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Effects of Food Lectins on the Transport System of Human Intestinal Caco-2 Cell Monolayers

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The effects of 16 lectins isolated from foodstuff on the transport system across human intestinal Caco-2 cell monolayers were investigated by using four fluorescent markers: lucifer yellow (LY) for the paracellular pathway, fluorescein (FL) for the monocarboxylic acid transporter-mediated pathway, rhodamine 123 for the P-glycoprotein-mediated efflux pathway, and calcein for the multidrug resistance associated protein-related efflux pathway. The transepithelial electrical resistance (TER) values for the monolayers were also measured. WGA from wheat germ, ABA from white mushroom, AOL from *Aspergillus oryzae*, and CSL3 from chum salmon eggs (each at 100 μg/mL) decreased the TER value by 20–40% which resulted in increased LY transport. These lectins, as well as such other lectins as SBA from soybean, RBA from rice bran, and Con A from jack bean, affected other transport pathways too. These results indicate that the lectins modulated the transepithelial transport system in different ways, probably because of their specific binding characteristics toward Caco-2 cell monolayers.

Key words: Caco-2 cell; intestinal transport; lectin; tight junction; transepithelial transport

Lectins are of non-immune origin and non-catalytic sugar-binding proteins that are widely distributed in most common foods in varying amounts.[1] Such lectins as the legume type are relatively stable against heat denaturation and proteolytic digestion; the digestive tract is therefore constantly exposed to the biologically active lectins contained in fresh and processed foods.[2,3] Since the epithelial surface of the intestines is extensively glycosylated, lectins interact with this surface and can induce physiological effects on humans and other animals, particularly when consumed in large quantities.

It is known that a high dose of uncooked or partially cooked kidney beans causes food poisoning.[4] The lectins contained in foods are therefore frequently regarded as anti-nutritional factors, although most of these adverse effects are limited to the legume lectins. Since the dietary intake of lectins is generally low, their activities have no measurable negative effects on nutritional value. Moreover, in some cases, small amounts of lectins may have such beneficial effects on a biological system as promoting gut regrowth after total parenteral nutrition, use as an oral vaccine adjuvant, and use in anti-cancer therapy.[3,4]

The absorption of nutrients and food factors across the intestinal epithelium occurs because of one or more different transport pathways such as passive paracellular transport, passive transcellular transport, and carrier-mediated transport.[5] The human colon adenocarcinoma cell line, Caco-2, has been used as an *in vitro* model of the human small intestinal epithelial system. Completely differentiated and polarized Caco-2 cell monolayers spontaneously exhibit various enterocyte characteristics, including the expression of brush border enzymes, nutrient transporters, and the formation of intercellular tight junctions (TJs).[6,7] Dietary substances and drugs have been found to affect intestinal absorption in model Caco-2 cell monolayers by using the following fluorescent markers: lucifer yellow (LY) for the paracellular pathway;[8,9] fluorescein (FL) for the monocarboxylic acid transporter (MCT)-mediated pathway;[10] rhodamine 123 (RH) for the P-glycoprotein (P-gp)-mediated efflux pathway;[11] and calcein (CA) for the multidrug resistance-associated protein (MRP)-mediated efflux pathway.[12] The transepithelial electrical resistance (TER) value across a Caco-2 cell monolayer reflects any effects on the TJ-mediated paracellular pathway.[9]

Various dietary components have been shown to regulate epithelial permeability by altering the expression and localization of TJ proteins.[5,13] We have shown in a previous study that several lectins, including soybean lectin (SBA), Japanese jack bean lectin (CGA), and wheat germ lectin (WGA), increased the transport of isoflavones but not their aglycons.[14] However, there is little information about the effects of lectins on intestinal transport systems.

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Abbreviations: BSA, bovine serum albumin; CA, calcein; CA-AM, calcein acetoxymethyl ester; CHCA, α-cyano-4-hydroxycinnamic acid; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; FL, fluorescein; Gal, D-galactose; HBSS, Hank's balanced salt solution; HEPES, *N*-(2-hydroxyethyl) piperazine-*N*′-2-ethanesulfonic acid; HPLC, high-performance liquid chromatography; IL, interleukin; LY, lucifer yellow; Man, D-mannose; MCT, monocarboxylic acid transporter; Me-α-Man*p*, methyl α-D-mannopyranoside; MES, 2-(*N*-morpholino) ethanesulfonic acid; MRP, multidrug resistance-associated protein; NEAA, non-essential amino acids; Neu5Ac, *N*-acetylneuraminic acid; P-gp, P-glycoprotein; Rha, L-rhamnose; RH, rhodamine 123; SDS, sodium dodecyl sulfate; SGLT, sodium-dependent glucose transporter; TER, transepithelial electrical resistance; TJ, tight junction; TNF-α, tumor necrosis factor-α

Table 1. Lectins Used in This Study

Lectin	Source	Sugar-binding specificity
Plant lectins		
Legume lectins		
SBA	Soybean (*Glycine max*)	GalNAc>Gal
Con A	Jack bean (*Canavalia ensiformis*)	Me-α-Man*p*>Man>Glc
CGA	Japanese jack bean (*Canavalia galadiata*)	Glc>Man>Rha
PSA	Garden pea (*Pisum sativum*)	Man>Glc>GlcNAc
PNA	Peanut (*Arachis hypogaea*)	Galβ(1,3) GalNAc>$GalNH_2$
Chitin-binding lectins		
RBA	Rice (*Oryza sativa*)	GlcNAc (oligomer>monomer)
WGA	Wheat (*Triticum aestivum*)	GlcNAc (oligomer>monomer), Neu5Ac
LEA	Tomato (*Lycopersicon esculetum*)	GlcNAc (oligomer)
STA	Potato (*Solanum tuberosum*)	GlcNAc (oligomer)
Monocot mannose-binding lectins		
ASA	Garlic (*Allium sativum*)	Man
DB1	Yam (*Dioscorea batatas*)	Man
Jacalin-realated lectin		
MAL	Banana (*Musa acuminata*)	Me-α-Man*p*>Man
Maltose-binding lectin		
DB3	Yam (*Dioscorea batatas*)	Mal
Fungal lectins		
ABA	White mushroom (*Agaricus bisporus*)	Galβ(1,3) GalNAc
AOL	(*Aspergillus oryzae*)	Fuc
Fish egg lectin		
CSL3	Chum salmon (*Oncorhynchus keta*)	Rha>Gal

GalNAc, *N*-acetyl-D-galactosamine; Gal, D-galactose; Man, D-mannose; Glc, D-glucose; GlcNAc, *N*-acetyl-D-glucosamine; Rha, L-rhamnose; Fuc, L-fucose; Me-α-Man*p*, methyl α-D-mannopyranoside; Mal, maltose; Neu5Ac, *N*-acetylneuraminic acid

We investigated in this present study the modulating effects of 16 lectins (Table 1) on the transport systems in human intestinal Caco-2 cell monolayers by using four fluorescent markers (LY, FL, RH and CA), and their TER values. The modulating activity of Con A for apical MRP2-mediated CA efflux was also examined based on its sugar-binding properties.

Materials and Methods

Materials. Dulbecco's modified Eagle's medium (DMEM), non-essential amino acids (NEAA), penicillin-streptomycin (10,000 units/mL and 10 mg/mL, respectively, in 0.9% NaCl), Hank's balanced salt solution (HBSS), lucifer yellow dilithium salt (LY), verapamil, and jack bean lectin (Con A) were purchased from Sigma (St. Louis, MO, USA). Rhodamine 123 (RH) and calcein acetoxymethyl ester (CA-AM) were procured from Molecular Probes (Leiden, The Netherlands), and fetal bovine serum (FBS) was obtained from JRH Biosciences (Lenexa, KS, USA). WST-1 assay kits and fluorescein (FL) were purchased from Dojindo Laboratories (Kumamoto, Japan). MK-571 was procured from Cayman Chemicals (Ann Arbor, MI, USA). Garden pea lectin (PSA) and white mushroom lectin (ABA) were obtained from J-Oil Mills (Tokyo, Japan), and potato lectin (STA), tomato lectin (LEA), and garlic lectin (ASA) were purchased from EY Laboratories (San Mateo, CA, USA). *Aspergillus oryzae* lectin (AOL) was kindly supplied by Gekkeikan (Koyto, Japan),[15] and peanut lectin (PNA) and methyl α-D-mannopyranoside were purchased from Wako Chemicals (Osaka, Japan). SBA, CGA, WGA,[14] banana lectin (MAL),[16] yam lectins DB1 and DB3,[17] and chum salmon egg lectin (CSL3)[18] were isolated as previously described. RBA was isolated by ammonium sulfate precipitation and affinity chromatography using a chitin column. The isolated lectins were subjected to SDS-polyacrylamide gel electrophoresis to check for their purity and stored at −20 °C until needed. All other chemicals used in this study were of analytical grade.

Cell culture. The Caco-2 human colon adenocarcinoma cell line was obtained from the American Type Culture Collection (Rockville, MD, USA). Cells at passage numbers 23–35 were cultured in DMEM with 10% (v/v) FBS, penicillin-streptomycin (50 IU/mL and 50 μg/mL, respectively), and 1% (v/v) NEAA, and maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air. The cells were sub-cultured at 70–80% confluency.

Cytotoxicity assay. Caco-2 cell monolayers were prepared by seeding on 96-well microtiter plates at a density of 1.0×10^5 cells/cm^2, and maintained for 18–21 d (the culture medium was replaced every 2–3 d).

The lectins were dissolved in HBSS containing 0.8 mM *N*-(2-hydroxyethyl) piperazine-*N*′-2-ethanesulfonic acid (HEPES, at pH 7.3). The cell monolayers were gently rinsed twice with HBSS, and then treated for 2 h with each lectin (200 μg/mL). The cytotoxicity after a treatment was determined by using a WST-1 assay kit. The WST-1 reagent was incubated with the cells for 2 h, after which the absorbance at 450 nm was measured by using a microtiter plate reader (Bio Rad, Tokyo, Japan).

LY, FL and RH transport experiments. Caco-2 cell monolayers were prepared by seeding on Transwell inserts with a 0.40-μm polycarbonate membrane 6.5 mm in diameter (Corning Costar, NY, USA) at a density of 1.0×10^5 cells/cm^2. The apical and basolateral compartments respectively contained 0.1 and 0.6 mL of the culture medium. The cell monolayers were maintained for 18–21 d (the culture medium was replaced every 2–3 d), and the integrity of the cell monolayers was evaluated by measuring the TER value with a Millicell-ERS instrument (Millipore, MA, USA). Cell monolayers with TER values of >500 Ω/cm^2 were used for the subsequent experiments.

Two different transport buffers were prepared for the transport experiments. HBSS containing 0.8 mM HEPES at pH 7.3 was used for the basolateral solution and HBSS containing 5 mM 2-(*N*-morpholino) ethanesulfonic acid (MES) at pH 6.0 was used for the apical solution. The cell monolayers on Transwell inserts were gently rinsed twice with transport buffer and incubated at 37 °C in the same buffer for 30 min. After incubation, the apical solution was removed, and 0.2 mL of HBSS at pH 6.0 containing 100 μM LY, 10 μM FL, and 10 μM RH with or without a lectin (4, 20, or 40 μg) was added to the apical side. After incubating for another 2 h, the basolateral solution was collected and analyzed by reversed-phase high-performance liquid chromatography (HPLC). The TER value of the monolayers was measured before adding a sample and after incubating for 2 h.

The basolateral solution was analyzed by reversed-phase HPLC on a Capcelpack ODS AG120 column (5 μm, 4.6 × 250 mm; Shiseido, Tokyo, Japan) at 40 °C with a flow rate of 1.0 mL/min. Solvent A was

10 mM sodium acetate at pH 7.0 and solvent B was 10 mM sodium acetate (pH 7.0)-acetonitrile (50:50, v/v). Linear gradient elution was used with 0 to 100% of solvent B over 15 min. A fluorescence detector was used for signal detection at an excitation wavelength of 450 nm and an emission wavelength of 540 nm. The respective retention times for LY, FL, and RH were approximately 3, 10, and 13 min under these conditions.

CA transport experiment. The cell monolayers were gently washed three times with HBSS containing 0.8 mM HEPES as the transport buffer at pH 7.3, and then loaded with CA by pre-incubating for 30 min at 37 °C with 2 μM CA-AM. After this pre-incubation, the cell monolayers were washed again and placed in new culture plates at 37 °C. A 0.2-mL amount of a sample solution (4, 20, or 40 μg of lectin or 10 μM MK-571 in the transport buffer) was next added to the apical compartment, and 0.8 mL of the transport buffer was added to the basolateral compartment.

After incubating for 2 h, the apical and basolateral solutions were each collected, and the cell monolayers were lysed with 0.4% Triton-X100. The CA fluorescence intensity was measured at an excitation wavelength of 480 nm and an emission wavelength of 530 nm by using a fluorescence plate reader (MTX Lab Systems, VA, USA).

The activity of Con A (100 μg/mL) for intracellular CA efflux was similarly examined in the presence of 50 mM D-mannose (Man), methyl α-D-mannopyranoside (Me-α-Man*p*), or D-galactose (Gal).

Data analysis. The results are expressed as the percentage of the control value and shown as the mean ± SD of three or four different determinations. A statistical comparison of non-normalized data was made by Student's two-tailed *t*-test, p values <0.05 being considered significant.

Results

Cytotoxicity of the lectins

Transport experiments could be achieved with Caco-2 cell monolayers up to 4 h without any problems under the conditions described. The Caco-2 cells were therefore incubated with lectins for 2 h to examine their cytotoxic activity against these cells. The lectins had no apparent cytotoxic effect, even at the highest concentration (200 μg/mL) used in this study (data not shown).

Effects of the lectins on TER and LY transport

The tightness of the intercellular junctions was evaluated by TER measurements; a decrease in TER would indicate an increase in paracellular transport, or vice versa (Fig. 1). The non-lectin protein, bovine serum albumin (BSA; 20–200 μg/mL), had no effect on the TER value. However, 0.1 mM sodium dodecyl sulfate (SDS), which is known to open TJs and increase paracellular transport due to membrane perturbations,[19] significantly decreased the TER value. The CGA, PNA, WGA, ABA, AOL, and CSL3 treatment at 100 μg/mL each decreased the TER value by 5–40% after a 2-h incubation. The initial TER values (Ω/cm^2; mean ± SD, $n = 12$) of the cell monolayers were 1129 ± 80 for BSA, 1038 ± 108 for SBA, 975 ± 45 for Con A, 800 ± 58 for CGA, 1047 ± 78 for PSA, 894 ± 112 for PNA, 964 ± 27 for RBA, 865 ± 92 for WGA, 719 ± 65 for LEA, 975 ± 68 for STA, 698 ± 58 for ASA, 644 ± 105 for DB1, 927 ± 37 for MAL, 735 ± 110 for DB3, 934 ± 74 for ABA, 1122 ± 100 for AOL, and 1168 ± 121 for CSL3. The relative TER values (%) in Fig. 1 were calculated by dividing the TER values of the monolayers treated with each lectin by that of the control monolayers without a treatment.

In the absence of a lectin, approximately 0.1% of LY, which is known to be transported by the paracellular pathway, was transported to the basolateral side from the apical side after incubating for 2 h. LY transport was slightly increased by treating with PNA, WGA, ABA, and AOL, and was significantly increased by treating with CSL3 (Fig. 2). Among the lectins examined, CSL3 from chum salmon eggs had the strongest effect on TJs in a dose-dependent manner; at 200 μg/mL, it decreased the TER value by approximately 40% and increased LY transport 2.5 times when compared with the control.

Effects of the lectins on FL transport

FL is transported by a MCT-mediated pathway under inward gradient conditions.[8] The apical-to-basolateral transport of FL was determined in the presence and absence of lectins (Fig. 3). BSA and α-cyano-4-hydroxycinnamic acid (CHCA, 1 mM), a selective MCT inhibitor,[20] were used for comparisons. In the absence of a lectin, approximately 20% of FL on the apical side was transported to the basolateral side after incubating for 2 h. FL transport was increased by treating with SBA, RBA, WGA, and CSL3 at 200 μg/mL, while it was decreased by 10% by MAL at 200 μg/mL.

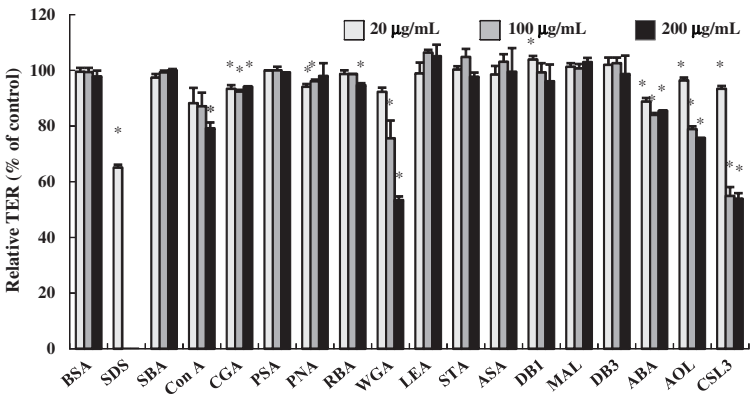

Fig. 1. Effects of Lectins on the TER Values of Caco-2 Monolayers.

The cell monolayer TER value was measured after incubating for 2 h with a lectin (20–200 μg/mL). BSA (20–200 μg/mL) and SDS (0.1 mM) were used as references. Results are expressed as the percentage of the control value without a lectin, and are the mean ± SD of three different determinations. $^*p < 0.05$ compared with the control value.

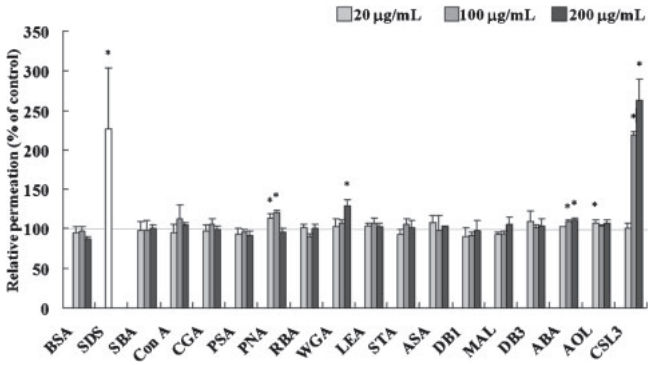

Fig. 2. Effects of Lectins on the LY Transport Across Caco-2 Cell Monolayers.

LY on the cell monolayer basolateral side was measured after incubating for 2 h with a lectin (20–200 μg/mL). BSA (20–200 μg/mL) and SDS (0.1 mM) were used as references. Results are expressed as the percentage of the control value without a lectin, and are the mean ± SD of three different determinations. $^{*}p < 0.05$ compared with the control value.

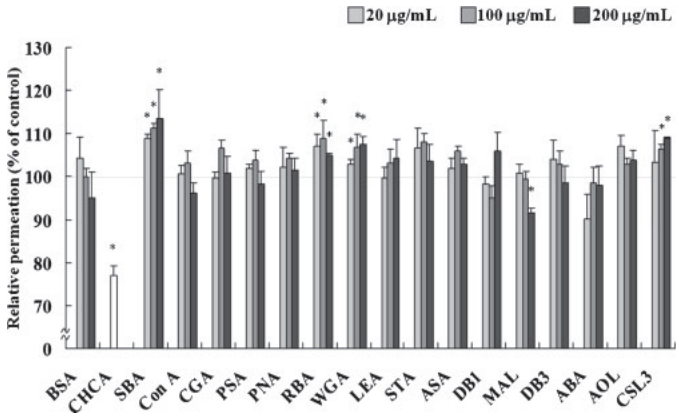

Fig. 3. Effects of Lectins on the FL Transport Across Caco-2 Cell Monolayers.

FL on the cell monolayer basolateral side was measured after incubating for 2 h with a lectin (20–200 μg/mL). BSA (20–200 μg/mL) and CHCA (1 mM) were used as references. Results are expressed as the percentage of the control value without a lectin and are the mean ± SD of three different determinations. $^{*}p < 0.05$ compared with the control value.

Effects of the lectins on RH transport

The apical-to-basolateral transport of RH was determined in the presence and absence of a lectin (Fig. 4). RH is absorbed by transcellular diffusion due to its hydrophobicity and hydrogen-bonding properties.[9] RH efflux from the cells to the apical side was due to P-gp; RH transport was thus an indicator of P-gp activity.[21] Approximately 8% of RH was transported to the basolateral side from the apical side after a 2-h incubation in the absence of a lectin. Verapamil (1 mM), a P-gp inhibitor,[22] increased RH transport by 15%. Treating with RBA, WGA, STA, AOL and CSL3 each significantly increased RH transport, whereas treating with ABA decreased RH transport.

Effects of lectins on the CA efflux from Caco-2 cell monolayers

CA is known to be transported to the apical side by MRP2, and to the basolateral side by MRPs in Caco-2 cell monolayers.[11,12] CA efflux from the Caco-2 cells to the apical compartment was greater than that to the basolateral compartment. Without a lectin, approximately 40% and 14% of the total CA was respectively transported to the apical and basolateral compartments. Treating with Con A, RBA, WGA, ABA, AOL, and CSL3 significantly decreased the apical CA efflux associated with an increase in the cellular retention of CA (Fig. 5A and C). In particular, Con A and RBA at 200 μg/mL decreased the apical efflux by approximately 40% and increased the cellular retention of CA by approximately 30%. CSL3 at 200 μg/mL significantly increased the basolateral CA efflux (Fig. 5B), and ASA and MAL slightly increased the apical CA efflux and reduced CA cellular retention. STA and DB3 decreased the levels (Fig. 5B). MK571 (10 μM), a selective MRP inhibitor,[23] respectively decreased the CA apical and basolateral efflux by 20 and 25%.

The modulating activity of Con A toward apical MRP2-mediated CA efflux was examined in the presence of specific sugars to explore the role of sugar binding in this activity (Fig. 6). Con A has specific binding affinity for Man and Me-α-Man*p*, but not for Gal.[24] Gal (50 mM) did not affect the apical CA efflux, which was decreased by the Con A treatment (Fig. 6A). In contrast, Me-α-Man*p* inhibited Con A to decrease the apical CA efflux, whereas Man showed marginal inhibition due to its weak affinity for Con A compared with Me-α-Man*p*. Similarly, Me-α-Man*p* canceled the effect of Con A on CA cellular retention, although Gal or Man showed little inhibitory effect toward Con A (Fig. 6C). There was a small increase in the basolateral CA efflux in the presence of Con A, although the effect of the lectin was much less than that for apical CA efflux and cellular CA (Fig. 6B). The addition of any sugar tested slightly increased the basolateral CA efflux.

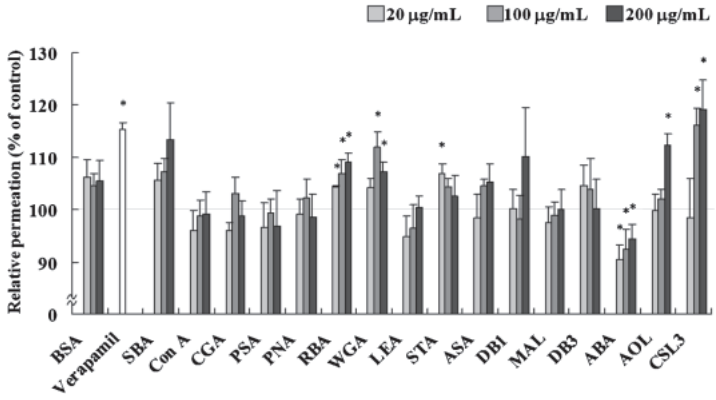

Fig. 4. Effects of Lectins on the RH Transport Across Caco-2 Cell Monolayers.

RH on the cell monolayer basolateral side was measured after incubating for 2 h with a lectin (20–200 μg/mL). BSA (20–200 μg/mL) and verapamil (0.1 mM) were used as references. Results are expressed as the percentage of the control value without a lectin and are the mean ± SD of three different determinations. $^{*}p < 0.05$ compared with the control value.

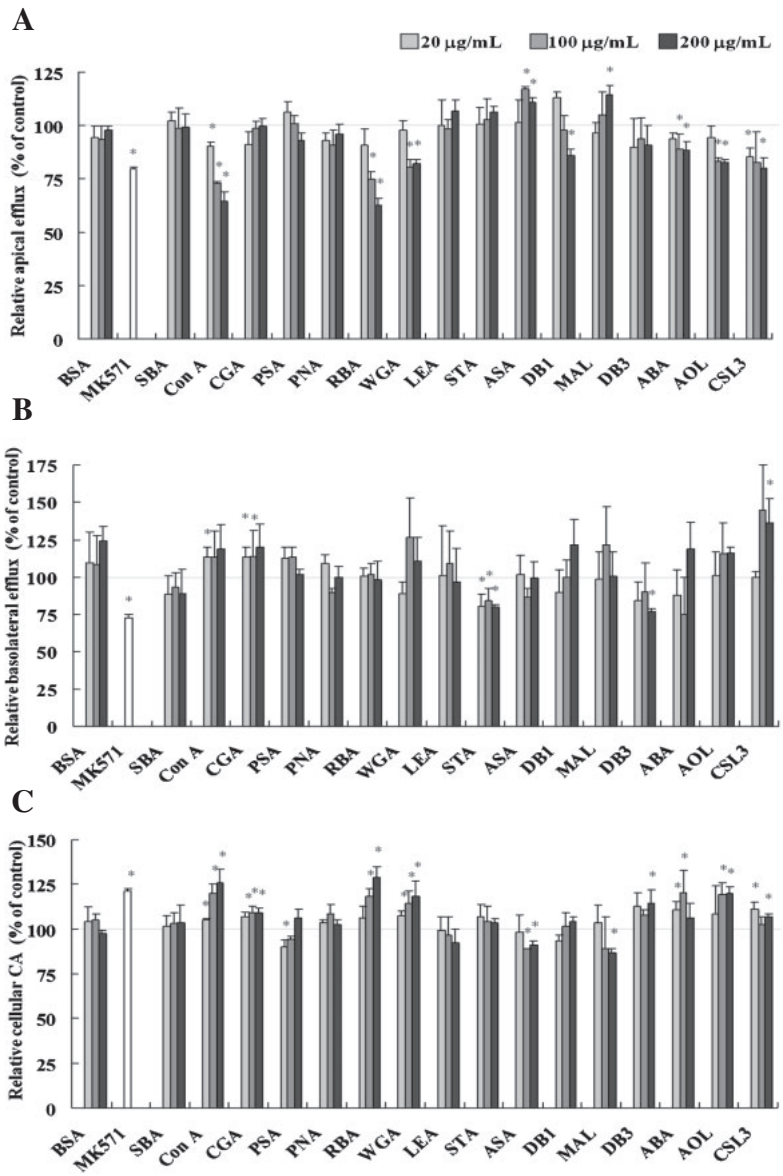

Fig. 5. Effects of Lectins on the Transport of CA in Caco-2 Cell Monolayers.

CA on the cell monolayer apical side (A), on the basolateral side (B), and cellular CA (C) were measured after incubating for 2 h with a lectin (20–200 μg/mL). MK-571 (10 μM) was used for a reference. Results are expressed as the percentage of the control value without a lectin and are the mean ± SD of three or four different determinations. $^{*}p < 0.05$ compared with the control value.

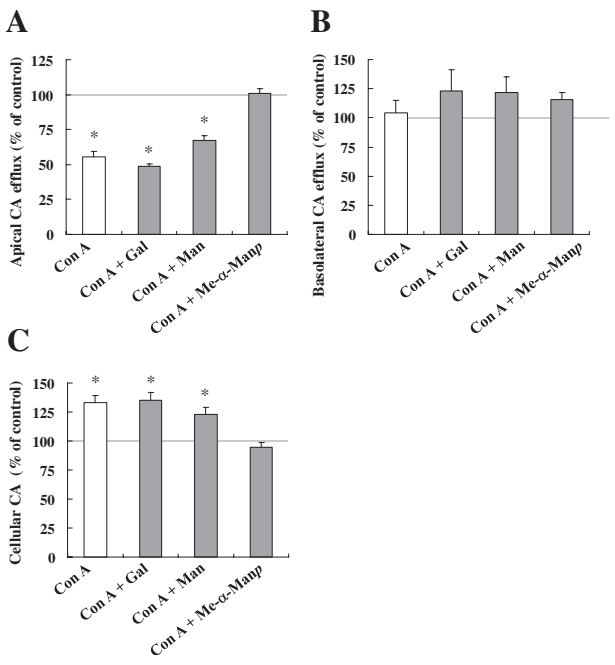

Fig. 6. Effects of Con A on the Intracellular CA Efflux from Caco-2 Cell Monolayers in the Presence of a Specific or Non-Specific Sugars.

Apical (A), basolateral (B), and cellular (C) CA were measured after incubating for 2 h with 200 μg/mL of Con A and 0–50 mM sugar. Results are expressed as the percentage of the control value without Con A and are the mean ± SD of three or four different determinations. $^{*}p < 0.05$ compared with the control value.

Discussion

We investigated the effects of 16 lectins with varying sugar-binding specificity on the transport system across Caco-2 cell monolayers by using four fluorescent markers, whose transport pathways were known, and measuring the TER values. CSL3 exhibited the most significant effects among the lectins examined. CSL3 has some binding affinity for L-rhamnose (Rha) and Gal and much higher binding affinity ($Kd = 2.6 \times 10^{-5}$ M) for globotriaosylceramide (Gb3; Galα1-4Galβ1-4Galβ1-Cer) which is located in lipid rafts on a cell surface.[25] In addition, CSL3 has a cytotoxic effect against the Gb3-expressing human colon cancer cell lines, Caco-2 and Lovo, which is mediated in a dose-dependent manner by an apoptotic pathway through the recognition of Gb3 on the cell surface.[26] Although CSL3 did not exhibit cytotoxicity under the conditions used in this study, its activity might have affected the transport system. The incubation period was therefore set to 2 h for all transport experiments to minimize the cytotoxic effect as much as possible.

WGA and RBA, which are GlcNAc specific, increased FL and RH transport and decreased the TER values and apical CA efflux. However, LEA and STA, which are also GlcNAc specific, exhibited only marginal effects. These differences may have been due to the fact that each lectin recognized distinct sugar chains and glycoconjugates in spite of apparently similar binding activity against simple sugars; for example, WGA, but RBA exhibits specificity toward *N*-acetylneuraminic acid (Neu5Ac).[27] In addition, WGA, LEA, and STA have their individual binding specificity to poly-*N*-acetyllactosamine-type oligosaccharides derived from human erythrocyte ghosts.[28] It has been also shown that other lectins, including SBA, Con A, CGA, PNA, ABA, and AOL, exhibited a characteristic effect on these transport pathways. These lectins have differing sugar-binding specificity: SBA for GalNAc, Con A for Man, CGA for Man, PNA for Gal, ABA for Gal, and AOL for Fuc.[1] These results indicate that a variety of lectins can interact with the epithelial surface of the intestines to modulate the transport system as other food components. Moreover, such lectins as WGA are known to be endocytosed by the epithelial cells of the small intestine.[3]

Such food components as fatty acids, oligo- and polysaccharides, amino acids, peptides, proteins, and flavonoids have been shown to modulate TJ permeability by intracellular signaling.[5,13] Several flavonoids, including quercetin, myricetin, and kaempferol, have enhanced TJ integrity by affecting cytoskeletal association, the expression of TJ proteins, or microdomains in the membranes of Caco-2 cell monolayers;[29] for example, chitosan has opened TJs by inducing intracellular redistribution of the TJ protein, claudin-4, and its subsequent degradation in lysosomes.[30] Regulation of the assembly, disassembly, and maintenance of TJ structures is influenced by several signaling proteins, including protein kinase C (PKC), mitogen-activated protein kinases (MAPKs), myosin light-chain kinase (MLCK), and the Rho family of small GTPases.[13] Capsaicin induces TJ opening by mediating cofilin dephosphorylation.[31] Such cytokines as tumor necrosis factor (TNF)-α[32] and interleukin (IL)-6[33] regulate the TJ-mediated paracellular pathway. It is worth noting that TNF-α and IL-6 have respective binding specificity for tri-mannose/chitobiose and sialyl T antigen,[34] suggesting that their lectin-like activity may have a role in transport regulation.

The proton-linked MCT-mediated transport system is important for the uptake into Caco-2 cell monolayers of a phenolic acid such as p-coumaric acid and the microbial metabolites of poorly absorbed polyphenols.[8,10] Catechins inhibit MCTs and also sodium-dependent glucose transporter (SGLT)1 in Caco-2 cells.[5] Ginsenosides have a potent effect on glucose uptake across Caco-2 cell monolayers by modulating the SGLT1 expression.[35] FL permeability is an indicator of MCT activity under inward-gradient conditions. CSL3 did not affect the MCT activity as strongly as it did its effects on other transport pathways.

P-gp is a transmembrane ATP-dependent drug pump that can transport a broad range of structurally unrelated compounds to the outside of cells.[36] Multi-drug resistance mediated by P-gp is believed to be one of the major causes for the failure of cancer therapy. However, P-gp is thought to prevent cytotoxic compounds in the environment and diet from entering the body of humans.[21] RH is a well-studied model substrate for P-gp[37] that is absorbed by transcellular diffusion and then effluxed to the apical side by P-gp. P-gp is a heavily *N*-linked glycosylated protein that is recognized by GlcNAc-specific lectins.[38] Indeed, WGA and RBA, typical GlcNAc-specific lectins, increased RH transport (Fig. 4), probably because of their inhibitory activity against P-gp. In contrast, ABA, which is specific for *O*-linked glycans, decreased RH transport, suggesting

that *O*-linked glycans mediated a P-gp-modulating pathway.

MRPs are *N*-linked glycosylated transmembrane ATP-dependent drug pumps that can transport various substrates to the outside of cells.[39] Conjugates of glutathione, glucuronide, and sulfate are well known MRP substrates. MRP activity can be examined by quantifying the CA efflux from Caco-2 cell monolayers. CA-AM is a non-fluorescent membrane-permeable substrate that is cleaved by intracellular esterases to the fluorescent membrane-impermeable metabolite, CA. CA-AM is a substrate for P-gp and MRPs, whereas CA is not a substrate for P-gp and is transported to the apical side by MRP2 and to the basolateral side by other MRP isoforms.[11,12] It is postulated that the effects of lectins on the transport system are attributable to their sugar-binding activity. The role of the sugar-binding activity of lectins was verified in this study with Con A which showed strong activity in apical MRP2-mediated CA efflux. As expected, the activity of Con A was inhibited by Me-α-Man*p*, but was not affected by Man (Fig. 6). These results suggest that the MRP-modulating activity of Con A was associated with its sugar-binding properties; the specificity of Con A for glycans that are linked to MRP2 activity is relatively high.

Besides the effects of lectins for a short period of incubation, we have previously conducted a proteomics analysis of Caco-2 cells that had been separately treated with SBA, Con A, and WGA at 10 μg/mL for 24 h.[40] Approximately 650 protein spots were visualized on each respective two-dimensional-electrophoresis gel (16 cm × 16 cm). This showed that the expression of five, eight, and six proteins was respectively regulated by treating with SBA, Con A and WGA without damaging the cells. Although these regulated proteins were specific for each lectin and did not include any transporters, they did include chaperones and cytoskeleton-related proteins. These results indicate that lectins affected Caco-2 cells in various manners.

Lectins are found in all organisms, many of which are used as food. Each lectin has such characteristic properties as sugar-binding specificity, stability against heat denaturation and protease digestion. We have shown in the present study that lectins contained in food-stuffs had varying modulating effects on the transport system of intestinal epithelial cells. Although the precise mechanisms underlying these modulating effects remain unclear, lectins may modulate the transport system through intracellular signaling, controlling the expression of various proteins, or assembling and disassembling cytoskeletal proteins, as has been reported for other food components. In fact, Zhao *et al.*[41] have reported that a high dose of SBA (0.1–0.2% of the total diet) reduced piglet intestinal epithelial tight junction protein occludin or ZO-1 expression and increased intestinal permeability, whereas a low dose of SBA (0.05%) had no such effect.

Despite the adverse effects of some dietary lectins, several studies have been performed in recent years on their possible uses for non-toxic oral dosing as a drug delivery agent, oral vaccine adjuvant, and anti-cancer therapy.[3,4] Furthermore, as the results of this study have suggested, an appropriate choice of lectins may make it possible to regulate the absorption of particular food factors.

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