Changes in Rectal Epithelial Cell Proliferation and Intestinal Bile Acids after Subtotal Colectomy in Familial Adenomatous Polyposis


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ABSTRACT
Subtotal colectomy and ileorectal anastomosis in familial adenomatous polyposis patients can induce temporary regression of adenomas in the rectum. The mechanism for this phenomenon is unclear. We evaluated the effect of colectomy on rectal mucosal proliferation, in relation to changes in bile acid metabolism. Four familial adenomatous polyposis patients were studied before and 3–6 months after surgery, and eight others 7–22 years postoperatively. Within 6 months after surgery, the size of the proliferative zone of the colonic crypts was found to be reduced (P < 0.05). The proliferative activity of total colonic crypts was not affected within this period. More than 7 years postoperatively, increased cell proliferation of total crypts (P < 0.02), as well as mid (P < 0.05) and basal (P < 0.05) crypt compartments, were observed compared to shortly after colectomy. In duodenal bile, deoxycholic acid was absent shortly after operation, whereas several years after operation only a small fraction (2%) was present. Fecal secondary bile acid excretion diminished after colectomy and did not change several years postoperatively. In postoperative stools only, small proportions of ursodeoxycholic acid were consistently found. As subtotal colectomy causes a temporary decrease in the length of the proliferative zone of rectal crypts toward a normal pattern, this may explain regression of rectal polyps. This temporary effect may be mediated, at least in part, by decreased amounts of cytotoxic secondary bile acids in the rectal lumen.

INTRODUCTION
FAP is an autosomal dominantly inherited disorder with a penetrance of nearly 100% (1). It is characterized by the presence of a hundred to thousands of adenomatous polyps in the entire large bowel. Malignancy will certainly develop in one or more of these polyps, if left untreated. The treatment of choice is a prophylactic surgical resection of the affected colon to prevent malignant degeneration of the adenomas.

In 1957, Hubbard (2) described a 9-year-old boy with FAP, in whom a subtotal colectomy with ileorectal anastomosis was followed by spontaneous regression of the remaining polyps in the retained rectum. This observation has been confirmed in larger numbers of patients since then (3–7). The subsequent chance of development of polyps increased as the duration between colectomy and follow-up increased (6). These studies demonstrate that exogenous factors can influence the rate of polyp development in this genetically determined disorder. Another indication of environmental modification in FAP has been presented recently by DeCosse et al. (8), who showed a beneficial effect of dietary fiber supplements on the number of rectal polyps in the retained rectum. The authors suggested that the consumption of extra bran might have resulted in decreased exposure of the rectal mucosa to the secondary bile acid deoxycholic acid.

Several studies (9–14) have demonstrated that intestinal bile acids may be involved in the development of colon carcinoma. Animal experiments have provided evidence that the secondary bile acids in particular, i.e., DCA and LCA, can act as promoters of colon carcinogenesis (15–18). The damage thus produced by bile acids in the colonic epithelium can induce an enhanced, presumably compensatory, colonic epithelial cell proliferation (19–21). An increase in epithelial proliferation is characteristic of subjects with a high risk for colon cancer (22–27) and has been postulated to represent a first step toward carcinogenesis (28). The methodology of epithelial cell proliferation determination is well established. The determination of epithelial cell proliferation is more objective than polyp counting, which is subject to interobserver variation and estimation of polyp size. Therefore, epithelial cell proliferation may be a better biomarker for the measurement of exogenous modification in FAP.

In order to gain insight into the possible mechanism for the reduction of rectal polyps after operation, the effect of subtotal colectomy and ileorectal anastomosis on rectal epithelial cell proliferation and duodenal as well as fecal bile acids in patients with FAP was studied.

MATERIALS AND METHODS

Patients

Twelve patients (median age, 33 years; range, 16–50 years) with clinically and histologically proven FAP were studied. Four of these patients (one male and three females; median age, 22 years; range, 16–27 years) were evaluated before as well as 3–6 months after subtotal colectomy and ileorectal anastomosis. The other eight patients (two males and six females; median age, 41 years; range, 23–50 years) were examined 7–22 years after operation. The median age at the time of colonic resection in this late postoperative group was 24 years (range, 16–36 years). In one of these patients, a proximal jejunal resection of 20 cm was carried out 4 years prior to this study, because of obstruction by adenomatous polyps. Another patient in this group was known to have severe protein loss from the small intestine, due to the presence of multiple polyps. All patients were in a clinically stable condition. Patients were consuming their habitual, unrestricted mixed Western diet during the test period. No antibiotic drug therapy was used at least 3 months prior to or during investigations.

The study was approved by the Medical Ethical Committee of the University Hospital of Groningen. All patients gave informed consent.

Biopsies of Colonic Mucosa

Rectal biopsies were taken using a flexible sigmoidoscope. Three biopsies were obtained from macroscopically normal appearing rectal mucosa with a miniforceps at 10 cm from the anal ring. Epithelial cell proliferation, expressed as the LI, was measured by immunohistochem-
No. of subjects
No. of columns/subject
No. of cells/colunm/subject
Total no. of cells counted
Total no. of labeled cells

Pre <6 months Post > 7 years

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>4</th>
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<td>21 ± 4</td>
<td>35 ± 8</td>
<td>26 ± 8</td>
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<td>47 ± 3</td>
<td>58 ± 7</td>
<td>60 ± 9</td>
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<td>3900</td>
<td>8139</td>
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<tr>
<td>256</td>
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* Mean ± SD. For data on statistical analysis see text.

Epithelial detection of BrdU (Serva, Heidelberg, Germany) incorporation as described by Welberg et al. (25). In brief, biopsies were cut into small pieces (1 mm²) for optimal diffusion of BrdU and incubated in 5 ml Hanks' balanced salt solution, pH 7.3, with 10% fetal calf serum (Dakopatts, Copenhagen, Denmark) and 500 μM BrdU for 1.5 h at 37°C. Biopsies were then frozen in Tissue Tek (Miles, Elkhart, IN) and stored at −80°C until further processing. Cryostat-cut sections (4 μm) were incubated with anti-BrdU mouse monoclonal antibody and subsequently with peroxidase-conjugated rabbit anti-mouse antibody (Dakopatts, Copenhagen, Denmark). The reaction product was visualized using 3-amino-9-ethyl-carbazole (EGA-Chemie, Steinheim/Albuch, Germany). Counterstaining was performed with celestin blue (Gurr; BDH Chemicals, Ltd., Poole, Dorset, England) and Mayer's haematoxylin (Sigma, St. Louis, MO). The LI was determined light microscopically in whole-length-cut colonic crypts by dividing the number of labeled cells by the total number of cells × 100%. The distribution of labeled cells was obtained by dividing crypts in three equal compartments. Consequently, labeling indices of basal, mid, and luminal compartments were determined. In addition, the relative distribution of proliferating cells, i.e., the distribution of labeled cells given as a percentage of total cells per crypt compartment, was determined.

Analysis of Duodenal Bile Acids

Duodenal bile was collected with the Entero-Test (HDC Corp., MountainView, CA) as described previously (29). In brief, an encapsulated, weighted, woolly string (Entero-Test) was swallowed between 4 and 6 a.m. with the end of the string stuck on the cheek of the fasting patient. After 4 h the string was withdrawn and stored at −20°C until further processing. The adsorbed duodenal bile acids were eluted with 0.5 M phosphate buffer, pH 7.0, and extracted from the buffer with diethyl ether solution, which was evaporated, reconstituted with 0.154 M NaCl and again filtrated. Three 5-ml filtrate samples were transferred to three XAD-2 columns (Serva), which were washed and cooled, filtrated, and rotary evaporated. The sediment was dissolved in 100 μl acetone and used for GLC analysis. In summary, 20 ml l M NaOH in 90% ethanol, boiling chips, and nordeoxycholic acid (200 μg, 10 mmol/l) were incubated with 3-amino-9-ethyl-carbazole (EGA-Chemie, Steinheim/Albuch, Germany) unless otherwise stated. In summary, 20 ml 1 M NaOH in 90% ethanol, boiling chips, and nordeoxycholic acid (200 μl, 10 mmol/l; Steraloids, Inc., Wilton, NH) as internal standard were added to an aliquot of 0.5 g of dry feces. The mixture was refluxed for 2 h, cooled, filtrated, and rotary evaporated. The sediment was diluted in 50 ml 0.154 M NaCl and again filtrated. Three 5-ml filtrate samples were transferred to three XAD-2 columns (Serva), which were washed beforehand with distilled water, methanol, and acetone and again with distilled water. Bile acids were eluted with methanol and dried under a stream of nitrogen at 50°C.

Enzymatic Assay. One of the three samples obtained after preparation was used for enzymatic determination of total 3α-hydroxy bile acid 3α-hydroxy steroid dehydrogenase (Sterognost-3α Pho; Nycomed AS, Oslo, Norway). The enzymatic analysis was carried out in duplicate.

Gas-Liquid Chromatography. The other two samples obtained after preparation were used for GLC analysis of the major bile acids (CDCA, CA, UDCA, UCA, LCA, and DCA), and the results of the duplicate analyses were averaged. For this purpose, acid steroids were extracted four times with 10 ml diethyl ether after the addition of 2.5 ml acetate buffer (0.1 M, pH 5.6), 81 mg ethylenediaminetetraacetic acid (BDH Chemicals, Ltd., Poole, Dorset, England), 0.15 ml 2-mercaptoethanol, 5 ml H₂O₂, and 0.4 ml 6 M HCl. The diethyl ether solution was evaporated under N₂, subsequently dissolved in 10 ml 10% methanol in diethyl ether, and then methylated with N-methyl-N-nitroso-p-toluenesulfonamide. Dried methylated bile acids were converted into their trifluoroacetates by the addition of 0.2 ml trifluoroacetic anhydride and incubated in closed tubes for 15 min at 37°C. After nitrogen evaporation the mixture was dissolved in 100 μl aceton and used for GLC analysis. GLC analyses were performed on a Becker Multigraph type 409 equipped with a 2-m glass column packed with 3% OV 210 (Chrompack, Middelburg, The Netherlands). Nitrogen was used as a carrier gas at a flow rate of 10 ml/min. The inlet and outlet temperature was 280°C. The oven temperature was set at 220°C for 5 min and increased to 260°C for 18 min, at a rate of 2°C/min.

Gas Chromatography-Mass Spectrometry. Characterization of fecal bile acids of one patient before as well as after subtotal colectomy was carried out using a Finnigan MAT 212 mass spectrometer (Finnigan MAT, Bremen, Germany) coupled to a Varian 3700 gas chromatograph (Varian, Palo Alto, CA) equipped with a 25 m x 0.2 mm OV-1 capillary column as described previously (32). Bile acids were analyzed as methyl-ester trimethylsilyl-ether derivatives (33).

Statistical Analysis

The paired, two-tailed Student's t test was used to compare the results of the four patients investigated both before and after subtotal colectomy with ileorectal anastomosis. Comparison of parameters studied in FAP patients within 6 months postoperatively and FAP patients more than 7 years postoperatively was made using the Wilcoxon's rank-sum test for unpaired data. Spearman's rank correlation was used for the analysis of fecal bile acid excretion and colonic cell proliferation in postoperative patients. The level of significance was set at P < 0.05. The running average of the LI for each patient was determined and was defined as the average values of the LI, after stepwise summation of the LI of individual crypts (25). The running average of the LI of all subjects reached a stable level, indicating that the number of columns counted per subject did not influence the results obtained.

RESULTS

Epithelial Cell Proliferation. In all four patients studied before as well as after operation and in six of eight patients studied more than 7 years after subtotal colectomy, biopsies were taken. Epithelial proliferation parameters are shown in Table 1. The average length of the crypts, expressed as number of cells per column, increased (P < 0.05) within 6 months after surgical resection compared to before surgery. This crypt length remained high more than 7 years after operation.

The mean LI of total colonic crypts in the four patients studied both before and 3–6 months after colonic resection was similar pre- and postoperatively (Table 2). Before colectomy, the labeled epithelial cells were nearly equally scattered throughout the basal and midcompartment, with a LI of 10.2 and 8.1%, respectively. Shortly after the operation the LI of the midcompartment significantly decreased in all patients, to the average of 5.1%. At the same time the LI of the basal compartment slightly increased, although it was not statistically significant. Few proliferating cells were seen in the luminal compartment both before and after surgical resection. The relative distribution of proliferating cells within the crypts thus showed a reduction in the size of the proliferating zone. The percentage...
of labeled cells found in the basal compartment changed from 52 ± 7% (SD) prior to surgery to 67 ± 10% (P < 0.05) shortly after operation, whereas in the midcompartment it changed from 44 ± 9% to 29 ± 5% (P < 0.05). The percentage of proliferating cells in the luminal compartment was not affected by surgical resection; specifically, the percentages were 5 ± 4% preoperatively and 4 ± 5% postoperatively.

The mean LI of total crypts in patients who were operated on more than 7 years before the study increased compared to that of patients studied within 6 months after colectomy: 8.6 ± 1.3% versus 6.0 ± 1.2% (P < 0.02; Fig. 1). An increase in labeling in patients more than 7 years after colectomy compared to shortly after subtotal colectomy was also found in the basal compartment (16.6 ± 2.2% versus 12.1 ± 3.3%; P < 0.05) and midcompartment (8.3 ± 2.2% versus 5.1 ± 1.3%; P < 0.05). The relative distribution of replicating cells within the crypt more than 7 years after resection was not significantly different from that of patients shortly after operation; specifically, relative distributions were 65 ± 6% in the basal compartment, 32 ± 6% in the midcompartment, and 4 ± 1% in the luminal compartment.

Duodenal Bile Acids. Three of the four patients studied both before and after subtotal colectomy and seven of eight patients more than 7 years after the operation swallowed the Entero-Test successfully. The percentage of DCA decreased from 12 ± 3% before to 0 ± 0% (P < 0.02) shortly after colonie resection (Fig. 2). The percentages of CDCA and UDCA increased relative to this decrease in DCA, although they were not statistically significant: 38 ± 6% before versus 49 ± 13% after colectomy and 3 ± 2% versus 5 ± 13%, respectively. The percentage of the other major bile acid, CA, was not significantly affected by subtotal colectomy: 46 ± 18% before versus 46 ± 13% shortly after colectomy. In the group tested more than 7 years after surgery the percentages of DCA and CA increased to 2 ± 1% and 62 ± 7%, relative to a decrease in the percentages of CDCA and UDCA to 34 ± 7% and 2 ± 1%, respectively. These changes did not reach a statistically significant level. Only traces of LCA were detected before as well as after surgery.

Fecal Parameters and Bile Acids. Table 3 shows fecal parameters in the three periods studied. Values of preoperative fecal parameters were within the range of values observed in healthy subjects as described previously (34). Compared to before surgery, an increase (P < 0.002) of fecal wet weight shortly after surgery coincided with a small nonsignificant decrease of the percentage fecal dry matter. Consequently the fecal dry weight rose from 26 (16–26) to 37 (30–39) g/24 h (P < 0.05). Fecal pH decreased, although not significantly. The above-mentioned fecal parameters determined more than 7 years postoperatively did not differ significantly from recorded shortly after the operation.

Total fecal bile acid excretion in 24 h measured enzymatically showed a very marked increase shortly after surgery (P < 0.05; Table 3). All fecal samples determined by GLC analysis showed increased total fecal bile acid levels compared to before surgery, although this difference in total bile acid excretion did not reach statistical significance. Unidentified 3α-hydroxy steroid bile acids, not determined by GLC analysis, will probably contribute to the difference between the two measurements. Since our main interest concerned potential changes in the metabolism of DCA and LCA, no attempts were made to identify all fecal bile acid species present in feces. Fecal excretion of the secondary bile acids DCA and LCA was drastically reduced shortly
after colonic resection \( (P < 0.05) \). Despite the fact that trends of increasing total and secondary bile acid excretions were observed several years after surgery, these were not statistically significant compared to observations made shortly after surgery. A reversal of the ratio of secondary to primary bile acids was found after colectomy (Fig. 3). Whereas DCA and LCA surgery. A reversal of the ratio of secondary to primary bile acids observed several years after surgery, these were not statistically significant compared to observations made shortly after surgery (Fig. 3). Fecal bile acid profiles showed two distinctive extra peaks in all patients after subtotal colectomy and in no patients before surgery. These two peaks were identified as UCA and UDCA by gas chromatography-mass spectrometry. These tertiary bile acids comprised about 5% each of fecal bile acid composition in postoperative patients.

In all postoperative patients the labeling index of total colonic crypts did not correlate with the fecal secondary bile acid excretion in 24 h \( (r = 0.25) \). And the LI of total colonic crypts did not correlate with total acid excretion \( (r = 0.22) \).

**DISCUSSION**

Proliferation of epithelial cells normally mainly occurs in the basal compartment or deeper one-third of the crypt (24–26, 35, 36). An ectopic expansion of the proliferative zone represents a phenotypic marker associated with the development of a precancerous state (23). It has been reported that in FAP patients, tritiated thymidine labeling occurred in the surface cells of polyps and occasionally in the surface cells of the intervening, macroscopically normal, flat mucosa (23, 35, 37, 38). In the present study, prior to colonic surgery a nearly evenly divided labeling throughout the basal and midcompartment of patients was observed. Subtotal colectomy induced an obvious decrease of proliferation in the midcompartment, thereby reducing the size of the proliferating zone within 6 months after surgery. Colonic resection thus induces a more quiescent proliferation state of the rectal mucosa. Lengthening of colonic crypts was found, following subtotal colectomy. This also shows that a more quiescent proliferative activity of the colon mucosa was achieved after colectomy, since Lipkin et al. (39) reported a larger number of cells per crypt column in low-risk populations compared to high-risk populations for colon cancer. This beneficial effect on rectal epithelial proliferation had largely disappeared several years after subtotal colectomy. More than 7 years after resection, proliferation in the midcompartment as well as in the basal compartment increased again. Concurrently, proliferation of total colonic crypts increased. On the other hand, the length of crypts remained the same. In healthy subjects older than 65 years, an overall increase of epithelial cell proliferation and an upward expansion of the proliferative zone has been described in contrast to younger subjects (36). All FAP patients studied were younger than 50 years. The development of labeling indices matching those of high-risk populations at longer follow-up is thus not only due to the effect of aging, but is primarily due to other, most probably genetic factors. The changes in proliferation pattern, described above, are in agreement with the observations by others of polyp regression and subsequent redevelopment in the rectum following subtotal colectomy.

The composition of the bile acid pool markedly changed shortly after surgical resection. In duodenal bile, DCA completely disappeared postoperatively. This finding is consistent with that of Spigelman et al. (40), who found DCA percentages in bile of colectomized FAP patients with a mean value of 1%. The reduction of secondary bile acid proportions and excretions in feces, as also described by others (41–44), accords with the absence of duodenal DCA. Since the bacterial flora in the colon is responsible for the degradation of the primary bile acids into secondary bile acids, the absence of the colon in patients with an ileorectal anastomosis is the most likely cause for the change in fecal steroids. Total fecal bile acid excretion was increased after colectomy in all patients studied. These results are different from early reports by Watne et al. (42, 43), in which unchanged total fecal bile acid excretion after colectomy was described. However, because removal of the colon results in a reduction of the area involved in intestinal bile acid absorption, increased fecal bile acid loss is to be expected under these conditions. Such an interruption of the enterohepatic circulation will lead to increased bile acid synthesis (45). This may even be enhanced by the almost complete absence of DCA in the bile acid pool, since this hydrophobic bile acid species is under physiological conditions in rats a very strong inhibitor of hepatic bile acid synthesis (46).

Dehydroxylation at the \( \alpha \) position is the main step in the conversion of primary bile acids by colonic bacteria. The finding in this study that colectomized FAP patients excrete large amounts of CA and CDCA and reduced amounts of LCA and DCA in their feces denotes a particular lack of \( \alpha \)-dehydroxy-
lase activity. The reduced fecal pH, although not statistically significant, should be taken into account in this respect, since it is proposed that a low colonic pH inhibits the 7α-dehydroxylation of bile acids (47). Instead of the 7α-dehydroxylation expected under physiological conditions, an inversion of the 7α-hydroxy group to the 7β-position occurred, a step catalyzed by microbial dehydrogenases. This resulted in the excretion of bile acids and neutral sterols in colon cancer patients and patients with adenomatous polyps. Cancer (Philad.), 39: 2533–2539, 1977.


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