Lectin Immunohistochemistry In Developing Rat Colon and Human Colonic Neoplasms

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Özet: *Fötal, meme emen, emzirme sonrası ve erişkin yaşlardaki 12 sıçanda giyco-protein içeriğine göre immünhistokimyasal olarak 5 adenom ve 5 kolon karsinomu saptandı. Peanut aglutinin (PNA) tip I ulex europeus agglotinin (UEA-I), Wheat germ agglutinin (WGA) ve soybean agglutinin (SBA) immünohistokimyasal analizlerde kullanıldı.*

Formalinle tespit edilip perafin bloklarla alınarak elde edilen kesitler peroksiden-antiperoksiden ve avitin-biotin antiperoksiden teknikleri ile boyadı.

PNA ve WGA ve SBA bağlanma örneklerinde anlamlı değişiklik olmadı. VEA-I kolon karsinomuna yoğun bir şekilde bağlandı, villa adenom dışında kolon adenomlarına bağlanmadı. PNA'da kolon karsinoma ve adenomlarına afinite gösterdi.

Kolon neoplazmlarında kolon mukozasında fötal karakteristikler gösteren değişiklikler olduğu ve bunun VEA-I ile başarılı bir şekilde gösterilebileceği kanısına varıldı.

Anahtar Kelimeler: Lektin, ulex europeus type-I aglutinin, wheat germ aglutinin, soybean aglutinin, peanut aglutinin, kolon neoplazmları

Summary: *Twenty rats of foetal, suckling, weanling and adult ages, five colonic adenomas and five colon carcinomas were detected immunohistochemically for their glycoprotein contents. Peanut agglutinin (PNA), ulex europeus agglutinin type-I (UEA-I), wheat germ agglutinin (WGA), and soybean agglutinin (SBA) were used for immunohistochemical assays.*

Sections from formalin fixed, paraffin embedded tissues were stained with peroxidase-antiperoxidase and avidin-biotin antiperoxidase techniques.

PNA and UEA-I lectin binding intensity decreased in rats with increasing age, whereas WGA and SBA binding patterns did not change significantly. While UEA-I did not bind to colonic adenomas, except a villous adenoma, it showed intense binding to colon carcinomas. Also PNA had affinity for colonic adenomas and carcinomas.

We suggested that, a return to foetal chracteristics in colon mucosa occurs in colonic neoplasms, and this property is successfully revealed by UEA-I.

Key Words: Lectin, ulex europeus type 1 agglutinin, wheat germ agglutinin, soybean agglutinin, peanut agglutinin, colon neoplasms.

Lectins are proteins that can bind carbohydrates, although they are not enzymes or proteins (1-5).

A normal glycosylation is of great importance in the development of cells and tissues. Diverse glycosylation can occur during neoplastic as well as inflammatory and reactive events. Sometimes such diversity can be determined before a macroscopic or microscopic lesion appears. Thus, identification of the carbohydrate structures of the cells becomes important in searching a precancerous tissue. Tissue carbohydrates show enhanced affinity to lectins in some periods of development whereas they lose their affinity in others. Those changes in affinity during normal development can also be seen in some neoplastic and inflammatory states. But, unlike neoplastic lesions, these changes are reversible in inflammatory conditions (4).

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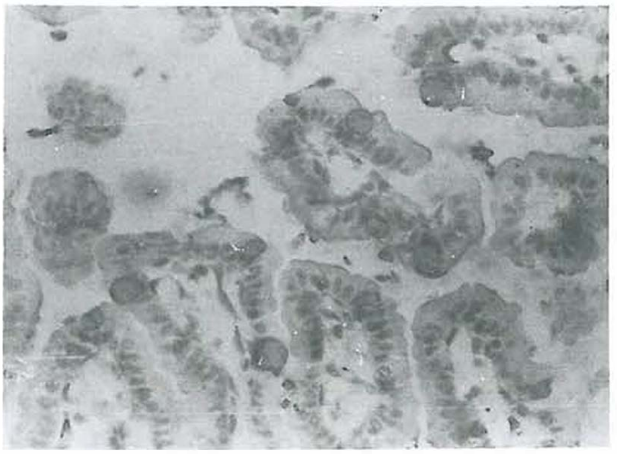

Fig 1: Anti UEA-1 immunostaining in foetal rat gut x 440

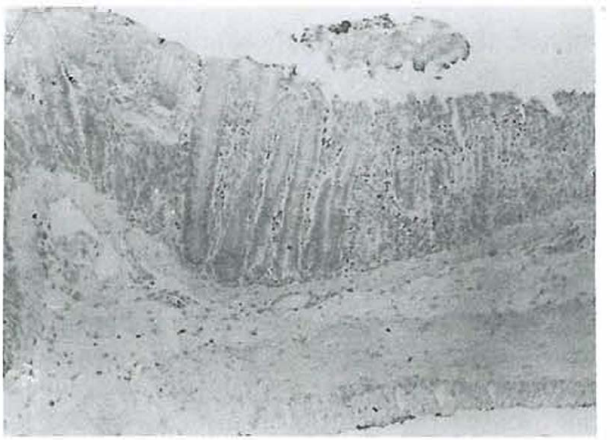

Fig 2: Anti UEA-1 immunostaining in adult left colon x 110

The peptid chains of the mucus of gastrointestinal mucosa are synthesized under genetic control in rough endoplasmic reticulum of the cells. Carbohydrate groups are attached to peptid chains in Golgi complex by the help of glycosyltransferases. Also this step is controlled genetically since glycosyltransferases are proteins. When cellular multiplication is overstimulated, the multiplied cells reach to the surface before they mature. Also the structure of mucus of these cells are different and does not have terminal sugar residues such as sialic acid and fucose. So these sugars show enhanced affinity for lectins. In normal states, peanut agglutinin (PNA) and ulex europaeus agglutinin type I (UEA 1) binding capacity of colon mucosa decreases by increasing age (6,7).

MATERIALS and METHODS

We examined 20 rats in foetal, suckling, weanling and adult ages, five adenomatous polyps and five colon carcinomas with respect to their affinity to bind lectins PNA, UEA 1, wheat germ agglutinin (WGA) and soybean agglutinin (SBA).

We applied peroxydase-antiperoxydase and avidin-biotin peroxydase immunostaining to the formalin fixed, paraffin embedded tissues. The lectin-peroxydase-antiperoxydase complex was conjugated with chromogen aminoaethylcarbazole (AEC) and slides were examined under light microscope.

RESULTS

UEA 1 and PNA binding capacity of rat colon decreased and eventually lost with increasing age. No UEA 1 binding was observed in adult rat colon. Lectin binding affinity of colonic mucosa shifted to the basal portion of crypts and especially to the right colon with increasing age (Fig. 1-2).

There was no difference in SBA and WGA lectin binding affinity of colon mucosa with increasing age.

UEA 1 remained always negative in tubular adenomas, so did PNA usually. A supranuclear binding was observed with all lectins in a villous adenoma. All, but one, colon carcinomas gave positive reaction with UEA 1 (Fig. 3). PNA

Fig 3: Anti UEA-1 immunostaining in a colon carcinoma x 440

stained the apical cytoplasmic borders of the cells weakly in colon carcinomas whereas WGA and SBA binding to the supranuclear portion of the cells had different intensities.

DISCUSSION

Our results agree with those in the literature which reported a decrease in PNA and UEA 1 binding affinity of rat colonic mucosa with increasing age (1). In some of the previous studies, colonic neoplasms developed in dimethylhydrazine treated rats, and even before a visible lesion has been discovered, a rise in PNA and UEA 1 binding affinity of the colonic mucosa was observed (2,8).

There was enhanced affinity for all lectins in a villous adenoma, while lectin binding was also prominent. This finding is significant since there was a similar binding intensity in colon carcinomas as well as villous adenoma which carries a high risk for malignant transformation. UEA1 expressed this property more successfully than other lectins.

Some authors reported that PNA binding to tissues increases with neuraminidase pretreatement (9,10). The results of these studies correlate well with the feature we determined, which is: 'The higher the malignant potential, the more intense PNA binding affinity'.

PNA and especially UEA 1 have a great importance in the assessment of neoplastic lesions of colon. In some studies it was stressed that, well diferentiated tumor cells possess more UEA 1 reactive molecules whereas progression of tumor and metastasis are associated with a decrease in UEA 1 affinity (2,8,11).

As a result we suggested that PNA and UEA 1 immunostaining can be used widely in the assessment of the neoplastic lesions of gastrointestinal tract after more information is gathered from vast study groups.

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