Effects of silkworm powder on glucose absorption by human intestinal epithelial cell line Caco-2

Junkyu Han[1], Susumu Inoue[2] and Hiroko Isoda[1]*

[1]Graduate School of Life and Environmental Sciences, University of Tsukuba,

Tennodai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan

[2]CB Japan, Co., Ltd,

Kamihiroya 459-1, Tsurugashima, Saitama 350-2203, Japan

*To whom correspondence should be addressed. FAX: +81-29-853-5776; E-mail:

isoda@sakura.cc.tsukuba.ac.jp

Abstract

Inhibitors of glucose absorption play an important role in the treatment of diabetes. In this research, we report that silkworm powder has inhibitory effects on glucose absorption in human intestinal epithelial cells. Silkworm powder inhibited α -glucosidase activation and glucose transporter (SGLT1) expression. These results suggest that silkworm powder can be used as a natural functional food for the prevention and alleviation of type-2 diabetes.

Key words: Silkworm powder, Caco-2 cells, Glucose absorption, α-glucosidase, Sodium-dependant glucose transporter, Type-2 diabetes

Introduction

Diabetes is complex in nature, and changing lifestyles, food habits and stress have further complicated it. In particular, type-2 diabetes is an increasingly common disease, which causes a number of life-threatening complications. The number of diabetics is increasing by 4-5% yearly with an estimated 40-45% of individuals over 65 years having either type-2 diabetes or impaired glucose tolerance [1]. Various approaches are

used to control type-2 diabetes via reduction of blood glucose level. Promising approaches are the use of (1) insulin sensitizers (such as glycogen synthase kinase 3 and protein tyrosine phosphatase-1B inhibitor), (2) inhibitors of gluconeogenesis (such as pyruvate dehydrogenase kinase inhibitor), and (3) inhibitors of disaccharide hydrolysis (such as acarbose, voglibose, and deoxynojirimycin).

The increase in the capacity of the intestine to absorb glucose through human intestinal epithelial cells is attributable to a combination of specific enzymes (such as α-glucosidase) and glucose transporters. In type-2 diabetes (noninsulin-dependent diabetes mellitus; NIDDM), there is an increase in the capacity of the intestine to absorb glucose. Moreover, sodium-dependent glucose transporters (SGLT1s) were found to be more abundant in human intestinal epithelium isolated from duodenal biopsies of type-2 diabetes patients than from healthy controls. This was determined from the threefold increases in SGLT1 mRNA measured [2].

In Asian countries including Korea, silkworm powder has long been favored as an antidiabetic agent, but its efficacy has not been tested using modern scientific methods [3]. In this study, we investigated the effects of silkworm powder on inhibition of α-glucosidase activity and on the expression of SGLT1 in human intestinal epithelial cells.

Materials and methods

Cell culture

Caco-2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Sigma), 1% penicillin-streptomycin, and 1% nonessential amino acids (Cosmo Bio). They were incubated in an atmosphere of 5% CO_2 at 37 ℃. The cells were passaged at a split ratio of 4 to 8 every 3 or 4 days. For the extraction of total RNA, cells were seeded onto Petri plates at a density of 1×10^6 cells per plate.

Preparation of silkworm powder solution

Silkworm powder (10g) were extracted at 105 ℃ with distilled water (100 ml) for 15 min. Extracted solution (10% (w/v)) were filterated with the filter unit (Millipore, pore size; 0.22 μm). Silkworm powder was obtained from CB Japan Co.

TER measurement

The condition of the Caco-2 cell monolayer was evaluated by measuring the

transepithelial electrical resistance (TER). TER is considered to be correlated with the change in paracellular permeability of the cell monolayer [4]. The cell monolayers on Millicell-HA were rinsed with DMEM and then set in a 12-well plate containing DMEM. DMEM was added to the apical side, and the TER was measured with a Millicell-ERS instrument (Millipore). TER, which can be used to evaluate the confluency of cells, become constant (600-700 $\Omega \times cm^2$) 3 weeks after seeding. After confirming that the Caco-2 cell monolayer has formed completely, we determined the α-glucosidase activity.

Measurement of α-glucosidase activation

To determine the α-glucosidase activity, the cells were plated at 2×10^5 cells/cm^2 onto a 12-mm polycarbonate transwell filter with 0.4 μm pores and a collagen-coated surface. The medium was replaced every three days until use. The cells were grown for 21 days before conducting the experiments. The culture medium was removed and both the apical and basal chambers were washed 3 times with 1 ml of phosphate-buffered saline (PBS). The culture medium in the apical chamber was replaced with 250 μl of PBS, 100 μl of 1% (w/v) soluble starch, 50 μl of porcine pancreatic α-amylase, and 100 μl of silkworm powder solution (0.1, 1% (w/v)). In the basal chamber, 500 μl of PBS was

added instead of culture medium. The assay plate was incubated at 37°C in a humidified atmosphere of 5% CO_2 for 2 h. After incubation, the monosaccharide (glucose) converted from disaccharide in the apical chambers was determined by the glucose oxidase (GOD) method [5, 6]. This α-amylase-added Caco-2 system was established as a useful model to evaluate the effects of α-glucosidase inhibitors on starch digestion [5, 7].

RNA extraction and reverse transcription (RT)

The silkworm powder solution treated plates were incubated at 37°C in a humidified atmosphere of 5% CO_2 for 2 h. After incubation, the total RNA of Caco-2 cells was isolated using ISOGEN (NIPPON GENE Co LTD, Tokyo). The total RNA was then quantified by measuring its absorbance at 260 nm using a UV spectrophotometer. Only RNA samples having a 260/280 ratio higher than 1.8 was used for the RT reaction. Template cDNA was obtained from total RNA by the SuperScript Ⅱ Reverse Transcriptase system (Invitrogen). Briefly, RNA was denatured at 65°C for 5 min, incubated with 1 μl oligo$(dT)_{12\text{-}15}$ primers, and chilled at 4°C. After adding 200 units of SuperScript Ⅱ Reverse Transcriptase, the reaction mix was incubated at 42°C for 60 min, then at 70°C for 10 min.

Real-time Qualitative PCR

For the quantification of mRNA, nested primers were designed using the 'Primer3 input' software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi/primer3_www.cgi). Quantitative PCR reactions were performed using a MiniOpticon instrument (BioRad) following the protocol of the iQ SYBR-Green Supermix system (BioRad). Briefly, the RT mix (2 μl) was used as template for the real-time PCR mix containing 0.5 μM forward (5'-CTTCACCATGGACATCTACGCCAA-3') and reverse (5'-CTGGTGATGGACTGGATGTAATCG-3') nested primers (2 μl each) and 2× SYBR-Green Supermix (10 μl). The amplification conditions were as follows: 10 s at 95°C, 30 s at 62°C and 30 s at 72°C for 34 cycles. At the end of the reaction, a melting curve analysis was carried out to check for the presence of primer-dimers [8].

Statistical analysis

All experiments were conducted at least in quadruplicate (n=4) and results are expressed as mean ±S.D. Data were analyzed by one-way ANOVA followed by Dunnett test (Stat-100, BIOSOFT, U.K) to determine significant differences among the means.

Results and Discussion

The inhibitory effect of silkworm powder on α-glucosidase activity in Caco-2 cells is shown in Fig. 1. Silkworm powder solution at 1% (w/v) concentration has a higher inhibitory effect on glucose conversion than acarbose (10 μM), a commercial α-glucosidase inhibitor. However, silkworm powder not showed porcine pancreatic α-amylase (data not shown). It has been reported that deoxynojirimycin (DNJ), a potent α-glucosidase inhibitor, is 2.7-fold more concentrated in silkworms than in mulberry leaves which are used to feed then [9]. Mulberry trees (*Morus alba L.*) are cultivated in China, Korea, and Japan, and their leaves are used to feed silkworms (*Bombyx mori L.*) [10]. The silkworm has special enzymes that can concentrate DNJ from mulberry leaves [11]. The structure of DNJ from silkworm powder was determined by variety of 1D and 2D NMR spectral data and HRFABMS, and the yield was 0.3% [3].

From this result, it can be hypothesized that DNJ components, which are concentrated by silkworms, inhibit glucose production from starch. Consequently, the converted glucose from disaccharide was decreased by silkworm powder in human intestinal epithelial cells. Next, we investigated the effects of silkworm powder on

SGLT1 mRNA expression by human intestinal epithelial cells. Results showed that silkworm powder solution (1%, w/v) inhibited the expression of SGLT1 mRNA (Fig. 2). Most of the SGLT1 inhibitors such as phlorizin inhibit the binding of glucose to SGLT1 [12]. However, silkworm powder inhibited the expression of the SGLT1 mRNA. Moreover, phlorizin reduces the barrier function of intestinal epithelial cells, such as by causing the diffusion of tight junctional protein (ZO-1) and decreasing the TER [13, 14].

From this result, it can be concluded that silkworm powder can be used as an inhibitor of glucose absorption in human intestinal epithelial cells. The inhibition mechanism by silkworm powder is composed of 2 stages. The first stage is the inhibition of glucose production from disaccharide through the inhibition of α-glucosidase activity, and the second stage is the inhibition of glucose transporter mRNA expression.

In conclusion, silkworm powder inhibited glucose absorption by human intestinal epithelial cells through the inhibition of α-glucosidase activity and SGLT1 expression.

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Figure 1

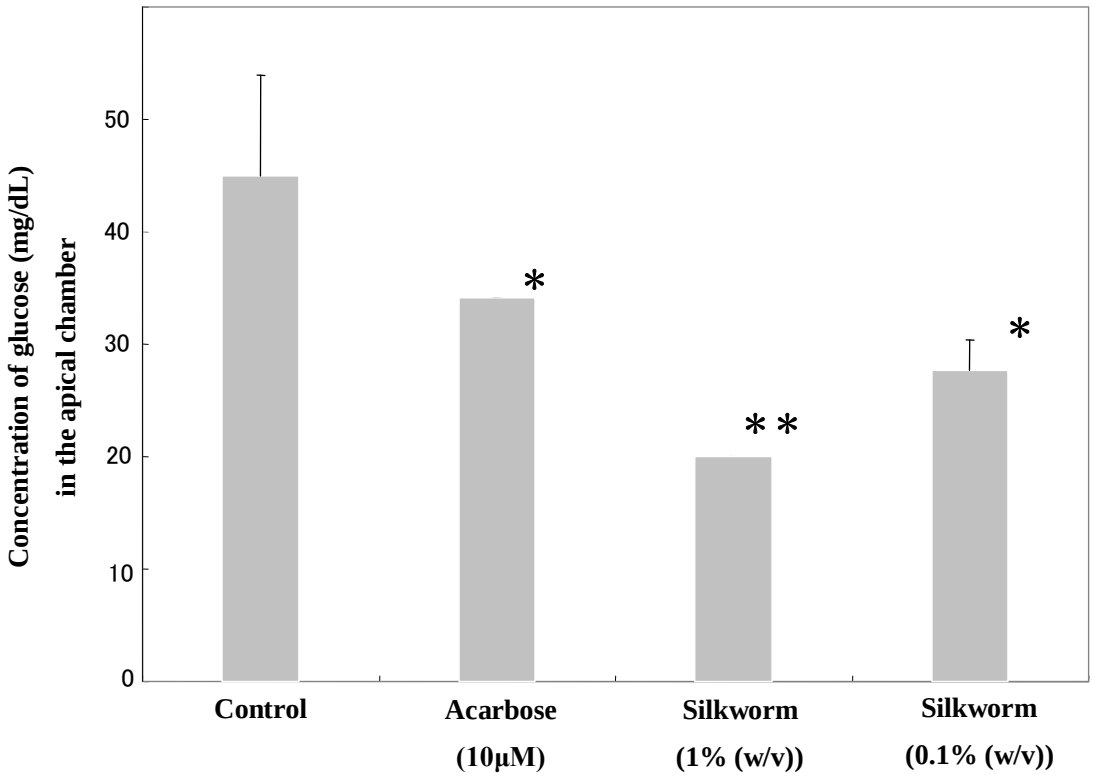
Concentration of glucose (mg/dL)
in the apical chamber
0
10
20
30
40
50
*
**
*
Control
Acarbose
(10μM)
Silkworm
(1% (w/v))
Silkworm
(0.1% (w/v))

Figure 2

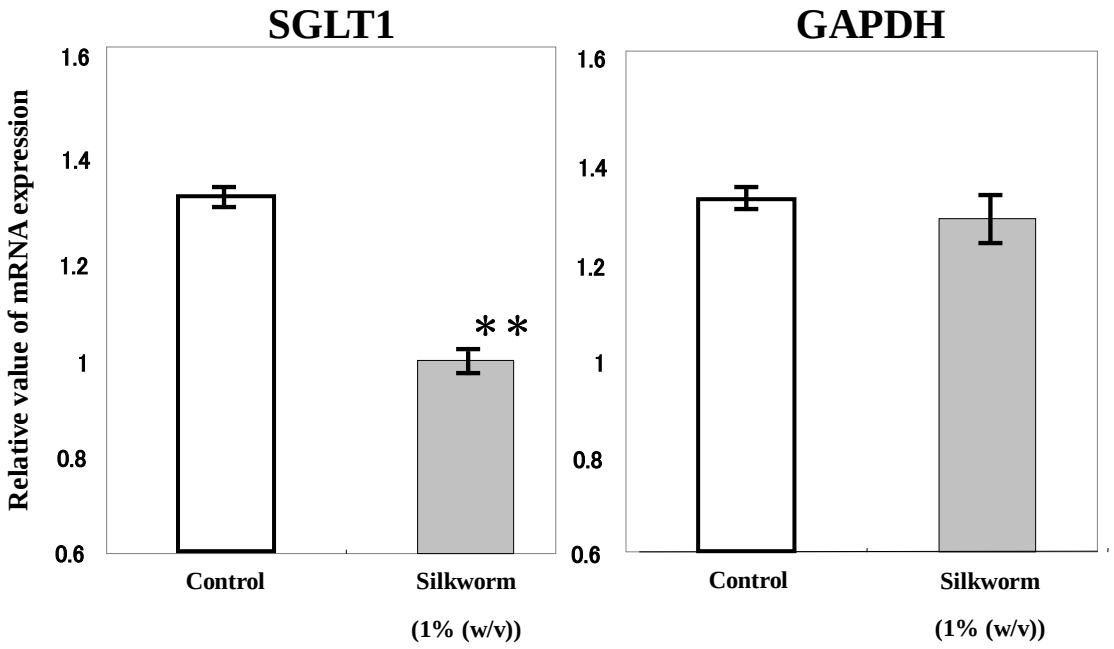
SGLT1
GAPDH
Relative value of mRNA expression
1.6
1.4
1.2
1
0.8
0.6
＊＊
Control
Silkworm
(1% (w/v))

Figure 1. Effect of silkworm powder solution on disaccharide hydrolysis by Caco-2 cells. Each bar represents the mean with S. D. (n=9). *$p < 0.05$, **$p < 0.01$ vs control.

Figure 2. Effect of silkworm powder solution on the expressions of SGLT1 and GAPDH mRNAs by Caco-2 cells. GAPDH was used as a housekeeping gene

Each bar represents the mean with S. D. (n=3). **$p < 0.01$ vs control.