Patients with adenomatous polyps and carcinomas have increased colonic mucosal prostaglandin E_2

S Pugh, G A O Thomas

Abstract
Colorectal carcinoma in humans and animal models is associated with increased synthesis of prostaglandin E_2 (PGE_2). PGE_2 synthesis was measured in normal and neoplastic human colorectal mucosa to investigate its role in the adenoma-carcinoma sequence. Paired mucosal biopsy specimens for PGE_2 synthesis and histological examination were obtained during 39 diagnostic colonoscopies. Twelve control patients in whom colonoscopies and histology were normal synthesised similar amounts of PGE_2 at all sites. Their results were (mean (SD) pg PGE_2/mg tissue) caecum 102.8 (15.9) (n=6), ascending colon 110.8 (24.3) (n=10), transverse colon 103.9 (19.5) (n=11), descending colon 102.9 (23.2) (n=12), sigmoid colon 96.4 (18.0) (n=12), and rectum 107.1 (17.6) (n=12). Nineteen patients had a total of 27 adenomatous polyps (rectum (1), sigmoid (22), descending (1), transverse (1), and ascending colon (1): histology – tubular (16), tubulo-villous (8), and villous adenomas (3)). The polyps (178.0 (55.0), n=27) synthesised more PGE_2 than controls (p<0.001), but the values in polyp-associated mucosa (mean (SD) 115.4 (21.9), n=15) were not different to control results. Eight patients had carcinomas (rectal (2), sigmoid (4), and caecal (2)) all of which were adenocarcinomas. The cancers (193.6 (40.2), n=8) synthesised more PGE_2 than control specimens (p<0.001), but were not different to polyps. Cancer-associated mucosa (140.3 (27.7) n=8) synthesised more PGE_2 than control and polyp-associated mucosa. Colorectal neoplasia is associated with a progressive increase in PGE_2 synthesis which may have a role in tumourigenesis and be a pathophysiological explanation for the beneficial effects of NSAIDs in animal models and human disease.

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Human and animal work in colorectal carcinogenesis has suggested an association with increased prostaglandin (PG) synthesis. Transformation from premalignant to a malignant state may be related to increased PG synthesis in susceptible individuals. Prostaglandins, in particular PGE_2, are produced in increased amounts by human colonic tumour cells. Prostaglandins may play a role in tumour development and progression; they modulate tumour cell growth and facilitate tumour cell progression. Animal models of colorectal cancer have shown increased prostaglandin production from carcinoma cells. In addition, it has been shown that non-cancerous or background mucosa in tumour bearing animals produces increased amounts of PGE_2. It has been suggested that the development of large bowel tumours may be related to the level of prostaglandin synthesis. The ability of certain NSAIDs to suppress colorectal tumourigenesis in animal models of colorectal cancer implies that PGs are involved.

We have, therefore, investigated the changes in colonic mucosal PGE_2 synthesis in the human adenoma-carcinoma sequence.

Methods

COLONOSCOPIC EXAMINATION
Thirty nine patients had a diagnostic colonoscopy performed by a single colonoscopist (SP). In each case, after giving informed consent, the patient was placed in the left lateral position and sedated by intravenous injection of individually titrated doses of diazepam (5-20 mg) and pethidine (0-100 mg) according to the patient’s age, weight, and clinical response. Patients underwent digital per-rectal examination and wherever possible a full diagnostic colonoscopy was performed using a standard flexible fibre optic colonoscope (Olympus).

Controls
Patients with normal colonoscopies had paired biopsy specimens taken from the caecum and ascending, transverse, descending, and sigmoid colons and rectum.

Colorectal neoplasia
Patients with polyps, or a carcinoma, had paired background biopsy specimens taken from normal looking mucosa at least 10 cm away from the lesion. The polyp or cancer was then biopsied. Polyps were removed by snare diathermy and retained for histological examination. In the case of suspected carcinoma multiple biopsy specimens were taken for histological examination.

HISTOLOGICAL EXAMINATION
Tissue specimens were placed in formalin and histological examination was undertaken by the Department of Histology, University College Hospital, London. Reports were issued by a consultant histopathologist.

PROSTAGLANDIN SYNTHESIS
Biopsy tissues for PGE_2 synthesis were transferred immediately in ice cold Tris buffer (pH 8.4) and processed within one hour. The method
of prostaglandin generation and validation is given elsewhere. Briefly, the sample was washed with Tris buffer, blotted dry on Whatman’s no 1 filter paper, and weighed using an electronic balance (Metler AC100). The specimen was then resuspended in 1 ml of Tris buffer and ultracentrifuged for exactly 15 seconds in a fixed speed Hawksley micro-haematocrit centrifuge (giving 10000 rpm). The supernatant was discarded and replaced with 0.05 ml Tris buffer and the biopsy was vortexed for exactly 60 seconds using a bench vortex mixer. The formation of PDs was stopped by the addition of 0.01 ml of indomethacin in ethanol (to give a final concentration of 10 μg/ml) and the total volume made up to 1 ml by the addition of 0.94 ml of Tris buffer.

MUCOSAL PGE$_2$ IN COLORECTAL POLyps AND CANCER

The biopsy tissue and additions were then recentrifuged as above and the supernatant divided into two aliquots and stored at -20°C. Aliquots were batch assayed by highly specific radioimmunoassay (anti-PGE$_2$ antibody, Sigma) for PGE$_2$ in duplicate and two dilutions, and with reagent and sample blanks. The final results are expressed as pg PGE$_2$/mg weight of tissue.

STATISTICAL ANALYSIS

The PG synthesis results were compared by Student’s $t$ test for unpaired data. Significance was defined at $p<0.05$ level. Correlations were analysed by regression analysis.

ETHICAL APPROVAL

Ethical approval was obtained from the Ethics Committee, University College London, and written informed consent was obtained from each patient.

Results

COLONSCOPIC AND HISTOLOGICAL FINDINGS

Controls

Twelve patients (seven men, five women; mean (SD) age 60.1 (15.4) years) had normal colonscoposcopic and histological examinations.

Polyps

Nineteen patients (nine men, 10 women; mean (SD) age 65.0 (12.3) years) had a total of 27 adenomatous polyps removed at colonoscopic examination.

Carcinomas

Eight patients (four men, four women; mean (SD) age 74.5 (16.7) years) had histologically proved colorectal carcinomas.

Age

There was no difference in sex ratios between the three clinical groups. Control patients and patients with polyps were similar in age. However, patients with colorectal cancer were older than the control group ($p<0.02$) but not older than patients with polyps.

Site and histology

As expected, most of the polyps were found in the distal colon. One was found in the rectum, 22 in the sigmoid, two in the descending, and one each in the transverse and ascending colon. There were two rectal carcinomas, four sigmoid carcinomas and two caecal carcinomas. Most polyps were tubular adenomas (16), eight were tubulo-villous adenomas, and three villous adenomas. The carcinomas were all adenocarcinomas.

PROSTAGLANDIN SYNTHESIS

Controls

Results of PGE$_2$ synthesis for different areas of the colon were (values mean (SD) pg PGE$_2$/mg tissue) caecum 102.8 (15.9) (n=6); ascending 110.8 (24.3) (n=10), transverse 103.9 (19.5) (n=11), descending 102.9 (23.2) (n=12), and sigmoid colons 96.4 (18.0) (n=12); and rectum 107.1 (17.6) (n=12). The distribution of the individual results are shown in Figure 1. There were no differences between areas in the ability to synthesise PGE$_2$, nor was there any trend in synthesis ability from right to left within the colon.

Patients with colorectal neoplasia

Biopsy specimens from the background mucosa of patients with polyps synthesised (mean (SD)) 115.4 (21.9) (n=15) pg PGE$_2$/mg tissue. Where-
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![Prostaglandin E2 (PGE2) synthesis in polyps, carcinomas, and their associated mucosa.](image)

Discussion

We have shown that PGE2 synthesis is increased in adenomatous polyps compared with control mucosa. Colonic adenocarcinomas synthesise yet more PGE2, and an increase is also found in cancer-associated background mucosa. Since 1971, when Jaffe et al. first showed that human colonic cancer cells produced increased amounts of PGE2 compared with normal tissue, there has been little work on the temporal relationship between human colonic cancer development and the changes in mucosal PGE2 synthesis, or indeed of PGE2 synthesis in premalignant adenomatous polyps. The association between PGs and carcinogenesis is unclear, but it is known that PGs are involved in proliferation and metastasis. It is also known that PGE2 has an effect on the immune system, and may reduce immunosurveillance and promote tumour development.

One of us, with others, has shown a persistent increase of PGE2 synthesis in the colonic mucosa of rats after treatment with the colonic carcinogen 1,2 Dimethylhydrazine (DMH) — a change which was found in background mucosa and in resulting polyps and cancers. Although the increase in PGE2 could be a secondary response to the carcinogen, the persistence of the increase long after the initial exposure suggests that it is not. Likewise, the presence of increased PG synthesis before detection of cancers suggests that such an increase is not a product of the tumour cell mass. Similarly, N'-methyl-N'-nitro-N-nitroguanidine (MNNNG) induced colonic carcinoma has been found to be associated with high levels of PGE2 synthesis at all stages of tumour formation compared with controls, and again significantly increased synthesis was shown before tumour development.

The suggestion that increased PGE2 synthesis may be important in colonic tumourigenesis has important therapeutic implications. Animal models have shown that certain non-steroidal anti-inflammatory drugs (NSAIDs) can reduce the number of tumours induced by various carcinogens. Among those actions of NSAIDs which may be anti-carcinogenic is their ability to inhibit PG synthesis. Indomethacin has been shown to enhance lymphocyte function and responses to mitogens in patients with colonic cancer, partly through suppression of monoocyte PGE2 synthesis. Indomethacin also has an effect on the cellular immune system that is not dependent on PGs.

The association of reduced PG synthesis, NSAIDs, and the suppression of tumourigenesis in animal models has prompted studies into the potentially beneficial effect of NSAIDs in human colorectal neoplasia. Initial anecdotal reports that sulindac (Clinoral, MSD) caused regression of polyps in patients with familial polyposis and Gardner’s syndrome have been confirmed in a controlled trial. The incidence of colorectal cancer is also reduced in regular users of aspirin.

There has been recent interest in the role of diet in colorectal carcinogenesis. There is evidence that the consumption of fish products rich in w-3 fatty acids may protect against colorectal cancer, perhaps through their ability to inhibit cyclo-oxygenase and reduce PG synthesis.

Our findings that PGE2 synthesis is increased in premalignant adenomatous polyps and in the background mucosa of those patients with colorectal cancer, suggests that PGE2 may be involved in the progression of the adenoma-carcinoma sequence. We postulate that mucosal PGE2 is increased in susceptible individuals in response to an unknown stimulus and that this promotes tumorigenesis. If this is the case, the use of NSAIDs and dietary changes to prevent the recurrence of colonic polyps and colorectal cancer deserves wider study in at risk groups.
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S Pugh and G A Thomas

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