**Epithelial Dysplasia of the Rabbit Colon Induced by Degraded Carrageenan**

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**ABSTRACT**

Colonic mucosal lesions, characterized by crypt abscesses and mononuclear cell infiltration, which resemble human ulcerative colitis can be induced in rabbits by short-term (7 to 8 weeks) administration of carrageenan according to our method. In this study experimental epithelial dysplasia of the colon was induced by the p.o. administration of λ-degraded carrageenan for a much longer period of time. Fifteen rabbits, sensitized i.m. with the same substance 1 week before, were subjected to 12 or 28 months of treatment with 1% carrageenan solution in drinking water. Histological examination disclosed chiefly mild inflammatory changes of the colonic mucosa in all animals and a focal but high-grade dysplasia (nonpolyloid) involving the mucosal epithelium in three of the five animals treated for 28 months.

The present observations suggested that epithelial dysplasia of the colon may be caused in association with inflammation and that the pathological condition produced by us can be a useful model of carcinoma in situ possibly resulting from inflammation.

**INTRODUCTION**

The risk of carcinogenesis is unquestionable in ulcerative colitis (1, 2). Mottet (3) detected tumors in 3.1% of ulcerative colitis patients. Several authors (1, 2–6) suggested that the risk of carcinogenesis is high in pancolorectal type cases and long-term of chronically persistent cases. Dysplasia often develops in the affected mucosa of ulcerative colitis patients, so that attention is called to the possible relationship between inflammation and carcinomatous changes (4, 7).

Experimental ulcerative colitis is of value in the investigation of the relationship of inflammation to dysplasia in ulcerative colitis patients (5, 6, 8–11). We have already induced experimental ulcerative colitis in rabbits by short-term (7 to 8 weeks) treatment with carrageenan (12, 13). In this rabbit experiment, we administered this agent on a long-term (12 or 28 months) basis to look into the possibility of colonic epithelial dysplasia development in association with chronic inflammation.

**MATERIALS AND METHODS**

Fifteen mature rabbits weighing about 2.2 kg were used. λ-Degraded carrageenan (1 mg; M, ~30,000) was dissolved in 1 ml of NaCl solution and injected into the trapezius muscle to sensitize the animals. One week after sensitization, administration of λ-degraded carrageenan, dissolved in drinking water at a concentration of 1%, was started. Ten rabbits were given the substance continuously for 12 months and the remaining five were treated for 28 months. They were sacrificed at the end of the treatment period and examined histopathologically. The histopathological findings were classified as negative, indefinite, and positive (low-grade or high-grade dysplasia) according to the criteria of Riddell et al. (7).

**RESULTS**

All rabbits began to discharge loose stool 2 weeks after the start of the treatment. The loose stool tended to worsen up to the seventh to eighth week but became almost constant thereafter. Five of the 15 rabbits discharged mushy stool containing obvious blood for 1 to 2 weeks in the first half of the seventh month, after which bloody stool was not seen.

The macroscopic observations in the large intestine of the rabbits treated for 12 months are described as follows. In 6 of the 10 rabbits the entire colon including the rectum had edematous changes and scattered erosions (Fig. 1). Histologically, the entire mucosa was atrophied, containing few glands; in addition, a moderate cellular infiltration was observed in the lamina propria (Fig. 2a). Furthermore, there was mild mononuclear infiltration in the lamina propria of the histological specimen obtained from the region where marked edematous changes were recognized macroscopically (Fig. 2b). The surface epithelial lesions seemed to be of the indefinite type. These changes were marked, especially in the region from the descending colon to the rectum.

The findings in the animals treated for 28 months are described as follows. Macroscopically, the entire colon including the rectum underwent edematous change and erosion in four of the five animals. Histologically, a moderate mononuclear cell infiltration was found in the colonic mucosa of these four rabbits; in addition, lesions of low-grade epithelial dysplasia were observed sporadically in the entire mucosa including the rectal mucosa (Fig. 3). The incidence of the lesions increased as the site of examination approximated the anus. In three of the animals in question the lamina propria had markedly edematous areas, which were devoid of glandular ducts at some sites, and the muscularis mucosae tended to be swollen. Furthermore, lesions which had changed into high-grade dysplasia discretely different from the surrounding mucosa were noticed sporadically in the conspicuously edematous areas near the anus (Fig. 4).

The epithelial cell density of these focal areas was high. Those epithelial cells were irregular in size and had lost nuclear polarity. Some of them had a hyperchromatic nucleus and basophilic cytoplasm (Fig. 5). As described above, low-grade and high-grade dysplasias of the epithelium were coexistent in the three rabbits, provided that the latter was found more frequently on the anal side than on the oral side of the colonic mucosa. Being heterogeneous lesions having no continuity to the surrounding mucosa, these atypical glandular ducts looked like carcinomas in situ grown on the flat mucosa.

**DISCUSSION**

A variety of methods have been proposed for producing experimental large intestinal lesions similar to those of human...
CARRAGEENAN-INDUCED EPITHELIAL DYSPLASIA

Fig. 1. Macroscopic changes in rabbit colonic mucosa induced by 12 months of carrageenan treatment. Edematous changes accompanied by sporadically scattered erosions are seen. (The entire colon including the rectum was involved.)

Fig. 2a. Histological changes of rabbit colonic mucosa induced by 12 months of carrageenan treatment. The defect in the surface epithelium, moderate cell infiltration, and sparse glands are suggestive of erosions. b, marked edematous changes in the mucosa. The mucosa is covered with a single layer of regenerated epithelium and epithelial glands are absent. × 100.

Fig. 3. Histological changes in the rabbit colonic mucosa induced by 28 months of carrageenan treatment (low-grade dysplasia). The nuclear polarity of the surface epithelial cells is lost and their cytoplasm is basophilic. The generated crypts in epithelium show a high cell density and a high nuclear density. × 100.

Fig. 4. Histological changes in rabbit colonic mucosa induced by 28 months of carrageenan treatment (high-grade dysplasia). A carcinoma is recognized in the regenerated surface epithelium. Being localized within the mucosa, it is considered a carcinoma in situ. × 100.

Ulcerative colitis, and diverse results have been presented. Among them, the lesions induced by p.o. carrageenan alone as reported by Watt and Marcus (6, 8, 9, 14) are known to have a relatively good resemblance to those of human ulcerative colitis. The point by which our technique can be distinguished from their method is that we introduced a sensitizing procedure.

The lesions induced by our technique cover the entire colon including the rectum as mentioned under "Results," and are macroscopically characterized by erosion, edema, epithelial depletion, cryptitis associated with mononuclear inflammatory cell infiltration, and mucin depletion. Histologically they are similar to the lesions of human ulcerative colitis. Those histopathological changes can be induced in 7 to 8 weeks. The lesions produced by this comparatively short-term administration include active colitis (12, 13).

On the other hand, the changes induced by long-term administration assume rather an inactive aspect. In fact, the lesions induced by 12-month treatment were strongly similar to those of long-standing human ulcerative colitis, and they were considered a good experimental model of colitis resulting from a prolonged...
inflammatory change. Moreover, the changes induced by 28 months of treatment included marked edema of the mucosa and epithelial regeneration. Besides, in most of the animals thus treated, dysplastic epithelium (carcinoma in situ) clearly different from the surrounding mucosa was generated in the region adjacent to the surface of the flat mucosa with inflammatory changes. The high-grade dysplasia, which originated in the inflamed mucosa, was different from conventionally induced experimental carcinoma of the colon which takes on a protruding form. In this sense the present findings may be interesting to investigators working in related fields.

In discussing the etiology of this dysplasia, consideration must be given to the effect of carrageenan as a carcinogenic factor (10, 11) and to the process of carcinogenesis in diseases, such as human ulcerative colitis, associated with inflammation as an underlying condition. Watt and Marcus (8) examined histologically the colorectal mucosa of animals with carrageenan-induced colitis and reported that the polypous lesion formed on the mucosa, which was accompanied by hyperplastic changes, had virtually no features characteristic of carcinoma. By administering degraded carrageenan to rats for 24 months, Wakabayashi et al. (5) produced malignant tumors of the rectum which were relatively large masses recognizable as adenocarcinomas with stalks or infiltrative squamous cell carcinomas. They postulated that these masses might be generated through metaplastic change secondary to colitis.

In our experimental animals neither polyps as protuberant lesions nor masses were formed. Actually, the lesions produced were localized within the mucosa and were considered to be carcinomas in situ developed in the so-called flat mucosa (Fig. 3). These observations disagreed with those reported by Wakabayashi et al. (5). Furthermore, while the inflammatory changes of the mucosa induced in the experiments of Wakabayashi et al. were not obvious, the lesions produced by us were thought to be due to dysplasia consequent upon mucosal inflammation. The presence of inflammatory changes of the mucosa may be responsible in large part for the development of dysplasia.

On the basis of their macroscopic investigations, Morson and Dawson (15) classified epithelial dysplasia associated with ulcerative colitis under two types, flat and adenomatous, and Blackstone (1) classified them under three types, mass, flat, and multiple protrusion. Riddell (4) classified atypical epithelium under five histological categories (adenomatous change, basal cell change, in situ anaplasia, clear cell change, and pancellular change) although they stated that these changes coexisted. Morson et al. (2, 15) classified dysplasia histologically as mild, moderate, and severe, according to the degree of atypia, and they surmised that only severe dysplasia may be precancerous. The lesions produced by us this time seemed to be high-grade dysplasia in situ originating in the flat mucosa, pursuant to the histopathological classification of Riddell et al. (7).

We believe that experimental epithelial dysplasia induced by degraded carrageenan will be useful for the elucidation of the pathogenesis of carcinoma in association with inflammatory diseases of the mucosa, including ulcerative colitis, in humans.

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