Morphological Changes of Rat Small Intestine After Short-Time Exposure to Concanavalin A or Wheat Germ Agglutinin

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ABSTRACT. Dietary lectins of gluten origin have been suggested to play an important role in the mechanisms leading to the characteristic morphology of the intestine found in patients with celiac disease. To further explore this issue we have used Wheat Germ Agglutinin (WGA) or Concanavalin A (Con A) to challenge rat small intestine and study the ultrastructural changes of such a treatment. Both lectins affected the enterocytes at the base of the villi more than those at the top. The morphological findings included disarrangement of the cytoskeleton, increased endocytosis and shortening of the microvilli. The interrelationship between the observed changes, and their relevance for similar morphological alterations found in patients with celiac disease are discussed. In conclusion, the morphological findings in our rat model resemble early changes in patients with celiac disease, thus supporting the idea that lectins or lectin-like substances are involved in the pathogenesis of this disease.

Plant lectins have been shown to bind specifically to glycoconjugates on cell membranes, including those of the intestinal mucosa (4). This gives them a role as potential activators of cellular processes as well as mediators of cellular damage (8, 14, 16). Plant lectins are also common components of our diet, thus enabling them to have various effects, primarily on the enterocytes (6). In accordance with this Weiser and Douglas proposed in 1976 that lectins could be responsible for the events leading to gluten intolerance (22). This idea is further supported by a number of different observations: 1) The finding that there is a modification of receptor sites for Wheat Germ Agglutinin (WGA) and Concanavalin A (Con A) in the small intestinal mucosa of patients with celiac disease (10). 2) A recent report demonstrating that a WGA-like lectin is a constituent of gluten and that patient with celiac disease have raised serum antibody levels to WGA (12). 3) Experimental studies pointing out similarities between the intestinal morphology of patients with celiac disease and the effects observed on organ cultures and rat intestines after exposure to lectins (9, 11, 14, 17). 4) We have showed that short-time exposure to WGA or Con A gave intestinal permeability changes mimicking those found in patients with celiac disease (19). The present study is a continuation of the latter work and presents the morphological changes of the rat intestinal mucosa. The morphological findings in this study further support the idea of lectins or lectin-like substances being involved in the pathogenesis of celiac disease.
MATERIALS AND METHODS

Animals and preparation of the intestine. Female, Sprague-Dawley rats (200 g, Anticimex, Stockholm, Sweden) were starved for 24 h before starting each experiment. The animals were anesthetized by an intraperitoneal injection of Ketalar/Rompun (4 : 1; 0.2 ml/100 g body weight) from Parke-Davis, England and Bayer AB, Sweden, respectively. The abdomen was opened up with a midline incision and the intestinal content gently pushed aside from a 10 cm-segment of the jejunum, 2–3 cm from the cecum. This segment was ligated with silk at both ends. Special care was taken to prevent bleeding from the intestine and to maintain a normal body temperature and dampness. In the control group 1 ml of Krebs-Ringer phosphate buffer with Ca²⁺, Mg²⁺ and 10 mM glucose, pH 7.3, (KRG) was instilled into the ligated part of the intestine. In the test situations, 1 mg of either Concanavalin A (Con A) from Pharmacia Fine Chemicals, Uppsala, Sweden or 1 mg of Wheat Germ Agglutinin (WGA) from Sigma, St. Louis, MO, USA was dissolved in the same volume of KRG medium and instilled into the ligated part of the intestine. The specificity of the Con A and WGA induced changes have been examined using mannan and β-chitobiose, respectively (data not shown). In all groups (6–12 animals), differently sized permeability markers were introduced together with the lectins. The effects of lectins on the intestinal mucosal permeability have been published elsewhere (19). 60 min after introduction of the lectins, 3–4 samples were cut out from different parts of each ligated intestine for further analysis with an electron microscope.

Electron microscopy. The intestines were fixed with 4 % (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at +4°C, and then stored in this fixative at 4°C until further processing. For the final preparation each aldehyde-fixed sample was divided into 3–4 tissue–blocks, rinsed in 0.1 M cacodylate buffer, post-fixed for 1 h in 1 % (w/v) OsO₄ (pH 7.2), and dehydrated with increasing concentrations of ethanol and finally embedded in Epon 812 (Polysciences, Inc, Warrington, PA, USA). Ultrathin sections were cut with a diamond knife in a LKB Ultratome (Stockholm, Sweden). These sections were stained with 4 % uranyl acetate for 1 h and 2.7 % lead citrate for 1 min. The stained sections were examined in a Philips EM 300 transmission electron microscope (Eindhoven, Holland) at an accelerating voltage of 60–80 kV (13).

RESULTS

Fig. 1 shows a schematic drawing of villi, indicating with numbers from where the different samples were taken. All samples from the same level showed similar morphology indicating a homogenous rather than a patchy effect of the lectins on the intestinal mucosa.

Fig. 2 shows electron micrographs of the apical part of villi from the jejunum of untreated (Fig. 2a) and lectin-treated rat small intestines (Fig. 2b). The untreated group had enterocytes with long, regular and well-separated microvilli, and intact glycocalyx. In the cytoplasm clearly defined core filaments were seen to reach into the apical part of the cells, and the terminal web was seen as an interrupted pair of bands. All enterocytes contained numerous mitochondria and well developed endoplasmic reticulum. Vesicles were found in the apical part of the cells, varying in number from one cell to another. The control group had this appearance throughout the whole villi (further graphs not shown). In the lectin-treated groups the enterocytes, at the top of the villi had long and regular microvilli. The cells had an
intact glycocalyx, and aggregated material was found outside of this layer. In the cytoplasm there was an increase of large heterophagosomes, membrane-bound vacuoles and lysosomal-like bodies, especially, in the apical part of the cells. Infiltration of lymphocytes, between the enterocytes, appeared to be most pronounced in the Con A-treated group.

Fig. 3 shows the intestinal mucosa from a more basal part of the villi (see Fig. 1). The lectin-treated groups showed minute alterations of their microvilli. However, they were more tight and closely packed in comparison with the controls. Furthermore, the lectin-treated groups had a thicker mucus layer. The cytoplasm looked swollen, especially in the sub-apical part of the enterocytes, whereas the terminal web structure, the mitochondria and the endoplasmic reticulum were all unchanged. In the cytoplasm we also found an increased number of cellular organelles, consisting, of multivesicular bodies and lysosomal-like bodies in the apical part of the cytoplasm, and large membrane-bound vacuoles usually located in the Golgi region of the cells.

Fig. 4 shows the intestinal mucosa from an even more basal region of the villi (see Fig. 1) of the lectin-treated groups. This figure reinforces the impression that the microvilli are closely packed, and furthermore, that the interspaces between opposite villi are remarkably reduced.

Fig. 5 shows the effects of WGA (a) and Con A (b) on the most basal part of the intestinal mucosa (see Fig. 1). The microvilli were shorter and more irregular, and the interspaces between the villi were filled with mucus and granulated material. These changes were accompanied by disorganization of the bundles of core filaments in the apical region of the enterocytes. In this region, as previously described, the cytoplasm was swollen and membrane-bound vacuoles and other vesicles were seen. A comparison between the two graphs gives the impression that the Con A-treated group (b) have shorter and more club-like microvilli. However, a quantitative analysis showed that the two lectins had similar effects on the length and width of the intestinal microvilli (Table I).
TABLE I. QUANTITATIVE EVALUATION OF THE EFFECTS OF CON A AND WGA ON INTESTINAL MICROVILLI. This table shows a quantitative evaluation of lectin-induced alterations of the microvilli on the enterocytes from the base of the villi.

<table>
<thead>
<tr>
<th></th>
<th>Length (µm)</th>
<th>Width (µm)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>1.44±0.03</td>
<td>0.12±0.01</td>
<td>44</td>
</tr>
<tr>
<td>Con A</td>
<td>0.52±0.02***</td>
<td>0.15±0.01***</td>
<td>28</td>
</tr>
<tr>
<td>WGA</td>
<td>0.59±0.03***</td>
<td>0.16±0.01***</td>
<td>19</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM. Statistical evaluations were performed with Student’s t-test for unpaired samples. *** P<0.001.

DISCUSSION

One of the most characteristic findings in patients with celiac disease is an alteration of the small intestinal morphology (1). In 1976 it was suggested that the cause of these morphological changes was related to the composition of the cell membrane of the enterocytes, enabling gluten to act as a toxic agent in an analogous way to plant lectins when they bind to cell membrane glycoconjugates (2, 22). Experimental support for this idea comes from the findings that: 1) Exposure to either Con A or WGA causes a decreased membrane surface area (14). 2) Treatment with kidney bean lectin leads to shortening of microvilli (17). 3) There exists a WGA-like lectin in gluten (12). 4) The intestinal permeability changes of rat intestinal mucosa after lectin challenge are similar to those found in patients with celiac disease (19). The present study is an extension of this latter work, showing the ultrastructural changes of the intestine after short-time exposure to WGA or Con A.

The basal part of the villi is well known to contain the most immature enterocytes with a progressive differentiation towards the top of the villi. In our study we found the most pronounced alterations of the microvilli in the basal part of the villi. A possible explanation for this finding could be a greater binding of WGA and Con A...
to the more immature enterocytes. This proposal, is supported by the observation that enterocytes at different sites of the villi exhibit distinct binding of lectins (4, 5). An alternative explanation could be a higher membrane turnover in the immature enterocytes, thus leading to a continuous appearance of new binding sites for lectins. Whatever the mechanism behind the more pronounced alterations of the immature cells, a continuous exposure to lectins would finally result in a changed morphology of all enterocytes as they move towards the top of the villi. This should be kept in mind when correlating our results of a short-time exposure (1 h), with the findings in untreated individuals with celiac disease that are continuously exposed to dietary lectins. The present results might mimick early intestinal changes in patients with celiac disease.

The transport of larger molecules through enterocytes occur to a major extent via endocytosis (21). In our investigation we found an increased number of membrane vesicles and heterophagosomes in the enterocytes after exposure to WGA or Con A as a sign of increased endocytosis. Similar results were also observed by other investigators (11, 14). These findings are in accordance with the ability of lectins to activate a large number of cellular processes in different types of cells, including endocytosis (3, 18). Furthermore, the remodulation of the cytoskeleton, which we observed in the terminal web region, might be of importance since there is little doubt that it plays a central role in endocytosis (20). If the lectins increase the rate of endocytosis far beyond normal, the capacity of the enterocytes to restore this loss of plasma membrane may not be sufficient and lead to a shortening and finally a total disappearance of the microvilli. An alternative explanation can be based on the suggestion that the terminal web is a tension-generating system that serves to anchor the microvilli in a rigid meshwork of filaments (15). The disruption of the terminal web after lectin binding to the cell membrane would then prevent the microvilli to retain their normal structure. Our finding of a disarrangement of the cytoskeleton might have a more general application, since it has recently been suggested that such a disarrangement is involved in the reactions behind enterotoxic diarrhoea (7). However, to determine the cause of Con A and WGA induced morphological changes more detailed analysis of the molecular events following lectin binding to the cell membrane of enterocytes have to be performed.

In summary we found that short-time lectin provocation affected the enterocytes at the base of the villi more than those at the top. The morphological findings included disarrangement of the cytoskeleton, increased endocytosis and shortening of the microvilli. The interrelationship between these changes and also their relation to those found in patients with celiac disease have been discussed.

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REFERENCES


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