Lectins as Endocytic Ligands: An Assessment of Lectin Binding and Uptake to Rabbit Conjunctival Epithelial Cells

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Purpose. To investigate the binding and uptake pattern of three plant lectins in rabbit conjunctival epithelial cells (RCECs) with respect to their potential for enhancing cellular macromolecular uptake.

Methods. Three fluorescein-labeled plant lectins (*Lycoperison esculentum*, TL; *Solanum tuberosum*, STL; and *Ulex europaeus* 1, UEA-1) were screened with respect to time-, concentration-, and temperature-dependent binding and uptake. Chitin (30 mg/ml) and L-α-fucose (10 mM) were used as inhibitory sugars to correct for nonspecific binding of TL or STL and UEA-1, respectively. Confocal microscopy was used to confirm internalization of STL.

Results. The binding and uptake of all three lectins in RCECs was time-dependent (reaching a plateau at 1–2 h period) and saturable at 1-h period. The rank order of affinity constants (k_m) was STL > TL > UEA-1 with values of 0.39 > 0.48 > 4.81 μM, respectively. However, maximal, specific binding/uptake potential was in the order UEA-1 > STL > TL with values of 53.7, 52.3, and 15.0 nM/mg of cell protein, respectively. Lectins showed temperature dependence in their uptake, with STL exhibiting the highest endocytic capacity. Internalized STL was visualized by confocal microscopy to be localized to the cell membrane and cytoplasm.

Conclusion. Based on favorable binding and uptake characteristics, potato lectin appears to be a useful candidate for further investigation as an ocular drug delivery system.

KEY WORDS: absorption; glycoprotein; mucoadhesives; ocular drug delivery; potato lectin; tomato lectin.

INTRODUCTION

Lectins are nonimmunoglobulin glycoproteins that recognize and bind to specific sugar moieties on the cell membrane. They are involved in a variety of biological processes, such as cell-cell and host pathogen interactions and innate immune responses (1). Lectins are usually derived from plants, animals, bacteria, or viruses, with plant lectins accounting for the majority of all lectins. Lectins are usually classified based on their saccharide specificity, although lectins in the same classification category, such as galactose-binding lectins, may have sugar binding patterns that are considerably different from one another (2). In addition, the glycosylation patterns in equivalent structures may vary among species (3). A more accurate classification, therefore, is based on homologies in the lectins' primary sequences and on three-dimensional structural similarities.

The conjunctiva is a thin, vascular membrane lining the inside of the eyelids, which participates in both ocular and systemic absorption of topically applied drugs (4,5). It may function as a protective barrier against the permeation of pathogens and exogenous drugs, in the exchange of nutrients and solutes with the cornea, and in the excretion of solutes and endogenous enzymes contributing to tear content (6). The dynamic nature of conjunctival epithelium has been demonstrated in the identification of active transport or uptake mechanisms for nucleosides, glutathione, dipeptides, and nanoparticles as well as in its permeability to hydrophilic molecules of up to ~40 kDa (7–11).

The binding of lectins to ocular surface epithelia, including the conjunctival epithelium, has been demonstrated in a variety of species including man (12). Lectin binding to the conjunctival epithelium has been shown to differ in binding intensity and cellular location (13). Nicholls *et al.* (14) demonstrated that compared to other lectins, those from both *Solanum tuberosum* (potato) and *Helix pomatia* (edible snail) bind more rapidly to unfixed rat ocular surface epithelia of both the cornea and conjunctiva and were retained longer (up to 3 h). In addition, these two lectins were later shown to cause minimal acute dermal irritancy to rabbits (15). Furthermore, Schaeffer *et al.* (16) demonstrated that lectin pretreatment of isolated rabbit corneas increased the binding of liposomes (containing gangliosides on their surface) to the cornea. This led to an increase in the flux of carbachol, entrapped within the liposome, to the cornea. Thus, identifying the lectin binding pattern in the conjunctival epithelium is important for understanding the biochemistry and pathophysiology of many ocular surface diseases such as dry ocular syndrome and allergic conjunctivitis (17).

The problem in delivering drugs to the eye is that only 1–2% of the instilled dose gets absorbed, as a result of the low residence time in the eye. Because lectins demonstrated prolonged ocular retention and enhanced drug delivery across epithelial tissues (14,18), it is worthwhile to investigate their binding and internalization kinetics as a means of using them as endocytic cell ligands to promote the ocular absorption of drug-loaded poly(lactide-co-glycolide) (PLGA) nanoparticles (10). The purpose of this study was to investigate the binding and uptake characteristics of three model plant lectins (*Solanum tuberosum*, STL; *Lycopersicon esculentum*, TL; and *Ulex europaeus*, UEA-1) in primary cultured rabbit conjunctival epithelial cells (RCECs), which have been demonstrated as an effective *in vitro* model for evaluating drug transport in the conjunctival epithelium (19,20).

MATERIALS AND METHODS

Materials

Fluorescently labeled *Solanum tuberosum* (potato) lectin (FITC-STL, molar ratio fluorescein/protein 3.2), FITC-*Lycopersicon esculentum* (tomato) lectin, FITC-*Ulex europaeus* 1 (UEA-1, gorse) lectin, and chitin hydrolysate (an inhibitor of potato and tomato lectins) were all obtained from Vector Laboratories (Burlingame, CA, USA). L-α-fucose (an inhibitor of UEA-1 lectin) was obtained from Sigma Chemical Co. (St Louis, MO, USA). Transwell filters (6.5-mm diameter, 0.4-μm pore size) were obtained from Costar (Cam-

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bridge, MA, USA). Culture media and supplies were purchased from Invitrogen (Carlsbad, CA, USA), and PC-1 serum-free culture medium was purchased from Biowhittaker (Walkersville, MD, USA). Madin-Darby canine kidney cells (MDCK) type II cells (NBL-2) were obtained from American Type Culture Collection (Manassas, VA, USA).

Animals and Tissue Preparation

Conjunctival tissue was isolated and prepared as described previously by Kompella *et al.* (19). Briefly, male Dutch-belted pigmented rabbits, weighing 2.0–2.5 kg, were obtained from Irish Farms (Norco, CA, USA) and handled in accordance with Guiding Principles in the Care and Use of Animals (DHEW publication, NIH 80-23). The animals were euthanized with an overdose of sodium pentobarbital solution (325 mg/kg) injected via the marginal ear vein. The eyeballs were excised, and then conjunctival tissues were carefully dissected.

Primary Air-Interfaced Culture of Rabbit Conjunctival Epithelial Cells

Rabbit conjunctival epithelial cells (RCECs) were harvested using a protocol developed by Saha *et al.* (20) and modified by Yang *et al.* (21). Briefly, following excision, the conjunctiva was washed in ice-cold Ca^{2+}/Mg^{2+}-free Hanks' balanced salt solution and treated with 0.2% protease (type XIV) for 60 min at 37°C in 95% air/5% CO_2 to dissociate the cells. The isolated cells were treated with Minimum Essential Medium (S-MEM) containing 10% fetal bovine serum (FBS) and 1 mg/ml deoxyribonuclease (DNAase I) to stop protease reaction. The cell pellet was washed, centrifuged at 100 × *g* for 10 min at room temperature, and filtered through a 40-μm cell strainer. The final cell pellet was resuspended in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 (DMEM/F12) medium supplemented with 100 U/ml penicillin-streptomycin, 0.5% gentamicin, 0.4% fungizone, 2 mM L-glutamine, 1% ITS^+ (6.5 μg/ml insulin, 6.5 μg/ml transferrin, 6.5 ng/ml selenious acid, 1.25 mg/ml BSA, and 5.35 mg/ml linoleic acid), 30 μg/ml bovine pituitary extract (BPE), 1 μM hydrocortisone, and 1 ng/ml epidermal growth factor (EGF). These cells were seeded on Transwell inserts precoated with rat collagen at a density of 1.2×10^6 cells/cm^2 and were cultured at 37°C in 5% CO_2 and 95% air. From day 2 onward, the growth medium was changed to PC-1 growth medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin-streptomycin, 0.5% gentamicin, and 0.4% fungizone. On day 4, cells were switched to an air-interface (i.e., nominally fluid-free on the apical surface of the cell layers) onward. On day 6 or 7, cells became confluent and were then used for experiments. MDCK (NBL-2) cells were propagated in Minimum essential medium Eagle containing 2 mM L-glutamine and Earle's salts, and supplemented with 1.5 g/L sodium bicarbonate, 0.1 mM nonessential amino acids, 1.0 mM sodium pyruvate, and 10% FBS. Every 2–3 days, the cell culture medium was changed until the cells were confluent and used afterwards.

Characteristics of Lectin Binding and Uptake

Model lectins from three species were used: *Solanum tuberosum* (potato lectin, STL), *Lycopersicon esculentum* (tomato lectin, TL), and *Ulex europaeus* (gorse lectin, UEA-1). The sugar specificity and molecular weight of each are provided in Table I. These lectins were examined with respect to their time-, concentration-, and temperature-dependent uptake and binding in primary cultured RCEC layers.

For time-dependent uptake experiments, RCECs were incubated at 37°C with 1 μM of FITC-STL, 1 μM FITC-TL, or 3.17 μM of FITC-UEA-1 lectin for various time intervals up to 2 h The concentration-dependency experiment was then carried out. To obtain the total binding and uptake in RCECs for each lectin, RCECs were incubated at 1 h with various lectin concentrations, ranging from 0.1 to 6.34 μM, in the absence or presence of an inhibitory sugar. Chitin (30 mg/ml) was used as an inhibitory sugar to correct for nonspecific binding of FITC-TL or FITC-STL. L-α-fucose (10 mM) was used as inhibitory sugar for FITC-UEA-1 lectin. The specific binding/uptake was obtained by subtracting the total binding/uptake, in the absence of inhibitory sugar, from nonspecific binding/uptake in the presence of excess amounts of the inhibitory sugar. The binding affinity parameters for each lectin (V_{max} and K_m values) were calculated from the specific binding/uptake curve fitted to Michaelis-Menten equation and analyzed using GraphPad Prism software (GraphPad Software Inc., San Diego, CA). The uptake/binding of various concentrations of FITC-STL (in the range 0.25–2 μM) in MDCK cells was determined to provide a comparison between the binding affinity of STL in MDCK cells and the affinity in RCECs. To evaluate the amount of lectin internalized (endocytosed) into RCECs, the uptake of various concentrations of fluorescently labeled lectins by RCECs at 4°C for 1 h, at a range of concentrations similar to that used for uptake experiments at 37°C, was evaluated. The uptake/binding curve obtained at 37°C was then subtracted from the uptake/binding curve at 4°C. This difference represented the active component of uptake (the amount internalized) and was expressed as nM lectin/mg total protein. The effect of lectins on the bioelectric parameters of RCECs was examined by incubating RCECs with lectins for 2 h then evaluating the change in both the transepithelial electrical resistance (TEER) and the potential difference (PD). The TEER and PD were measured using a Voltohmmeter electrode (EVOM,

Table I. Model Plant Lectins and Their Affinity Parameters in RCEC*

Lectin name	Source and abbreviation	Mw (kDa)	Sugar specificity	V_{max} (nM/mg protein)	K_m (μM)
Solanum tuberosum	Potato (STL)	100	*N*-actyl glucosamine oligomers	52.3 ± 2.33	0.39 ± 0.06
Lycopersicon esculentum	Tomato (TL)	100	*N*-acetyl glucosamine oligomers	15.0 ± 0.76	0.48 ± 0.07
Ulex europaeus 1	Gorse (UEA-1)	63	α-L-fucose	53.7 ± 2.19	4.81 ± 0.53

* Values represent mean ± SEM (n = 6). V_{max} and K_m values for STL in MDCK cells (chosen as control) were 37.1 ± 5.9 nM/mg protein and 0.38 ± 0.14 μM, respectively.

World Precision Instruments, Sarasota, FL, USA). The cell layer resistance was obtained by subtracting the total resistance from the blank filter resistance, corrected for the specific Transwell surface area, and expressed as $\Omega \cdot cm^2$. Similarly, the PD was calculated and expressed as mV. Binding and uptake data are presented as mean ± SEM (n), where n is the number of observations.

Confocal Microscopy

We used confocal laser scanning fluorescent microscopy to confirm the binding and internalization of lectins in RCECs. Briefly, RCECs were incubated for 30–60 min at 37°C with 1 μM FITC-STL, fixed with 3.7% formaldehyde for 20 min, permeabilized with 0.5% Triton-X 100 for 15 min, treated with 0.1 mM TRITC-phalloidin for 45 min, mounted on a cover slide using Prolong anti-fade kit (Molecular Probes Inc., Eugene, OR, USA), and viewed under the confocal microscope. In another set of experiments to confirm the specificity of lectin internalization/binding in RCEC rather than the fluorophore FITC, RCECs were incubated at 37°C for 1 h with FITC-STL in the presence of excess amount of the inhibitory sugar, chitin, at 30 mg/ml. After RCECs were permeabilized, they were stained with fluorescent TRITC-phalloidin at 1 μM for 40 min to label actin filaments around the cell periphery. Confocal images (X-Y and X-Y gallery) of stained RCECs were viewed using FITC and TRITC filters on a Zeiss 510 LSM microscope (Carl Zeiss, Thornwood, NY, USA).

RESULTS

The average transepithelial electrical (TEER) and potential difference (PD) values for RCEC culture (day 6 and 7) obtained in all uptake experiments were 0.90 ± 0.18 $k\Omega \cdot cm^2$ and 2.9 ± 0.85 mV, respectively. Treatment of RCECs with lectin during uptake/binding did not significantly alter the TEER and PD values (data not shown).

Characteristics of Lectin Binding and Uptake

As shown in Table I, three model plant lectins with variable molecular weight and substrate specificity were chosen for this study. Figure 1 shows that the binding and uptake of all three lectins in RCECs was time-dependent (reaching a plateau at 1–2 h period). Because most reported studies used 1-h period to evaluate lectin binding, we have chosen an incubation period of 1 h for all further binding/uptake studies. The binding/uptake of STL was first studied in MDCK cells (as a control) at 37°C to evaluate its binding parameters (V_{max} and K_m) and the suitability of chitin as a lectin binding/uptake inhibitor at the concentration used. As shown in Fig. 2A, the specific binding/uptake of STL to MDCK cells was concentration dependent with V_{max} and K_m values of 37.1 ± 5.9 nM/mg protein and 0.38 ± 0.14 μM, respectively. The specific STL binding/uptake curve was deduced by subtracting the nonspecific binding/uptake obtained in the presence of excess chitin from the total binding/uptake obtained in the absence of chitin. As shown in Fig. 2B, the specific binding/uptake of the three plant lectins to RCECs increased in a concentration-dependent manner when incubated at 37°C and followed a Michaelis-Menten equation fit. The specific binding/uptake curves were similarly deduced as in Fig. 2A

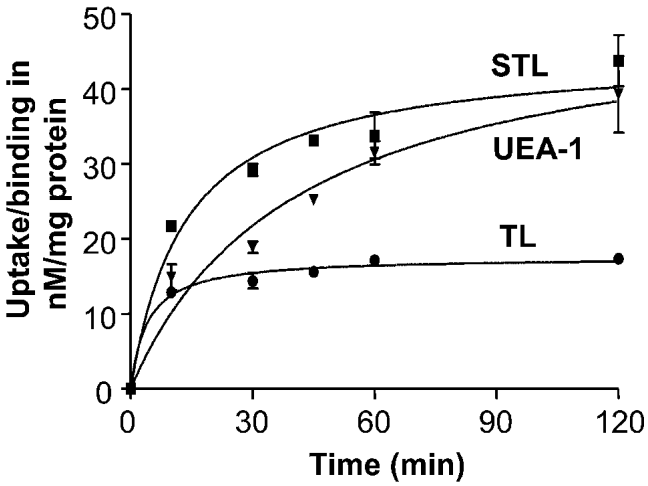

Fig. 1. Effect of incubation time on uptake/binding of FITC-lectins in RCECs. The concentrations of FITC-STL and FITC-TL used were 0.5 and 0.71 μM, respectively, whereas the concentration of FITC-UEA-1 was 3.17 μM. Uptake/binding of lectins was carried out at 37°C up to 2 h period. Symbols (■, ●, ▼) represent mean ± SEM (n = 6).

(total and non-specific curves not shown). As shown in Table I, STL and TL had higher affinity constants (K_m values corresponding to 0.39 and 0.48 μM, respectively) than UEA-1 lectin, whereas STL and UEA-1 had higher maximal uptake/binding values (V_{max} corresponding to 52.3 and 53.7 nM/mg protein, respectively) than TL. Figure 3A shows that all three lectins display temperature-dependent uptake, with higher cell association at 37°C than at 4°C. The uptake difference between the two temperatures represents the internalized (endocytosed) amount of lectins in RCECs (Fig 3B). STL had the highest amount of internalization and the optimal affinity parameters of the three lectins in 1 h uptake/binding study. Therefore, STL was considered the best lectin for future ocular studies.

Confocal Microscopy

Confocal microscopy demonstrates lectin binding and internalization into RCECs. Figure 4A shows that STL staining (green) was abundant in the subapical and intermediate layer of RCECs (see also Fig. 4E). STL was distributed on the cell periphery, below the cell membrane, and in the cytoplasm (either in a diffused or punctate manner). STL staining was absent when RCECs were simultaneously treated with chitin (at 30 mg/ml) for 1 h, indicating that it is due to lectin uptake/binding rather than FITC staining (Fig. 4B). STL stain co-localized with phalloidin (red stain), indicating that it was staining the cell membrane (Fig. 4D). Figure 4C is a confocal image of RCECs treated with phalloidin only (red stain, as control). Figure 4E is an x-y confocal image gallery of RCEC layers sectioned at 0.5-μm increments. It shows the distribution and staining of both STL (green) and phalloidin (red) stain in both the subapical and intermediate layers of RCECs.

DISCUSSION

Summary of Findings

Our studies determined the binding/uptake characteristics of three model plant lectins in a rabbit conjunctival epi-

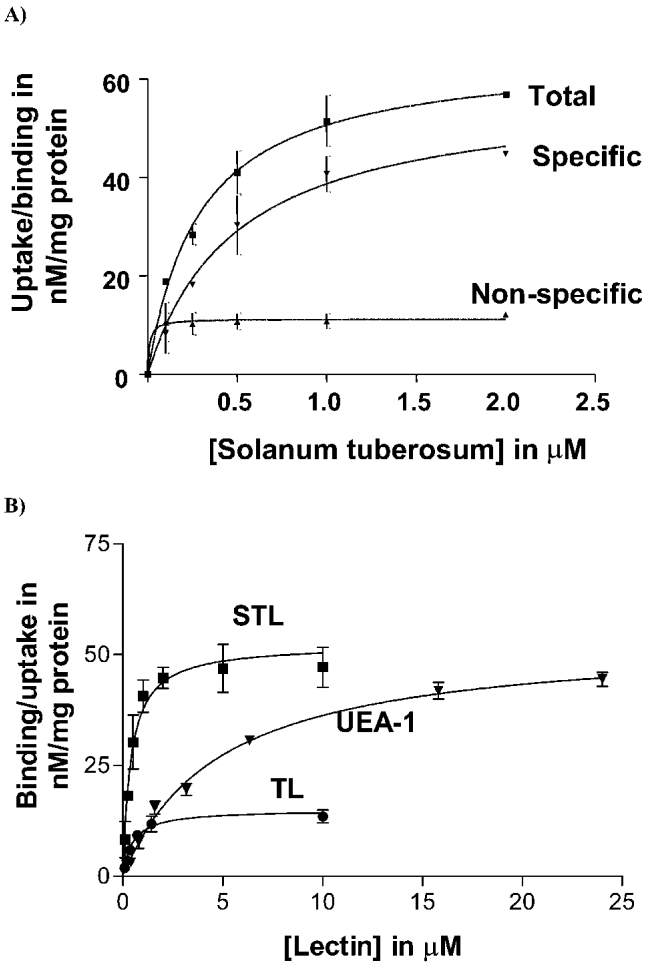

Fig. 2. Effect of concentration on specific binding/uptake of FITC-lectins in RCECs. (A) MDCK were incubated at 37°C for 1 h using various concentrations for STL ranging from 0.1 to 2 μM, in the presence or absence of the inhibitory sugar chitin (30 mg/ml), to obtain the total and nonspecific binding/uptake curves as described in the "Materials and Methods" section. Symbols are (■) total, (▼) specific, and (▲) nonspecific values and represent mean ± SEM (n = 6). B) RCECs were incubated for 1 h with various concentrations of FITC-lectins (STL, TL, and UEA-1) ranging from 0.1 to 31.6 μM at 37°C, in the presence or absence of an inhibitory sugar, to obtain specific binding/uptake for each lectin as described in the "Materials and Methods" section (nonspecific and total binding/uptake curve not shown). Symbols (■, ●, ▼) represent mean ± SEM (n = 6).

thelial cell culture model. These three lectins differ in their molecular weight and in their sugar specificity, with UEA-1 binding to L-fucose residues, and TL and STL binding to *N*-acetylglucosamine oligomer residues. Cytochemical and immunofluorescence studies have shown that both UEA-1 and STL display intense and rapid binding on the conjunctival epithelium surface (13,14). Tomato lectin was chosen in this study to evaluate whether lectins with similar sugar specificity and size (with regards to STL) have similar uptake/binding pattern. We have shown that all three lectins (STL, TL, and UEA-1) display a time-dependent binding/uptake pattern at 37°C in RCECs, with a maximal binding/uptake occurring at around 1–2-h period, depending on the type of lectin. We have also shown that these three lectins exhibit specific, saturable binding/uptake pattern in RCECs, with affinity constants ranging from 0.39 to 4.81 μM. This binding/uptake was specific, as supported by the marked inhibition in the presence of competing sugars such as *N*-acetylglucosamine and L-α-fucose. Of the three lectins, STL displayed optimal binding/uptake potential to RCECs, with favorable affinity parameters (K_m of 0.39 ± 0.06 μM and V_{max} of 52.3 ± 2.3 nM/mg protein). We ranked the lectins' binding affinity to RCECs as STL > TL > UEA-1. The higher V_{max} uptake/binding value for potato lectin compared to tomato lectin could be attributable to differences in the molecular structure and conformation of the two lectins. It is interesting to note that UEA-1 lectin, the smallest of all lectins used that binds to L-fucose residues, has a similar V_{max} value to STL but a lower K_m value than either STL or TL. This supports the notion that other factors are involved in the uptake/binding of these lectins. In addition, all three lectins demonstrated temperature dependence in their uptake/binding pattern. STL and UEA-1 were endocytosed to a greater degree than TL. The ranking of their capacity for internalization was STL > UEA-1 > TL. Lectin internalization could be facilitated by additional protein-protein interaction between the lectin and membrane proteins. This is very likely, as chitin (the competing oligosaccharide) inhibited lectin uptake up to 60–85%, indicating that a fraction of the lectin binding to the cell sur-

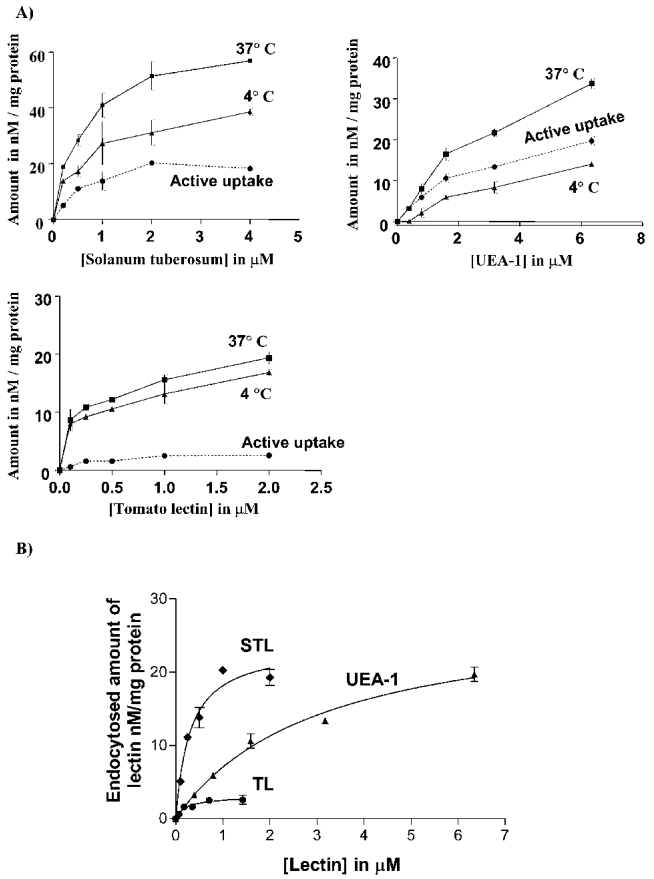

Fig. 3. Amount of FITC-lectin internalized in RCECs. Uptake of various concentrations of FITC-labeled lectins (with a similar range as that done at 37°C in RCECs) was carried at 4°C for 1 h The uptake/binding curve obtained at 37°C was then subtracted from that at 4°C to obtain the active component of uptake (amount of lectin internalized), which is expressed as nM lectin/mg protein. Symbols (■, ●, ▼) represent mean ± SEM (n = 6).

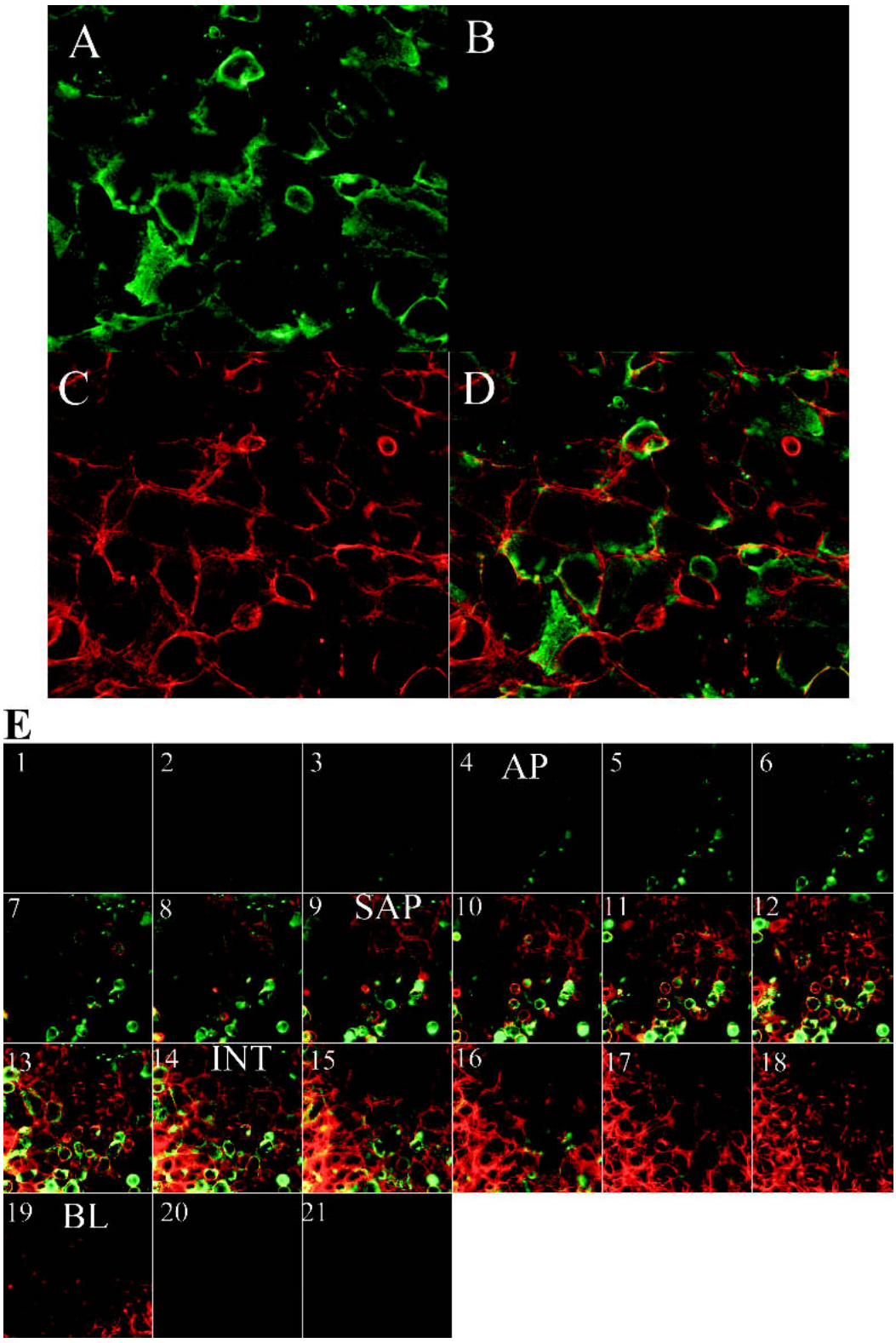

Fig. 4. Confocal microscopy image of RCECs after STL uptake. Confocal x-y image of RCECs following 1 h incubation with FITC-STL (green). (A) Control; (B) in the presence of chitin (inhibitory sugar); (C) shows RCECs stained with phalloidin only (red); (D) shows double stain image of both (A) and (C) in RCECs; (E) represents an x-y gallery image of RCECs stained with FITC-STL and phalloidin.

face is mediated by nonglycosidic interactions. The binding and internalization of STL (potato lectin) was also confirmed using confocal microscopy, with STL staining mostly distributed at the cell membrane and in a diffuse and punctate manner in the cytoplasm. PD and TEER values did not change in RCECs following a 2-h incubation with lectins, which excludes the perturbation of tight junctions and ion transport properties by lectins.

Lectins Binding in Other Epithelial Cells

Lehr and co-workers (22) have shown that lectin binding to isolated pig enterocytes was dependent on the incubation time reaching equilibrium in 1 h. This is consistent with our findings concerning the uptake/binding of three lectins in RCECs, although certain lectins reached binding equilibrium at 2-h period. In contrast, Wirth *et al.* (23) reported that binding of STL to the carcinoma cell lines HT-29 and Caco-2 cells was independent of incubation time up to 4 h. This difference could be attributed to slow binding kinetics of STL to these cell lines or to a negligible number of binding sites in these cell types. This is a plausible explanation, as differences in the binding capacity of the same lectin to various epithelial cell types have already been reported. For instance, Tuori *et al.* (24) showed that lectins such as *Griffonia simplicifolia* agglutinin-I-B_4 and *Ricinus communis* agglutinin had marked reactivity toward the conjunctival epithelium compared with the corneal epithelium. The nonlinear binding/uptake pattern for lectins is consistent with that reported for Caco-2 cells and human alveolar epithelial cells (25,26). The affinity constant values for lectins obtained in these cell systems are comparable to those obtained in RCECs. For instance, Haltner *et al.* (25) reported K_m value for TL in the range 1–2 μM in Caco-2 cells. In these studies, lectins displayed both temperature dependence and temperature independence. The absence of temperature dependence in some lectins was explained either by lack of endocytosis (only binding) or by transport of the internalized lectins into acidic intracellular compartments, where they were degraded. Indeed, accumulation of wheatgerm agglutinin-BSA conjugate within lysosomal compartments followed by subsequent proteolytic degradation was demonstrated in Caco-2 cells (27). In our findings, there was no significant difference in TL cell association at both 37°C and 4°C, indicating that the lectin may have been degraded within acidic compartments in RCECs. In fact, Lehr and coworkers (22) showed that tomato lectin binding to isolated, fixed pig enterocytes was reduced in an acidic environment. Thus, the greater amount of STL that was accumulated in RCEC compared to other lectins points to its higher endocytic rate and intracellular stability. We have measured the uptake/binding of STL at lower pH (pH range 4–5) and did not notice any significant difference in binding/uptake in RCECs (data not shown). Several investigators have demonstrated the binding and internalization of lectins in various epithelial cells using confocal microscopy (19,28). These lectins were shown to stain the plasma membrane and cytoplasm, with some intensely stained granular regions indicative of vesicular endocytosis. Our confocal microscopy findings with STL staining of RCECs are in agreement with these reports. Lectin endocytosis may be occurring by adsorptive endocytosis as suggested by Lehr and Lee (29) for tomoto lectin in Caco-2 cells.

Earlier studies with tomato lectin showed that it stimulated immune response in mice following oral absorption and raised fears about the safety of lectin use in human (30). Recently, in *in vivo* studies, no evidence of irritancy was observed when STL was instilled into the eye of rats or injected intradermally into rabbits (14,15). This is in concert with our observation of no change in the TEER and PD of RCECs during uptake/binding studies with STL, indicating that it has no deleterious effect on tight junctions and ion transport properties. In addition, we have supporting data indicating lack of cytokine induction by STL (in comparison with histamine as a positive control) following 24-h treatment of RCECs (data not shown here)

Several investigators successfully used UEA-1 lectin to target histochemical markers (HRP and biotin) to mouse intestinal M cells and enhance their subsequent absorption across intestinal epithelial barrier (31,32). The authors postulated that lectins could serve as "receptors" for targeting of lectin-antigen conjugates to the mucosal immune system. In addition, Wirth *et al.* (28) demonstrated increased adhesion/transport of doxorubicin in Caco-2 cells following conjugation to wheatgerm agglutinin lectin. Furthermore, lectin conjugates were demonstrated to deliver and express a reported gene in human airway epithelial cells (33). In the eye, the only report to our knowledge is by Schaeffer *et al.* (16), in which ganglioside-containing liposomes with entrapped carbachol enhanced carbachol flux across isolated rabbit corneas when pretreated with wheat-germ agglutinin. Because potato lectin was shown to prolong drug retention in the eye and was well tolerated, we believe that our future conjugation studies with drug-loaded nanoparticles will shed significant findings for its role in ocular drug delivery.

CONCLUSIONS

Of the three lectins studied, *Solanum tuberosum* lectin (potato) appears to be the best candidate for further examination. This is attributed to optimal binding properties, greater endocytic capacity, and good tolerance in conjunctival epithelial cells. In addition, this lectin displayed prolonged retention to rabbit conjunctival surface as observed by Nicholls *et al.* (14). These findings should set the stage for further studies aimed at usingg *Solanum tuberosum* lectin as a coating agent or conjugates of biodegradable PLGA nanoparticles to promote their absorption of therapeutic drugs into the conjunctival epithelium and the anterior segments of the eye.

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