Lectin-mediated bioadhesion: binding characteristics of plant lectins on the enterocyte-like cell lines Caco-2, HT-29 and HCT-8

Franz Gabor*, Martina Stangl, Michael Wirth
Institute of Pharmaceutical Technology, the University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Received 2 December 1997; accepted 3 February 1998

Abstract

In order to take advantage of the biorecognition between lectins and specific carbohydrates for targeted drug delivery, fluorescein-labelled lectins of different carbohydrate specificities were screened for binding to human colorectal carcinoma cell lines by flow cytometry and confocal microscopy. The lectin-binding rate increased as follows: Dolichos biflorus agglutinin, DBA<peanut agglutinin, PNA<Lens culinaris agglutinin, LCA<Solanum tuberosum lectin, STL<Ulex europaeus isoagglutinin I, UEA-I<wheat germ agglutinin, WGA (Caco-2); PNA<UEA-I<WGA (HT-29); DBA<UEA-I<WGA (HCT-8), thus reflecting the glycosylation pattern of the cells. Compared to the BSA-binding capacity of the cells, the extent of nonspecific binding was strongly dependent on the type of cell line and lectin under investigation being lower than 2% in the case of WGA, STL and UEA-I/Caco-2 and HT-29 cells. Whereas 50% of DBA was bound nonspecifically to Caco-2 cells, the interactions DBA/HCT-8 and PNA/HT-29 were due to nonspecific binding. By competitive inhibition of lectin-adhesion to the cells upon addition of the complementary carbohydrate, specificity of lectin-binding was confirmed except for the interaction of DBA/HCT-8 and PNA/HT-29. Following on from this work, we consider WGA-, STL- and UEA-I-mediated drug delivery to be a promising approach for peroral bioadhesive formulations adhering to absorptive enterocytes. © 1998 Elsevier Science BV. All rights reserved.

Keywords: Caco-2; HT-29; Lectin; Bioadhesion; Flow cytometry

1. Introduction

At the present, one of the most promising strategies for successful delivery of perorally administered drugs is the concept of bioadhesion. Due to the intimate contact and the fixation of bioadhesive drug delivery systems at the mucosal epithelium the diffusion pathway of the drug is shortened, its dilution by luminal liquids is reduced and the drug concentration gradient is increased. Thus enhancement of the absorption rate is thought to result in improved bioavailability.

In the case of buccal, nasal, rectal and vaginal administration the feasibility of the concept was confirmed by mucoadhesive formulations composed of hydrophillic polymers such as polyacrylic acid and cellulose derivatives [1]. Studies with oral mucoadhesive drug delivery systems were less successful due to the short turnover time of the mucus layer ranging from 90 to 240 min [2] and inactivation of the formulation by shed-off mucus prior to the contact with the mucosal epithelium [3,4].

Since the mucus gel layer acts as a link between

*Corresponding author. Tel.: +43 1 313368041; fax: +43 1 31336779; e-mail: franz.gabor@univie.ac.at
the absorptive epithelium and the formulation alone, a second generation of bioadhesives was proposed that directly adhere to the absorptive enterocyte in a specific manner like a lock–key mechanism [5]. In this context, especially, nontoxic lectins of plant origin being part of the regular diet of man gained attention in the field of bioadhesion. Lectins are known to be proteins of nonimmune origin that are capable of binding to particular oligosaccharides [6]. Taking advantage of this specific interaction between lectin and peculiar oligosaccharide moieties being part of the glycocalyx of the absorptive enterocyte, lectins appear to be attractive carriers for oral drug delivery.

Hitherto, tomato lectin (TL) from *Lycopersicum esculentum* was found to bind specifically to both monolayers of human Caco-2 cells and isolated porcine enterocytes [7]. Despite significant cross-reactivity with pig gastric mucin, the uptake rate of TL in rat gut tissue was found to be 11-times higher than bovine serum albumin and 20-times higher than PVP indicating improved absorption of a conjugated drug [8]. Also, mucin-binding of TL–polystyrene latex conjugates was investigated resulting in an interaction two-times stronger than bovine serum albumin–polystyrene conjugates [9,10].

Since there is less knowledge about the interaction between lectins and enterocytes, it is the purpose of the present paper to outline the binding characteristics of a panel of selected lectins with different carbohydrate-specificity. Cell-binding of the N-acetyl-galactosamine-specific lectin from horse gram seeds (*Dolichos biflorus* agglutinin, DBA) and the α-1-fucose-specific lectin from furze seeds (*Ulex europaeus* isoagglutinin I, UEA-I) was investigated, since these lectins selectively adhered to rabbit cecal M cells [11]. As N-acetyl-glucosamine containing glycoproteins were isolated from the brush border membranes of rat small intestinal cells [12], wheat germ agglutinin (WGA) from *Triticum vulgare* and *Solanum tuberosum* lectin (STL) from potato tubers were chosen. Both, peanut agglutinin (PNA) from *Arachis hypogea* exhibiting galactosamine-specificity as well as the lentil lectin from *Lens culinaris* (LCA) recognizing α-mannose-containing oligosaccharide moieties were also included since adherence of *Salmonella typhimurium* to the intestinal epithelium is correlated with the presence of binding sites for these lectins [13,14].

To investigate the lectin binding-characteristics of intestinal cells, studies were performed on three in vitro cell culture systems derived from human colon carcinomas, the Caco-2, the HT-29 and the HCT-8 cell line. The Caco-2 cell line exhibits structural and functional features similar to intestinal epithelial cells and it is the most popular model in drug discovery and development [15]. In comparison to Caco-2 cells, HT-29 cells are less differentiated but they form mucus-producing goblet cells upon cultivation in methotrexate-containing medium [16]. When cultured on filter supports, HCT-8 cells also form polarized monolayer developing apical brush border microvilli.

### 2. Materials and methods

#### 2.1. Chemicals

The fluorescein labelled lectins from *Triticum vulgare* (molar ratio fluorescein/protein \(F/P\)=3.2), *Solanum tuberosum* \(F/P=2.9\), *Dolichos biflorus* \(F/P=5.2\), *Arachis hypogea* \(F/P=4.7\), *Ulex europaeus* (Ulex europaeus isoagglutinin I, \(F/P=4.0\)) and *Lens culinaris* \(F/P=6.3\) were purchased from Vector laboratories (Burlingame, USA) and contained >98% active conjugate and no free fluorescein. Tissue culture reagents were from Biowhittaker (Workingham, UK).

N,N’,N”-Triacetylchitotriose, N-acetyl-\(n\)-galactosamine, \(n\)-galactosamine, α-1-fucose and \(n\)-mannose were from Sigma (St. Louis, MO, USA); all other chemicals were of analytical grade and purchased from Merck (Darmstadt, Germany).

#### 2.2. Tissue culture

The colon carcinoma cell lines HT-29, HCT-8 and Caco-2 were obtained from the American Type Culture Collection (Rockville, ML, USA). Cells were grown in culture medium consisting of RPMI-1640 with 10% fetal calf serum, 4 mM \(l\)-glutamine and 75 \(\mu\)g/ml gentamycine in a humidified 5% \(CO_2\)/95% air atmosphere at 37°C and subcultured by trypsination.
2.3. Viability of cells

Prior to each experiment, viability of the cells was determined by trypanblue exclusion by viable cells. Viability of every cell preparation exceeded 85% as determined by counting the stained cells.

2.4. Determination of the lectin-binding capacity of colon carcinoma cells

Using a 96-well microtiter plate, 50 μl of cell-suspension (3×10³ cells) in PBS were resuspended with 100 μl PBS. After addition of 50 μl of a dilution series of labelled lectins in PBS (16, 8, 4, 2, 1, 0.5, 0.25, 0.12 μg/well), cells were incubated overnight at 4°C. Cells were collected by centrifugation (200 g, 5 min) and 130 μl of the supernatant were discarded. After addition of 130 μl PBS, washing was repeated twice in the same manner. Cells were resuspended in 1.8 ml Cell Pack and assayed by flow cytometry.

Negative controls were included in every experiment consisting of unlabelled cells for estimation of autofluorescence. Each concentration was tested in triplicate and the dilution experiments were repeated at least twice in each case.

As a control for estimation of nonspecific binding, aliquots were prepared as above but using a dilution series of fluorescein labelled bovine serum albumin (F/P=12) instead of the lectins.

2.5. Carbohydrate-binding specificity of the lectins

For assessment of carbohydrate-specific binding of the lectins to Caco-2, HT-29 and HCT-8 cells, 50 μl of cell-suspension (3×10³ cells) in PBS, 100 μl of a dilution series of the lectin-specific carbohydrate (0.1–3000 μg, serial dilution) and 50 μl of a solution containing 1 μg STL (HT-29, Caco-2, HCT-8), 0.25 μg WGA (HT-29, Caco-2, HCT-8), 4 μg PNA (HT-29, Caco-2), 2 μg PNA (HCT-8), 8 μg DBA (HT-29, Caco-2), 10 μg DBA (HCT-8), 2 μg LCA (HT-29, Caco-2), 5 μg LCA (HCT-8), 1 μg UEA-I (HT-29, Caco-2) or 3.3 μg UEA-I (HCT-8) were processed as described above.

2.6. Flow cytometry

Flow cytometric measurements were carried out using an Epics XL–MLC analytical flow cytometer (Coulter, FL, USA). Cell-bound fluorescence intensity of the single-cell suspension was determined using a forward versus side scatter gate for the inclusion of single cell populations and exclusion of debris and cell aggregates. Fluorescence was detected at 515 nm (10 nm bandwidth) and the mean channel number of the logarithmic fluorescence intensities of individual peaks was used for further calculations. Amplification of the fluorescence signals was adjusted to put the autofluorescence signal of unlabelled cells in the first decade of the four-decade log range resulting in a gain of 570 for each experiment. For each measurement 5000 cells were accumulated.

2.7. Confocal microscopy

Cells were stained by incubation of 150 μl cell-suspension (2×10⁵/ml PBS) with 150 μl solution of the labelled lectin (100 μg/ml PBS) for 1 h at 4°C. Cells were spun down (5 min, 4°C, 200 g), washed twice as described above but using 200 μl PBS and mounted for microscopy. Confocal images of fluorescent labelled cells were obtained using a Zeiss Axiovert confocal microscope. Transmission light and fluorescence pictures were acquired at 40× magnification and the black level (background offset) of the green fluorescence detector was adjusted to eliminate any autofluorescence of unstained cells.

3. Results

3.1. Lectin-binding capacity of colon carcinoma cell lines

For potential use of plant lectins in bioadhesive devices, their interaction with Caco-2, HT-29 and HCT-8 cells was investigated by both flow cytometry and confocal microscopy.

In order to estimate the lectin-binding capacity of the cells, increasing amounts of fluorescent labelled lectins were allowed to interact with a fixed number of cells. Only the amount of cell-associated lectin was estimated by flow cytometric detection of the fluorescein conjugated analogues.

Upon incubation of increasing amounts of the lectins with the cells, the mean cell-associated fluorescence intensity increased independent of the type
of cell line and lectin (Fig. 1). Binding of increasing amounts of WGA to HT-29 cells resulted in increasing cell-bound fluorescence intensity ranging from 0.27±0.0 to 195.4±0.3 (mean±SD, n=3) as compared to the autofluorescence of HT-29 cells yielding 0.2±0.01 (n=18). Even a low lectin binding rate to HT-29 resulted in a rather high shift of relative fluorescence intensity from 0.23±0 to 5.51±0.3 at

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

Fig. 1. Saturation analysis of lectin-binding sites on Caco-2 (A), HT-29 (B) and HCT-8 cells (C) with fluorescein-labelled lectins in comparison to BSA related to an apparent F/P ratio=1 (mean±SD, n=3).
maximum UEA-I binding to HT-29 cells. Similarly, HCT-8 associated relative fluorescence intensity increased from 0.53±0 to 112.7±3.3 upon incubation with labelled WGA in comparison to 0.16±0.02 as derived from the autofluorescence of HCT-8 cells. The autofluorescence of Caco-2 cells was estimated to be 0.17±0.02 in contrast to the increasing relative fluorescence intensity ranging from 0.31±0 to 351.44±20 upon increasing WGA-binding and 0.17±0 to 2.05±0.3 upon increasing DBA-binding.

This concentration dependent adhesion might be attributed to specificity of interaction, but the amount of cell-bound lectin is quite different. Considering the different extents of labelling of each individual lectin, for comparison of the data obtained the mean relative cell-associated fluorescence intensity of each individual lectin was related to an apparent conjugation number of 1 mol fluorescein/mol lectin. In general, the WGA-binding capacity of all cell lines exceeded that of all other lectins under investigation by far. As calculated from the saturation experiments (Table 1), the WGA-binding capacity of Caco-2 cells exceeded that of HCT-8 cells by about three times. The amount of HT-29 bound WGA was about 1.7-fold higher than that of HCT-8 cells. In comparison to cell-binding of WGA (100%), the amount of cell-associated STL diminished by 50% (HT-29), 76% (Caco-2) and 89% (HCT-8), respectively. Considerably high lectin-binding to colon carcinoma cells was also observed in the case of UEA-I as indicated by 34% (Caco-2) and 37% (HCT-8) of the WGA-binding capacity. The LCA-binding capacity of the cells was considerably lower and was found to be 9% (Caco-2), 8% (HT-29) or 12% (HCT-8) of cell-bound WGA. The amount of cell-bound lectin of both DBA and PNA was determined to be lower than 5% of the WGA-cell interaction.

The results of saturation analysis of lectin receptors located at the cell surface of the carcinoma cells were confirmed qualitatively by confocal microscopy of the cells preincubated with fluorescein-labelled lectins. For detection of membrane-bound lectin, cells were incubated for 1 h at 4°C in order to minimize metabolic activity of the cells and to prevent possible internalization of cell-bound lectins. In accordance with the rather low interaction of DBA with the cells as determined by saturation analysis, Caco-2 cells lacked fluorescent staining with labelled DBA (Fig. 2). Upon preincubation of Caco-2 cells with labelled PNA, a dot-like, small fluorescent ring was observed on the fluorescent image, which refers to the membrane section, as could be seen from the transmission image. Accordingly, the membrane area of Caco-2 cells was intensively stained by fluorescein-conjugated WGA indicating a rather strong interaction between the cell surface and the N-acetylglucosamine binding lectin.

### Table 1

<table>
<thead>
<tr>
<th>Lectin</th>
<th>HT-29 cells</th>
<th>Caco-2 cells</th>
<th>HCT-8 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>WGA</td>
<td>30.55</td>
<td>54.87</td>
<td>17.62</td>
</tr>
<tr>
<td>STL</td>
<td>15.34</td>
<td>13.04</td>
<td>2.00</td>
</tr>
<tr>
<td>PNA</td>
<td>0.03</td>
<td>3.33</td>
<td>0.85</td>
</tr>
<tr>
<td>DBA</td>
<td>0.60</td>
<td>0.39</td>
<td>0.10</td>
</tr>
<tr>
<td>LCA</td>
<td>2.41</td>
<td>5.00</td>
<td>2.20</td>
</tr>
<tr>
<td>UEA-I</td>
<td>0.69</td>
<td>18.46</td>
<td>6.57</td>
</tr>
</tbody>
</table>

WGA (wheat germ agglutinin), STL (*Solanum tuberosum* lectin), PNA (peanut agglutinin), DBA (*Dolichos biflorus* agglutinin), LCA (*Lens culinaris* agglutinin), UEA-I (*Ulex europaeus* iso-agglutinin I).

3.2. Specificity of binding

To estimate the extent of lectin-binding to the cells due to nonspecific protein–cell interactions, the colon carcinoma cells were incubated with a dilution series of fluorescein labelled bovine serum albumin.

Assuming that the BSA-binding capacity of HT-29 cells represents the maximum nonspecific interaction between HT-29 cells and proteins, 13% of DBA-, 5% of LCA-, 0.3% of WGA-, 0.8% of STL- and 14% of UEA-I-binding rate to HT-29 cells is derived from nonspecific protein-binding (Table 2). Since the binding rate of PNA is lower than that of BSA, the PNA/HT-29 interaction is due to nonspecific interaction. In a similar way binding of DBA to HCT-8 cells is attributed to a nonspecific binding mechanism (Fig. 1). On the other hand, very low contribution of nonspecific interactions to lectin-binding to colon carcinoma cells was observed exhibiting 0.1% (WGA/Caco-2), 0.4% (UEA-I/Caco-2), 0.5% (STL/Caco-2), 0.6% (WGA/HCT-8), 1.5% (LCA/Caco-2) and 1.8% (PNA/Caco-2, UEA-I/HCT-8) of
total lectin binding to the colon carcinoma cells. Nonspecific binding exerts higher influence on the HCT-8 binding of WGA, LCA and PNA as indicated by the extent of nonspecific interaction of 6, 8 and 12%, respectively, whereas 50% of DBA was bound to Caco-2 cells nonspecifically.

Specificity of lectin-binding to the colon carcinoma cells was investigated by competitive inhibition of the lectin-binding to the cell surface. Depending on the amount of the corresponding carbohydrate added, the carbohydrate-combining site of the lectin is blocked partially and inhibited from binding to the cell surface. According to preliminary experiments and in order to establish optimum conditions for the competitive assay, the concentration of the lectins was chosen such that about 50% of maximum lectin-binding to the cells was achieved.

Upon addition of increasing amounts of carbohydrate appropriate for specific inhibition of the lectin-binding to the cells the amount of cell-bound labelled lectins decreased indicating the extent of carbohydrate-mediated binding of the lectins (Fig. 3). Except for the interaction PNA/HT-29 and DBA/HCT-8 the lectins exhibited carbohydrate-mediated binding to the three cell lines under investigation. This result is in accordance with the estimation of the extent of nonspecific binding as the BSA-binding rate was as high as that for PNA and DBA in the case of HT-29 and HCT-8 cells, respectively.

For comparison of the data obtained, the amount of the corresponding carbohydrate was calculated from the plots (Fig. 3) as necessary for 50% inhibition of lectin-binding to the cells (IC_{50}). According to the saturation analysis, the number of WGA-binding sites on Caco-2 cells exceeded that of HT-29 cells followed by HCT-8 cells as confirmed by the slope of the inhibition curve. Due to the low inhibitory potency of N-acetylglucosamine resulting in rather poor decrease of cell-bound fluorescence intensity, N,N',N''-triacetylcobaltotriose was used in
Cell-associated mean relative fluorescence intensities (mean±SD, n=3) after incubation of $3\times10^4$ cells with fluorescence labelled WGA, STL, DBA, LCA, UEA-I and PNA as compared to fluorescent labelled bovine serum albumin (BSA).

<table>
<thead>
<tr>
<th>Micrograms protein/3×10^4 cells</th>
<th>DBA</th>
<th>LCA</th>
<th>PNA</th>
<th>WGA</th>
<th>STL</th>
<th>UEA-I</th>
<th>BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HT-29 cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.21±0.0</td>
<td>0.33±0.0</td>
<td>0.04±0.0</td>
<td>18.4±0.2</td>
<td>0.11±0.0</td>
<td>0.34±0.0</td>
<td>0.04±0.0</td>
</tr>
<tr>
<td>2</td>
<td>0.46±0.0</td>
<td>0.90±0.1</td>
<td>0.04±0.0</td>
<td>28.1±0.3</td>
<td>1.02±0.1</td>
<td>0.55±0.0</td>
<td>0.05±0.0</td>
</tr>
<tr>
<td>4</td>
<td>0.82±0.0</td>
<td>1.86±0.1</td>
<td>0.04±0.0</td>
<td>39.5±0.9</td>
<td>10.93±0.2</td>
<td>0.87±0.0</td>
<td>0.06±0.0</td>
</tr>
<tr>
<td>8</td>
<td>1.21±0.0</td>
<td>3.07±0.3</td>
<td>0.05±0.0</td>
<td>48.8±1.7</td>
<td>20.38±2.4</td>
<td>1.16±0.0</td>
<td>0.16±0.0</td>
</tr>
<tr>
<td><strong>Caco-2 cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.05±0.0</td>
<td>1.28±0.1</td>
<td>3.04±0.3</td>
<td>20.00±0.8</td>
<td>0.10±0.0</td>
<td>10.34±0.1</td>
<td>0.03±0.0</td>
</tr>
<tr>
<td>2</td>
<td>0.08±0.0</td>
<td>3.26±0.1</td>
<td>4.01±0.4</td>
<td>44.54±1.9</td>
<td>0.61±0.0</td>
<td>14.23±1.1</td>
<td>0.03±0.0</td>
</tr>
<tr>
<td>4</td>
<td>0.13±0.0</td>
<td>5.32±0.1</td>
<td>4.92±0.4</td>
<td>72.26±5.4</td>
<td>9.47±0.9</td>
<td>20.64±0.6</td>
<td>0.05±0.0</td>
</tr>
<tr>
<td>8</td>
<td>0.22±0.0</td>
<td>7.25±0.1</td>
<td>5.85±0.5</td>
<td>88.83±7.5</td>
<td>19.30±1.5</td>
<td>25.18±1.5</td>
<td>0.11±0.0</td>
</tr>
<tr>
<td><strong>HCT-8 cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.04±0.0</td>
<td>0.20±0.0</td>
<td>0.75±0.0</td>
<td>6.89±0.3</td>
<td>0.99±0.2</td>
<td>2.32±0.2</td>
<td>0.04±0.0</td>
</tr>
<tr>
<td>2</td>
<td>0.06±0.0</td>
<td>0.60±0.0</td>
<td>1.14±0.0</td>
<td>14.69±1.4</td>
<td>2.25±0.8</td>
<td>5.48±0.4</td>
<td>0.07±0.0</td>
</tr>
<tr>
<td>4</td>
<td>0.08±0.0</td>
<td>1.30±0.1</td>
<td>1.42±0.0</td>
<td>24.66±0.7</td>
<td>3.19±0.8</td>
<td>8.66±0.5</td>
<td>0.10±0.0</td>
</tr>
<tr>
<td>8</td>
<td>0.10±0.0</td>
<td>2.40±0.2</td>
<td>1.66±0.1</td>
<td>31.36±1.6</td>
<td>3.49±0.3</td>
<td>11.13±0.1</td>
<td>0.20±0.0</td>
</tr>
</tbody>
</table>

Considering different fluorescein/protein ratios of labelled lectins and BSA, the data given represent the mean cell-bound fluorescence intensity related to an apparent $F/P$ ratio=1.

Considering different lectins as suitable tools for drug delivery systems, the binding characteristics of a panel of lectins exhibiting different carbohydrate specificities was elucidated using their fluorescence labelled derivatives.

Employing colon carcinoma cells as a model for absorptive enterocytes, an overview of lectin-binding to the cells was attained by confocal microscopy of cells preincubated with labelled lectins. As deduced from the fluorescent images (Fig. 2), the membrane of the cells was stained intensively by the N-acetylgalactosamine-specific lectins WGA and STL. UEA-I, LCA and PNA accumulated at the membrane of the cells to a minor, but still remarkable, extent, whereas DBA-staining of the cells was hard to observe.

The qualitative results of confocal microscopy
were confirmed by quantification of cell-bound lectin upon flow cytometry of the stained cells. The binding rate of the lectins was strongly dependent on the type of cell line used for saturation analysis. By weight, the amount of Caco-2 bound lectin increased in the following manner: DBA (3%)<PNA (25%)<LCA (38%)<STL (100%)<UEA-I (141%)<WGA (420%). Vice versa, the glycosylation pattern of the Caco-2 glycoalyx cells might be described by high amounts of N-acetylglucosamine containing oligosaccharides, followed by fucosyl- and mannosyl- and rather minor amounts of galactosamine- and N-
acetyl-galactosamine-residues. Despite similar carbohydrate specificity of WGA and STL, these lectins exhibited different binding rates to Caco-2 cells. This discrepancy might be due to the fact that WGA is a dimeric protein, each subunit containing two or four carbohydrate-combining sites, whereas the STL-subunit contains only one binding site resulting in lower cell-bound fluorescence intensity. Additionally, STL exists as a monomer–dimer system in aqueous solution [6]. In comparison to Caco-2 bound STL (100% by weight), the lectin-binding pattern of HT-29 cells follows the order of precedence: WGA (234%) > STL (117%) > LCA (18%) > UEA-I (5%) > DBA (4.6%) > PNA (0.2%). Compared to Caco-2
Fig. 3. (continued)

cells, the lectin binding rate of HCT-8 cells decreases by about a third. The amount of lectin-binding sites on HCT-8 cells decreases as follows: WGA (135%) > UEA-I (50%) > LCA (17%) > STL (15%) > PNA (6%) > DBA (0.7%).

Among all lectins and cell lines under investigation, the binding rate of WGA was still the highest followed by UEA-I and STL. Similar results were obtained by staining of fixed enterocytes in rabbit Peyer’s patches [17].

As observed by saturation analysis the relative cell-bound fluorescence intensity increased concurrently with increasing lectin concentration, indicating specificity of interaction between the lectins and the
Table 3
Amounts of complementary carbohydrate (μg) as necessary for 50% inhibition of lectin-binding to 3×10^5 colon carcinoma cells

<table>
<thead>
<tr>
<th>Lectin/competitive carbohydrate</th>
<th>HT-29 cells</th>
<th>Caco-2 cells</th>
<th>HCT-8 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 μg WGA/N,N',N''-triacetylchitotriose</td>
<td>6.2</td>
<td>13.5</td>
<td>2.3</td>
</tr>
<tr>
<td>1 μg STL/N,N',N''-triacetylchitotriose</td>
<td>4.2</td>
<td>2.9</td>
<td>23.1</td>
</tr>
<tr>
<td>1 μg UEA-I/fucose</td>
<td>8.5</td>
<td>88.9</td>
<td>87.13</td>
</tr>
<tr>
<td>4 μg PNA/galactosamine</td>
<td>-</td>
<td>58.8</td>
<td>56.43</td>
</tr>
<tr>
<td>2 μg LCA/mannose</td>
<td>329</td>
<td>427</td>
<td>776</td>
</tr>
<tr>
<td>8 μg DBA/N-acetylgalactosamine</td>
<td>164</td>
<td>201</td>
<td>-</td>
</tr>
</tbody>
</table>

glycocalyx. On assaying specificity of interaction between the colon carcinoma cells and the lectins, two approaches were followed: The amount of labelled bovine serum albumin adhering to the cells was attributed to nonspecific binding. On the other hand, the extent of specific binding was determined by competitive inhibition of the lectin-binding by the complementary carbohydrate.

Using the BSA-binding capacity of the cells as a reference for the extent of nonspecific binding, the interaction DBA/HCT-8 and PNA/HT-29 is a nonspecific one. In contrast, the extent of nonspecific interaction was lower than 1% in the case of WGA/Caco-2, WGA/HT-29, WGA/HCT-8, STL/Caco-2, STL/HT-29, UEA-I/Caco-2 and lower than 2% in the case of LCA/Caco-2, PNA/Caco-2 and UEA-I/HCT-8, respectively. Concerning the rest of lectin-cell interactions, nonspecific binding was found to contribute 5–13% to the total amount of cell-binding with the exception of DBA/Caco-2 yielding 50% (Table 2).

On the other hand, with the exception of the interactions DBA/HCT-8 and PNA/HT-29, adhesion of the lectins to the colon carcinoma cells was found to be specific, since the mean cell-bound fluorescence intensity decreased as the concentration of the complementary carbohydrate increased. But affinity of the lectins to the glycocalyx of the cells was quite different, as expressed by the amount of carbohydrate necessary for 50% inhibition of lectin-binding to the cells. Referring to the specific monosaccharide for comparison of lectin-affinity to the cells, the affinity of the N-acetylglucosamine-combining lectins WGA and STL is at least three orders of magnitude higher than that of the other lectins under investigation, but the exact value still being dependent on the type of cell line used (Table 3). In contrast to the rather low LCA-binding capacity of the colon carcinoma cells, the affinity of LCA was found to be higher than that of DBA and UEA-I, followed by PNA.

According to these observations, the N-acetylglucosamine specific lectins WGA and STL as well as the fucose-combining lectin UEA-I provide for promising tools for lectin-mediated drug delivery. They exhibit low nonspecific proteinaceous interactions with the surface of the enterocytes and high affinity to the carbohydrate-combining sites to oligosaccharides located at the cell surface. Moreover the WGA-, STL- and UEA-I-binding capacity of the colon carcinoma cells was the highest of the lectins under investigation and seems to be sufficient for utilization in drug delivery. Further criteria such as toxicity and gastrointestinal stability must be considered when selecting lectins as specific bioadhesive excipients. As wheat germ contains about 300 mg WGA/kg [18] and about 50 mg STL was isolated from 1 kg of potato tubers [19], peroral toxicity might be negligible. But at present, there is no information available about peroral toxicity of UEA-I. All lectins under investigation were found to resist proteolytic degradation as determined by SDS-PAGE [20]. In view of the fact that specific adhesion of WGA and STL on the surface of Caco-2 and HT-29 cells is followed by intracellular accumulation of the lectins [21], this trafficking offers new perspectives for drug delivery. Recently, the bioadhesive and endocytic potential of TL was confirmed by translocation of 23% of orally administered TL-conjugated nanoparticles into the systemic circulation. In contrast, the systemic uptake was 0.5% in the case of hapten-blocked nanospheres [22]. But a problem possibly encountered with peroral administration of lectins that has still not been elucidated might be the interaction with the mucous gel layer covering the absorptive enterocytes.
5. Conclusion

The interaction of WGA, STL and UEA-I with the enterocyte-like colon carcinoma cell lines is characterized by low nonspecific binding as compared to BSA, high specific binding and affinity due to the carbohydrate-combining activity of the lectins and a high density of WGA-, STL- and UEA-I-binding sites on the surface of absorptive enterocytes. Additionally, proteolytic stability of the lectins permits peroral administration of lectin-containing cytoadhesive formulations. Being part of the regular diet of man, WGA and STL are without any obvious toxic effects, whereas peroral UEA-I-toxicity was not examined up to now. According to the cytoinvasive behaviour of WGA, STL and UEA-I, new perspectives for successful therapy are offered by means of these lectins in drug delivery devices.

Acknowledgements

The authors are grateful to Dr. G. Hamilton, University Clinic of Surgery, the University of Vienna, for kind permission of free disposal to the flow cytometer.

References

[16] T. Lesuffleur, A. Barbat, E. Dussaulx, A. Zweibaum, Growth adaption to methotrexate of HT-29 human colon carcinoma cells is association with their ability to differentiate into columnar absorptive and mucus-secreting cells, Cancer Res. 50 (1990) 6334–6343.