Sulindac Causes Regression of Rectal Polyps in Familial Adenomatous Polyposis

DENIS LABAYLE, DANIEL FISCHER, PHILIPPE VIELH, FRANCOIS DROUHIN, ALEX PARIENTE, CHRISTIAN BORIES, OLIVIER DUHAMEL, MICHEL TROUSSET, and PIERRE ATTALJ

Département Médico-Chirurgical des Maladies de l’Appareil Digestif, Centre Hospitalier Louise Michel, Evry; Département de Pathologie, Institut Curie, Paris; Service de Gastroentérologie, Centre Hospitalier La Source, Orléans; Service de Gastroentérologie, Centre Hospitalier de Béziers, Béziers; Service de Gastroentérologie, Centre Hospitalier de Beauvais, Beauvais; Service de Gastroentérologie, Centre Hospitalier d’Argenton, Argenteuil; and Service de Gastroentérologie, Centre Hospitalier de Bicêtre, Le Kremlin-Bicêtre, France

In familial adenomatous polyposis, sulindac-induced polyp regression has been reported by several authors. In this study, the goal was to confirm these results by a randomized, placebo-controlled, double-blind crossover study in 10 patients with rectal polyps that had been previously treated by colectomy and ileorectal anastomosis. Patients received sulindac, 300 mg/day, or placebo during two 4-month periods separated by a 1-month wash-out phase. One patient was not compliant and was excluded. With sulindac, the authors observed a complete (6 patients) or almost complete (3 patients) regression of the polyps. With placebo, the authors observed an increase (5 patients), no change (2 patients), and a relative decrease (2 patients) in the number of polyps. The difference between sulindac and placebo was statistically significant (P < 0.01). In biopsy specimens of polyps and normal rectal mucosa of 6 patients, the authors conducted an immunohistochemical study of the cellular proliferation index using the Ki 67 monoclonal antibody (Ki 67 index), at the beginning and at the end of each treatment period. They were not able to show a sulindac-induced modification of the Ki 67 index. The authors conclude that sulindac is effective in inducing the regression of rectal polyps in familial adenomatous polyposis.

The inevitable development of colorectal cancer in patients with familial adenomatous polyposis (FAP) justifies preventive colectomy or coloproctectomy. The cumulated incidence of rectal cancer after ileorectal anastomosis is 5% and 59% after 5 years and 23 years, respectively (1). These features reinforce the need for periodic endoscopic controls that allow removal or destruction of polyps with hot biopsy forceps, diathermy snare, or laser photocoagulation (2).

In 1983, Waddell and Longhry (3) first reported the regression of colorectal polyps in FAP patients treated with sulindac, a nonsteroidal antiinflammatory drug. Since this report, similar results have been obtained by several investigators in uncontrolled trials of the drug (4–7).

The aim of the present study was to assess the efficacy of sulindac on the regression of rectal polyps after subtotal colectomy in FAP in a randomized, placebo-controlled, double-blind crossover study. To investigate the effects of sulindac at the cellular level, we also conducted an immunohistochemical study using the Ki 67 monoclonal antibody in six patients.

Methods

Patients

Patients of either sex with FAP, older than 18 years, who had undergone prior colectomy with ileorectal anastomosis and had polyps in the rectum were included in the study. Patients with histories of peptic ulcer disease or of liver cirrhosis and blood creatinine levels >120 μmol/L were excluded.

The initial clinical, endoscopic, and histological features of the 10 patients included into the study are shown in Table 1. There were 2 women and 8 men, mean age 36.9 years (range 24–52); 2 men were brothers. The time interval
between colectomy and inclusion into the trial ranged from 1 to 18 years (mean, 5.9 years). In 2 patients, the disease had already evolved to cancer (of the transverse colon in 1, of the sigmoid colon in 1) at the time of diagnosis. Associated extracolonic lesions were gastric polyps (4 patients), duodenal polyps (3 patients), ileal polyps (3 patients), and extradigestive tumors (3 patients: 1 osteoma, 2 desmoid tumors and dermoid cysts). At the time of inclusion, histology of rectal polyps was assessed in all patients: 9 patients had tubular adenomas without dysplasia, 1 patient had tubulovillous adenomas without dysplasia. All patients gave written informed consent to participate in the study.

### Clinical Study

This multicenter study (six participating hospitals) was double-blind, and each patient served as his or her own control. For each patient, two 4-month treatment periods were separated by a 1 month period without treatment ("wash-out" period). During each treatment period, patients were given sulindac, 100 mg, or an identical-appearing placebo, three times per day. For each patient, the treatment sequence was randomly predetermined and was unknown to the patient, the prescriptor, or the endoscopist. Hence, 5 patients took sulindac during the first 4 months then switched to placebo after 1 month of wash out (group 1), and 5 patients took first placebo then sulindac (group 2). During the course of the study, no other medication (particularly other nonsteroidal antiinflammatory drugs or pros taglandins) was taken by the patients.

Flexible rectoscopy was performed on all patients at the beginning and at the end of each 4-month treatment period. Most patients underwent additional endoscopic procedures during the treatment periods. For each individual patient, all endoscopic procedures were performed by the same endoscopist with the same endoscope. Bowel preparation with polyethylene glycol 4000 (2 L PO 6 hours before the procedure) allowed a complete and satisfactory exploration of the rectum and of the anastomotic region in all cases. The endoscopist used a semiquantitative classification for each examination: grade 1, no polyp; grade 2, < 5 polyps; grade 3, 5–10 polyps; grade 4, 11–20 polyps; grade 5, > 20 polyps.

Digestive tolerance to the drug was appreciated on symptoms, and renal tolerance was assessed using the blood creatinine level at the beginning and at the end of each treatment period.

### Immunohistochemical Study

The immunohistochemical study was conducted as follows. Endoscopically removed polyps and normal colonic mucosa biopsy specimens were frozen in embedding medium (Tissu-Tek, Miles Scientific, Elkhart, IN). Cryostat sections (5 μm) were immediately prepared and fixed in cold acetone (−20°C. 10 minutes), air-dried, and stored at −80°C until used. For immunostaining, sections were washed (1–2 minutes) and incubated for 30 minutes with the Ki 67 (Dakopatts, Copenhagen, Denmark) murine monoclonal antibody (dilution, 1:10) (8); Slides were then incubated successively with the biotinylated horse anti-mouse antibody and the avidin-biotinylated peroxidase complex (Vector Laboratories, Burlingame, CA). Incubations were performed at room temperature. Washings and dilutions were performed with phosphate-buffered saline, pH 7.6. Staining with the diaminobenzidine substrate was developed until labeling was clearly detectable. Control slides were incubated with an unrelated monoclonal antibody. Quantification of Ki 67 staining (Ki 67 index) was achieved using an ocular micrometry on a Leitz microscope (Orthoplan). For each patient, the Ki 67 index was determined using an ocular magnification of 40 x with an eyepiece grid (Leitz). Ki 67 index was based on a count of 200 consecutive glandular cells and was expressed as a percentage of labeled cells per 100 cells.

Statistical analysis of the results was performed using the Wilcoxon test.

### Results

#### Clinical Study

All patients but one completed the study. Patient 7 was excluded from the statistical analysis because of a lack of compliance with treatment. Results of groups 1 and 2 are shown in Figures 1 and 2, respectively.

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<table>
<thead>
<tr>
<th>No. of polyps</th>
<th>Size of polyps (mm)</th>
<th>Histology of rectal polyps</th>
<th>Gastric polyps</th>
<th>Small intestinal polyps</th>
<th>Mesenchymal tumors</th>
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<tbody>
<tr>
<td>1 M 24</td>
<td>4</td>
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<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>2 M 39</td>
<td>7</td>
<td>TA</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
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<td>+</td>
<td>−</td>
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<tr>
<td>4 M 36</td>
<td>4</td>
<td>TA</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>5 M 32</td>
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<td>TA</td>
<td>+</td>
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<td>9 F 30</td>
<td>4</td>
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<td>−</td>
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<td>−</td>
</tr>
<tr>
<td>10 M 33</td>
<td>6</td>
<td>TA</td>
<td>−</td>
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</table>

TA, tubular adenomas; TVA, tubulovillous adenomas.
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Figure 1. Results of group 1 (5 patients), receiving sulindac then placebo.

Group 1 included patients 2, 3, 6, 8, and 10. At the time of inclusion, 2 patients were grade 5, 1 was grade 4, and 2 were grade 3. At the end of the 4-month period with sulindac, we observed a complete regression of the polyps in 4 patients and a decrease from grade 5 to grade 2 in the fifth patient. A reappearance of the polyps or an increase in their numbers was observed at the end of the wash-out period in 2 patients and after 4 months of placebo in 2 other patients. One patient still had no polyp at the end of the trial.

Group 2 included patients 1, 4, 5, and 9. At the time of inclusion, 2 patients were grade 5, 1 was grade 3, and 1 was grade 2. At the end of the first period (placebo), we observed an increase in the number of polyps in 1 patient, no change in 1 patient, and a decrease from grade 5 to grade 3 in 2 patients (patients 4 and 9). However, these 2 latter patients were again classified grade 5 by the endoscopist 1 month later, at the beginning of the second treatment period. After 4 months of sulindac, 2 patients were grade 1 and 2 were grade 2.

Figure 3 compares placebo and sulindac periods when combining the results of the 9 patients who completed the study; sulindac was significantly more effective than placebo ($P < 0.01$).

Immunohistochemical Study

Results of the immunohistochemical study conducted on biopsy specimens of polyps and rectal mucosa, at the beginning and at the end of each treatment period, in 6 patients (3 from group 1 and 3 from group 2) are shown in Table 2. No differences were found between Ki 67 indices measured in the 6 patients or in the sequential samples obtained from each patient during the course of the study.

Table 2. Ki 67 Indices, Expressed As Percentage of Cycling Cells, in Six Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Biopsy specimen</th>
<th>Placebo</th>
<th>Sulindac</th>
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<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>T</td>
<td>T'</td>
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<tr>
<td>1</td>
<td>N</td>
<td>16</td>
<td>15</td>
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<td></td>
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<td>18</td>
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<td>P</td>
<td>19</td>
<td>17</td>
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<td></td>
<td>P</td>
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</table>

N, normal mucosa; P, rectal polyps; T, beginning of treatment period; T', end of 4-month treatment period.
Discussion

We report the results of the first randomized trial showing the efficacy of sulindac on the disappearance of colorectal polyps in FAP. In spite of the small number of patients, the difference between sulindac and placebo was statistically significant. Regression of the polyps with sulindac was achieved in < 4 months in the majority of cases. Digestive and renal tolerance was excellent, and no adverse effect was observed with the dosage chosen.

After switching to placebo, polyps reappeared in < 4 months in 4 of 5 patients. As could be expected, the number of polyps either increased or did not change in most of the patients receiving placebo. The transient decrease in the number of polyps in 2 patients during the placebo period may be explained by an error in the counting of the polyps because of their small size (< 2 mm in both patients) (Table 1). Spontaneous regression of polyps has been reported in FAP, but it occurred immediately after subtotal colectomy (9,10), which was not the case in these 2 patients (4 years for each).

Our results confirm previously published observations (4–7). Since Waddell and Longhry’s report in 1983 (3), 22 cases of FAP polyposis regression with sulindac have been reported; 17 of these patients had previously undergone colectomy with ileorectal anastomosis, and 5 patients had not undergone operations. The reported doses of sulindac ranged from 150 to 450 mg/day (6); for our study we chose the most commonly prescribed dose (300 mg/day). Regression seemed to be influenced neither by the initial numbers (6,7) nor by the sizes (5) of the polyps. With respect to the number of polyps, this seems to be confirmed by our study: 3 of the 5 patients who were initially grade 5 had complete regression of their polyps with sulindac.

Polyp regression in our patients was always rapid, occurring in < 4 months. In previous reports, the time required for polyp disappearance ranged from 2 to 8 months when mentioned.

In our group 1, recurrence of the polyps was quick (< 4 months in four of five patients) after stopping sulindac. Cherneau et al. (7) found recurrence in 4 of 7 patients after 4 months with no treatment; one patient had no recurrence after 19 months but had received 5-fluorouracil, cisplatin, and mitomycin during that time.

Most studies investigating the biochemical and molecular effects of prostaglandin inhibitors on cell multiplication have been performed in vitro using indometacin. Bayer et al. (11) have shown that indometacin inhibits the C phase of the cell cycle (S phase of the cell cycle). Indometacin has been shown to be capable of inducing regression of some animal (13,14) and human (15,16) tumors in vitro, but not of colonic polyps in vivo (3). This is probably because of its low colonic concentrations (17).

Biochemical effects of sulindac in vivo are caused by a sulfide metabolite derived after sulforeduction (18,19). This metabolite is mostly formed in the colon, where anaerobic bacterial enzymes are present (18). Concentrations of the sulfide metabolite are low in the duodenum and high in the colon (18).

The cellular effects of sulindac are still unknown. In 6 patients, we calculated the percentage of cycling cells in rectal polyps and normal mucosa biopsy specimens at the beginning and the end of each treatment period using an immunohistochemical method based on the use of the Ki 67 monoclonal antibody (8). We were not able to show a sulindac-induced modification of the Ki 67 index, but it should be emphasized that Ki 67 antibody binds to a nuclear antigen expressed in cycling cells but not in resting cells (20). Therefore, Ki 67 immunostaining may be observed during the S phase but also during the G1-, G0-, and M phases of the cell cycle (8,20). Risio et al. (21) recently described a technique, based on the use of 5-bromodeoxyuridine, that should enable investigators to determine whether or not sulindac interferes with the S phase of the cell cycle in vivo.

In conclusion, the results of this randomized, double-blind, crossover study show that sulindac (300 mg/day) induces a significant, and often complete, regression of FAP rectal polyps. Nevertheless, three problems remain unsolved. The quick recurrence of polyps after stopping sulindac emphasizes the need for a maintenance treatment. Long-term or intermittent administration of sulindac in young patients could result in digestive and/or renal complications. The sulfide metabolite of sulindac has been synthesized, and a study of the effects of its rectal administration (enema) in FAP patients with ileorectal anastomosis and rectal polyps could be justified, with the aim of reducing the risk of adverse effects. It is yet too early to establish a correlation between the regression of polyps with sulindac and a diminution of the malignant transformation incidence.

References