Occurrence of dysplasia and adenocarcinoma after experimental chronic ulcerative colitis in hamsters induced by dextran sulphate sodium

M Yamada, T Ohkusa, I Okayasu

Abstract
In this study, long term dextran sulphate sodium administration was studied to ascertain whether colorectal carcinoma could be produced in patients with long standing ulcerative colitis. Simultaneously, changes in the intestinal microflora were analysed. Low grade to high grade dysplasia was seen in three of the five hamsters treated with 1% dextran sulphate sodium solution for 100 days, while no dysplasia was detected in the eight animals concomitantly treated with metronidazole, an anti-anerobic microbial agent, which prevents colonic ulceration. In these two groups, none of the animals developed colorectal cancer over 100 day period. In a group treated for 180 days, seven of the eight animals had dysplasia, and one had two adenomas. Furthermore, four of the eight animals had adenocarcinoma in the transverse colon; they were protruding well differentiated adenocarcinoma in one and non-protruding lesions infiltrating into the muscularis propria in three. The three non-protruding infiltrating adenocarcinomas were classified to be well differentiated adenocarcinoma in one and mucinous adenocarcinoma in two, resembling the type of cancer which complicates ulcerative colitis in man.

(Gut 1992; 33: 1521–1527)

In man, it is clinically well known that colon carcinoma often develops as a complication of long standing and extensive ulcerative colitis disease, or colitis associated with dysplasia.1–3 Ulcerative colitis can be induced experimentally in guinea pigs,6,7 rabbits,8,9 and hamsters10 with carrageenan, sulphated amylopectin, or dextran sulphate sodium mixed in drinking water. We have been studying experimental ulcerative colitis induced with dextran sulphate sodium and previously reported experimental chronic ulcerative colitis.10 This study, we have investigated whether long term dextran sulphate sodium administration produced colorectal carcinoma as a model of colorectal carcinoma seen in patients with longstanding ulcerative colitis. Because it has been reported that metronidazole prevents the occurrence of ulcerative colitis like lesions,11 and we had obtained similar findings,12 we also studied whether or not this drug could prevent not only ulcerative colitis, but cancer development as well. Changes in the intestinal microflora were also analysed.

Methods
ANIMALS
Syrian hamsters, males (Nippon Bio-supp Center, Tokyo, Japan), eight to nine weeks of age, were kept at our animal laboratory centre. These hamsters were used for the induction of experimental ulcerative colitis. The rodents were kept under standard laboratory conditions and were allowed free access to animal chow (MF, Oriental Food Inc, Tokyo, Japan). Dextran sulphate sodium treated or non-treated drinking water was provided ad libitum.

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Induction of chronic ulcerative colitis by dextran sulphate sodium in hamsters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Treatment*</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1% DSS</td>
</tr>
<tr>
<td>AM</td>
<td>1% DSS+MNZ</td>
</tr>
<tr>
<td>B</td>
<td>1% DSS</td>
</tr>
<tr>
<td>Control</td>
<td>water only</td>
</tr>
</tbody>
</table>

*Hamsters received distilled water containing the agent described ad libitum.† The severity of ulcerative colitis was scored on a scale from 0 to 3 (graded pathology index; 0 normal; 1 focal inflammatory cell infiltration including polymorphonuclear leucocytes; 2 inflammatory cell infiltration including polymorphonuclear leucocytes, focal loss of gland and crypt abscesses; 3 mucosal ulceration; ‡Number positive/total.

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Incidence of dysplasia, carcinoma and squamous metaplasia of the large intestine produced in hamsters by dextran sulphate sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Dysplasia</td>
</tr>
<tr>
<td>Caecum</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>3</td>
</tr>
<tr>
<td>AM</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
</tbody>
</table>

* Detailed pathology: two of four mucinous carcinoma; two of four well differentiated adenocarcinoma

Departments of lst Internal Medicine, and Pathology School of Medicine, Tokyo Medical and Dental University, Tokyo, Japan
M Yamada
T Ohkusa
I Okayasu

Correspondence to:
Masahiro Yamada MD, First Department of Internal Medicine, School of Medicine, Tokyo Medical and Dental University, Yushima 1-5-5, Bunkyo-ku, Tokyo 113, Japan.

Accepted for publication 11 May 1992
Co, Ltd, Tokyo, Japan) ad libitum for 100 days. Group AM (eight animals) received both 1% dextran sulphate sodium solution and metronidazole (MNZ; Shionogi Pharmaceutical Co, Ltd, Osaka, Japan). The latter was given at 1 mg/ml solution starting three days before the start of dextran sulphate sodium administration, for 100 days. Group B (eight animals) received 1% dextran sulphate sodium solution for 180 days. The control group (eight animals) received only distilled water for 180 days.

OBSERVATION OF COLITIS
Faecal findings for colitis were studied. The occurrence of loose faeces was recorded visually. Occult blood in the faeces was evaluated using the orthotolidine and guaiac methods (Occult blood slides; Shionogi Pharmaceutical Co, Ltd, Osaka, Japan).

BACTERIOLOGICAL ANALYSIS OF INTESTINAL MICROFLORA
The bacteriological analysis of intestinal microflora was performed using experimental methods which were essentially the same as those established by Mitsuoka et al. Briefly, after freshly voided stool specimens were collected, the samples were homogenised and diluted with an anaerobic dilution solution (decimal dilutions up to 10⁶ were prepared). Serial dilution volumes (10⁻¹, 10⁻², and 10⁻³) of the specimen were spread on the following agar media: trypticase soy blood agar BBL (Microbiology Systems, Cockeysville, Md, USA) for all aerobes and facultative anaerobes, DHL (Eiken Chemical Co, Ltd, Tokyo, Japan) for members of the family Enterobacteriaceae and Pseudomonas spp, TATAC (prepared in our laboratory) for Enterococcus spp, PEES (prepared in our laboratory) for Staphylococcus spp, modified LBS (prepared in our laboratory) for Lactobacillus spp, BS (prepared in our laboratory) for Bifidobacterium spp, modified VS (prepared in our laboratory) for members of the family Veillonellaceae, NBBT (prepared in our laboratory) for members of the family Bacteroidaceae, BL (prepared in our laboratory) for all lactic acid-producing bacteria, EG (prepared in our laboratory) for most of the obligate and facultative anaerobes, and CCFA (Eiken Chemical Co, Ltd, Tokyo, Japan) for Clostridium difficile. The plates inoculated for the recovery of obligate anaerobes were incubated in an anaerobic chamber (80% nitrogen, 10% hydrogen, and 10% carbon dioxide) for 48–72 hours at 37°C. The media used for the isolation of aerobes and facultative species were incubated in air for 48 hours at 37°C. After incubation, morphologically distinct colonies were described, enumerated, isolated, and identified. Identification was performed in most cases at family and genus levels using standard bacteriologic techniques. In our study, the lowest detection limit was 2×10⁴ organism per gram of faeces. The incidence of each bacterial group was calculated. Statistical analyses of the logarithms of the viable counts and the detection frequency were carried out by Student’s t test.
Figure 3: Microphotograph of the transverse colon in group AM of 1% dextran sulphate sodium with metronidazole for 109 days. No inflammatory changes except for mild oedema are found. (Haematoxylin and eosin).

Figure 4: Low grade dysplasia of the colonic mucosa of a hamster with chronic colitis induced by 100 day administration of 1% dextran sulphate sodium. (group A, Haematoxylin and eosin).

Figure 5: Adenoma of the transverse colon of a hamster with chronic colitis induced by 180 day administration of 1% dextran sulphate sodium (Haematoxylin and eosin).

MORPHOLOGICAL ANALYSIS
All analyses were performed on coded sections. After the body weight was measured, blood was promptly collected from the hamsters under anaesthesia with diethyl ether. After the hamsters were killed, the gastrointestinal tract, liver, pancreas, spleen, mesenteric lymph nodes, kidneys and lungs were isolated and fixed in 10% formalin solution (pH 7-2). Histological examinations were performed with haematoxylin and eosin staining after paraffin sections of the colon were made. The severity of ulcerative colitis was graded on a scale from 0-3 and expressed as the pathological index: (0) normal; (1) focal inflammatory cell infiltration including polymorphonuclear leucocytes; (2) inflammatory cell infiltration, focal loss of gland, and crypt abscess; (3) mucosal ulceration.

STATISTICAL ANALYSIS
All data were expressed in terms of the mean plus standard deviation (mean (SD)). Statistical significance of any difference between the control and experimental groups was determined by Student’s t test and the χ² method.

Results

PROPERTIES OF STOOL
Except for one hamster of group AM, which had diarrhoea, none of the animals in the control group AM showed faecal abnormalities and all tested negative for faecal occult blood. All animals in group A and group B had black soft stool, diarrhoea, or soft mucous haemoschezia, and these animals all became and remained positive for faecal occult blood from three to nine days after the beginning of dextran sulphate sodium administration to the day when they were killed. (Table 1).

MACROSCOPIC FINDINGS
In the control group, there were no abnormalities in the gastrointestinal tract from the oesophagus to the rectum. In group AM, which was treated with dextran sulphate sodium and metronidazole, erosion and mucosal oedema were seen in the area from the caecum to the rectum in three of the eight animals, while the remaining five showed no abnormalities in the colon. In group A, which was treated with dextran sulphate sodium for 100 days, erosion, ulceration and bleeding (Fig 1) were seen in all the animals throughout the large intestine, but mainly in the caecum and transverse colon. Two of the five animals also had protruding lesions (Fig 1) in the caecum and colon. As with group A, all the animals in group B treated with dextran sulphate sodium for 180 days had erosion, ulceration, and bleeding throughout the entire colon, but mainly in the caecum and transverse colon. In addition to these findings, reddish tumour-like protrusions (Fig 2) were observed in the caecum or transverse colon of seven of the eight animals. In these lesions, the wall of the intestinal tract was hard and the lumen was stenotic, macroscopically suggestive of cancer. There were no
abnormalities in the oesophagus, stomach or small intestine in groups A and B.

HISTOPATHOLOGICAL FINDINGS
Histological observation showed infiltration of inflammatory cells, including polymorphonuclear leucocytes and multiple erosive lesions (score, 1–3), but only in the large intestine of the hamsters in group A, AM, and B (Table I). Despite long term treatment with dextran sulphate sodium, erosion was found only in four of the eight group AM animals and was localised to the caecum. These findings indicated that the occurrence of inflammation was significantly inhibited in group AM which received metronidazole, in comparison with group A, which did not receive it (Table 1). Furthermore, dysplasia was not observed in group AM or in the control group (Table II, Fig 3). In group A, which was treated with dextran sulphate sodium for 100 days, three of the five animals had low grade to high grade dysplasia (Fig 4) in the caecum, rectum, and transverse colon, but none had carcinoma (Table II). In group B, which was treated with dextran sulphate sodium for 180 days, one of the eight animals had two tumour like adenomas in the caecum and rectum, respectively (Fig 5). Seven of these had low grade to high grade dysplasia in the caecum and four had dysplasia in the colon and rectum (Fig 6, Table II). Furthermore, in group B, four of the seven animals with dysplasia had cancer in the transverse colon – namely, one had a protruding lesion with tubular adenocarcinoma, which was classified as well differentiated adenocarcinoma (Fig 7), and the remaining three had non-protruding lesions with a few tubular structures, resulting in cancer cells invading into the muscular layer. One of the latter three animals was found to have well differentiated adenocarcinoma (Fig 8) and the other two, mucinous adenocarcinomas (Fig 9). In addition, three of these eight animals in group B also developed squamous metaplasia in the rectum, but not squamous cell carcinoma.

EXAMINATION OF INTESTINAL MICROFLORA
The number of Bacteroidaceae was significantly increased in group A after dextran sulphate sodium administration, while that of this species was significantly decreased in group AM soon after the beginning of metronidazole administration (Fig 10). The number of Bacteroidaceae then increased again after dextran sulphate sodium administration started, although the increase was not statistically significantly different from the baseline count before metronidazole administration. This suggests that metronidazole inhibited the growth of Bacteroidaceae. The number of Enterobacteriaceae was significantly increased after dextran sulphate sodium administration in both groups A and AM. The number of Lactobacilli was significantly increased to group AM (Table III). In comparison with the results obtained before dextran sulphate sodium administration, the number of Bacteroidaceae was significantly increased in group B and the control group. The
increase was greater in group B, which was treated with dextran sulphate sodium than in the control group (Table IV).

With regard to other bacteria, the numbers of *Enterococci* and *Eubacteria* were significantly decreased in the control group, and *Bifidobacteria*, in group B (Table IV).

**Discussion**

Many published studies have shown that the risk of developing cancer is higher in ulcerative colitis with extensive involvement of the colon existing over 10–20 years, when compared with the general population. Moreover ulcerative colitis patients with colorectal cancer have a worse prognosis in comparison with non-colitic or non-familial polyposal colorectal cancer patients. Therefore, it is important to develop an experimental model of ulcerative colitis with cancer in order to understand the mechanism of cancer development.

Since Marcus and Watt reported the use of degraded carrageenan for the induction of experimental ulcerative colitis in 1969, various sulphated polysaccharides, such as degraded carrageenan, sulphated amylpectin and sodium lignosulphonate have been shown to be useful for the induction of ulcerative colitis. We recently reported the induction of experimental ulcerative colitis with dextran sulphate sodium, which is a sulphated polysaccharide. The present study further tested the adequacy of this model by investigating whether colorectal cancer would occur during prolonged dextran sulphate sodium induced colitis. Hamsters were selected as subjects because this species has a longer digestive tract, which is easier to observe than in the mouse. In this study, the animals treated with 1% dextran sulphate sodium solution for 180 days all developed ulcerative colitis like lesions, and dysplasia was also seen in seven of these eight animals. Of the seven animals with dysplasia, one had two adenomas, and four had cancer of the large intestine. The histopathological types of these cancers were found to be well differentiated adenocarcinoma and mucinous adenocarcinoma, similar to the findings in cancers complicating ulcerative colitis in man. Although squamous metaplasia was seen in three of the eight animals, no squamous cell carcinoma developed. Such squamous metaplasia was localised to the rectum. Although low grade to high grade dysplasia was seen in three of the five animals treated with 1% dextran sulphate sodium for 100 days, these animals had no cancer. That cancer was produced in group B animals treated for 180 days indicates that the risk of cancer rises when inflammation persists for a long period of time in a manner comparable with human ulcerative colitis. Assuming that human and hamster life span are 100 and three years, respectively, 100 and 180 days for hamsters are equivalent to about nine and 16 years for humans, respectively. A length of 180 days for hamsters, during which period the group developing cancer received 1% dextran sulphate sodium continuously, corresponds to the time needed for the appearance of cancer in human ulcerative colitis. In addition to our findings, the occurrence of tumours in the large intestine after the administration of a sulphated polysaccharide was reported by Wakabayashi *et al.*, who used degraded carrageenan, and by Hirono *et al.*, who used dextran sulphate sodium. Wakabayashi *et al.* stated that squamous cell carcinoma, adenocarcinoma and adenoma developed in rats after ulcerative colitis like lesions. Hirono *et al.* observed adenoma and adenocarcinoma as well as papilloma in rats fed 5% dextran sulphate sodium added diet for 102–215 days. Papilloma and squamous cell carcinoma did not develop in our experiment. The difference may be because of the species used.
Based on the results obtained by Wakabayashi, Hirono, and our laboratory it is considered that dysplasia occurring in a ulcerative colitis like lesion progresses to adenoma and further to colorectal cancer. This progression is similar to the dysplasia carcinoma sequence seen in the course of cancer development in human ulcerative colitis. We also detected mucinous adenocarcinoma in two of the eight animals treated for 180 days. This type of cancer also frequently complicates human ulcerative colitis, suggesting that dextran sulphate sodium induced experimental ulcerative colitis is a useful model for the clarification of the mechanism of cancer associated with human ulcerative colitis.

Protruding adenocarcinoma having adenomatous components was observed in one of the four animals of developing cancer of the large intestine in our present study. The underlying mechanism may be explained by the theory of the adenoma carcinoma sequence. In contrast, cancers in the remaining three had neither adenomatous components nor protruding surfaces, but infiltrated into the muscular layer. These cancers seemed to be the progression of dysplasia in flat mucosa.

Finally, the examination of changes in intestinal microflora revealed that Bacteroidaceae species significantly increased in number in the presence of experimental ulcerative colitis, as seen in several other studies. Watt and Marcus postulated that changes in intestinal microflora by degraded carrageenan would induce ulcerative colitis like mucosal lesions. Their hypothesis was supported by Onderdonk and colleagues who found that carrageenan induced colitis could be prevented by pretreatment with metronidazole, which reduces intestinal anaerobes in guinea pigs. They also reported that administration of Bacteroides vulgatus isolated from caecal microflora of carrageenan induced colitis produced caecal ulceration in germ free guinea pigs. In the present study, it is suggested that metronidazole has the inhibitory effect on the growth of Bacteroidaceae in group AM treated with 1% dextran sulphate sodium in combination with metronidazole for 100 days, although its effect is still not clear. In this group, the occurrence of dysplasia as well as experimental ulcerative colitis was also inhibited. Our results also suggest that the increase of Bacteroidaceae may have some relation to the occurrence of ulcerative colitis like lesions and dysplasia induced by dextran sulphate sodium. In recent years, the promotive effect of bile acid on the occurrence of colorectal cancer has attracted our attention. As bile acid is decomposed by intestinal bacteria, the increase of Bacteroides is considered to have some relation to cancer development. Such a relation is also supported by the fact that cancers produced by various sulphated polysaccharides are localised within the large intestine. Tendencies of decrease of Bifidobacterium and increase of Clostridium other (Clostridia apart from Clostridium perfringens) were reported in the intestinal microflora of patients of colon adenomas or cancers. Further, the increase of Bacteroidaceae and decrease of Enterobacteriaceae were reported in the intestinal microflora of people who live in high risk area of colon cancer. It was also proposed that Clostridia, which are able to produce unsaturated bile acids, are implicated in the causation of large bowel cancer. It was also proposed that Clostridium, which are able to produce unsaturated bile acids, are implicated in the causation of large bowel cancer. In the present study, however, the increase of Clostridium other were not seen. Instead, the animal group developing cancer showed the decrease of Bifidobacterium and the increase of Bacteroidaceae in microflora. Detection incidence of Clostridium other was very low in both groups with or without metronidazole administration in our experiment. Accordingly, it is difficult to identify whether Clostridia have a critical role for development of colorectal cancer in this experiment. These data suggest that the change of intestinal microflora has a causal relationship to the development of colon cancer.

In conclusion, dextran sulphate sodium induced experimental ulcerative colitis very closely resembled human ulcerative colitis with respect to the pathology as well as the process of cancer development.

### Table III

<table>
<thead>
<tr>
<th>Organisms (Log10)</th>
<th>Before (n=5)</th>
<th>After 100 days (n=5)</th>
<th>1% DSS*</th>
<th>After 100 days (n=5)</th>
<th>1% DSS+MNZ**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td>6.1 (0.80)</td>
<td>6.7 (0.83)</td>
<td>5.5 (0.53)</td>
<td>7.1 (0.73)</td>
<td></td>
</tr>
<tr>
<td>Enterococci</td>
<td>[100]</td>
<td>[100]</td>
<td>[100]</td>
<td>[100]</td>
<td></td>
</tr>
<tr>
<td>Streptococci</td>
<td>3.7 (0.67)</td>
<td>4.9 (3.91)</td>
<td>3.6 (0.76)</td>
<td>4.1 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Yeasts</td>
<td>3.0 (0.60)</td>
<td>4.7 (3.15)</td>
<td>3.9 (0.64)</td>
<td>3.3 (0.64)</td>
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</tr>
<tr>
<td>Lactobacilli</td>
<td>7.3 (0.56)</td>
<td>8.9 (3.35)</td>
<td>8.0 (0.52)</td>
<td>9.4 (0.59)</td>
<td></td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>7.6 (0.40)</td>
<td>6.2 (1.3)</td>
<td>8.0 (0.52)</td>
<td>7.5 (0.63)</td>
<td></td>
</tr>
<tr>
<td>Escherichia</td>
<td>7.1 (0.56)</td>
<td>7.3 (4.49)</td>
<td>7.7 (0.75)</td>
<td>7.0 (0.50)</td>
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</tr>
<tr>
<td>Clostridia</td>
<td>7.1</td>
<td>3.2</td>
<td>7.4 (0.32)</td>
<td>4.9 (3.0)</td>
<td></td>
</tr>
</tbody>
</table>

*Log10 (No. of organisms/gram faeces) (mean ± SD); † Detection incidence in total number of hamster (percentages in square brackets); ‡ P<0.05, § P<0.001. No superscript means no significant differences; † ‡ DSS: dextran sulphate sodium; ** MNZ: metronidazole 1 mg/ml in distilled water.

### Table IV

<table>
<thead>
<tr>
<th>Organisms (Log10)</th>
<th>Before (n=8)</th>
<th>After (n=8)</th>
<th>1% DSS*</th>
<th>After (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td>6.5 (0.40)</td>
<td>6.6 (0.68)</td>
<td>6.5 (0.28)</td>
<td>8.2 (1.7)</td>
</tr>
<tr>
<td>Enterococci</td>
<td>[100]</td>
<td>[100]</td>
<td>[100]</td>
<td>[100]</td>
</tr>
<tr>
<td>Streptococci</td>
<td>7.5 (0.71)</td>
<td>6.8 (0.55)</td>
<td>7.1 (0.59)</td>
<td>6.5 (2.1)</td>
</tr>
<tr>
<td>Yeasts</td>
<td>3.6 (1.1)</td>
<td>4.2 (0.75)</td>
<td>3.0 (0.54)</td>
<td>3.8 (0.92)</td>
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<tr>
<td>Lactobacilli</td>
<td>9.3 (0.76)</td>
<td>8.6 (0.90)</td>
<td>9.0 (0.40)</td>
<td>8.4 (1.0)</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>4.1 (2.5)</td>
<td>6.5 (2.7)</td>
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<tr>
<td>Escherichia</td>
<td>8.7 (0.48)</td>
<td>7.1 (1.21)</td>
<td>8.7 (0.41)</td>
<td>7.6 (3.1)</td>
</tr>
<tr>
<td>Clostridia</td>
<td>4.4 (0.9)</td>
<td>5.5 (2.8)</td>
<td>3.8 (2.6)</td>
<td>3.0 (1.7)</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>7.2 (1.0)</td>
<td>8.1 (0.59)</td>
<td>7.3 (1.4)</td>
<td>9.1 (0.84)</td>
</tr>
</tbody>
</table>

*Log10 (No. of organisms/gram faeces) (mean ± standard deviation); † Detection incidence in total number of hamster (percentages in square brackets); ‡ P<0.05, § P<0.001. No superscript means no significant differences. † ‡ DSS: dextran sulphate sodium.
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This work was presented in part at the IX World Congresses of Gastroenterology, Sydney, Australia, 26-31 August, 1990. The authors thank Professor N Aoki, Emeritus Professor R Nakaya, and Dr D Nagata for helpful suggestions and corrections in the preparation of the manuscript.


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*Gut* 1992 33: 1521-1527
doi: 10.1136/gut.33.11.1521