Inflammopharmacology 16 (2008) 36–39
0925-4692/08/010036-4
DOI 10.1007/s10787-006-1543-3
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Research Article

Effects of nimesulide, a cyclooxygenase-2 selective inhibitor, on colitis induced tumors

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Received 20 July 2006; revised 21 August 2006; accepted 4 September 2006

Abstract. Cyclooxygenase-2 (COX-2) is known to suppress sporadic colorectal cancer, but effect of selective COX-2 inhibitor in UC-associated neoplasia is still unknown. This study investigated effect of a selective COX-2 inhibitor on colorectal carcinogenesis in experimental murine UC. Chronic colitis was induced in mice by four cycles of administration of dextran sulfate sodium (DSS) (i.e., 5% DSS for 7 days and distilled water for the following 14 days), and the mice were sacrificed 120 days after the end of the fourth cycle. The mice were divided into the following five groups: Group A, served as a disease control; Group B, received a diet mixed with 400 ppm of nimesulide (NIM), a selective COX-2 inhibitor, during the whole period; Group C, received NIM during the four cycles of DSS administration; Group D, received NIM for 120 days from the end of the fourth cycle; Group E, served as a normal control. In Group D, NIM significantly suppressed the occurrence of dysplasia and/or cancer. The results show that NIM inhibited both dysplasia and cancer in DSS-treated mice, thus showing that NIM has preventive effects on the remission phase of carcinogenesis.

Key words: Dextran sulfate sodium – ulcerative colitis – dysplasia – cancer – cyclooxygenase-2

Introduction

Patients with UC exhibit an increased risk for the development of cancer of the colon and rectum. Cyclooxygenase-2 (COX-2) inhibitors are well known to suppress sporadic colorectal cancer, but it has been unknown whether selective COX-2 inhibitors exhibit a preventive effect in ulcerative colitis (UC)-associated neoplasia. And a few investigators have described the appearance of dysplasia and/or cancer in mice by simple repeated administration of non-genotoxic colon carcinogen dextran sulfate sodium (DSS) (Cooper et al., 2000; Okayasu et al., 2002).

Materials and methods

Animals

Seven-week-old female BALB/c mice (CLEA Japan, Tokyo, Japan) weighting 20–25 g were used in this study.

Induction of experimental colitis

DSS with molecular weight of 5000 was obtained from Meitou Sangyou (Osaka, Japan). Mice were given water containing 5% DSS on the indicated days instead of tap water.

Protocol for induction of colorectal tumors and experimental procedures

Mice were divided into the following 5 groups. Mice in Groups A–D received four cycles of DSS administration (i.e., 5% DSS for 7 days and distilled water for the following 14 days) and were sacrificed 120 days after the end of the fourth cycle (204 days after the start of the first cycle). Group A (n = 25, a disease control) was fed a normal diet. Group B (n = 20) received the same diet mixed with 400 ppm of NIM for the whole experimental period (204 days). Group C (n = 17) received a diet mixed with 400 ppm of NIM during the four cycles of DSS administration (84 days). Group D (n = 30) received a diet with 400 ppm of NIM for 120 days commencing from the end of the fourth cycle. In Group E (n = 10) the mice did not receive any agents throughout the study and served as a normal control group.

Evaluation of severity of clinical colitis

Disease activity index (DAI) was determined in all animals during the first administration cycle of DSS by scoring body weight, haemoccult...
Pathologic examination

After sacrifice, the entire colorectum from the colorectal junction to the anal verge was excised and rinsed in phosphate-buffered saline (PBS). The specimen was opened longitudinally and examined for gross lesions without the use of any magnification and all gross lesions were recorded. The colon was divided into three equal portions (proximal, middle and distal). Subsequently, each segment was submitted as three transverse sections for histological processing. All slides were stained with hematoxylin and eosin. Then we performed pathological evaluation according to the histological criteria outlined by the Inflammatory Bowel Disease-Dysplasia Morphology Study Group (Riddell et al., 1983).

Evaluation of COX-2 immunohistochemistry

Localization and expression of COX-2 in the intestinal mucosa were assessed by the labeled streptavidin biotin method using the primary polyclonal rabbit antibody (1 in 50 dilution in PBS) against COX-2 (Cayman Chemical, Ann Arbor, MI, USA) and a LSAB KIT (DAKO, Carpinteria, CA, USA) with microwave accentuation (Brazowski et al., 2005; Yamauchi et al., 2002).

PGE$_2$ levels

An ELISA (EIA; Cayman Chemical) was used for quantitation of mucosal PGE$_2$. The contents of the proximal segment of colon were stored at −70°C and used later for measurement of PGE$_2$ by EIA kit according to the manufacturer’s instruction. PGE$_2$ concentration (in μg) was normalized per g of protein.

Statistical analysis

All results were expressed as mean ±SE. Comparisons were done using one-way ANOVA followed by Tukey-Kramer’s test. Categorical data were analyzed by chi-squared test. Statistical significance was defined as $P < 0.05$.

Results

Changes of DAI score

The DAI score gradually increased from day 2 (0.248 ± 0.074) to day 8 (2.932 ± 0.179) and usually reverted to normal by day 21 (0.026 ± 0.021). The administration of NIM significantly increased the DAI score from day 2 (0.52 ± 0.06) to day 7 (3.05 ± 0.12). However, NIM did not change the DAI score significantly during the following 14 days.

Incidence of dysplasia and/or cancer, distribution and tumor size

All gross lesions had the shape of the sessile type and were observed in the middle or distal colon. The mean size of gross lesions in Groups A (disease control), B (oral NIM administration during the whole experimental period), C (NIM administration during the four cycles of DSS) and D (NIM administration for 120 days from the end of the fourth cycle) was 4480 ± 469.9 μm, 2560 ± 416.3 μm, 4100 ± 70.7 μm and 2350 ± 106.1 μm, respectively. The incidence of dysplasia and/or cancer is presented in Figure 1. In Group A, the incidence of dysplasia and/or cancer was 28%. When NIM was used, the occurrence of dysplasia and/or cancer tended to be suppressed. In Group D, NIM significantly suppressed the occurrence of dysplasia and/or cancer.

COX-2 immunohistochemistry

COX-2 immunohistochemistry showed diffuse cytoplasmic overexpression in dysplastic and cancer cells (Fig. 2). Dysplastic cells exhibited weak positivity for COX-2 compared to cancer cells. Although the normal colon showed little COX-2 expression, the lesion-free colon in the disease control mice showed diffuse COX-2 expression.

PGE$_2$ levels

Mucosal PGE$_2$ concentration was significantly higher in Group A (15.19 ± 1.75 μg/g protein) than in Group E (4.25 ± 1.16 μg/g protein). In the experiments, mucosal PGE$_2$ was significantly lower in Group B (8.80 ± 1.66 μg/p protein) and D (8.70 ± 2.31 μg/p protein).

Discussion

In this study, we evaluated the effect of NIM on colitis induced tumors using murine UC model induce by simply DSS repeated, and NIM showed suppressive effects on development of dysplasia and/or cancer in DSS mice colitis model which has had relevance to long standing UC in human.

Although the type of cell that expresses COX-2 and immunostaining pattern have been still controversial, previous studies have described strong immunohistochemical COX-2