

## Effect of Ginsenosides on Glucose Uptake in Human Caco-2 Cells Is Mediated through Altered Na<sup>+</sup>/Glucose Cotransporter 1 Expression

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In this study, we measured the effect of ginsenosides on glucose uptake using the Caco-2 cell system. At submicromolar concentrations, these compounds exhibited marked effects on the rate of glucose transport across the differentiated Caco-2 cell monolayer. Compound K (CK), the main intestinal bacterial metabolite of the protopanaxadiol ginsenosides, significantly enhanced the steady-state glucose transport rate to about 50% of the control sample rate (from  $1.54 \pm 0.09$  to  $2.25 \pm 0.15$  nmol/min). Conversely, the protopanaxatriol ginsenoside Rg1 inhibited glucose transport to about 70% of the original rate (from  $1.54 \pm 0.09$  to  $1.02 \pm 0.05$  nmol/min). Consistent with the effect on glucose uptake rate, CK and Rg1 conferred a significant and paralleled alteration on both the protein and mRNA expression levels of the Na<sup>+</sup>/glucose cotransporter 1 (SGLT1) gene. Unlike SGLT1, there is no significant alteration on the protein or mRNA levels of GLUTs in CK- or Rg1-treated cells. Taken together, our results demonstrate that ginsenosides CK and Rg1 elicited potent enhancing and suppressing effects, respectively, on glucose uptake across human intestinal Caco-2 monolayer through modulation of SGLT1 expression.

**KEYWORDS:** Ginsenosides; glucose; Na<sup>+</sup>/glucose cotransporter; Caco-2 cells

### INTRODUCTION

Ginseng is the most widely used herbal medicine with a long history in Asian countries. Ginseng exerts a wide range of beneficial effects such as antiaging, improving cognitive performance, and enhancing metabolic functions (1–3). Ginseng is often taken for years without evidence of adverse effects or toxicity (1–3). The main active components in ginseng are ginsenosides, a range of triterpenoid saponins with a backbone of steroidal structures. Classified into protopanaxadiol and protopanaxatriol saponins according to their structures, approximately 30 different ginsenosides have been isolated and identified to date (4). With similarity to the structure of steroid hormones, the ginsenosides are generally believed to contribute to ginseng's pharmacological actions (1, 2).

One prominent physiological property of ginseng is its hypoglycemic activity (5–9). Historical records reveal that ginseng has been used clinically to treat diabetes (10). Ginseng was shown to decrease postprandial glycemia in both nondiabetic and type 2 diabetic subjects (5). The aqueous extract of ginseng was shown to be capable of producing hypoglycemia

both in glucose-loaded healthy animals and in animals with experimentally induced diabetes (6, 11, 12). The proposed mechanisms for the hypoglycemic effect of ginseng may involve enhanced insulin secretion (13), increased insulin sensitivity (6), and reduced glucose absorption (14) or a combination of these modes. Despite extensive literature documenting the glucoregulatory activity of ginseng, the mechanisms responsible for ginseng's hypoglycemic effect still need to be elucidated.

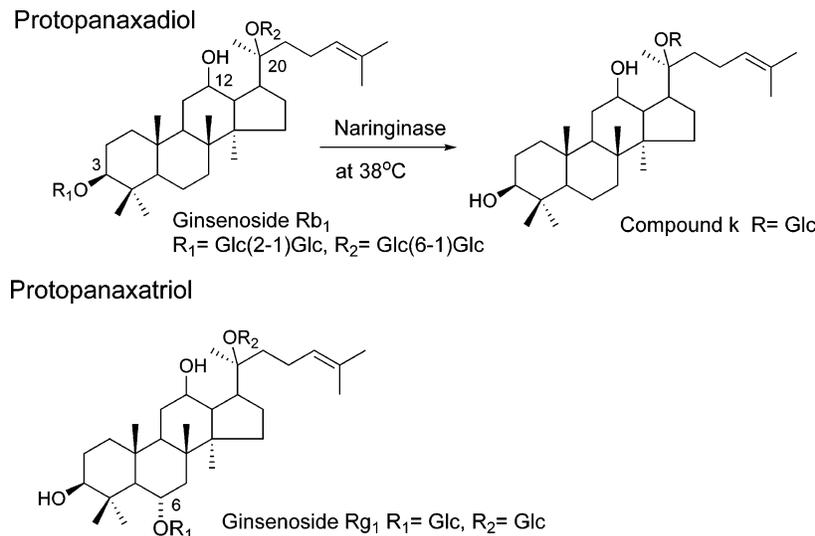
In this study, ginsenosides and the human intestinal Caco-2 cell system were used in an attempt to clarify the effect of ginseng on glucose uptake in intestinal cells. The Caco-2 cells originated from human colonic adenocarcinoma but will spontaneously differentiate into an enterocyte-like phenotype after 2 weeks. These cells form monolayers with well-developed tight junctions and have been evaluated in detail as an in vitro model system for the study of both transcellular nutrient and drug transport in the intestinal lumen (15, 16). Caco-2 cells were shown to express proteins involved in absorption of sugar nutrients, including the Na<sup>+</sup>/glucose cotransporter (SGLT1) and facilitative transporters GLUT1, GLUT2, GLUT3, and GLUT5 (17, 18). This cell line constitutes a unique human in vitro model for the study of sugar transport activity and regulation of the expression of sugar transporters (19, 20).

Here, we demonstrated that ginsenosides compound K (CK) and Rg1 display significant but opposite effects on the rate of glucose transport across the Caco-2 cell monolayer. The effect

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**Figure 1.** Structure of ginsenosides Rb<sub>1</sub>, CK, and Rg<sub>1</sub>. Ginsenoside Rb<sub>1</sub> was converted to CK by naringinase digestion as described under Materials and Methods.

on glucose transport coincides very well with both the SGLT1 protein and mRNA levels in this cell system. Our results suggest that ginsenosides, at least in CK and Rg<sub>1</sub>, display significant effects on SGLT1 gene expression, which in turn modulates glucose uptake activity in human intestinal cells.

## MATERIALS AND METHODS

**Extraction and Isolation of Ginsenosides.** Dried roots of *Panax notoginseng* (5.05 kg) were extracted repeatedly with EtOH (10 L × 5) at room temperature. The combined EtOH extract was evaporated in vacuo to yield dark-brown syrup (778.19 g). The syrup was partitioned between *n*-hexane and 80% MeOH to give an *n*-hexane layer (20.01 g), a MeOH layer (741.41 g), and an insoluble layer (16.77 g). The MeOH layer was chromatographed on an Amberlite XAD-4 column by elution with H<sub>2</sub>O, 50% MeOH/H<sub>2</sub>O, and 100% MeOH, gradually, to give three fractions: H<sub>2</sub>O fraction (202.02 g; fraction 1), 50% MeOH/H<sub>2</sub>O fraction (68.35 g; fraction 2), and 100% MeOH fraction (471.04 g; fraction 3). Fraction 2 was chromatographed on Lobar (RP-18, 75% MeOH/H<sub>2</sub>O) to give ginsenoside Rb<sub>1</sub> (5.53 g) (21). Fraction 3 was subjected to SiO<sub>2</sub> column chromatography and eluted with CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O (65:35:10) and MPLC (C-8, 75% MeOH/H<sub>2</sub>O) to give ginsenoside Rg<sub>1</sub> (29.54 g, **Figure 1**) and ginsenoside Rb<sub>1</sub> (25.14 g) (21, 22).

**Enzymatic Hydrolysis of Ginsenoside Rb<sub>1</sub> with Naringinase.** Ginsenoside Rb<sub>1</sub> (1.00 g) was dissolved in 100 mL of phosphate–citrate buffer (pH 4.0) and incubated with 1.0 g of naringinase (300 units/g; Sigma, St. Louis, MO). After incubation at 38 °C for 3 days, the products were extracted with EtOAc. The EtOAc extract was chromatographed on Lobar (RP-18, 90% MeOH/H<sub>2</sub>O) to yield compound K (0.28 g, **Figure 1**) (23). The compound was identified by fast atom bombardment mass spectrometry (FAB-MS) and <sup>1</sup>H and <sup>13</sup>C NMR spectra, and the purity was assessed by HPLC to be about 99%.

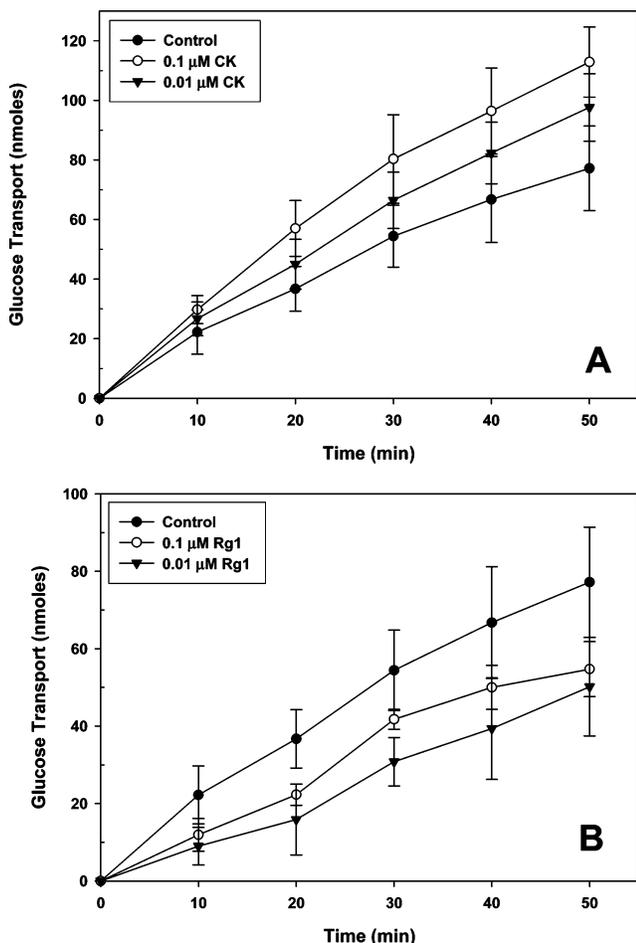
**Cell Culture.** The established human intestinal epithelial Caco-2 cell line was obtained from American Type Culture Collection (Rockville, MD). Cells were routinely grown in Dulbecco's modified Eagle medium (DMEM) containing 4.5 g/L glucose, 0.584 g/L glutamine, 10% fetal bovine serum, 3.7% sodium bicarbonate, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 1% nonessential amino acids. The medium was changed every 2 days and the cells were inspected daily. For transport experiments, the cells were seeded into polycarbonate filter cell culture chamber inserts (Transwell, 24-mm diameter, 3 μm; Costar, Corning Inc., Corning, NY) as described (24). The cells were left to differentiate for 15–17 days after confluency; the medium was regularly changed three times a week. The integrity of the Caco-2 cell monolayers and the full development of the tight junctions were monitored before every experiment by determining the

transepithelial electrical resistance (TEER) of filter-grown cell monolayers by use of a commercial apparatus (Millicell ERS; Millipore, Bedford, MA). Only cell monolayers with TEER values of 400–600 Ω·cm<sup>2</sup> were used for experiments.

**Glucose Uptake Assay.** In measuring the transport of glucose across the Caco-2 monolayer, both side of the transwells were washed with incubation buffer consisting of 80 mM NaCl, 100 mM mannitol, 20 mM Tris-HCl, pH 7.4, 3 mM K<sub>2</sub>HPO<sub>4</sub>, 1 mM CaCl<sub>2</sub>, and 1 mg/mL BSA as described (25). The cell monolayer was preincubated in the incubation buffer at 37 °C for 1 h and replaced with fresh medium right before transport experiment. Transport experiment was started by replacing the incubation solution on the apical side to solution containing 25 mM D-glucose in which 0.2 μCi/mL D-[<sup>14</sup>C]glucose (60 mCi/mmol, American Radiolabeled Chemicals, ARC, St. Louis, MO) was added. Samples were taken from the apical chamber in order to measure the initial donor concentration. At designated time intervals, 10 μL aliquots of solution were removed from the basolateral side and replaced with equal volumes of incubation buffer. The uptake of L-[<sup>14</sup>C]-glucose was measured simultaneously to correct for D-glucose taken up passively. All samples were analyzed by use of a liquid scintillation counter (TopCount, Packard BioSciences, Meriden, CT). Results are expressed as the steady-state rate of glucose transport (nanomoles per minute) across the Caco-2 monolayers in mean ± SD (*n* = 3–5). Differences between means of groups were assessed by the *t*-test.

**Western Blot Analysis.** Human Caco-2 cells were plated on culture dishes (6 cm diameter) at a density of 1 × 10<sup>6</sup> cells/dish and designated as day 0. The cells were left to undergo differentiation for 8 days prior to treatment with ginsenosides for another 2 days. The cells were then washed and lysed in 0.2 mL of lysis buffer [1% NP-40, 50 mM Tris-HCl, pH 7.4, 180 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethanesulfonyl fluoride (PMSF), 1 mM NaF, and 10 mM Na<sub>3</sub>VO<sub>4</sub>] for 30 min at 4 °C. Protein concentration of the samples were measured by the bicinchoninic acid (BCA) protein assay kit according to the manufacturer's protocol (Pierce, Rockford, IL). Equal amount of protein samples of cell lysate (20 μg) were separated by sodium dodecyl sulfate (SDS)–10% polyacrylamide gel electrophoresis (PAGE) and transblotted onto poly(vinylidene difluoride) (PVDF) membrane. Immunoblotting was performed with anti-human antibodies for GLUT1, GLUT2, GLUT3, SGLT1, and α-tubulin (Abcam, Cambridge, MA). Signals were visualized with an enhanced chemoluminescence kit (ECL, Amersham, U.K.) followed by exposure to X-ray films.

**Quantitative Analyses of Glucose Transporter Transcripts.** Relative levels of glucose transporters expressed in human Caco-2 cells were determined by real-time quantitative PCR (Q-PCR). Total RNAs were isolated from the cultured human cells by use of TRIzol reagent (Invitrogen, Irvine, CA). RNA (1 μg) was reverse-transcribed at 37 °C



**Figure 2.** Effect of ginsenosides on glucose transport in Caco-2 cells. The cell monolayer was pretreated with ginsenosides for 24 h before the assays were carried out. Glucose transport was measured as described under Materials and Methods. (A) CK enhanced glucose uptake; (B) Rg1 inhibited glucose uptake. Results represent mean  $\pm$  SD ( $n = 3-5$ ).

for 60 min in a 20  $\mu$ L transcription mixture containing dNTP (0.5 mM), oligo-dT (0.1  $\mu$ g), RNasin (10 units), 1 $\times$  PCR buffer (20 mM Tris-HCl, pH 8.3; 2.5 mM MgCl<sub>2</sub>; 50 mM KCl), and 100 units of Moloney murine leukemia virus reverse transcriptase (Invitrogen). Q-PCR was performed on the Applied Biosystems 7500 system with predeveloped Taqman gene expression assays (Applied Biosystems, Foster City, CA). The reaction mixtures (20- $\mu$ L total volume) contained 2  $\mu$ L of serially diluted cDNA, 10  $\mu$ L of Taqman universal PCR master mix (Applied Biosystems), and 1  $\mu$ L of either SGLT1 (assay ID Hs00165793\_ml), GLUT1 (assay ID Hs00197884\_ml), GLUT2 (assay ID Hs00165775\_ml), GLUT3 (assay ID Hs00359840\_ml), or GAPDH primer mix (assay ID Hs99999905\_m1, Applied Biosystems). Two independent triplicate experiments were performed for the selected genes, and the obtained threshold cycle (Ct) values were averaged. According to the comparative Ct method described in the ABI manual, gene expression was normalized to the expression of the housekeeping gene GAPDH, yielding the  $\Delta$ Ct value. The average  $\Delta$ Ct value obtained from untreated control samples was then subtracted from the average  $\Delta$ Ct value of each corresponding sample subjected to treatment, yielding the  $\Delta\Delta$ Ct value. The specific gene expression level, normalized to the GAPDH gene, and relative to the control sample, was calculated by  $2^{-\Delta\Delta C_t}$ . Data were normalized to untreated control samples arbitrarily set at 1. Relative quantitation was performed by use of software from the manufacturer (Applied Biosystems). All of the information about primer sequences and probes used is available from the web page of Applied Biosystems.

**Table 1.** Effect of Ginsenosides on Glucose Transport in Human Caco-2 Cells<sup>a</sup>

compd	concn ( $\mu$ M)	transport rate (nmol/min)	percentage	significance <sup>b</sup>
control		1.54 $\pm$ 0.09	100	
CK	0.1	2.25 $\pm$ 0.15	146.10	**
CK	0.01	1.93 $\pm$ 0.09	125.52	*
Rg1	0.1	1.16 $\pm$ 0.10	75.58	**
Rg1	0.01	1.02 $\pm$ 0.05	66.67	**

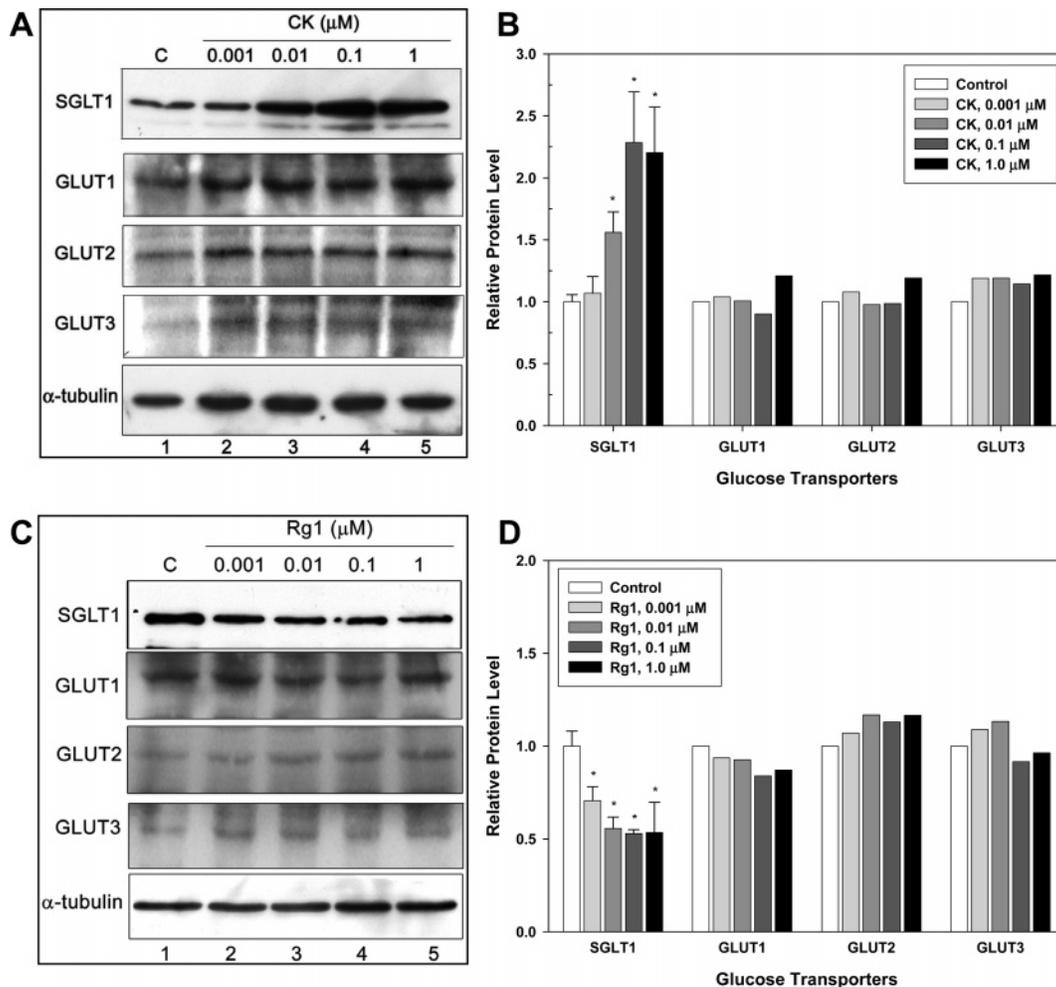
<sup>a</sup> Values are the average rate of glucose transport calculated from results shown in Figure 2. <sup>b</sup> \* $p < 0.05$ ; \*\* $p < 0.01$ .

## RESULTS

Two ginsenosides, CK and Rg1 (Figure 1), belonging to the protopanaxadiol and protopanaxatriol categories, respectively, were used in this study.

**CK and Rg1 Modulate Glucose Uptake Activity in Caco-2 Cells.** By use of confluent and differentiated Caco-2 cell monolayer, the effect of CK and Rg1 on glucose uptake in human Caco-2 cells was studied. The cells were pretreated with ginsenosides CK or Rg1 for 48 h. A saturated glucose concentration was used in this study to minimize the rapid metabolic effect of glucose in cells. To eliminate nonspecific absorption, the uptake of L-glucose was measured simultaneously to correct for D-glucose taken up passively. The Caco-2 cell monolayers with intact tight junctions exhibited a steady-state rate of glucose transport of 1.54  $\pm$  0.09 nmol/min. As shown in Figure 2 and Table 1, CK exerted a marked stimulatory effect on glucose transport across Caco-2 monolayers. The rate of glucose transport was significantly enhanced to 2.25  $\pm$  0.15 (46%) and 1.93  $\pm$  0.09 (26%) nmol/min in cells incubated in 0.1 and 0.01  $\mu$ M CK, respectively. Conversely, the protopanaxatriol-type ginsenoside Rg1 conferred a significant inhibitory effect on glucose uptake activity. The steady-state rate of glucose transport in the cell reduced to 76% and 67% of the original control rate, down from 1.54  $\pm$  0.09 to 1.16  $\pm$  0.10 and 1.02  $\pm$  0.05 nmol/min with 0.1 and 0.01  $\mu$ M Rg1, respectively.

**Ginsenosides Modulate Protein Expression Level of SGLT1.** The remarkable effect of ginsenosides on the rate of glucose uptake suggested that these compounds may exert their effect through modulating the expression of glucose transporters. Caco-2 cells were found to express many glucose transporters, including SGLT1, GLUT1, GLUT2, GLUT3, and GLUT5. For the substrate specificity of glucose, we analyzed the expression of SGLT1, GLUT-1, GLUT-2, and GLUT-3. The results shown in Figure 3 demonstrated that CK exerted a marked enhancing effect on the SGLT1 protein expression level. CK-mediated induction of SGLT1 is concentration-dependent. With increased CK concentrations, the SGLT1 protein level significantly increased up to 130% greater than control (Figure 3). CK also induced GLUT-1, -2, and -3 protein levels to a lesser and not significant extent of about 10–20% as compared to the control samples. In contrast to CK treatment, the SGLT1 protein level was significantly decreased in Rg1-treated cells. As the Rg1 concentration increased to 1  $\mu$ M, the SGLT1 expression was gradually reduced to 50% of the original level (Figure 3C,D). In addition to SGLT1, Rg1 also inhibited the expression level of GLUT1 to about 80% of the original level. The expression of GLUT-2 and -3 were mildly increased up to about 10–20% by Rg1, but not significantly. These results suggest that the observed effects on the rate glucose transport were mainly due to increased or decreased SGLT1 protein expression levels mediated by CK or Rg1, respectively.



**Figure 3.** Effect of CK and Rg1 on SGLT1 and GLUTs protein expression level. Cell extracts were prepared from differentiated Caco-2 cells treated with increasing concentration of CK or Rg1 for 24 h. Immunoblots were analyzed by use of antibodies against SGLT1 and GLUTs. Relative protein expression levels of SGLT1 and GLUTs were normalized with respect to the level of  $\alpha$ -tubulin and expressed as relative ( $x$ -fold) changes in comparison to the untreated control samples, which were arbitrarily set to 1.0. (A) Effect of CK on glucose transporters protein expression level. (B) Quantitative analysis of the results shown in panel A. (C) Effect of Rg1 on glucose transporters protein expression level. (D) Quantitative analysis of the results shown in panel C. Results for GLUTs are the average of two independent experiments. Results for SGLT1 expression level represent mean  $\pm$  SD ( $n = 3$ ) ( $*p < 0.05$ ).

#### Ginsenosides Modulate mRNA Expression Level of SGLT1.

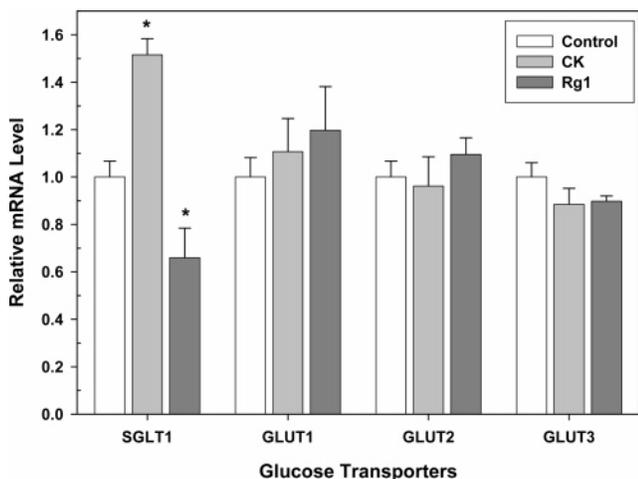
Q-PCR was used to analyze the effect of ginsenosides on mRNA levels of SGLT1 and GLUTs. The results shown in **Figure 4** indicated that, at 0.1  $\mu$ M, CK up-regulated and Rg1 down-regulated the SGLT1 mRNA to a significant level by 50% and 35% compared with the control, respectively. The expression of GLUT1, GLUT2, and GLUT3 mRNA was not significantly altered by these ginsenosides. Both CK and Rg1 slightly but not significantly induced the mRNA level of GLUT1 to around 10–20%. These results demonstrated that CK and Rg1 conferred parallel alterations on SGLT1 transcript and protein levels. Taken together, our results indicated that CK and Rg1 modulate SGLT1 gene expression at the level of transcription or mRNA stability in Caco-2 cells.

#### DISCUSSION

The use of ginseng is popular worldwide; however, scientific data supporting the claimed benefits are not always available. The hypoglycemic activity of ginseng is one of the most studied effects. Although numerous *in vitro* and animal studies have repeatedly showed the hypoglycemic effects of ginseng, the efficacy evidence of ginseng is still inconclusive (26, 27). It has been shown that ginseng of different species or even

different batches of the same species displayed quite variable gluoregulatory effects (26, 27). A likely explanation for the variable hypoglycemic effects of different ginseng preparations is the marked difference in the profile of ginsenosides (26, 28, 29). Studies using ginseng preparations in which the composition was not well-characterized would likely yield inconclusive results. In addition, ginsenosides may display very different, even opposite pharmacological effects (1, 2). Most of the previous studies on ginseng were carried out with extracts composed of ginsenosides mixtures. To avoid any ambiguous effect, purified ginsenosides were used in this study.

In this study, we analyzed the effect of ginsenosides on glucose transport in the differentiated Caco-2 cells. We found that the ginsenosides CK and Rg1 displayed marked but opposite effects on glucose uptake in the Caco-2 intestinal cell system. Our results showed that CK, a protopanaxadiol produced from ginsenoside Rb1 by intestinal bacteria, significantly enhanced glucose uptake. Conversely, the protopanaxatriol type ginsenoside Rg1 significantly inhibited glucose uptake. The Rg1-mediated inhibitory effect on rate of glucose transport was dose-independent (**Figure 3C,D**). At 0.01  $\mu$ M, Rg1 displayed a more potent suppressive effect on glucose transport than at 0.1  $\mu$ M Rg1. These results imply that the maximum effect on glucose



**Figure 4.** Effect of CK and Rg1 on SGLT1 and GLUTs mRNA levels: real-time Q-PCR analysis of the relative mRNA levels of SGLT1 and GLUTs in differentiated Caco-2 cells treated with 0.1  $\mu$ M CK or Rg1 for 24-h. Experiments were carried out and analyzed as described under Materials and Methods. Results represent mean  $\pm$  SD ( $n = 3$ ) ( $*p < 0.05$ ).

transport was reached at 0.01  $\mu$ M Rg1. It was shown that ginseng extract attenuates postprandial glycemia in a time-dependent but dose-independent manner (30).

Ginseng preparations have been shown to modulate glucose uptake in different tissues or cells. The standardized ginseng extract G115 was shown to enhance glucose uptake in rabbit brain tissue (31). Several ginsenosides showed stimulative effects on glucose transporter in sheep erythrocytes and (32). In contrast, ginseng extract has been shown to significantly inhibit glucose uptake in human and rat gastrointestinal tract (14). The differential effects may be due to the difference between ginseng preparations and different glucose transporters specifically expressed in different tissues or cell types. The finding that CK enhances glucose uptake suggests that certain ginsenosides may contribute to an unintended hyperglycemic effect. Interestingly, total protopanaxatriol ginsenosides were observed to inhibit glucose uptake in primary renal tubular cells, which express another isoform of  $\text{Na}^+$ /glucose cotransporter, SGLT2 (33).

In addition to changes in glucose uptake rate, we found that CK and Rg1 conferred significant and parallel alterations in SGLT1 mRNA and protein levels in Caco-2 cells (Figures 3 and 4). The SGLT1 is the primary carrier protein responsible for transport of glucose from the lumen of the intestine across the brush border membrane of intestinal epithelial cells (34). Since there was no significant change in the expression levels of GLUT1, GLUT2, or GLUT3, we conclude that the altered glucose uptake rate across the Caco-2 cell monolayer is primarily through modulation of SGLT1 gene expression level. The close correlation in the magnitude of the increases or decreases in SGLT1 mRNA and protein suggests that the exposure of the cells to CK or Rg1 leads to an increase or decrease in abundance of the SGLT1 transcript, and that in turn gives rise to a corresponding increase or decrease in the levels of SGLT1 protein. Thus, in human Caco-2 cells, the CK- or Rg1-mediated expression of SGLT1 is primarily controlled at the level of mRNA abundance.

In conclusion, we showed that ginsenosides CK and Rg1 displayed opposite effects on glucose transport across the Caco-2 cell monolayer, which correlates significantly with SGLT1 gene expression. These results suggest that ginsenosides CK and Rg1

altered SGLT1 gene expression, which in turn modulate glucose uptake activity in the intestinal cell system. This study provides a novel mechanism for ginsenosides in regulating glucose uptake in human cells and showed that some ginsenosides might display opposite pharmacological effects. This study may also provide an opportunity to develop Rg1 as antidiabetic agents or acting as dietary adjuncts to existing therapies.

#### ABBREVIATIONS USED

SGLT1,  $\text{Na}^+$ /glucose cotransporter 1; GLUT, glucose transporter; CK, compound K; Q-PCR, real-time quantitative polymerase chain reaction.

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