Gastrointestinal polyposis syndromes

Keller, J.J.

Citation for published version (APA):
Chapter 2

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Josbert J. Keller, Francis M. Giardiello

Department of Pathology, Academic Medical Center, Amsterdam, The Netherlands
Department of Medicine, Johns Hopkins University Hospital, Baltimore, MD, USA

Cancer Biology and Therapy 2003, in press
Models of Anti-Cancer Therapy

Chemoprevention Strategies Using NSAIDs and COX-2 Inhibitors

Johbert J. Koller¹,²
Francis M. Ghadri²

¹Department of Pathology; Academic Medical Center; Amsterdam, The Netherlands
²Department of Gastroenterology; Division of Medicine; Johns Hopkins University School of Medicine; Johns Hopkins Hospital; Baltimore, Maryland USA

ABSTRACT

The chemopreventive efficacy of NSAIDs against colorectal cancer has been established. However, NSAIDs may decrease the incidence of carcinomas of the esophagus, stomach, breast, lung, prostate, urinary bladder and ovary. The clinical use of these agents is limited to patients with familial adenomatous polyposis (FAP), which may benefit from chemopreventive treatment with sulindac or the selective COX-2 inhibitor celecoxib. The mechanism of chemoprevention by NSAIDs is still a matter of debate. COX-2 inhibition explains at least part of the observed effects, however, other targets have been proposed. Furthermore, the effect of NSAIDs is incomplete and molecular mechanisms related to resistance need to be studied. Also, more effective chemopreventive regimens are needed; combination therapy appears attractive, potentially increasing efficacy and decreasing toxicity. This review discusses molecular and clinical aspects of chemoprevention of the adenoma-carcinoma sequence with NSAIDs or COX-2 inhibitors.

INTRODUCTION

The chemopreventive potential of nonsteroidal anti-inflammatory drugs (NSAIDs) against colorectal carcinoma (CRC) has been documented by four lines of evidence:
1. epidemiological studies;
2. animal studies;
3. in vitro experiments; and
4. clinical trials.

Also, NSAIDs may decrease the incidence of carcinomas of the esophagus, stomach, breast, lung, prostate, urinary bladder and ovary. Although the chemopreventive activity of NSAIDs appears established, debate continues about the mechanisms underlying these effects. Inhibition of cyclooxygenase-2 (COX-2) seems a partial explanation but many alternative targets have been proposed. Despite studies favoring chemoprevention by NSAIDs, the clinical use remains limited to individuals with familial adenomatous polyposis (FAP). Additional patients who may benefit from NSAIDs-based chemoprevention are those with hereditary non-polyposis colorectal cancer (HNPPC), other hereditary colorectal cancer syndromes, and sporadic adenomatous polyps. This review discusses the current progress and future perspectives for chemopreventive strategies for colorectal cancer prevention with NSAIDs and COX-2 inhibitors.

ADENOMA-CARCINOMA SEQUENCE AND MECHANISM OF CHEMOPREVENTION

Adenoma-Carcinoma Sequence. The adenoma-carcinoma sequence is a multi-step model describing the transition of normal colorectal mucosa into invasive carcinoma. Subsequent pre-cursor stages are aberrant crypt foci (ACF), small tubular adenomas, and large adenomas with high-grade dysplasia. Colorectal carcinogenesis is driven by an accumulation of molecular alterations, inactivating tumor-suppressor genes, and activating oncogenes (Fig. 1).

Initiation of the adenoma-carcinoma sequence requires oncogenic activation of the Wnt-pathway, in which APC and β-catenin are essential components. Wild type APC binds β-catenin, enabling phosphorylation and degradation of β-catenin. Inactivation of APC, or activating mutations in β-catenin result in nuclear accumulation of non-phosphorylated β-catenin and β-catenin/TCF-4 mediated transcription of Wnt-target genes. Inactivation of APC is found in most CRCs; the remaining cases are likely to harbor β-catenin mutations. FAP patients have an inherited germline mutation in one of the APC alleles, accelerating the initiation of the adenoma-carcinoma sequence, which results in the development of numerous adenomas at young age.
Progression of the adenoma-carcinoma sequence is characterized by additional mutations in a consecutive order (Fig. 1): activating K-RAS mutations, LOH at 18q (SMAD locus), and inactivation of TPT53. Concomitantly, the expression of important cell regulatory proteins is altered, amongst others cyclooxygenase-2 (COX-2), which regulates the conversion of arachidonic acid into prostaglandins. The iso-enzyme COX-1 is considered the housekeeping enzyme and constitutively expressed. Expression of COX-2 is absent under physiological conditions but is increasingly induced during consecutive stages of the adenoma-carcinoma sequence. The role of this enzyme in colorectal carcinogenesis was established by Oshima et al., showing that COX-2 deficiency partly suppressed the adenoma phenotype of Apcmin mice. The tumor-promoting effect of COX-2 is in part mediated by binding of increased levels of PGE2 to its receptor EP2, resulting in resistance to apoptosis, increased angiogenesis, and disturbed cell adhesion. In adenomas, both epithelial and stromal COX-2 overexpression can be found. The significance of stromal overexpression is unclear: a "landscaping" role has been suggested.

Two distinct pathways of colorectal carcinogenesis are recognized, based on morphological and molecular-genetic differences (Fig. 1). In FAP and most sporadic CRCs, carcinogenesis is characterized by chromosomal instability leading to frequent loss of heterozygosity (LOH) throughout the genome. The cause of chromosomal instability is yet unknown. Mutations in mitotic checkpoint genes and the APC gene, independent of its Wnt-regulatory function, may be involved. In contrast, carcinogenesis in HNPCC and ~10% of sporadic CRCs is characterized by mismatch repair (MMR) deficiency, caused by inactivation of one of the MMR genes, most often hMLH1 or hMSH2. HNPCC patients have a germline MMR-gene mutation. Inactivation of the wild type allele results in a hypermutable state with unstable replication of repetitive DNA sequences, reflected by microsatellite instability (MSI) and frameshift mutations in growth regulatory genes. Thus, MMR-deficiency accelerates the progression of the adenoma-carcinoma sequence. In sporadic CRC, MSI may result from hypermethylation of CpG-islands at the promoter region of hMLH1, silencing its expression. Oncogenic activation of the Wnt-pathway in MMR-deficient CRC is characterized by a relative excess of frameshift APC mutations, and β-catenin mutations. Further hallmarks of MMR-deficient CRCs are mutations in genes containing simple repeated sequences in their coding region, such as TGF-β-II receptor, mutated in ~80% of CRC with MSI, and BAX, mutated in ~50% of CRC with MSI. Of note, there is less COX-2 expression in CRCs with MSI than in CRCs with MSI-negative. Differences between carcinogenesis in HNPCC and FAP may have implications for chemopreventive strategies.

Mechanism of Chemoprevention (See Fig. 2). The chemopreventive effect of NSAIDs appears mediated by induction of apoptosis. This is supported by the observations that NSAIDs induce apoptosis in:

1. CRC cell lines;
2. CRCs of carcinogen treated rats;
3. normal intestinal mucosa of Apcmin mice;
4. normal rectal mucosa of FAP patients.

Whether NSAIDs suppress proliferation remains controversial. NSAIDs inhibit proliferation of CRC cells in vitro and decrease the proliferation rate in tumors from carcinogen treated rats and Apcmin mice. However, there was no effect of sulindac on the extent and localization of proliferation in the normal colorectal mucosa of FAP patients, although decreased rectal epithelial proliferation was reported after rectal administration of sulindac. Also, decreased proliferation was observed in the duodenum of FAP patients on sulindac. The absence of an effect on proliferation in normal colorectal mucosa does not exclude an anti-proliferative effect of NSAIDs in adenomas.

COX-2 appears the most important molecular target of NSAIDs. These agents are known for COX inhibitory capacity and COX-2 is involved in early stages of the adenoma-carcinoma sequence. Evidence that COX-2 inhibition has chemopreventive potential is based on:

1. In vitro studies;
2. studies with different mouse models of FAP and carcinoma treated rats;
3. a randomized trial showing that the selective COX-2 inhibitor celecoxib caused adenoma regression in patients with FAP.
Furthermore, prostaglandin levels were decreased in the colorectal mucosa of FAP patients with adenoma regression on sulindac, indicating that COX-2 is a chemopreventive target of NSAIDs. Of note, NSAIDs not only inhibit COX-2 activity, but also reduce COX-2 expression. This may be explained by a positive feedback mechanism via PGE2 and its receptor EP2.

Evidence for COX-2 independent mechanisms of NSAID chemoprevention also exists. NSAIDs induce apoptosis in cells lacking COX-2, and prostaglandins do not rescue cells from NSAIDs-induced apoptosis. Concentrations of NSAIDs required for growth inhibition of tumor cells exceed the COX-2 inhibiting dose. Furthermore, sulindac appears more powerful than celecoxib (see below), indicating that COX-2 inhibition alone may not suffice.

Other proposed targets of chemoprevention include RAS, NF-κB, PPARs, Bcl-XL, FAK, NAG-1, and β-catenin. But in vivo support is lacking for most of these, and doses used in laboratory settings often exceed lethal human concentrations. A postulated target is β-catenin. This oncogene effectuates the activation of the Wnt-pathway, which is crucial for the initiation of the adenoma-carcinoma sequence. Thus, reducing nuclear β-catenin levels could be important in preventing the adenoma-carcinoma sequence. In vitro and in vivo studies show that sulindac and indomethacin affect the expression or localization of β-catenin. Sulindac and indomethacin inhibited the nuclear accumulation of β-catenin in colon cancer lines, directly affected Wnt-regulated transcription, and altered the expression of the Wnt-targets cyclin D1, Met, and CD68. In addition, sulindac and other NSAIDs downregulated the expression of β-catenin in normal mucosa and adenomas of ApoE mice, and prevented nuclear accumulation of β-catenin in tumors from carcinoenabled rats and in adenomas from FAP patients. Also, the sulfone metabolite of sulindac, which lacks COX-inhibitory capacity, reduced nuclear β-catenin in a CRC cell line, suggesting that the effect of sulindac against nuclear β-catenin is COX-2 independent.

In vitro and animal studies using ApcMin mice and cancer cell line xenografts in nude mice point to the anti-angiogenic activity of NSAIDs. This effect appears to be both COX-2 dependent and independent, and may be mediated by downregulation of important pro-angiogenic growth factors, such as VEGF and bFGF. The extent to which inhibition of angiogenesis contributes to the chemopreventive effects of NSAIDs is unknown.

Partial Inhibition of Neoplasms. Sulindac and other NSAIDs do not completely prevent the development of colorectal cancer. Understanding the mechanism of resistance is of clinical importance to define the limitations of NSAIDs based chemoprevention. In vitro and in vivo studies point to selective inhibition of certain tumor cells by sulindac and other NSAIDs. Molecular alterations possibly involved in selectivity are RAS-mutations, BAX-mutations, p27kip1 inactivation, and SRC-mutations.

RAS mutations may affect the sensitivity to NSAIDs, although findings are not uniform. For example, while some studies have reported that K-RAS-transformed rat intestinal cells were relatively resistant to sulindac-induced apoptosis, in addition, K-RAS mutations were found in sulindac-resistant adenomas from FAP patients. These data suggest that acquisition of a K-RAS mutation may be critical in blunting chemoprevention by sulindac. Others, however, found no effect of activated K-RAS on the growth inhibitory capacity of sulindac. Also, sulindac specifically inhibited transformation of rat intestinal cells mediated by activated H-Ras, and H-Ras transduced rat intestinal cells appeared more sensitive to COX-2 inhibition induced apoptosis. In addition, tumors from carcinoenabled rats displayed fewer K-Ras and H-Ras mutations after treatment with piroxicam or sulindac. Finally, sulindac directly inhibits oncogenic RAS-signaling in vitro. Taken together, data about RAS activation and NSAIDs seem contradictory and need further study.

In vitro, wild type BAX is a precondition for NSAIDs-induced apoptosis. Also, this effect appeared dependent on changes in the ratio between BAX and Bcl-XL. Thus, BAX mutations may be involved in sulindac/NSAID resistance. This observation could have important implications for the use of NSAIDs as chemopreventive
agents, since BAX mutations are frequently found in HNPPC related tumors. The role for p21<sub>FMF</sub> in the chemopreventive action of sulindac was established by the finding that adenomas from Apc<sup>1638T</sup> mice with inactivation of p21<sub>FMF</sub> (APC/p21 mice) did not respond to sulindac treatment. Finally, preliminary data suggest that NSAIDs promote the invasive potential of activating-SRC mutation containing cells. Further studies are needed to elucidate whether and how treatment with NSAIDs or selective COX-2 inhibitors induce specific pathways of carcinogenesis, while preventing others.

### Biomarkers

Biomarkers are needed to monitor and/or predict the effect of chemopreventive therapy for colorectal neoplasia. In addition, biomarkers may provide surrogate endpoints to assess the efficacy of treatment. Ideally, a biomarker is involved in carcinogenesis and constitutes a target for chemopreventive therapy. Alterations in the expression or presence of such a biomarker in the normal colorectal mucosa should predict treatment response.

Presently, the most promising biomarker for NSAID chemoprevention is NSAIDs-induced downregulation of prostaglandin levels in the normal colorectal mucosa. These levels can predict regression of adenomas and suppression of the development of adenomas by sulindac in FAP patients with adenomas and presymptomatic individuals respectively. However, the usefulness of prostaglandin levels is limited since they did not predict the development of a breakthrough carcinoma. Also, sulindac induced downregulation of interstitial prostaglandin levels appeared independent of the

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**Table 1** FAP Patients Treated with Sulindac, Indomethacin or Celoxics for Colorectal Adenomas

<table>
<thead>
<tr>
<th>First author</th>
<th>Ref.</th>
<th>Treatment dose/day</th>
<th>Duration</th>
<th>Patients</th>
<th>Effect on (role) rectal polyps</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gniadek*</td>
<td>93</td>
<td>sulindac 300 mg</td>
<td>9 months</td>
<td>11</td>
<td>44% in rectal polyp number after 9 months.</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>35% in polyp size after 9 months</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>maximal effect at 6 months (p&lt;0.001 vs placebo)</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>recurrence after discontinuation of treatment</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td>Nogawa*</td>
<td>43</td>
<td>sulindac 60 mg</td>
<td>6 months</td>
<td>11</td>
<td>regression of adenomas in 3 patients</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 patient dropped out (necrosis)</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td>Laboyle*</td>
<td>40</td>
<td>sulindac 300 mg</td>
<td>4 months</td>
<td>10</td>
<td>disappearance of polyps in 6 patients; number of polyps in 3 patients (p&lt;0.01 vs placebo)</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>recurrence after discontinuation of treatment</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td>Gniadek*</td>
<td>78</td>
<td>sulindac 150-300 mg/day</td>
<td>4 years</td>
<td>21</td>
<td>young asymptomatic FAP patients (primary prevention)</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td>Steinbach*</td>
<td>49</td>
<td>sulindac 300 mg</td>
<td>6 months</td>
<td>32</td>
<td>12% in colorectal polyp number (p&lt;0.33 vs placebo)</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12% in colorectal polyp burden (p&lt;0.09 vs placebo)</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>28% in colorectal polyp number (p&lt;0.003 vs placebo)</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td>Feminboh</td>
<td>91</td>
<td>sulindac 300 mg</td>
<td>6 months</td>
<td>29</td>
<td>complete regression of rectal polyps in 28 patients</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 patient did not respond and subsequently developed CRC</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td>Spagnesi*</td>
<td>39</td>
<td>sulindac 200 mg</td>
<td>2 months</td>
<td>20</td>
<td>number of polyps (p&lt;0.01)</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td>Goldnach*</td>
<td>41</td>
<td>sulindac 300 mg</td>
<td>4 months</td>
<td>17</td>
<td>77% in colorectal polyp number (p&lt;0.007)</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td>Muller</td>
<td>87</td>
<td>sulindac 300 mg</td>
<td>4 months</td>
<td>10</td>
<td>regression of polyps in 9 patients</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td>Inama</td>
<td>88</td>
<td>sulindac 150mg/day</td>
<td>3 months</td>
<td>10</td>
<td>partial regression in 3 patients</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td>Charnow</td>
<td>86</td>
<td>sulindac 200-300 mg</td>
<td>4 months</td>
<td>8</td>
<td>disappearance of polyps in 7 patients, 1 patient dropped out of trial</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td>Ishikawa</td>
<td>90</td>
<td>sulindac 100-300 mg</td>
<td>6-24 months</td>
<td>6</td>
<td>number of polyps in 3 patients after 6 months</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td>Laboyle*</td>
<td>85</td>
<td>sulindac 300 mg</td>
<td>2 months</td>
<td>3</td>
<td>disappearance in 2 patients, clinical complete regression in 1 patient</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td>Hicato</td>
<td>89</td>
<td>indomethacin 100 mg sup</td>
<td>4-8 weeks</td>
<td>6</td>
<td>&gt;50% in polyp number</td>
<td>no adverse events due to sulindac</td>
</tr>
<tr>
<td>Delibe</td>
<td>92</td>
<td>nimesulide 2 mg/kg</td>
<td>2.5 months</td>
<td>7</td>
<td>no effect</td>
<td>no adverse events due to sulindac</td>
</tr>
</tbody>
</table>

*Indicates that patients were treated with sulindac or indomethacin. + indicates individual patients who received sulindac or indomethacin.
Clinical response in another study with FAP patients, and in ApcMin mice. Another biomarker examined was sulindac-induced apoptosis. This was a correlation between treatment response and the effect of sulindac on the compartmentalization of apoptosis throughout the crypt. However, sulindac did not affect rectal epithelial apoptosis in young FAP patients without adenomas. Further studies, using more refined methods for apoptosis detection, are needed to evaluate the clinical potential of this marker. Additional insights in the mechanisms of chemoprevention will enable the future development of accurate biomarkers.

NSAIDS AND COX-2 INHIBITORS FOR COLON CANCER PREVENTION

Chemoprevention of FAP. Familial adenomatous polyposis (FAP) is an autosomal-dominant disorder caused by a germline mutation of the Apc gene. FAP patients develop innumerable colorectal adenomas and colorectal cancer is inevitable. Prophylactic surgery is indicated and colectomy with an ileorectal anastomosis (IRA) is a surgical option. After colectomy, these patients are still at increased risk for the development of rectal stump cancer. Prevention of rectal stump adenomas, and of adenomas in young pre-symptomatic FAP patients are major targets for chemopreventive treatment in FAP.

Reports of FAP patients treated with sulindac, indomethacin or selective COX-2 inhibitors are listed in Tables 1 and 2. Endpoints are number and size of adenomas measured by flexible sigmoidoscopy or colonoscopy. All studies report complete or partial regression of adenomas after 3-6 months of treatment with sulindac 300-400 mg a day. However, doses of sulindac 150-200 mg a day appeared ineffective. A non-randomized controlled study showed the efficacy of rectal administration of sulindac 300 mg a day against rectal adenomas. Also, indomethacin suppositories regressed rectal polyps in a non-controlled series of patients at a dose of 100 mg, but not 50 mg a day.

Four randomized, placebo controlled trials have established that sulindac 300-400 mg a day and celecoxib 800 mg a day reduce the number and size of adenomas in the rectum of FAP patients. Giardiello et al. found a 65% decrease of rectal adenomas after 6 months of sulindac 300 mg a day. The most conservative recalculation of the semiquantitative measured response reported by Labayle et al. reveals a 78% decrease of adenomas after 4 months of sulindac 400 mg a day. Combining these randomized studies, patients treated with sulindac 300-400 mg a day (n = 21) showed a 71% decrease of rectal adenomas after 4-6 months, compared to a 23% decrease in 27 patients treated with celecoxib 800 mg a day after 6 months. When comparing the efficacy of these agents, the surgical status of FAP patients should be accounted, since patients with subtotal colectomy have greater polyp regression on sulindac than those with an intact (non-operated) colon.

The potency of sulindac may be related to its metabolism. Sulindac is a pro-drug (sulindac sulfide), which is converted into sulindac sulfide, the active COX-inhibitory metabolite, and sulindac sulfone. This conversion occurs in the colon under influence of colonic bacteria, possibly resulting in high local concentrations of these compounds. Furthermore, the "inactive metabolite" sulindac

**Table 2** FAP Patients Treated with Sulindac for 12 Months or More

<table>
<thead>
<tr>
<th>First author</th>
<th>Ref.</th>
<th>Treatment</th>
<th>dose/day</th>
<th>Duration</th>
<th>Outcome</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cruz-Correa</td>
<td>93</td>
<td>sulindac</td>
<td>300-400 mg</td>
<td>12-36 months</td>
<td>within 6-12 months: polyps disappeared in 3 patients, continuous suppression during long term treatment</td>
<td>1 patient dropped out (gastric bleeding)</td>
</tr>
<tr>
<td>Waddell</td>
<td>94</td>
<td>sulindac</td>
<td>300-400 mg</td>
<td>12-36 months</td>
<td>polyps disappeared in 3 patients (after 24-47 months)</td>
<td>1 patient dropped out (gastric bleeding)</td>
</tr>
<tr>
<td>Torell</td>
<td>95</td>
<td>sulindac</td>
<td>200 mg</td>
<td>12-124 months</td>
<td>polyps disappeared in 4 patients (after 24-47 months)</td>
<td>1 patient dropped out (gastric bleeding)</td>
</tr>
<tr>
<td>Rigou</td>
<td>96</td>
<td>sulindac</td>
<td>200 mg</td>
<td>12-36 months</td>
<td>continuous suppression during long term treatment</td>
<td>1 patient dropped out (gastric bleeding)</td>
</tr>
<tr>
<td>Winde</td>
<td>97</td>
<td>sulindac</td>
<td>50-300 mg</td>
<td>up to 48 months</td>
<td>polyps disappeared in all patients within 1 year</td>
<td>2 patients with mild gastritis</td>
</tr>
</tbody>
</table>
Chemoprevention of colon cancer

Table 3  Breakthrough carcinomas in the rectal stump from FAP patients during treatment with sulindac.

<table>
<thead>
<tr>
<th>Author</th>
<th>Ref</th>
<th>Treatment</th>
<th>Clinical course; histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thorson</td>
<td>100</td>
<td>sulindac 300 mg/day</td>
<td>Initially good response on sulindac. At 15 months: flat ulcerative rectal lesion; Pathology: moderately differentiated adenocarcinoma. Surrounding rectal mucosa: 2 flat adenomas; severe dysplasia.</td>
</tr>
<tr>
<td>Niv</td>
<td>101</td>
<td>sulindac 450 mg/day</td>
<td>Initially good response on sulindac. At 28 months: 2 cm polypoid rectal tumor with large pelvic mass (unresectable); Pathology: moderately differentiated adenocarcinoma.</td>
</tr>
<tr>
<td>Giordello</td>
<td>50</td>
<td>sulindac 300 mg/day</td>
<td>Initially complete response on sulindac. At 35 months: flat ulcerative rectal lesion, without concomitant polypoid adenomas; Pathology: adenocarcinoma.</td>
</tr>
<tr>
<td>Tonali</td>
<td>95</td>
<td>sulindac 200 mg/day</td>
<td>Initially partial response on sulindac. At 85 months: large (1.5 cm) only slightly elevated rectal polyp; severe dysplasia. At 106 months: flat depressed and eroded rectal lesion (2x1.5 cm). Pathology: depressed mucinous carcinoma. Surrounding rectal mucosa: 2 large flat adenomas.</td>
</tr>
</tbody>
</table>

Sulindac (sulindac) has shown chemopreventive potential in animal studies, although a limited (not statistically significant) effect against adenomas in FAP patients was observed. In a randomized placebo-controlled trial, FAP patients treated with sulindac were followed during 12 months, monitoring polyph number and size every 3 months. Polyp regression was most pronounced after 6 months. Between 6 and 9 months of treatment a small increase in the number and size of adenomas occurred. After discontinuation of treatment, at 9 months, a rapid further recurrence of adenomas was noted (Fig. 3). Also, reports about long-term administration of sulindac (Table 2) showed (partial) regression of polyphs during continuous treatment. In one investigation, sulindac reduced the number and size of polyps after 6 months, but not after prolonged treatment (12–124 months), possibly because of the relative low dose of sulindac (200 mg per day administered). Thus, 1. sulindac causes regression of adenomas, without complete suppression; 2. sulindac resistant adenomas may develop within 9 months treatment; 3. continuous chemopreventive treatment is needed.

Whether the development of CRC (true endpoint) in FAP patients is delayed or prevented by sulindac or COX-2 inhibitors is not known. Reports of resistant adenomas and breakthrough carcinomas during treatment with sulindac (Table 3) point to important limitations. Interestingly, distinct macroscopic and microscopic features of sulindac resistant adenomas after long-term treatment have been described. Macroscopically, these resistant rectal adenomas appeared reddish, and flat or only slightly elevated. At microscopy, large sulindac resistant polyphs had an increased thickness of the mucosal lamina propria, and almost all crypts carried dysplastic epithelium over the full length of the crypts. Smaller polyphs showed features of low-grade flat adenoma or depressed adenoma. Moreover, Oshima et al. reported a flat and regressed appearance of polyphs from Apc mice which were deficient for COX-2, or which had been treated with a COX-2 inhibitor. Taken together, sulindac may induce a flat growth pattern of adenomas, distinct from the conventional polyoid neoplasia found in FAP.

The importance of flat lesions in FAP patients treated with sulindac may be reflected by reports of breakthrough rectal stump carcinomas in FAP patients on sulindac as listed in Table 3. Three carcinomas appeared as flat ulcerative lesions. One rectal stump carcinoma appeared as a relatively small luminal polypoid mass, but had already expanded deeply into the pelvis, with invasion of the sacrum. Taken together, carcinomas reported in patients on sulindac follow a predominantly flat but infiltrating growth pattern, potentially masking their existence. Recognition of this entity is of importance during surveillance endoscopy in patients treated with sulindac or other NSAIDs.

A recent randomized, double-blind, placebo-controlled study addressed the effect of sulindac on the development of adenomas in young FAP patients genotypically affected but phenotypically unaffected (without adenomas). Sulindac did not prevent the development of adenomas. Importantly, suppression of protooncogenes in the normal rectal mucosa of FAP patients on sulindac could discriminate between responders and non-responders, providing a useful biomarker to monitor the effect of treatment in these patients.

Chemoprevention of Other Hereditary Colorectal Cancer Syndromes. Patients with hereditary non-polyposis colorectal cancer (HNPPC) are at high risk to develop CRC and may benefit from chemopreventive treatment. Studies, investigating chemoprotection with NSAIDs in HNPPC are underway but are difficult to conduct since these patients do not develop numerous adenomas which can serve as an intermediate endpoint. Whether surrogate endpoints, such as prostaglandin levels, proliferation, or apoptosis will be useful in HNPPC awaits further study. Of note, animal studies do not support the efficacy of NSAIDs or COX-2 inhibitors in HNPPC. The administration of piroxicam in HNPPC-deficient mice showed an unexpected increase of adenomas. Others found that aspirin and sulindac did not affect intestinal tumor burden in Apc mice whereas a specific COX-2 inhibitor only decreased the number of adenomas in the small intestine, without affecting colon adenomas in this mouse model. Also, HNPPC-related MSI-positive CRCs express less COX-2 than MSI-negative CRCs and up to 50% of MSI positive tumors have mutations in the BAX gene, which are potentially involved in sulindac resistance.

Other hereditary colon cancer syndromes for which chemopreventive treatment might be of importance are the hamartomatous polyposis syndromes, i.e., Peutz-Jeghers syndrome and juvenile polyposis syndrome. To date, one patient with juvenile polyposis treated with sulindac has been described, showing regression of 2 pre-existing...
adenomatous polyp. Whether hamartomatous polyps are sensitive for treatment with NSAIDs has not been evaluated. Recently, COX-2 expression was reported in Peutz-Jeghers hamartomas.106 Also, we found COX-2 expression in Peutz-Jeghers syndrome related carcinomas (unpublished), providing a rationale for further investigation of NSAIDs chemoprevention against carcinogenesis in this syndrome.

Chemoprevention of Sporadic Adenomas. Case-control studies107,108 and cohort studies109-111 have shown that regular use of aspirin at a dose of ~300 mg a day decreases the adenomas-risk 30-50%, and decreases the incidence of CRC. Studies evaluating the effect of NSAIDs and COX-2 inhibitors in patients with previous adenomatous polyps are ongoing112 and results will direct potential chemopreventive strategies against sporadic CRC. Investigations of the efficacy of NSAIDs against pre-existing sporadic adenomas failed to show a convincing effect. In two studies, including one randomized placebo-controlled trial, there was no effect of sulindac 300-400 mg a day against sporadic adenomas;112,113 possibly due to difficulties in the study design.114 Another evaluated the regression of 20 histologically proven sporadic adenomas after 4 months of sulindac 300 mg a day.115 Nine adenomas (45%) disappeared, and 4 adenomas (20%) showed >40% decrease of polyp diameter. Small adenomas (<6 mm) were more likely to respond. After 4 months, remaining polyps were removed. One of the non-responding polyps contained a focus of carcinoma, whereas another polyp that apparently disappeared after treatment with sulindac developed into a rectal carcinoma 16 months after discontinuation of therapy.116 This report illustrates that sulindac can cause incomplete regression of adenomas leaving neoplastic cells which can progress to invasive carcinoma, and that medical therapy with NSAIDs can not replace polyectomy. One non-randomized study showed that 8-12 months of treatment with sulindac 300 mg a day decreased the presence of ACF in 4 normal controls, 6 patients with adenoma, and 1 patient with carcinoma.117 Thus, NSAIDs may interfere with the earliest stages of the adenoma-carcinoma sequence.

Side Effects. Sulindac is the most effective and extensively studied chemopreventive drug available. However, COX-2 inhibitors are attractive alternatives since they cause less toxic gastrointestinal side effects.118 Thus far, most FAP patients have been treated with sulindac; reported side effects are listed in Tables 1 and 2. The extent and degree of side effects appears limited. However, two reports are of particular concern. Serious side effects were noted in 5 out of 6 Japanese FAP patients treated with sulindac.119 and long-term use of sulindac orally caused rectal erosions in 6 out of 12 FAP patients.99 Previously, ileal erosions and pouchitis were also attributed to sulindac.119 This toxicity compelled dose reduction, which may impair the chemopreventive efficacy. The clinical implications of these lesions await further study.

LESSONS FROM ANIMAL MODELS

Investigations using carcinogen treated rats and mouse models for FAP (ApcMin mice, Apc1638N mice, Apc8576 mice) have demonstrated the chemopreventive potential of many different NSAIDs. These include sulindac, indomethacin, piroxicam, aspirin, and ibuprofen, and the selective COX-2 inhibitors celecoxib, rofecoxib, nabumetone and meloxicam.53,54,36,46-48,55,56,66,67,74,130-132 A dose-dependent effect of NSAIDs and COX-2 inhibitors exists against both the initiation and promotion of the adenoma-carcinoma sequence, and pre-existing tumors. Of note, there are regional differences in sensitivity for chemopreventive treatment. In the Apc1638N mouse, the chemopreventive effect of piroxicam and celecoxib appeared most pronounced in the distal parts of the small intestine.43 The effect of piroxicam in AOM treated rats appeared more prominent in the proximal compared to the distal colon.122 The latter observation corresponds to the findings of Guldenschuh et al., who reported a 85% decrease of adenomas in the proximal compared to a 62% decrease in the distal colon of sulindac-treated FAP patients with an intact colon.41 Also, the response to sulindac was greater in FAP-patients after colectomy with IRA.44 These differences may reflect the exposure of the mucosa to carcinogenic agents and/or bile-salts, or the site where sulindac is converted into active metabolites.

Animal models can be used to compare the chemopreventive potential of combinatorial regimens. The combination of agents with different mechanisms of action and nonoverlapping toxicities could enhance the chemopreventive efficacy and also allow dose reductions, decreasing toxicity. Piroxicam and the ODC inhibitor difluoromethylornithine (DFMO) were more effective together than either agent alone and resulted in a significant number of mice with a complete response (no polyps).123 Also, sulindac with an EGFR-knase inhibitor almost completely prevented the formation of polyps and permitted a 75% reduction in the dose of sulindac, potentially abolishing the toxicity of long term NSAID use.124 The development of chemopreventive combination therapy is an important area for further research.

CONCLUSIONS AND FUTURE PERSPECTIVES

The effect of sulindac and celecoxib against colorectal adenomas is incomplete and data showing chemoprevention of colorectal carcinoma in FAP are not available. The FDA has approved celecoxib 800 mg a day as a treatment option for chemoprevention in FAP, usually in patients with retained rectum. In view of the limited effect of celecoxib treatment with sulindac 300 mg a day could be considered for patients with an insufficient response to celecoxib. Currently, there is no medical treatment for primary chemoprevention of FAP. Chemoprevention of HNPC and other hereditary colon cancer syndromes should be restricted to experimental settings. Results from ongoing trials will resolve whether NSAIDs or COX-2 inhibitors are useful for chemoprevention against sporadic adenoma and CRC development. The clinical application of chemopreventive agents requires the development of surrogate endpoint biomarkers to monitor the effect of treatment. Identification and evaluation of biomarkers is therefore an important area of further research.

Further progress depends on increased understanding of the mechanisms underlying chemoprevention. Although the role of COX-2 is established, inhibition of this enzyme cannot explain all observed effects. Further insights in the involved pathways will enable more specific targeting of key molecules, and define the limitations of NSAIDs-based chemoprevention, providing a rationale for selecting patients likely to respond. Understanding of the molecular basis of carcinogenesis will lead the future development of chemopreventive regimens. Presumably, more than one pathway needs to be targeted to achieve adequate results. Combination therapy appears attractive, potentially increasing efficacy and decreasing toxicity. Finally, the identification of novel agents and the modification of existing chemopreventive drugs125,126 is likely to contribute to future chemopreventive strategies against the development of colorectal carcinoma.
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Acknowledgements
We thank Professor Offerhaus for support and helpful comments.

References

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