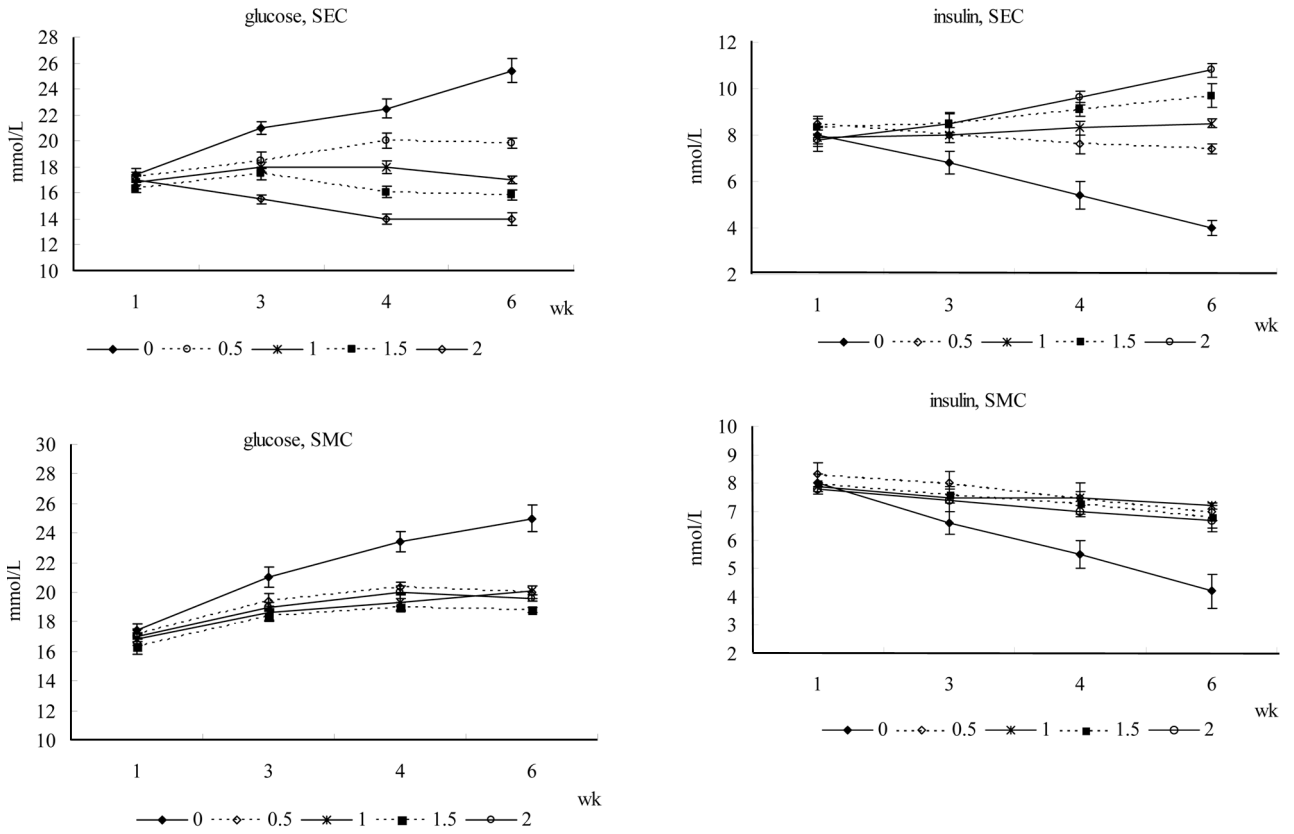

Figure 1. Plasma level of glucose (mmol/L) and insulin (nmol/L) in mice that consumed 0, 0.5, 1, 1.5, 2 g/L SEC or SMC at 1, 3, 4, and 6 wk. Data are mean ± SD ($n = 15$).

and type IV collagen ($P < 0.05$). SMC treatments also significantly decreased albumin and type IV collagen levels ($P < 0.05$), but there was no dose-dependent effect. The effect of SEC or SMC intake on renal TGF-beta1 level and PKC activity is presented in Fig. 5. SEC or SMC intake significantly suppressed renal TGF-beta1 production and PKC activity ($P < 0.05$); however, only SEC treatments showed dose-dependent effect on TGF-beta1 level. The effect of SEC and SMC on mRNA expression of renal TGF-beta1 is shown in Fig. 6. SEC treatments caused dose-dependently downregulation in mRNA expression of renal TGF-beta1 ($P < 0.05$). SMC treatments also down-regulated TGF-beta1 mRNA expression, but there was no dose-dependent effects.

4 Discussion

Our previous study has indicated that the dietary intake of SEC or SMC at one dosage, 1 g/L, for 4 wk could effectively reduce plasma glucose and increase plasma insulin in diabetic mice [7]. Our present study further observed that SEC intake dose-dependently increased insulin release; however, it seems only SMC retarded insulin decline in diabetic mice. Further study is necessary to examine whether SEC is able to repair STZ-treated pancreatic beta cells. On the other hand, we found the dietary intake of SEC or SMC alleviated body weight loss and urine output, as well as markedly decreased plasma BUN and CCr in diabetic mice. SEC and SMC are cysteine derived peptides, and may act as precursors for GSH biosynthesis and subsequently upregulate GPx activity [7]. Our present study also found SEC and SMC dose-dependently increased renal GSH content and attenuated renal oxidative damage in diabetic mice. These results strongly revealed that SEC or SMC could protect kidney against diabetic associated injury. Furthermore, we found that antioxidative, antiinflammatory, and antifibrogenic effect from SEC were dose-dependent. Although SMC did not exhibit dose-dependent protection, the intake of this agent seemed also to delay the deterioration of diabetic associated renal injury. The results of our present study once again supports that SEC and SMC, two compounds naturally derived from *Allium* plants, are potent antidiabetic agents.

IL-6 and TNF-alpha, proinflammatory cytokines, were central mediators for the regulation of several biomarkers such as c-reactive protein and von Willebrand factor [18, 19], which consequently contributed to the progression of

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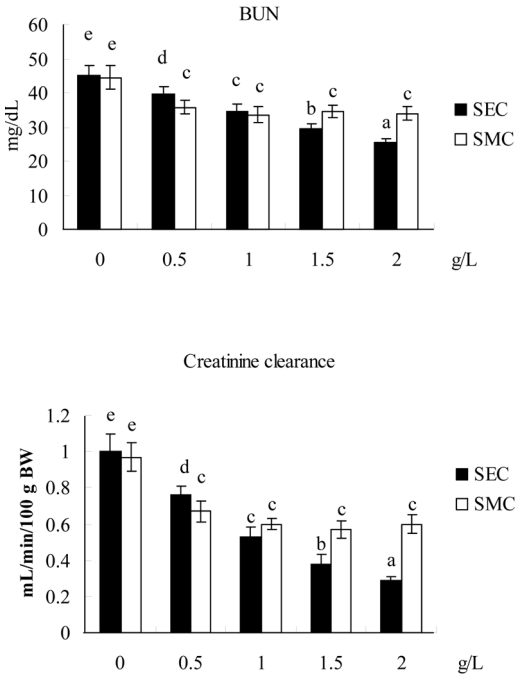

Figure 2. Plasma urea nitrogen (BUN, mg/dL), and CCr (mL/min *per* 100 g body weight) in mice that consumed 0, 0.5, 1, 1.5, 2 g/L SEC or SMC for 6 wk. Data are mean ± SD ($n = 15$). [abcde]Means among bars without a common letter differ, $P < 0.05$.

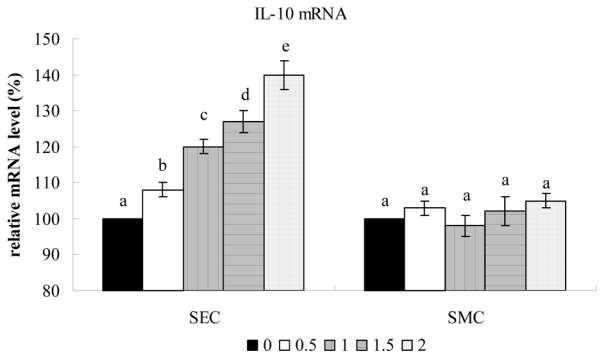

Figure 3. mRNA expression of renal IL-10 in mice that consumed 0, 0.5, 1, 1.5, 2 g/L SEC or SMC for 6 wk. Data are mean ± SD ($n = 15$). [abcde]Means among bars without a common letter differ, $P < 0.05$.

inflammation, endothelial dysfunction, and coagulation. Thus, the suppression on IL-6 and TNF-alpha could retard or alleviate inflammation and improve endothelial dysfunction. Our present study found that the dietary intake of either SEC or SMC could effectively decrease renal IL-6 and TNF-α levels in diabetic mice. These results indicated that these compounds are potent antiinflammatory agents. IL-4 and IL-10, antiinflammatory and immunosuppressive cytokines, could downregulate the production of proinflammatory cytokines such as IL-1 and TNF-α from monocytes

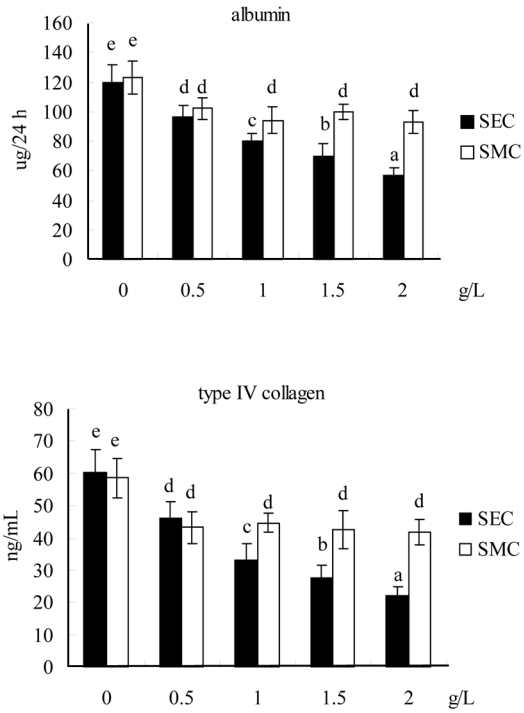

Figure 4. Urinary level of albumin (μg/24 h) and type IV collagen (ng/mL) in mice that consumed 0, 0.5, 1, 1.5, 2 g/L SEC or SMC for 6 wk. Data are mean ± SD ($n = 15$). [abcde]Means among bars without a common letter differ, $P < 0.05$.

[20, 21]. Thus, the elevation of IL-4 and IL-10 from SEC supplement as observed in our present study might contribute to alleviate renal inflammatory stress. The inhibitory effect of IL-10 on ischemic and nephrotoxic renal injury has been reported [17]. Our present study found that SEC treatments dose-dependently elevated mRNA expression of IL-10, which indicated that the effect of SEC occurred at the level of transcription. These results not only revealed the antiinflammatory action mode of SEC on renal tissue, but also observed the dose-dependent antiinflammatory effect of SEC. Therefore, our present study suggested that SEC and SMC could alleviate diabetic renal inflammation *via* suppressing the release of IL-6 and TNF-α release, and increasing renal IL-4 and IL-10 levels.

It is known that the TGF-beta1 is one of the key regulators of ECM genes in mesangial cells. Elevated glomerular TGF-beta1 level have been shown to make a significant contribution to the pathogenesis of diabetic glomerular lesions and fibrosis because TGF-beta1 could induce the expression of type IV collagen and fibronectin in mesangial cells [22–24], and further caused renal dysfunction such as albuminuria. The suppressive effect from SEC and SMC on the mRNA expression of TGF-beta1 partially explained the less production of TGF-beta1, which consequently diminished type IV collagen biosynthesis, alleviated albuminuria, and decreased BUN and Cr. The results of TGF-beta1

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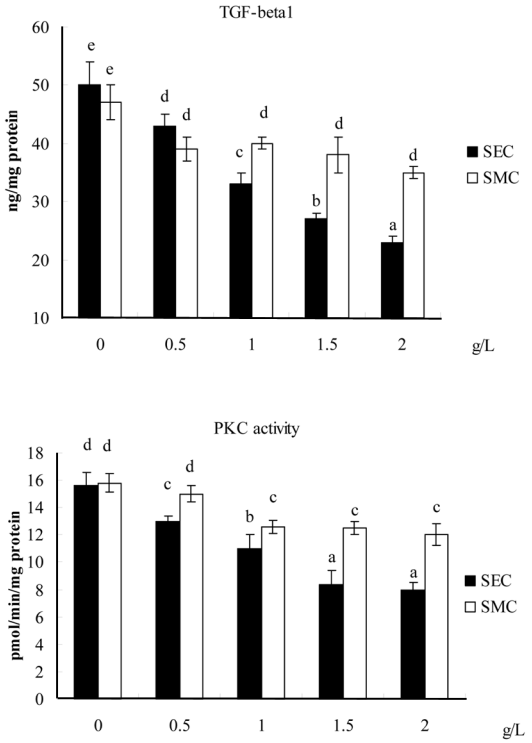

Figure 5. Renal TGF-beta1 level (ng/mg kidney) and PKC activity (pmol/min/mg protein) in mice that consumed 0, 0.5, 1, 1.5, 2 g/L SEC or SMC for 6 wk. Data are mean ± SD ($n = 15$). [abcde]Means among bars without a common letter differ, $P < 0.05$.

mRNA expression also supported the direct and dose-dependent effects of SEC and/or SMC on renal tissue. Furthermore, the suppressive effect from SEC or SMC on TGF-beta1 production also suggested that these agents might be useful for improving other fibrosis-associated diseases such as liver fibrosis and cystic lung disease.

Our present study found SEC and SMC effectively suppressed renal PKC activity. This finding partially explained why renal TGF-beta1 level was reduced, and once again supported that SEC and SMC were effective antifibrogenic agents against diabetic renal injury. So far, downregulation of TGF-beta1 signaling provides a useful therapeutic strategy for diabetic kidney diseases. Since SEC and SMC could effectively suppress renal PKC activity and renal TGF-beta1 overproduction, these two agents may be helpful for prevention and/or therapy for diabetic kidney diseases. Further study is necessary to ensure whether SEC or SMC could be considered as a PKC inhibitor.

Both SEC and SMC are hydrophilic peptide derivates and naturally formed in *Allium* foods such as garlic; however, the kidney protection from these two agents was somehow different. Apparently, the ethyl group of SEC and methyl group of SMC played important role in determining their bioactivities. Benevenga *et al.* [25] indicated that excessive SMC consumption might cause cytotoxicity.

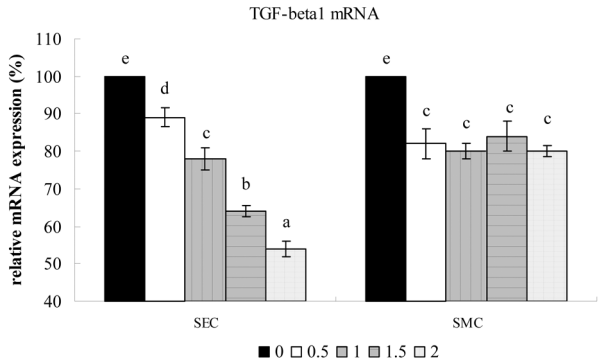

Figure 6. mRNA expression of renal TGF-beta1 in mice that consumed 0, 0.5, 1, 1.5, 2 g/L SEC or SMC for 6 wk. Data are mean ± SD ($n = 15$). [abcde]Means among bars without a common letter differ, $P < 0.05$.

Thus, the safety of SMC needs further study before it is applied for human. On the other hand, Krest *et al.* [26] reported that SMC content was 33–487 mg/100 g fresh garlic or 4–623 mg/100 g stems or roots of *Allium* plants, dependent on the species or vegetation period. Thus, it may not be appropriate to obtain these compounds by supplementing the diet with garlic or other *Allium* plants.

In summary, SEC and SMC provided antioxidative, anti-inflammatory, and antifibrogenic protection for kidney against diabetic associated renal injury. These agents also suppressed renal PKC activity and TGF-beta1 production, which consequently decreased type IV collagen production. Furthermore, marked dose-dependent effect from SEC for these above functions was observed. The impact of SEC and/or SMC on mRNA expression of renal IL-10 and/or TGF-beta1 revealed that effects of these agents occurred at the level of transcription. Therefore, the supplement of SEC or SMC might be helpful for the prevention or treatment of diabetic kidney diseases.

5 References

[1] Wang, S. N., LaPage, J., Hirschberg, R., Role of glomerular ultrafiltration of growth factors in progressive interstitial fibrosis in diabetic nephropathy, *Kidney Int.* 2000, *57*, 1002–1014.

[2] Brownlee, M., Biochemistry and molecular cell biology of diabetic complications, *Nature* 2001, *414*, 813–820.

[3] Strippoli, G. F., Di Paolo, S., Cincione, R., Di Palma, A. M., *et al.*, Clinical and therapeutic aspects of diabetic nephropathy, *J. Nephrol.* 2003, *16*, 487–499.

[4] Matteucci, E., Malvaldi, G., Fagnani, F., Evangelista, I., Giampietro, O., Redox status and immune function in type I diabetes families, *Clin. Exp. Immunol.* 2004, *136*, 549–554.

[5] Fukushima, S., Takada, N., Hori, T., Wanibuchi, H., Cancer prevention by organosulfur compounds from garlic and onion, *J. Cellular Biochem.* 1997, *27*, 100–105.

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[6] Jones, M. G., Hughes, J., Tregova, A., Milne, J., *et al.*, Biosynthesis of the flavour precursors of onion and garlic, *J. Exp. Bot.* 2004, *55*, 1903 – 1918.

[7] Hsu, C. C., Yen, H. F., Yin, M. C., Tsai, C. M., Hsieh, C. H., Five cysteine-containing compounds delay diabetic deterioration in Balb/cA mice, *J. Nutr.* 2004, *134*, 3245 – 3249.

[8] Hsu, C. C., Lin, C. C., Liao, T. S., Yin, M. C., Protective effect of s-allyl cysteine and s-propyl cysteine on acetaminophen-induced hepatotoxicity in mice, *Food Chem. Toxicol.* 2006, *44*, 393 – 397.

[9] Iwano, M., Kubo, A., Nishino, T., Sato, H., *et al.*, Quantification of glomerular TGF-beta 1 mRNA in patients with diabetes mellitus, *Kidney Int.* 1996, *49*, 1120 – 1126.

[10] Sharma, K., Ziyadeh, F. N., Alzahabi, B., McGowan, T. A., *et al.*, Increased renal production of transforming growth factor-beta1 in patients with type II diabetes, *Diabetes* 1997, *46*, 854 – 859.

[11] Hayashida, T., Schnaper, H. W., High ambient glucose enhances sensitivity to TGF-beta1 via extracellular signal – regulated kinase and protein kinase Cdelta activities in human mesangial cells, *J. Am. Soc. Nephrol.* 2004, *15*, 2032 – 2041.

[12] Toyoda, M., Suzuki, D., Honma, M., Uehara, G., *et al.*, High expression of PKC-MAPK pathway mRNAs correlates with glomerular lesions in human diabetic nephropathy, *Kidney Int.* 2004, *66*, 1107 – 1114.

[13] Lowry, O. H., Rosebrough, N. J., Farr, A. L., Protein determination with the Folin phenol reagent, *J. Biol. Chem.* 1951, *193*, 265 – 275.

[14] Jain, S. K., Palmer, M., The effect of oxygen radical metabolites and vitamin E on glycosylation or proteins, *Free Rad. Biol. Med.* 1997, *22*, 593 – 596.

[15] Mizuno, S., Kurosawa, T., Matsumoto, K., Mizuno-Horikawa, Y., *et al.*, Hepatocyte growth factor prevents renal fibrosis and dysfunction in a mouse model of chronic renal disease, *J. Clin. Invest.* 1998, *101*, 1827 – 1834.

[16] Koya, D., Haneda, M., Nakagawa, H., Isshiki, K., *et al.*, Amelioration of accelerated diabetic mesangial expansion by treatment with a PKC beta inhibitor in diabetic db/db mice, a rodent model for type 2 diabetes, *FASEB J.* 2000, *14*, 439 – 447.

[17] Deng, J., Kohda, Y., Chiao, H., Wang, Y., *et al.*, Interleukin-10 inhibits ischemic and cisplatin-induced acute renal injury, *Kidney Int.* 2001, *60*, 2118 – 2128.

[18] Mohamed-Ali, V., Armstrong, L., Vlark, D., Bolton, C. H., Pinkney, J. H., Evidence for the regulation of levels of plasma adhesion molecules by inflammatory cytokines and their soluble receptors in type 1 diabetes, *J. Intern. Med.* 2001, *250*, 415 – 421.

[19] Tomita, M., Dragoman, M., Worcester, H., Conran, P., Santoro, T. J., Proinflammatory cytokine genes are constitutively overexpressed in the heart in experimental systemic lupus erythematosus: a brief communication, *Exp. Biol. Med.* 2004, *229*, 971 – 976.

[20] Berger, S., Ballo, H., Stutte, H. J., Immune complex-induced interleukin-6, interleukin-10 and prostaglandin secretion by human monocytes: a network of pro- and anti-inflammatory cytokines dependent on the antigen:antibody ratio, *Eur. J. Immunol.* 1996, *26*, 1297 – 1301.

[21] Schneider, C. P., Schwacha, M. G., Chaudry, I. H., The role of interleukin-10 in the regulation of the systemic inflammatory response following trauma-hemorrhage, *Biochim. Biophys. Acta* 2004, *1689*, 22 – 32.

[22] Cohen, M. P., Sharma, K., Guo, J., Eltayeb, B. O., Ziyadeh, F. N., The renal TGF-beta system in the db/db mouse model of diabetic nephropathy, *Exp. Nephrol.* 1998, *6*, 226 – 233.

[23] Ziyadeh, F. N., Hoffman, B. B., Han, D. C., Iglesias-De La Cruz, M. C. *et al.*, Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-beta antibody in db/db diabetic mice, *Proc. Natl. Acad. Sci. USA* 2000, *97*, 8015 – 8020.

[24] Schena, F. P., Gesualdo, L., Pathogenetic mechanisms of diabetic nephropathy, *J. Am. Soc. Nephrol.* 2005, *1*, S30 – S33.

[25] Benevenga, N. J., Yeh, M. H., Lalich, J. J., Growth depression and tissue reaction to the consumption of excess dietary methionine and S-methyl-L-cysteine, *J. Nutr.* 1976, *106*, 1714 – 1729.

[26] Krest, I., Glodek, J., Keusgen, M., Cysteine sulfoxides and alliinase activity of some Allium species, *J. Agric. Food Chem.* 2000, *48*, 3753 – 3760.

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