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Colon Cancer Chemoprevention: Clinical Development of Aspirin as a Chemopreventive Agent

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Abstract We have studied aspirin as a potential chemopreventive for colorectal cancer, completing Phase I studies on aspirin pharmacology and potential biomarker assays (prostaglandins, PGE_2 and $PGF_{2\alpha}$ and cyclooxygenase modulation) in normal human subjects. These studies have determined the optimal dose of aspirin for future Phase IIa and IIb chemopreventive trials in high-risk cohorts of patients for colon cancer. Aspirin's effects on rectal prostaglandins are prolonged, detectable even after aspirin and its metabolite are removed from the plasma. Aspirin-mediated inhibition of prostaglandin production in the human rectal epithelium may be related to direct suppression of cyclooxygenase transcription and not to enzyme inactivation by acetylation. A systematic method to monitor adherence (self-report, telephone contact, pill count, and microelectronic monitoring) has been established for future trials. Strategies to improve recruitment of high-risk cohorts have been developed. Phase IIa non-randomized studies with aspirin at 81 mg in high-risk cohorts (resected Duke's A colon cancer, Duke's C colon cancer treated with adjuvant therapy and disease-free at 5 years, history of colon adenomas $>$ 1 cm, two or more first-degree relatives with colon cancer, and familial adenomatous polyposis and hereditary non-polyposis colorectal cancer syndromes) are currently being conducted for surrogate end-point biomarker (prostaglandins, cyclooxygenase, cellular mucins, and proliferation) modulation. J. Cell. Biochem. Suppls. 28/29:148–158.

Key words: aspirin; colon cancer chemoprevention; cyclooxygenase isoforms (COX-1 and COX-2); NSAIDs; prostaglandins (PGE_2 and $PGF_{2\alpha}$); surrogate end-point biomarkers (SEBs)

Colon cancer is the second leading cause of death from cancer in the developed world, with an estimated 94,100 new cases and 46,600 deaths in the United States in 1997 [1]. Early detection and prompt surgery in the early stages of colon cancer offer the only hope of long-term cure. Cytotoxic chemotherapy for advanced disease is not curative and offers only short-term palliation.

It is, therefore, logical to devise strategies to decrease the incidence of this common malignancy through primary, secondary, and tertiary prevention approaches. Primary prevention is aimed at lifestyle and dietary modification to eliminate potential mutagens and carcinogens from the diet. The American Cancer Society Committee on Diet, Nutrition and Prevention has emphasized the importance of lifestyle (balance of caloric intake, physical activity), and dietary changes (limited amounts of meat and increased fiber, fruits, and vegetables) to decrease the incidence of various types of cancer [2]. Secondary prevention attempts to reduce incidence by earlier detection of precursor lesions at the endoscopic and molecular level. The American Cancer Society has outlined guidelines for colon cancer screening in the "normal-risk" and "high-risk" groups of pa-

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tients with digital rectal examination, fecal occult blood screening, and sigmoidoscopic surveillance.

Tertiary prevention or chemoprevention uses pharmacological approaches with drugs to arrest
the progression of colon cancer at the earliest possible stage of its development. Epidemiological and experimental evidence support drug-based suppression of colon carcinogenesis. Major advances in molecular genetics over several years help to better define high-risk persons. In the future, it may be feasible to do genetic susceptibility screening, although currently only about 15–20% of colon cancer has a known genetic basis. The Chemoprevention Branch of the National Cancer Institute has initiated a step-wise development of potential cancer chemopreventives from basic science research to hypothesis generation, methods development, and controlled clinical trials [3].

Substances with potential chemopreventive activity are identified based on human cancer epidemiology, with an emphasis on diet assessment, geographic, dietary and environmental variation, and differences in cancer incidence among similar regional populations. Clinical development of chemopreventive agents differs in many ways from therapeutic drug testing. No clinically or radiologically defined abnormalities are available to monitor drug effects. Frequent dosing schedules are undesirable. Treatment adherence over long time periods is an important problem that must be systematically addressed.

The use of surrogate end-point biomarkers (SEBs) as predictors of cancer occurrence has several advantages, including lesser expense and time to completion and fewer subjects. SEBs should have differential expression in normal and abnormal tissues, correlate between degree of change and stage of carcinogenesis, be easily accessible and measurable, and demonstrate detectable modulation by chemopreventive agents. Several such potential SEBs are being evaluated in clinical trials [4–6].

Several classes of drugs, including non-steroidal anti-inflammatory drugs (NSAIDs), calcium, antioxidants, polyamine inhibitors (difluormethylornithine, DFMO), dithiolthiones (oltipraz), antioxidants, polyphenols (ellagic acid), and micronutrients, are being evaluated as suitable colon cancer chemopreventives [7]. Sufficient evidence exists in the published literature, based on epidemiological, experimental, and molecular data, to develop NSAIDs as chemopreventive agents to prevent, retard, or inhibit colorectal cancer in risk-prone cohorts. We and others have recently reviewed this literature [7,8]. Several questions must be addressed before this approach can be implemented in large clinical trials. The authors summarize the current basis for the development of NSAIDs as cancer chemopreventives and, in particular, the progress made with aspirin as a colorectal cancer chemopreventive.

NSAIDS AS POTENTIAL COLON CANCER CHEMOPREVENTIVE AGENTS: WHAT IS THE EVIDENCE?

Evidence From Epidemiological Studies

Numerous published epidemiological studies have shown a 40–50% reduction in the incidence of colon cancer in individuals on aspirin [9]. The optimal dose and frequency, duration of administration, and anti-carcinogenic mechanism of aspirin are not deducible from these studies. Nevertheless, these studies are important and informative and provide a basis for future step-wise clinical trials.

Evidence from In Vitro and In Vivo Experimental Studies

Several types of experimental colon cancer models are available to study colon carcinogenesis and the effects of chemopreventive agents. These include animal models of chemically induced colon cancer, aberrant crypt foci (ACF) assay, immortalized colon cancer cell lines, the Multiple Intestinal Neoplasia or *Min* mouse (a murine model of familial adenomatous polyposis), rat intestinal epithelial cells (RIE), and genetic "knockout models" of cyclooxygenase-1 and -2 (COX-1 and COX-2). Using these models, we found evidence that NSAIDs suppress carcinogenesis in a variety of experimental conditions, and verified the important role of COX-2 in colorectal carcinogenesis. Quantitative changes in COX-2 expression have been linked to changes in key steps in carcinogenesis. Restoration of these COX-2 alterations by NSAIDs have been recently shown in some elegant molecular studies [10–12]. In addition to demonstrating the chemopreventive efficacy of NSAIDs, studies in these models have provided critical information on colon carcinogenesis.

Animal models of colon cancer. Several of the NSAIDs (aspirin, sulindac, sulindac sul-

fone, ibuprofen, piroxicam, curcumin) have demonstrated anti-carcinogenic effects in animal models of chemical colon cancer [13–17], showing significant reduction in the number and size of these colonic tumors. But the optimal dose and schedule of drug administration and the ideal NSAID for human chemoprevention trials cannot be inferred from these studies.

ACF assays. ACF are microscopically identifiable lesions seen on whole-mount segments of colonic mucosa from carcinogen-treated rodents [18]. ACF may represent a precursor lesion of colon cancer. A rapid ACF bioassay has been developed to screen potential chemopreventives and surrogate markers, involving measurement of ACF growth rates in rats exposed to carcinogens. A good correlation between long-term rodent assay systems and the ACF assay has been established. Piroxicam (0.125 g/kg body weight) inhibits azoxymethane-induced ACF and colon cancer in male Fischer 344 rats. Inhibition of colon tumors and ACF regression was demonstrated in this model [19].

Transfection models. Tsujii and DuBois, 1995 [10] successfully transfected RIE cell lines to overexpress COX-2. These cell lines demonstrated increased adhesion to extracellular matrix proteins and failure of apoptosis. Sulindac sulfide reversed these phenotypic changes and restored apoptosis in this model [10]. Further studies are in progress on the molecular basis of these changes in cellular adhesion in RIE cells (DuBois, personal communication).

Genetic and "knockout" models. The *Min* mouse is a murine model of human familial adenomatous polyposis (FAP). It carries a mutation of the murine homologue of the *Apc* gene [20]. This model is useful in studying the carcinogenic effects of environmental influences, effects of chemopreventives, and SEB modulation in a genetically cancer-prone model.

C57BL/6J-*Min*/ + (Min) mice, a strain with a dominant *Apc* mutation, showed a significant reduction in the number of tumors, decreased amounts of prostaglandin E_2 (PGE_2) and restoration of apoptosis when fed sulindac compared to control mice [11]. Using the *Min* model, we have introduced mutations in other genes and analyzed such modifying influences on the *Apc* gene. For example, creating a knockout of the COX-2 gene in the *Min* mouse decreased the formation of polyps in this model [12]. Introducing a null mutation of the COX-2 gene into this model caused a reduction in the size and number of polyps [12]. COX-2 inhibition by sulindac and a newer, selective COX-2 inhibitor (MF tricyclic) caused a decrease in the size and number of tumors. The selective COX-2 inhibitor was more effective than sulindac in polyp suppression [12].

Evidence From Human Clinical Controlled and Uncontrolled Studies

Waddell et al., 1989, first observed that sulindac causes human polyp regression when used in an individual with FAP and desmoid tumor [21]. It caused a dramatic reduction in the size and number of polyps, but the polyps recurred when the drug was discontinued. In a crossover controlled study of 5 patients with FAP and previous ileorectal anastomosis, sulindac decreased the number of adenomatous polyps in the treatment arm compared to the placebo arm [22].

In a randomized, double-blind study of 22 patients with FAP (including 18 patients without colectomy), Giardiello et al., 1993, administered sulindac (150 mg twice daily) or placebo for 9 months [23]. Polyp number and size were evaluated every 3 months. A statistically significant decrease in mean number of polyps was observed in the sulindac arm. At 9 months, the number of polyps had decreased to 44% of the baseline values and diameter of the polyps had reduced to 35% of baseline values. But an increase in the number and size of polyps was noted in the sulindac-treated arm 3 months after cessation of drug. Contrary to earlier studies, complete regression of polyps was not observed in this study and sulindac was not considered a suitable alternative to colectomy in FAP patients. In another controlled study of 24 patients, polyps were visualized by video assessment following sulindac administration, and proliferation rates were measured by bromodeoxyuridine labelling, showing a reduction in number and size of polyps and proliferation indices [24].

Spagnesi et al. 1994, administered sulindac at 200 mg per day for 60 days to FAP patients who had undergone ileorectal anastomosis [25]. The number and size of polyps by colonoscopy and proliferation indices (labelling index, percentage of labelled cells per crypt compartment by [^{3}H]thymidine incorporation, and autoradiography) were assessed. Although sulindac caused significant reduction in the number and size of polyps as shown in earlier studies, prolif-

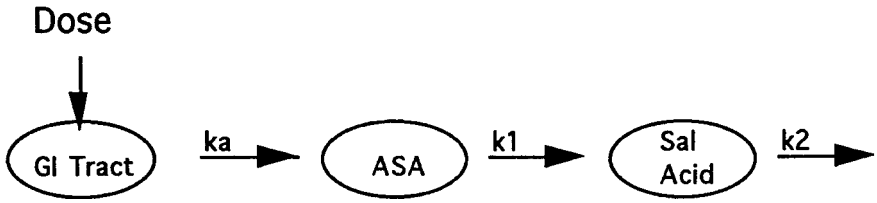

Fig. 1. Compartmental model for aspirin (ASA) absorption, metabolic elimination, and salicyclic acid (SA) elimination.

eration indices were not influenced. There have also been case reports of the occurrence of rectal cancer in FAP patients treated with sulindac after ileo-rectal anastomosis [26]. These observations suggest that the effects of sulindac are not completely protective in FAP patients. Rectal administration of sulindac has also been effective in inducing polyp regression [27].

Pilot studies on the effects of sulindac in sporadic colon polyps have also been published. In a small, uncontrolled 6-month trial in patients with sporadic colon polyps, 400 mg/day of sulindac or 20 mg/day of piroxicam were given to 5 and 2 patients, respectively. Two additional patients on piroxicam were withdrawn because of side effects (bleeding gastric ulcer and rash). A 6-mm polyp disappeared in one patient on sulindac. One polyp showed partial regression in a piroxicam-treated subject. But there was no significant regression in sporadic polyps in this small study [28]. In a double-blind placebo-controlled study, 44 asymptomatic patients with sporadic colon polyps (>1 cm) were randomly selected from among 162 patients screened by colonoscopy. Sulindac at 150 mg twice daily was administered to one arm (22 subjects) and placebo was given to the other arm (22 subjects). After a short follow-up of 4 months with colonoscopy, there was no difference in the two arms. This suggested that sulindac's effects on sporadic colon polyps and FAP may be different [29].

CLINICAL DEVELOPMENT OF ASPIRIN AS A COLON CANCER CHEMOPREVENTIVE

We have been working on the development of aspirin as a potential colorectal cancer chemopreventive over the last 5 years.

Determination of Optimal Aspirin Dose and Aspirin/Salicylic Acid Pharmacokinetics: Phase I Aspirin Dose De-Escalation Trial

Phase I trials of aspirin pharmacokinetics and modulation of prostaglandin PGE_2 and $F_{2\alpha}$ ($PGF_{2\alpha}$) as potential surrogates for drug effect have been completed. This trial was targeted to identify a single, non-toxic, once-daily schedule that modulates a potential surrogate endpoint biomarker (SEB) in the human rectal epithelium.

To define an optimal schedule for aspirin as a chemopreventive for colorectal cancer, the effects of a single, daily dose of 648 mg aspirin on rectal PGs in 15 normal, healthy subjects was studied initially [30–31], followed by a dose de-escalation trial with various doses of aspirin (324, 162, 81, 40.5 mg) involving a total of 65 normal human subjects. Diet was controlled and adherence was closely monitored by telephone interviews, pill counts, and microelectronic monitoring. An effective chemopreventive dose in these subjects has been defined, and clinical prevention trials and analytical methodologies have been refined [32].

Serial rectal biopsies were done at baseline, 2 and 24 h after the first aspirin dose and 24 h after day 14 of aspirin. The rectal biopsy specimens were assayed for PGE_2 and $PGF_{2\alpha}$. On each occasion, 8 rectal biopsies were obtained. Serial blood samples were obtained and assayed for aspirin and salicyclic acid concentration by high-performance liquid chromatography (HPLC) after a single dose and at steady state.

The pharmacokinetic parameters of acetylsalicylic acid and salicyclic acid are detailed in Table I [32]. The acetylsalicylic acid half-life ranged from 0.38 to 0.60 h; the salicyclic acid half-life ranged from 1.61 to 3.34 h. Salicyclic acid had a half-life sixfold longer than acetylsalicylic acid, an area under the curve (AUC) 8- to 52-fold higher, and maximum concentrations that were two- to sevenfold higher. Plasma aspirin and salicylate levels were undetectable 24 h after first aspirin dose or after multiple daily doses for 2 weeks, yet PGE_2 and $PGF_{2\alpha}$ levels were suppressed. The plasma pharmacokinetics of aspirin and salicylic acid measured in the

study were consistent with previously published data [33].

Significant suppression of gut PGE_2 and $PGF_{2\alpha}$ was demonstrated in 80% of the 15 normal subjects treated with a single dose of 648 mg. PGs were suppressed at 24 h when no aspirin or salicylic acid concentrations were detectable in plasma in any of our subjects. In a subsequent trial, the pharmacokinetics and the effects of lower doses (324, 162, 81, and 40.5 mg) of aspirin upon rectal PGs were studied. Since PGs were suppressed 24 h after the 14th dose, the rectal biopsies after 14 daily doses were performed at 28 and 76 h after the final dose. Data from a total of 65 subjects (10 receiving placebo, groups of 10 each receiving 40.5, 81, 162, or 324 mg of aspirin, and the group of 15 who previously received 640 mg) were available for analysis. The lowest doses of aspirin taken daily for 14 days to significantly suppress both PGE_2 and $PGF_{2\alpha}$ were 81 and 40.5 mg, respectively. Based on this data, we recommend that aspirin at 81 mg once daily is sufficient for colorectal cancer chemoprevention [32]. Aspirin is a potent inhibitor of rectal PG synthesis long after aspirin and salicylic acid have been cleared from the plasma as measured by HPLC [32].

Development of Prostaglandins (PGs) as Potential SEBs of Drug Effect

The arachidonic acid cascade involves the conversion of arachidonic acid to PGs and thromboxanes by cyclooxygenase (COX) and to leukotrienes, hydroxyacids, and lipoxins by lipoxygenase [34]. PGs are widely distributed in mammalian tissue and are important as signalling molecules in several cellular homeostatic mechanisms [34]. Elevated levels of PGs have been detected in certain animal and human tumors [35–37]. Earnest et al., 1992 [38] described higher PG levels in tissues obtained from colonic adenomas and tumors when compared to normal adjacent colonic tissue. Highest values were seen in tumor tissue [38]. PGs have been shown to cause cellular proliferation in certain tissues [39] but not in others [40]. Therefore, although high PG levels have been detected in certain tumor types, a causal or mechanistic link has not yet been established between PGs and tumor development and/or proliferation.

Since PGs may have a role in colonic carcinogenesis and are modulated by NSAIDs, measurements of PGs in human colorectal tissue

TABLE I. Pharmacokinetic Parameters of Acetylsalicylic Acid (Aspirin) and Salicylic Acid After a Single Dose*

Acetylsalicylic acid dose (mg)	No. of subjects	Mean BMI (kg/m^2)	Mean ± standard deviation					
			AUC (μg/mL) × h	$t_{1/2}$ (h)	C_{max} (μg/mL)[a]	T_{max} (h)[b]	V_d(L)[c]	CL_{TB} (L/min)[d]
A. Aspirin								
648	14	24.3	6.56 ± 4.44	0.50 ± 0.23	5.16 ± 3.21	0.72 ± 0.38	93.9 ± 46.5	2.69 ± 2.1
324	9	22.9	2.87 ± 1.38	0.38 ± 0.08	3.76 ± 2.00	0.45 ± 0.22	55.3 ± 17.7	1.94 ± 0.8
162	10	25.4	1.92 ± 0.77	0.40 ± 0.19	2.17 ± 1.08	0.55 ± 0.21	71.4 ± 58.5	1.71 ± 0.7
81	10	23.9	0.98 ± 0.54	0.60 ± 0.40	1.13 ± 1.00	0.53 ± 0.23	106.1 ± 86.0	2.07 ± 1.5
40.5	10	22.9	0.67 ± 0.85	0.41 ± 0.40	0.68 ± 0.38	0.47 ± 0.21	49.6 ± 31.3	2.11 ± 1.5
B. Salicylic acid								
648	14	24.3	243.94 ± 64.3	3.10 ± 0.83	37.24 ± 12.41	1.98 ± 0.84		
324	9	22.9	149.15 ± 59.1	2.88 ± 0.89	25.68 ± 5.87	1.12 ± 0.43		
162	10	25.4	42.76 ± 14.51	2.46 ± 1.20	10.94 ± 3.16	1.25 ± 0.45		
81	10	23.9	16.34 ± 8.61	1.61 ± 0.83	4.76 ± 1.96	1.43 ± 0.73		
40.5	10	22.9	5.44 ± 4.42	3.34 ± 3.04	1.63 ± 1.13	0.93 ± 0.40		

*The total number of subjects for this aspect of our study was 53. Ten subjects taking placebo did not complete the pharmacokinetic data collection; two subjects (one taking 648 mg and one taking 324 mg) had incomplete pharmacokinetic data. BMI, body mass index; AUC, area under the curve from time zero extrapolated to infinity; $t_{1/2}$, terminal half-life. Reproduced from Ruffin et al. [32] with permission of the publisher, Oxford University Press, Journal of Cancer Institute.

[a]Peak measured concentration.

[b]Time to peak measured concentration.

[c]Volume of distribution of the terminal excretion phase. Not measured for the metabolite salicylic acid.

[d]Total-body clearance. Not measured for the metabolite salicylic acid.

may serve as a suitable SEB of drug effect. Can PGs be reliably measured in biopsied human tissue? We and others have directly addressed this issue in human tissue samples [32,41]. Measurement of PGs varies with regard to assay methodology. There is also biological variation in PG levels. In two published studies, there was good reproducibility in PG levels when multiple biopsies were homogenized and assayed repeatedly. But wide variation occurred in prostaglandin levels when assayed separately, suggestive of biological rather than methodological variation [41,42].

Should we measure endogenous prostglandins or in vitro synthesis of prostaglandins? Several different assay methods are available, including measurement of exogenous synthesis of PGs, as in ex vivo culture of colonic biopsies incubated with [^{14}C] arachidonic acid and measurement of radiolabelled products [43,44]; and measurement of endogenous synthesis of PGs, by snap-freezing freshly obtained minimally manipulated tissue, assaying it within an hour of procurement [42,45], and either adding indomethacin to the snap-frozen tissue [41] or homogenizing it at 0°C before incubation at high temperature to stop PG production [45]. Assay recovery may vary with extraction techniques (formic acid/radioimmunoassay method [41] vs. chloroform extraction and gas chromatography-mass spectroscopy method [46]). We have also encountered significant differences in PG levels between different batches of PG assay kits [32]. Currently there is no accepted uniform method of PG assay in human tissue; hence, valid comparison between laboratories is not possible.

We did quantitative assays of colorectal mucosal PGE_2 and $PGF_{2\alpha}$ by ELISA technique in 65 normal subjects before and after aspirin administration [32]. The mean baseline concentration of colorectal mucosal PGE_2 was 25.8 pg/µg protein (range 2.7–125.7 pg/µg protein). The mean baseline concentration of $PGF_{2\alpha}$ in biopsy specimens was 12.1 pg/µg protein (range 0.3–161.3 pg/µg protein). PGE_2 and $PGF_{2\alpha}$ levels showed significant variation between batches, but no gender variation was detected in the final analysis. Age, sex, race, diet, source of tissue, method of assay, timing of biopsy, amount of trauma during biopsy, and several other factors may explain these wide variations. Further work is required to precisely determine the reason for this variation in PG levels in human colorectal tissue.

Development of Cyclooxygenase Isoforms (COX-1 and -2) as Potential SEBs of Drug Effect

Two isoforms of cyclooxygenase (COX-1 and -2) have different functions in mammalian tissue. COX-1 has important "house-keeping" functions in normal homeostatic mechanisms. COX-2 is a growth factor inducible enzyme involved in inflammation and mitogenesis. Recent studies highlight the possible role of COX-2 in tumorigenesis. COX-2 mRNA is not detectable in normal mouse and human colonic epithelium but is up-regulated in colonic adenomas and carcinomas [47–49]. COX-2 expression increases quantitatively with growth of adenomas in "knockout" colon cancer models [12]; its overexpression in rat intestinal epithelial cells manifests with phenotypic alterations (changes in cell adhesion and resistance to apoptosis) that favor neoplastic growth [10]. Adenoma growth is suppressed in both knockout models and animal models of familial adenomatous polyposis by sulindac [11,12]. Suppression of COX-2 expression by selective COX-2 inhibitors decreases tumors in animal models of colon cancer [50]. Hence, quantitation of COX-2 protein and/or mRNA may serve as a useful SEB for colorectal chemoprevention trials. Further, it would be logical to develop selective inhibitors of COX-2 as chemopreventive agents.

Quantitative assays of COX-1 and -2 protein by Western immunoblot and densitometric quantitation were initiated as part of a strategy to develop potential SEBs of drug effect. Preliminary data previously published in abstract form is reviewed here [51,52]. In a preliminary study, COX-1 protein was quantified by Western immunoblot and image densitometry in 9 subjects both before administration of 80 mg of aspirin and 28 h after the first dose, as well as 28 and 76 h after day 14 of aspirin. COX-1 protein was reduced post-aspirin administration in 5/9, 4/7, and 3/9 of the subjects, respectively, during these time frames. Low-dose aspirin appears to reduce the immunodetectable COX-1 protein in the human rectal epithelium in only a percentage of the subjects studied [51]. We have now extended our analysis to include about 40 normal-risk and 43 high-risk human subjects; the data is being analyzed for publication. Preliminary analysis shows no significant alteration of COX-1 protein by aspirin when analyzed by image densitometry [52]. Since COX-1 is expressed in normal human gut and not signifi-

cantly modulated by low doses of aspirin in the human rectal epithelium, it may not be a suitable SEB to pursue in future human colorectal chemoprevention trials. In contrast, since COX-2 is not expressed in most normal human rectal or colonic tissue and is probably involved in colorectal tumorigenesis, it may be more logical to pursue and develop COX-2 protein/mRNA quantitation as a potential SEB of drug effect. Such studies are in progress.

Development of Lectins as SEBs

Lectins are naturally occurring agglutinins, derived from plant seeds, that may serve as markers of epithelial proliferation (*Amaranthine,* ACA) or differentiation (*Dolichos biflorus,* DBA). In normal colonic tissue, both bromodeoxyuridine (BrdU) incorporation and lectin (ACA) labeling were confined to the lower half of the colonic crypt. In adenomatous polyps, BrdU incorporation was found in 21.68 ± 0.58% of nuclei and labeling was diffuse throughout the polyp. ACA labeled 74.4 ± 3.88% of the glands and was similarly diffuse. ACA labeling of glycoconjugates consistent with hyperproliferative colon epithelia correlated with BrdU labeling [53–55]. We used human colonic tissue from 5 high-risk subjects (4 with adenomatous polyps, one with previously resected colon cancer) to examine whether aspirin modulates labeling by lectin markers ACA and DBA. A single daily dose of 81 mg aspirin for 28 days modulated ACA labelling of the crypts. Aspirin did not affect crypt labelling by the lectin DBA in this study [56]. Further studies are being done to analyze for possible differential modulation in high-risk and normal-risk subjects.

Development in Understanding Mechanisms of NSAID-Based Cancer Chemoprevention

NSAIDs have been assumed to prevent cancer development and/or progression because they suppress PG synthesis. But the precise mechanism is not known. New insights on how NSAIDs may suppress cellular proliferation and growth indicate that their effects may be mediated through both PG-dependent and PG-independent pathways. Abnormalities in apoptotic mechanisms (programmed cell death) can lead to cancer development and progression. NSAIDs can influence programmed cell death (apoptosis) through PG-independent pathways [57,58], and can reduce the proportion of cells in the S phase and increase cells in the G_0/G_1 phase in HT-29 colon adenocarcinoma cells [57]. Aspirin and piroxicam can slow the cell cycle by decreasing levels of cyclin-dependent kinases [57,58]. Sulindac sulfone, an inactive metabolite of sulindac, affects cell proliferation by its effects on the apoptotic path [59,60] without any effect on PG synthesis. These studies indicate that PG suppression may not be crucial for tumor suppression. Further understanding of the apoptotic pathways may lead to the development of molecular SEBs and specific targeted therapy.

Development of Methods to Improve Adherence to Drug Treatment

Assessment of adherence to prescribed treatment (or experimental drug regimens) is essential for accurate interpretation of chemoprevention SEB data. We have developed and implemented a program of adherence monitoring, including subject self-reporting, pill counting on a regular basis, pharmacokinetic drug sampling (when applicable or available), and microelectronic pill cap monitoring. In microelectronic monitoring, a microprocessor embedded in a pill cap records the date and time the pill cap is opened. Study subjects are carefully instructed as to time of day and frequency of drug administration. As a preliminary evaluation of this methodology, 24 women taking tamoxifen were monitored over a 3-month period. Self-report and pill count methods showed high adherence (>95%) but electronic monitoring revealed a marked reduction in adherence (approximately 80%) [61].

The same methods were used to assess adherence in the aspirin Phase I chemoprevention trial reported above [62,63]. Since the time of drug treatment was limited (2 weeks) and subjects were paid volunteers, full adherence by self-report and pill-count assessments was expected. Adherence, despite intensive written and verbal instructions and contact with subjects, was poor. Subjects missed doses, took extra doses beyond the 14-day treatment period without informing investigators, split doses instead of taking doses daily, and even shared the drug with roommates! Eighteen of 63 (29%) subjects missed a dose. Only 9/63 (14%) subjects completed the entire trial taking every dose within ± 2 h of the agreed-upon time for drug administration. Thirteen of 63 (21%) subjects took doses beyond 14 days of treatment

even when told that the trial was to be completed after 14 days. Rectal biopsy data from subjects taking extra aspirin doses beyond 14 days were removed from the preliminary analysis. Forgetfulness is the single most common source of nonadherence.

Rate of adherence depends on the definition of adherence, but it is extremely difficult to provide a general definition of adherence for chemoprevention trials because of differences in pharmacokinetics of the drugs. Moreover, the ideal SEB of cancer incidence that needs to be modulated is not known for many chemopreventive agents. For example, aspirin has diverse effects on prostaglandin metabolism, apoptotic pathways, and cell cycle and proliferation. We do not know the SEB which affects cancer incidence; different doses and schedules probably modulate each of these SEBs. Until we know the key elements to modulate, the definition of adherence will depend on the marker being studied. A run-in period will eliminate the poorly compliant individuals. A proactive promotion of adherence strategies is essential. Although adherence monitoring and adherence enhancement are crucial to the accuracy and success of a chemoprevention trial, the strategies required to improve adherence are not well understood.

Development of Strategies to Improve Recruitment Into Future Phase II Colorectal Cancer Chemoprevention Trials

Recruitment of subjects into cancer prevention trials is a challenging task. We have tested various methods to improve recruitment including personal contact, prepaid mail-in cards, telephone contact, university and local newspaper advertising, and community organizations. Distance, transportation, unwillingness for endoscopy, and misunderstood eligibility criteria were some factors cited by eligible subjects who were not willing to enroll. Recruitment improves if more than one method is used (unpublished observations).

Development of Aspirin Chemoprevention Trials in Cohorts of High-Risk Subjects

Phase IIa surrogate modulation trials with a single, daily dose of 81 mg aspirin are underway, testing SEB modulation (prostaglandins, COX-2) in high-risk categories (defined as two or more first-degree relatives, history of polyps >1 cm, or a history of surgically resected Duke's A or B colon cancer or Duke's C colon cancer treated with adjuvant therapy and disease-free for 5 years). This trial has been completed and data is being analyzed.

FUTURE PHASE IIB AND III TRIALS WITH NSAIDS IN HIGH-RISK COHORTS

Before Phase IIb and III trials are executed, we have to address the following issues:

Is aspirin a candidate colon cancer chemopreventive? Currently, we do not have answers to this question and others. The authors believe that aspirin is a good choice based on strong epidemiological and experimental supportive data, easy over-the-counter availability, popularity, and probable easy acceptance due to decades of familiarity. Moreover, aspirin has a well-documented positive effect in secondary prevention of myocardial infarction in subjects with coronary artery disease, reduces primary mortality from coronary artery disease (Nurses' Health Study), and reduces cerebrovascular events in carotid occlusive disease. None of the other NSAIDs have been tested in these medical conditions. Moreover, the 81 mg dose that modulates gut prostaglandins is commonly used for cardiovascular prophylaxis. Since coronary artery disease is the commonest cause of adult mortality in the United States, a large number of subjects at high risk for colon cancer are likely already on aspirin or will require aspirin in the future. Hence, it would be reasonable to identify a dual-action drug, and aspirin fits that role. But it is necessary to demonstrate its efficacy in colon cancer risk reduction prospectively before this approach can be applied in routine health care.

Would a selective COX-2 inhibitor be a better choice? Evidence to date suggests that the mitogen-inducible isoform of cyclooxygenase, COX-2, has an important role in colon carcinogenesis. Selective inhibition of COX-2 would be a logical option based on this data. This would also avoid the gastrointestinal side effects associated with COX-1 inhibition. Further work is necessary before firm conclusions can be drawn.

Would a combination of chemopreventives be more effective? Based on experience with cytotoxic drugs, where non-crossreactive drugs with different mechanisms of action are used in combination with greater efficacy, it would seem reasonable to extend this idea to chemoprevention. Conclusive studies are currently lacking.

SUMMARY

Chemopreventive intervention with NSAIDs continues to evolve. Advances in molecular carcinogenesis, mechanisms of drug action, and SEB modulation and validation will provide further information on newer effective strategies to inhibit neoplastic transformation and reduce the incidence of colorectal cancer.

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