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Research Article

Antiglycative and anti-VEGF effects of *s*-ethyl cysteine and *s*-propyl cysteine in kidney of diabetic mice

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Antiglycative and antivascular endothelial growth factor (VEGF) effects of *s*-ethyl cysteine (SEC), and *s*-propyl cysteine (SPC) in kidney of diabetic mice were examined. SEC and SPC at 1 and 2 g/L were added to the drinking water for 12 wk. Results showed that diabetic mice with SEC or SPC intake had significantly higher final body weight, lower kidney weight, lower levels of plasma glucose, urinary albumin (UA), and urinary creatinine (UC) ($p < 0.05$), in which dose-dependent effects were observed in reducing plasma glucose, UA, and UC ($p < 0.05$). The intake of these compounds significantly and dose-dependently decreased the levels of plasma glycated hemoglobin (HbA1c), renal carboxymethyllysine and urinary glycated albumin ($p < 0.05$). SEC or SPC intake significantly and dose-dependently diminished renal aldose reductase (AR) activity and enhanced glyoxalase I (GLI) activity ($p < 0.05$); also significantly decreased renal sorbitol and fructose concentrations ($p < 0.05$). The intake of SEC or SPC significantly lowered renal VEGF level ($p < 0.05$), and caused dose-dependent downregulation in AR mRNA expression, and upregulation in GLI mRNA expression ($p < 0.05$). Our present study suggests the supplement of SEC or SPC might be helpful for the prevention or treatment of diabetic kidney diseases *via* alleviating renal glycative injury.

Keywords: Aldose reductase / Glycation / *s*-Ethyl cysteine / *s*-Propyl cysteine / VEGF

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1 Introduction

Diabetic renal injury, or so-called diabetic nephropathy, is one of diabetic complications, which exacerbate the severity and the mortality of diabetes. Nonenzymatic glycation with the formation of Maillard reaction products, also known as advanced glycation endproducts (AGEs) such as glycated hemoglobin (HbA1c), N^{ε}-carboxymethyllysine (CML), and glycated albumin has been implicated in the pathogenesis of diabetic nephropathy and other complications of diabetes [1 – 3]. Particularly, it is indicated that the accumulation of CML and glycated albumin contributed to the deterioration of diabetic nephropathy [3, 4]. Thus, any agent with antiglycative effect may potentially prevent or delay the progression of diabetic renal injury. In addition, AGEs could promote the expression of various growth factors including vascular endothelial growth factor (VEGF), which further induces microvascular permeability and causes hyperfiltration, albuminuria, and glomerular hypertrophy [5, 6]. Thus, inhibition of VEGF has been considered as a potential therapeutic approach to prevent and attenuate diabetic microvascular complications.

It is well known that hyperglycemia enhances glucose metabolism *via* the polyol pathway [1, 2]. Aldose reductase (AR), the first and rate-limiting enzyme in the polyol pathway, reduces glucose to sorbitol, which could be further metabolized to fructose by sorbitol dehydrogenase (SDH), the second enzyme in the polyol pathway [7, 8]. It is reported that the flux through SDH and elevated fructose level may increase AGEs formation, which contributes to diabetes-induced microvascular abnormalities [9]. Glyoxalase I (GLI), part of the glyoxalase system presented in the

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Abbreviations: AGE, advanced glycation endproduct; **AR,** aldose reductase; **CML,** carboxymethyllysine; GAPDH, glyceraldehyde-3-phosphate dehydrogenase**GLI,** glyoxalase I; **HbA1c,** glycated hemoglobin; **SDH,** sorbitol dehydrogenase; **SEC,** *s*-ethyl cysteine; **SPC,** *s*-propyl cysteine; **VEGF,** vascular endothelial growth factor

cytosol of cells, could metabolize physiological reactive α-carbonyl compounds such as glyoxal and methylglyoxal, and consequently decreased the available precursors for AGEs formation [10]. Because AR, SDH, and GLI are involved in diabetic-associated glycation reactions, any agent with the ability to affect the activity and/or mRNA expression of these enzymes might potentially mediate these glycation reactions.

s-Ethyl cysteine (SEC) and *s*-propyl cysteine (SPC) are hydrophilic cysteine-containing compounds naturally formed in *Allium* plants such as garlic and onion [11, 12]. Our past diabetic animal study observed that the supplementation of SEC and SPC effectively decreased blood glucose, increased insulin, improved haemostatic balance, and attenuated oxidative and inflammatory stress in diabetic mice [13]. Furthermore, our another animal study found that SEC intake dose-dependently decreased urinary albumin (UA) and renal type IV collagen, diminished protein kinase activity and downregulated mRNA expression of renal transforming growth factor (TGF)-beta1 [14]. Thus, our previous studies already supported that SEC and SPC might be helpful for the prevention or treatment of diabetic kidney diseases. However, it remains unknown that SEC or SPC could inhibit or attenuate the production of AGEs and VEGF in kidney. Also, the information regarding the impact of these compounds upon activity and mRNA expression of enzymes involved in polyol pathway is also lacked.

The major purpose of this study was to investigate the antiglycative and anti-VEGF effects of SEC and SPC in kidney of diabetic mice. The influence of these compounds upon renal activity and mRNA expression of AR, SDH, and GLI was also evaluated.

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; and five mice were housed in one cage. The use of mice was reviewed and approved by Chung Shan Medical University animal care committee. To induce diabetes, mice with body weight (BW) at 22.3 ± 0.6 g were treated with streptozotocin (STZ, 40 mg/kg BW in 0.1 mol/L citrate buffer, pH 4.5) ip for five consecutive days. The blood glucose level was monitored on d 2, 5, and 10 from the tail vein by using a one-touch blood glucose meter (Lifescan). Mice with fasting blood glucose level ≥250 mg/dL were used for this study. After diabetes was induced, mice were divided into several groups. SEC (99%) and ascorbic acid (99.5%) were purchased from Sigma Chemical (St. Louis, MO, USA). SPC (99%) was supplied by Wakunaga Pharmaceutical (Hiroshima, Japan). Ascorbic acid was used for comparison. SEC, SPC, or ascorbic acid at 0, 1, and 2 g/L was added to the drinking water.

2.2 Experimental design

All mice had free access to food and water at all times. Consumed water volume and feed were recorded. BW and plasma glucose level were measured weekly. Urine was collected from mice housed in metabolic cages for 24 h. After 12 wk supplementation, mice were killed with carbon dioxide. Blood and kidney were collected. Plasma was separated from erythrocytes immediately. Kidney weight (KW) was measured. The protein concentration of plasma or kidney sample was determined by the method of Lowry *et al*. [15] using BSA as a standard.

2.3 Biochemical analyses

Plasma glucose level was measured by a glucose HK kit (Sigma Chemical, St. Louis, MO, USA). Plasma HbA1c level was measured by using a DCA2000 analyzer (Bayer-Sankyo, Tokyo, Japan). Urine albumin was measured by a competitive ELISA assay according to the manufacturer's instruction (Exocell, Philadelphia, PA, USA). Glycated albumin was determined by affinity chromatography on phenylboronate agarose to separate nonglycated (unbound) from glycated (bound) albumin *via* eluting the bound fraction with 0.3 mol/L sorbitol. The concentration of creatinine was measured by using a creatinine-test kit (Wako Pure Chemical Industries, Osaka, Japan). Creatinine clearance (CCr) was calculated and expressed as mL/min/100 g BW.

2.4 Determination of renal carboxymethyllysine (CML) and pentosidine

The method described in Inagi *et al*. [16] was used to detect CML. Kidney cortex at 100 mg was minced, followed by adding excess $NaBH_4$ in 0.2 mol/L borate buffer (pH 9.1) for reduction. Proteins were precipitated by adding 20% trichloroacetic acid and centrifugation at 2000 × *g* for 10 min. The pellet was washed with 1 mL 10% trichloroacetic acid. After drying, the pellet was acid hydrolyzed in 500 μL 6 N HCl for 16 h at 110°C in screw-cap tubes purged with nitrogen. Hydrolysates were dried, and rehydrated in water, and used for measuring CML by RP HPLC. Pentosidine was analyzed by a HPLC method [17] in which a C18 RP column was equipped.

2.5 Activity of aldose reductase (AR), sorbitol dehydrogenase (SDH), and glyoxalase I (GLI)

The method of Nishinaka and Yabe-Nishimura [18] was used to measure AR activity. Renal cortices were separated from the medulla, and glomeruli were isolated by differen-

tial sieving with stainless steel meshes under sterile condition. Both glomeruli and medulla were washed with ice-cold PBS (pH 7.4). After sonication, sample was centrifuged and the supernatant was used for analysis. AR activity was measured in both glomeruli and medulla by monitoring the decrease in absorbance at 340 nm due to NADPH oxidation. SDH activity was assayed according to the method of Ulrich [19] by mixing 100 μL kidney homogenate, 200 μL NADH (12 mM), and 1.6 mL triethanolamine buffer (0.2 M, pH 7.4), and monitoring the absorbance change at 365 nm. The method of McLellan and Thornalley [20] was used to assay GLI activity. Renal tissue, 50 mg, was homogenized in 1 mL NaPB containing 0.02% Triton X-100. After centrifugation at 20 000 × *g* for 20 min at 4°C, supernatant was collected. GLI activity was assayed by monitoring the increase in absorbance at 240 nm due to the formation of *S*-(D)-lactoylglutathione.

2.6 Measurement of renal vascular endothelial growth factor (VEGF) level

Renal cortex was prepared for VEGF assay by homogenization in 1 mL of lysis buffer, containing 50 mmol/L Tris HCl, pH 7.5, 150 mmol/L NaCl, 0.05% NP-40, proteinase inhibitor cocktail, and 0.5 mmol/L PMSF. After refrigeration for 15 min, centrifugation at 10 000 × *g* for 10 min, supernatant was used for assay. VEGF was measured by using a quantitative ELISA method (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instruction.

2.7 Determination of renal sorbitol and fructose content

Kidney (100 mg) was homogenized with PBS (pH 7.4) containing U-[^{13}C]-sorbitol as an internal standard. After precipitating protein by ethanol, the supernatant was lyophilised. The content of sorbitol and fructose in each lyophilized sample was determined by LC with MS/MS, according to the method of Guerrant and Moss [21].

2.8 Semiquantitative reverse transcription-PCR (RT-PCR) for mRNA expression

Semiquantitative RT-PCR was used to examine the mRNA expression of AR, GLI and VEGF in order to demonstrate the effect of SEC and SPC on the level of transcription. Total RNA was isolated from kidney tissue using the SV Total RNA Isolation kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. Total RNA (2 μg) was used to generate cDNA. PCR was carried out in 50 mL of reaction mixture containing Taq DNA polymerase buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl, 200 mM dNTP, 2.5 mM $MgCl_2$, 0.5 mM of each primer) and 2.5 U Taq DNA polymerase. The primers for PCR were synthesized based on previously published primer sequences [22 – 24]. AR: forward, 5′-CCC AGG TGT ACC AGA ATG AGA-3′, reverse, 5′-TGG CTG CAA TTG CTT TGA TCC-3′; GLI: forward, 5′-CGT GAG ACA GCA AGC AGC TAG A-3′, reverse, 5′-ACC ATG AGG CAT AGG CAT ACC C-3′; VEGF, forward, 5′-AGG CGA GGC AGC TTG AGT TA-3′, reverse, 3′-CAG CCT GGC TCA CCG C-5′; glyceraldehyde-3-phosphate dehydrogenase (GAPDH): forward, 5′-TGA TGA CAT CAA GAA GGT GGT GAA G-3′, reverse, 5′-CCT TGG AGG CCA TGT AGG CCA T-3′. The target concentration was expressed relative to the concentration of a reference housekeeping gene, GAPDH. The cDNA was amplified under the following reaction conditions: 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min. Twenty eight cycles were performed for GAPDH, and 35 cycles for AR, GLI, and VEGF. The corresponding PCR products were analyzed by 1% w/v agarose gel electrophoresis and revealed with ethidium bromide. Finally, quantitative analysis was performed with a BAS 2000 BIO-image analyzer (Fuji Photo Film, Tokyo, Japan). In this present study, mRNA level was calculated as percentage value of the control group (without SEC or SPC treatment).

2.9 Statistical analysis

The effect of each measurement was analyzed from 15 samples ($n = 15$) in each group. All data were expressed as mean ± SD. Statistical analysis was done using analysis of variance (ANOVA), and posthoc 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, 6, and 11 are presented in Table 1. There was no significant difference among groups ($p > 0.05$). Ascorbic acid, a known hydrophilic antioxidant, was used in this study for comparison. This agent significantly reduced plasma glucose and HbAlc only ($p < 0.05$), but failed to affect other measurements ($p > 0.05$). The effects of SEC and SPC on BW, KW, plasma glucose, UA, and urinary creatinine (UC) levels are presented in Table 2. Diabetic mice with SEC or SPC intake had significantly higher final BW, lower KW, lower levels of plasma glucose, UA, UC, and CCr ($p < 0.05$), in which dose-dependent effects were observed in reducing plasma glucose, UA, UC, and CCr ($p < 0.05$). The effects of SEC and SPC on the level of AGEs in plasma, kidney, and urine are shown in Table 3. The intake of these compounds significantly and dose-dependently decreased the levels of plasma HbA1c, renal CML, and urinary glycated albumin ($p < 0.05$). SEC or SPC intake also significantly reduced renal pentosidine ($p < 0.05$), but there was no dose-dependent effect. Renal activity of AR, SDH, and GLI is shown in

Table 1. Feed intake (FI, g/mouse/d) and water intake (mL/mouse/d) of mice consumed 0, 1, and 2 g/L SEC, SPC, or ascorbic acid at 1, 6, and 11 wk

FI g/mouse/d	WI mL/mouse/d			Time, wk		
	1	6	11	1	6	11
SEC						
0	5.2 ± 0.4	4.8 ± 0.3	4.3 ± 0.6	7.3 ± 0.9	6.8 ± 0.7	7.0 ± 0.6
1	5.0 ± 0.5	4.7 ± 0.5	4.6 ± 0.7	6.8 ± 0.8	6.6 ± 1.1	6.5 ± 1.0
2	5.3 ± 0.3	4.5 ± 0.6	4.1 ± 0.3	7.1 ± 0.7	6.7 ± 0.8	6.6 ± 0.7
SPC						
0	5.4 ± 0.4	5.0 ± 0.6	4.7 ± 0.5	6.9 ± 1.1	7.1 ± 0.5	6.6 ± 1.2
1	5.6 ± 0.5	4.4 ± 0.3	4.5 ± 0.6	6.5 ± 0.6	6.8 ± 1.0	7.1 ± 0.7
2	5.3 ± 0.6	4.9 ± 0.5	4.3 ± 0.4	6.8 ± 0.5	6.7 ± 0.9	6.8 ± 0.6
Ascorbic acid						
0	5.1 ± 0.3	5.3 ± 0.5	4.9 ± 0.2	7.0 ± 0.8	7.0 ± 0.7	6.7 ± 0.8
1	5.0 ± 0.5	5.2 ± 0.4	4.4 ± 0.5	6.4 ± 0.7	6.6 ± 0.8	6.6 ± 1.0
2	5.5 ± 0.4	5.1 ± 0.6	4.6 ± 0.4	6.7 ± 1.0	7.2 ± 1.1	6.5 ± 0.9

Data are mean ± SD, $n = 15$; $p < 0.05$.

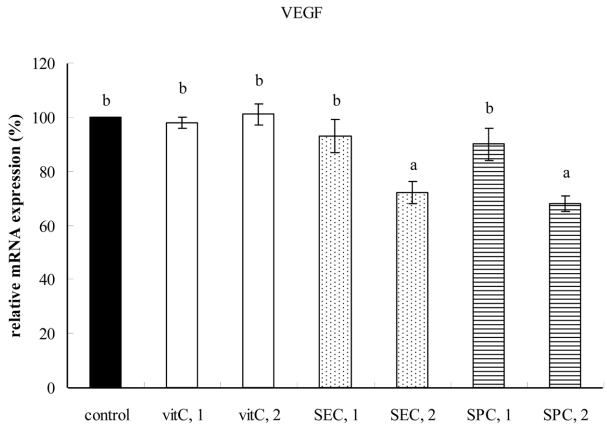

Figure 1. Renal VEGF protein level in mice consumed 0, 1, and 2 g/L SEC, SPC, or ascorbic acid (vitC) for 12 wk. Data are mean ± SD ($n = 15$). (a–d) Means among bars without a common letter differ, $p < 0.05$.

Table 4. SEC or SPC intake significantly diminished AR activity in glomeruli and medulla, and enhanced GLI activity ($p < 0.05$); however, these two compounds did not affect renal SDH activity ($p < 0.05$). SEC or SPC intake also significantly and dose-dependently decreased renal sorbitol and fructose concentrations (Table 5, $p < 0.05$). Renal VEGF protein level is presented in Fig. 1. The intake of SEC and SPC significantly and dose-dependently lowered renal VEGF level ($p < 0.05$). The effects of SEC and SPC on mRNA expression of renal AR, GLI, and VEGF are shown in Fig. 2. SEC and SPC treatments caused dose-dependently downregulation in AR mRNA expression and upregulation in GLI mRNA expression ($p < 0.05$). SEC or SPC treatment downregulated VEGF mRNA expression only at high dose ($p < 0.05$).

4 Discussion

The anti-oxidative protection of ascorbic acid against diabetic nephropathy has been reported [25]. However, the study of Melhem *et al*. [26] and our present study revealed that ascorbic acid was not an effective agent against diabetic renal glycative injury. Our previous study has indicated that the dietary intake of SEC and SPC in diabetic mice alleviated BW loss and urine output, decreased plasma BUN and CCr, suppressed renal protein kinase C

Table 2. BW (g/mouse), KW (g/mouse), plasma glucose (mg/dL), UA (mg/L), UC (mg/dL), and CCr (mL/min/100 g BW) levels in mice consumed 0, 1, and 2 g/L SEC, SPC, or ascorbic acid for 12 wk

Time, wk	BW 1	BW 12	KW 12	Plasma glucose 12	UA 12	UC 12	CCr 12
SEC							
0	21.6 ± 1.0$^{a)}$	11.4 ± 0.9$^{a)}$	293 ± 34$^{b)}$	503 ± 30$^{d)}$	108.7 ± 8.9$^{c)}$	25.7 ± 2.6$^{c)}$	1.41 ± 0.19$^{c)}$
1	21.2 ± 0.8$^{a)}$	15.7 ± 0.8$^{b)}$	233 ± 20$^{a)}$	391 ± 18$^{b)}$	82.5 ± 4.6$^{b)}$	19.6 ± 1.7$^{b)}$	0.92 ± 0.10$^{b)}$
2	22.3 ± 1.1$^{a)}$	16.0 ± 0.6$^{b)}$	219 ± 25$^{a)}$	290 ± 21$^{a)}$	67.0 ± 3.1$^{a)}$	15.3 ± 1.2$^{a)}$	0.56 ± 0.08$^{a)}$
SPC							
0	21.5 ± 0.8$^{a)}$	12.0 ± 1.0$^{a)}$	288 ± 28$^{b)}$	494 ± 25$^{d)}$	113.4 ± 10.2$^{c)}$	27.0 ± 2.4$^{c)}$	1.39 ± 0.22$^{c)}$
1	22.4 ± 1.2$^{a)}$	15.7 ± 0.7$^{b)}$	240 ± 16$^{a)}$	402 ± 22$^{b)}$	83.6 ± 6.2$^{b)}$	21.2 ± 1.0$^{b)}$	0.85 ± 0.16$^{b)}$
2	22.7 ± 1.3$^{a)}$	15.2 ± 0.8$^{b)}$	227 ± 21$^{a)}$	309 ± 14$^{a)}$	61.9 ± 5.0$^{a)}$	16.8 ± 1.3$^{a)}$	0.41 ± 0.10$^{a)}$
Ascorbic acid							
0	22.3 ± 1.0$^{a)}$	11.5 ± 0.9$^{a)}$	290 ± 25$^{b)}$	500 ± 34$^{d)}$	117.4 ± 11.0$^{c)}$	26.7 ± 2.1$^{c)}$	1.35 ± 0.24$^{c)}$
1	21.9 ± 0.9$^{a)}$	12.3 ± 0.5$^{a)}$	281 ± 19$^{b)}$	465 ± 20$^{c)}$	103.4 ± 9.2$^{c)}$	28.3 ± 1.7$^{c)}$	1.20 ± 0.18$^{c)}$
2	22.0 ± 0.7$^{a)}$	12.5 ± 0.6$^{a)}$	278 ± 23$^{b)}$	460 ± 24$^{c)}$	105.8 ± 8.8$^{c)}$	26.1 ± 2.3$^{c)}$	1.24 ± 0.20$^{c)}$

Data are mean ± SD, $n = 15$.
a–c) Means in a column without a common letter differ, $p < 0.05$.

Table 3. Level of plasma HbA1c, renal CML, pentosidine and urinary glycated albumin in mice consumed 0, 1, and 2 g/L SEC, SPC, or ascorbic acid for 12 wk

	Plasma HbA1c (%)	Renal CML pmol/mg	Pentosidine pmol/mg	Urine Glycated albumin μg/mL
SEC				
0	9.2 ± 0.8[c)]	70.4 ± 3.2[c)]	1.80 ± 0.21[b)]	1047 ± 129[c)]
1	6.8 ± 0.5[b)]	55.7 ± 1.9[b)]	1.40 ± 0.13[a)]	897 ± 71[b)]
2	5.3 ± 0.7[a)]	43.1 ± 2.4[a)]	1.28 ± 0.10[a)]	676 ± 32[a)]
SPC				
0	9.7 ± 0.6[c)]	68.7 ± 4.0[c)]	1.72 ± 0.24[b)]	1105 ± 108[c)]
1	7.1 ± 0.7[b)]	51.8 ± 3.5[b)]	1.34 ± 0.08[a)]	913 ± 56[b)]
2	5.2 ± 0.4[a)]	40.3 ± 2.6[a)]	1.22 ± 0.11[a)]	690 ± 41[a)]
Ascorbic acid				
0	10.1 ± 1.0[c)]	71.3 ± 4.8[c)]	1.85 ± 0.23[b)]	1089 ± 114[c)]
1	7.8 ± 0.4[b)]	68.4 ± 4.1[c)]	1.76 ± 0.15[b)]	1096 ± 93[c)]
2	8.0 ± 0.6[b)]	70.7 ± 5.0[c)]	1.81 ± 0.19[b)]	1107 ± 106[c)]

Data are mean ± SD, $n = 15$.
a – c) Means in a column without a common letter differ, $p < 0.05$.

Table 4. Activity of AR, SDH, and GLI in kidney from mice consumed 0, 1, 2 g/L SEC, SPC, or ascorbic acid for 12 wk

	AR (glomeruli) nmol/min/mg protein	AR (medulla) nmol/min/mg protein	SDH U/g protein	GLI nmol/min/mg protein
SEC				
0	2.41 ± 0.23[c)]	2.75 ± 0.31[c)]	8.4 ± 0.67[a)]	172 ± 13[a)]
1	1.60 ± 0.15[b)]	1.88 ± 0.22[b)]	7.9 ± 0.41[a)]	218 ± 9[b)]
2	1.26 ± 0.08[a)]	1.31 ± 0.14[a)]	7.5 ± 0.33[a)]	247 ± 15[c)]
SPC				
0	2.31 ± 0.27[c)]	2.90 ± 0.28[c)]	8.2 ± 0.52[a)]	180 ± 12[a)]
1	1.75 ± 0.10[b)]	2.01 ± 0.20[b)]	7.6 ± 0.48[a)]	221 ± 16[b)]
2	1.58 ± 0.11[b)]	1.78 ± 0.11[b)]	8.0 ± 0.39[a)]	258 ± 11[c)]
Ascorbic acid				
0	2.20 ± 0.25[c)]	2.83 ± 0.24[c)]	8.7 ± 0.60[a)]	177 ± 15[a)]
1	2.26 ± 0.14[c)]	2.70 ± 0.26[c)]	8.6 ± 0.71[a)]	190 ± 19[a)]
2	2.31 ± 0.22[c)]	2.68 ± 0.19[c)]	9.0 ± 0.59[a)]	184 ± 10[a)]

Data are mean ± SD, $n = 15$.
a – c) Means in a column without a common letter differ, $p < 0.05$.

activity, and renal TGF-beta1 mRNA expression [13, 14]. Therefore, we concluded that SEC and SPC, cysteine-containing compounds naturally derived from *Allium* plants, were potent agents for attenuating diabetic renal injury. Our present study further found that SEC and SPC effectively improved glycemic control, decreased the formation of glycative biomarkers in blood and kidney, diminished renal AR activity, enhanced GLI activity, and reduced renal VEGF production, which consequently improved renal functions and BW loss in diabetic mice. These findings once again support that SEC and SPC could protect kidney against the development or deterioration of diabetic nephropathy *via* their antiglycative and anti-VEGF effects.

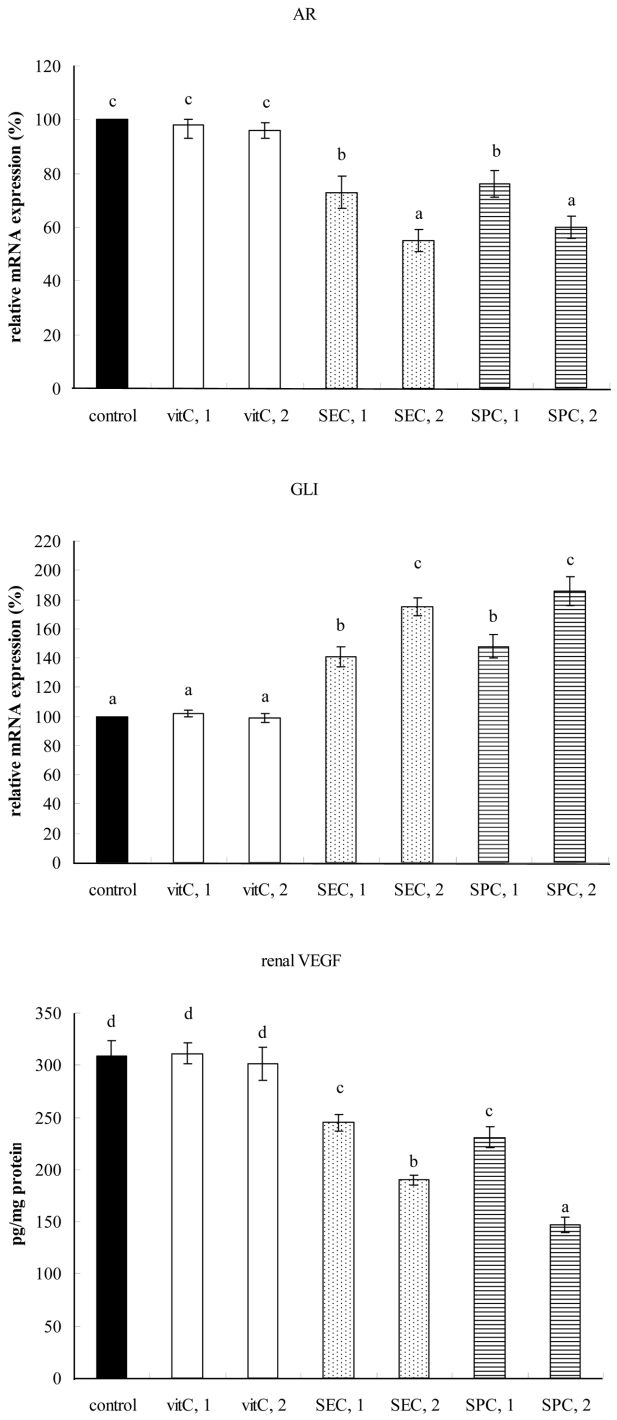

Figure 2. mRNA expression of renal AR, GLI, and VEGF in mice consumed 0, 1, and 2 g/L SEC, SPC, or ascorbic acid (vitC) for 12 wk. Data are mean ± SD ($n = 15$). (a – c) Means among bars without a common letter differ, $p < 0.05$.

Plasma HbAlc, renal CML and pentosidine, and urinary glycated albumin are biomarkers for evaluating glycative

injury in diabetic progression. It has been documented that the elevation or accumulation of these AGEs in plasma or organs means diabetic deterioration, and favors the development of diabetic complications [1, 3, 4]. Our two previous studies observed that SEC and SPC could nonenzymatically inhibit low density lipoprotein (LDL) glycation, and suggested that these compounds might possess greater affinity to water soluble molecules such as glucose; that is, they might compete with the protein part of the LDL particle for glucose, which would allow them to interfere glycative reactions occurred between glucose and the LDL protein particles [27, 28]. Our present animal study further found that SEC and SPC effectively decreased plasma HbAlc, renal CML, pentosidine, and urinary glycated albumin levels, which not only diminished renal glycative injury but also alleviated systemic glycative stress. This finding suggested that these compounds were effective agents to retard glycation reactions and reduce AGEs formation.

AR and SDH are two enzymes involved in polyol pathway, in which AR is the first and rate-limiting enzyme in this pathway. It is reported that the increased activity and expression of these enzymes facilitate the formation of sorbitol and fructose. Then, fructose and its metabolites promote nonenzymatic glycation of cellular proteins and lipids in kidney and other organs, which exacerbate glycative injury [29, 30]. Thus, these enzymes have been considered as therapeutic target for improving diabetic nephropathy. Our present study found that SEC and SPC effectively reduced AR activity in cortex and medulla fractions, and dose-dependently downregulated the mRNA expression of this enzyme in kidney, which consequently led to lower production of sorbitol and fructose. This finding implied that these compounds could decrease AGEs formation in kidney *via* suppressing polyol pathway. On the other hand, GLI could metabolize physiological reactive carbonyl compounds, the major precursors of AGEs in tissues [10]. Other studies have indicated that increased activity or expression of GLI could inhibit AGEs formation *via* decreasing their precursors [31, 32]. Our present study found that SEC or SPC intake enhanced the activity and mRNA expression of GLI, which suggested that these agents could decrease AGEs formation *via* metabolizing carbonyl intermediates occurred in the Maillard reactions. Obviously, besides nonenzymatically antiglycative action, SEC and SPC could diminish renal glycative stress *via* mediating AR and GLI.

SEC and SPC dose-dependently decreased renal VEGF level; however, the suppressive effect of these compounds on VEGF mRNA expression was observed only at high dose treatment (2 g/L). Obviously, the decline of renal VEGF protein level was not due to the direct effect of these compounds on mRNA expression of VEGF. It is known that renal VEGF could be regulated by oxidative stress, AGEs, cytokines, and TGF-beta1 [6, 33]. Our previous study already indicated that SEC could markedly improve renal oxidative stress and blunt renal TGF-beta1 expression [14]. Our present study further found that SEC and SPC could reduce the AGEs levels in plasma and kidney. Thus, the decreased renal VEGF protein level from SEC or SPC treatment should be partially ascribed to the antiglycative and anti-inflammatory actions of these agents. Renal VEGF level is particularly relevant to the pathogenesis of diabetes associated albuminuria [5]. Since renal VEGF level has been decreased by SEC or SPC treatment, the observed alleviated albuminuria in our present study could be explained. Although both SEC and SPC are amino acid derivates, and naturally formed in *Allium* foods such as garlic, the kidney protection from these two agents was somehow different. SEC was more effective in reducing AR activity; but SPC was greater in decreasing renal VEGF level. Apparently, the ethyl group of SEC and propyl group of SPC played important roles in determining their bioactivities such as affinity or reaction rate. Further study is necessary to examine the relationship between structure and function for these compounds.

In summary, SEC and SPC provided antiglycative and anti-VEGF protection for kidney against diabetic renal injury. These agents markedly suppressed renal AR activity and enhanced GLI activity, which consequently contributed to decreasing AGEs formation. The impact of SEC and SPC on mRNA expression of renal AR and GLI revealed that the effects of these agents occurred at the level of transcription. Therefore, the supplement of SEC or SPC might be helpful for the prevention or treatment of diabetic kidney diseases.

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