Lectin Binding In Colorectal Mucosa

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Abstract

Lectin binding of goblet cell mucin in human colorectal mucosa was studied in patients with irritable bowel syndrome, colorectal malignancy and ulcerative colitis using plant lectins, Dolichos biflorus agglutinin (DBA) and peanut agglutinin (PNA). Normal colorectal mucosa demonstrated a strong binding with DBA (100%) but did not bind to PNA at all. Colonic carcinomas showed strong PNA binding (7 of 15 biopsies) while DBA binding was absent in 14 of 15 biopsies. The transitional mucosa showed reduced or absent DBA binding in 6 and positive PNA binding in 2 of 15 biopsies. During the active phase of ulcerative colitis, there was a loss of DBA binding in 10 of 15 biopsies, which was restored during remission in all. One biopsy with severe dysplasia showed PNA binding. It is concluded that normal colorectal mucosa binds DBA strongly but not PNA. Malignant tissue and transitional mucosa reveal PNA binding often, with decreased DBA binding. In ulcerative colitis DBA binding decreases during phases of activity.

Key words: Lectin binding of colonic mucin, ulcerative colitis, colorectal cancer, irritable bowel syndrome.

Introduction

Lectins are proteins that bind non-covalently to specific carbohydrates. Using plant lectins, alteration of the colonic mucin in its carbohydrate structure has been recently described in colorectal cancers (CC), in mucosa adjacent to cancers, the transitional mucosa (TM) and in ulcerative colitis (UC). The lectin derived from Dolichos biflorus (DBA) binds to the mucin of the well-differentiated goblet cells found in the upper colonic crypt, but not to the mucin of the immature cell in the lower crypt. DBA does not bind to the mucins secreted by the majority of colon cancers. The lectin derived from peanut (PNA) does not bind to the goblet cell mucin of any normal colonic mucosa, but was found to bind to the mucin secreted by all the colon cancer tissues and transitional mucosa studied.

The appearance of receptors for PNA binding in glycoconjugates has been suggested to be a common occurrence in malignant transformation. Preliminary work on biopsies from patients with ulcerative colitis also revealed alterations in lectin binding. In an animal model, a diffuse alteration in glycoprotein structure, related to PNA binding, was found in that part of the murine colon that subsequently developed cancer. We therefore decided to study the lectin binding of mucins in normal colorectal mucosa and in mucosa of patients with UC and CC.

Material and Methods

During proctosigmoidoscopic examinations, eight normal rectal biopsies were obtained from eight patients with irritable bowel syndrome (IBS) who had normal barium enema, and 30 biopsies from ten patients with UC during different phases of activity. Fifteen colorectal resection specimens (6 colon, 9 rectum) obtained from patients suffering from colorectal cancer were also studied; the cancer affected, transitional and apparently normal mucosa (6 cm from the edge of the tumour) were separately studied.

All the biopsies were fixed in Bouin's fixative and the operated specimens were fixed in 10 percent formal-buffered saline.

Staining Method: Haematoxylin-eosin staining of tissue specimens was performed in all cases. Lyophilised preparations of fluorescein-isothiocyanate (FITC) conjugates of DBA and PNA were obtained from Vector Laboratories, Burlingame, California, USA. An FITC-lectin solution was prepared with a concentration of 0-2 mg protein/ml reconstituted with phosphate-buffered saline (PBS) at pH 7-4. Serial sections of 6 μ thickness were cut from each block. The paraffin-embedded sections were deparaffinised in xylene and rehydrated through grades of alcohol and placed in PBS (pH 7-4) for 5 minutes. The sections were then incubated with 50 μl of FITC-lectin solutions for 20 min at room temperature. The excess solution was then rinsed off with PBS (pH 7-4). The sections were then mounted with PBS-glycerol (pH 8-0). To confirm the lectin specificity, 50 μl of FITC-lectin was incubated with 50 μl of the specific inhibitory sugar before application to the tissue section. The specificity of the sugar was demonstrated by the abolition of specific fluorescence on the tissue section. N-acetyl galactosamine (Gal NAc) 0-2 M was used to inhibit binding by DBA, and galactose (Gal) 0-3 M to inhibit PNA binding.

The slides were then examined under a fluorescence microscope. The fluorescence was graded for DBA binding as: absent (none of the crypts showing fluorescence), decreased (less than 25% of the crypts showing fluorescence) and strong (more than 75% of the crypts showing fluorescence). For PNA binding fluorescence was recorded as absent or present as most of the slides were negative for PNA binding and in those where it was positive, there was not much variation in the intensity.

The biopsies from patients with UC were graded as showing mild, moderate or severe activity or complete
remission with quiescence. The dysplasia was graded as mild, moderate or severe. Severe dysplasia was equivalent to carcinoma in situ.

Results

FITC-DBA binding was observed in all biopsies from eight patients with IBS in the upper crypts while FITC-PNA binding was not seen in any biopsy. Similar findings of lectin-binding were observed in 13 biopsies from "normal" mucosa from patients with colorectal cancer (Fig).

In UC patients, the results were correlated with the severity of the disease. There was a reduction of DBA binding in severe cases (11 of 15 biopsies). As remission set in, the DBA binding was restored to normal pattern. The differences in DBA binding between remission and activity (p < 0.01) and between severe to moderate activity and mild activity (p < 0.05) were significant by chi squared analysis. The difference between remission and mild activity was not significant. FITC-PNA binding was observed in only two of the 30 biopsies from patients with UC (Table). One of those two biopsies revealed severe activity of UC while the other showed severe dysplasia (carcinoma in situ) during remission. In the latter, the adjacent non-dysplastic mucosa showed strong FITC-DBA binding. No PNA binding was observed in the supra-nuclear portion of epithelial cells. There was no significant difference in PNA binding between the three groups of UC.

Of the 15 adenocarcinomas, 11 were mucin-secreting type, of which six were colloid cancers. The single case where FITC-DBA binding was positive had colloid cancer. FITC-PNA binding was observed in all seven cases with mucin-secreting carcinoma, five of whom had colloid cancers. Neither DBA nor PNA binding was seen in four patients with cancers of the mucin-secreting variety, of whom one had colloid cancer. The mucin non-secreting type of cancers also did not show either DBA or PNA binding. PNA binding was seen in two cases with mucin-secreting cancer in the transitional mucosa; in these patients the cancerous tissue was also positive for PNA binding.

Table: FITC-lectin binding in normal and abnormal colorectal biopsies

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<tr>
<th></th>
<th>DBA +ve</th>
<th>DBA -ve</th>
<th>PNA +ve</th>
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<td>8</td>
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(No of biopsy specimen in parenthesis)

Discussion

Early work identified the normal pattern of positive DBA binding and negative PNA binding in normal human colorectal mucosa. A similar pattern was seen in all our patients with irritable bowel syndrome.

In patients with ulcerative colitis, Boland et al correlated the abnormalities of lectin binding with subsequent clinical outcome and dysplasia but not with the severity of the disease activity. In the 3 year follow up period, four patients developed dysplasia and two of these biopsies had decreased DBA binding and positive PNA initially. They felt that the above changes in lectin binding if substantiated in long term studies may identify UC patients at risk for development of cancer. The present study revealed that decreased DBA binding may be related to the activity of the disease, being restored to normality when the activity decreases. The only biopsy with severe dysplasia was positive for PNA binding.

DBA binding was absent in 14 of 15 colonic cancers, while PNA binding was seen in seven of these. These results differ to some extent from those of Boland et al who found that only 14 of 21 patients were negative for DBA binding while all biopsies were positive for PNA binding. There were six colloid carcinomas in the present series and three in Boland's material. In Boland's study all three had normal DBA binding besides PNA binding. Hence one would expect more cases showing DBA binding in the present series but the
results were contrary to this expectation. Similarly, the PNA positivity was not 100 percent as reported by Boland. The reasons for the above differences are not known.

The transitional mucosa showed decreased or no DBA binding in six of 15 biopsies and positive PNA binding in two biopsies. These results again are different from Boland's study where all 14 biopsies from transitional mucosa showed decreased DBA binding and positive PNA binding.

Brausius[8] has suggested that PNA binding may be an indication of the presence of the Thomas-Friedenreich (T) antigen, which has been recognized as a colonic tumour marker. This antigen has the disaccharide specific for PNA binding in its poly saccharide residue. Pfleiderer[7] have confirmed that PNA stains the T-blood group antigen in rectal mucosal biopsies with dysplasia and is indicative of de-differentiation of mucin-producing cells, irrespective of whether it is caused by increased cell turnover or by dysplasia.

It is concluded that in normal colorectal biopsies, upper crypts show FITC-DBA binding and PNA binding is not observed in any portion. Biopsies from patients with UC show decreased or no DBA binding during severe activity, which becomes positive again during recovery from the acute episode. Positive PNA binding if substantiated in a larger number of patients may be a marker for dysplasia and possibly for future development of cancer. In cancer tissues there is a tendency for absence of DBA binding and positive PNA binding. The transitional mucosa shows intermediate features of lectin binding between cancerous and normal tissues.

References

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