

Phase I Trial of Exisulind (Sulindac Sulfone, FGN-1) as a Chemopreventive Agent in Patients with Familial Adenomatous Polyposis¹

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ABSTRACT

Exisulind (sulindac sulfone; FGN-1), a metabolite of sulindac without known effects on prostaglandin synthesis, can promote apoptosis and inhibit tumorigenesis in preclinical systems. We performed a Phase I trial of this compound in patients with familial adenomatous polyposis (FAP) to examine the tolerability and safety of this drug in the cancer chemoprevention setting. Six patients each were treated with exisulind at doses of 200, 300, and 400 mg p.o. twice a day. Reversible hepatic dysfunction was noted in four of six patients treated at the 400-mg p.o., twice-a-day dose level, but in only one to two of six patients treated at each of the lower dose levels. The serum half-life of exisulind was 6–9 h; little drug accumulation was noted over time. A nonsignificant trend toward increased apoptosis in polyps was noted at the maximum tolerated dose, but no decrease in polyp numbers or significant effects on cellular proliferation was noted. After treatment, polyps tended to display a “halo” appearance grossly and mucinous differentiation histologically. The maximum safe dose of exisulind is 300 mg p.o. twice a day in patients with subtotal colectomies. Reversible hepatic dysfunction limits further dose escalation. A decrease in polyp numbers could not be demonstrated, but the

trend toward increased apoptosis at the MTD and the observation of mucinous change histologically suggest that further investigation of drugs of this class might be warranted.

INTRODUCTION

Colorectal cancer is the third leading cause of cancer death among both men and women, and the American Cancer Society has estimated that this disease accounted for over 131,000 new cancer cases and nearly 55,000 deaths in the United States in 1997 (1). It is thought that the process of colorectal carcinogenesis involves the accumulation of genetic changes in concert with progression from normal-appearing mucosa to adenomatous polyps, higher grade dysplasia, and eventually, frank malignancy (2). This process is in many ways mimicked and accelerated in FAP,³ a heritable autosomal dominant condition resulting in the development of large numbers of colorectal polyps and the inevitable progression to colorectal cancer. Affected individuals have germ-line mutations in the *APC* gene in band q21 of chromosome 5 (3, 4). Somatic mutations in the *APC* gene are often found in sporadic colon and rectal cancer, so that FAP may be a model for the sporadic disease in which preventive strategies may be tested. One such strategy involves the administration of nonsteroidal anti-inflammatory drugs or their derivatives.

Considerable epidemiological evidence suggests that nonsteroidal anti-inflammatory drugs can reduce the incidence of colorectal cancer (5–7). In addition, the nonsteroidal anti-inflammatory drug sulindac has been shown to cause regression of colonic and rectal polyps in patients with FAP (8). However, sulindac and other currently approved nonsteroidal anti-inflammatory drugs are associated with irritation of the upper gastrointestinal tract. This toxicity is attributed to the inhibition of COX1, whereas it is postulated that the cancer chemopreventive activity of these agents is attributable to the inhibition of the inducible enzyme COX2 (9). Although relatively specific COX2 inhibitors are under investigation, there is evidence that the chemopreventive activity of nonsteroidal anti-inflammatory drugs is not solely attributable to COX inhibition. In *Min/+* mice, which are heterozygous for a germ-line mutation in the murine *APC* gene, sulindac can decrease the incidence of intes-

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³ The abbreviations used are: FAP, familial adenomatous polyposis; COX, cyclooxygenase; bid, twice a day; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; AUC, area under the plasma concentration-time curve, extrapolated to time infinity; MTD, maximum tolerated dose.

tinal adenomas. However, results are mixed with respect to the correlation of this activity with an alteration in prostaglandin E₂ or leukotriene B₄ levels in intestinal tissue (10, 11). Among the two major metabolites of sulindac is exisulind (also known as sulindac sulfone or FGN-1). This compound does not inhibit either COX1 or COX2 in preclinical systems (12, 13). Yet, it exerts chemopreventive activity in chemically induced rodent colon and breast cancer (12, 14), suggesting the existence of a novel mechanism of action unrelated to the inhibition of COX1 or COX2. On the basis of these preclinical observations, we performed a Phase I trial of exisulind in patients with FAP. The objectives of our study were to assess the safety and tolerability of exisulind, to determine the maximum safe dose of this agent in patients with FAP who had previously undergone subtotal colectomy with ileorectal anastomosis, and to examine the effects of treatment with exisulind on the natural history of FAP in this patient population. This report details the safety and pharmacokinetic results of this study as well as the effects of exisulind therapy on polyp numbers, histology, cellular proliferation, and apoptosis.

PATIENTS AND METHODS

Subject Selection

This trial was open to patients with a clinical diagnosis of FAP who had undergone a subtotal colectomy 3 or more years prior to study entry. Patients were required to be ≥ 18 years of age, to have at least five rectal polyps at the time of study entry, and to give informed consent to participation. Fertile women were required to have a negative pregnancy test and to be nonlactating, and all fertile participants were required to use adequate contraception during the course of the study. Patients were not allowed to take nonsteroidal anti-inflammatory drugs for 2 weeks prior to study entry, and the use of such drugs during the course of the study was prohibited. Patients with known hypersensitivities to nonsteroidal anti-inflammatory drugs were excluded from participation, as were patients who had been documented to be resistant to a course of therapy with sulindac. Potential participants underwent pre-study upper endoscopy, and subjects with active peptic ulcer disease were excluded from further participation. Also excluded from participation were patients who had gastrointestinal problems that were felt by the investigators to be likely to interfere with absorption of the study drug or assessment of toxicity, patients in whom a proctectomy was planned prior to completion of participation in the study, and patients who had used another investigational medication within 1 month of study entry. FAP patients who had hepatic or renal dysfunction that was significant in the opinion of the investigators or that was associated with an elevation of the aspartate aminotransferase or alanine aminotransferase to >40 units/l were excluded, as were patients with a hemoglobin value of <10 g/dl, a platelet count of $<100 \times 10^9$ /liter, or with any laboratory abnormality of grade 2 or worse, based upon the Common Toxicity Criteria (version 1.0) of the National Cancer Institute. Patients with a prior history of malignancy, with the exception of nonmelanoma skin cancer, were excluded.

Conduct of the Clinical Trial

Prior to study entry, informed consent to participate was obtained, and potential participants underwent eligibility screen-

ing and baseline studies, including a medical and surgical history, physical examination, flexible sigmoidoscopy, esophago-gastroduodenoscopy, serum pregnancy test (if appropriate), serum chemistries, urinalysis, bleeding time, and complete blood count with differential. Eligible patients were entered in cohorts of six into successively higher dose levels according to an empirically designed dose-escalation scheme. The dose of exisulind was never escalated in an individual patient to a dose higher than the starting dose. The planned dose levels were 200 and 400 mg p.o. bid and higher, but dose escalation was halted after dose-limiting toxicity was noted at the 400-mg p.o. bid dose level. An additional, intermediate dose level of 300 mg p.o. bid was then added; six patients were treated at this dose level. A minimum of 4 months of treatment was necessary for a patient to be considered evaluable, unless dose-limiting toxicity was experienced. Escalation to the next higher dose level occurred only after all patients treated at all lower dose levels had been treated for a minimum of 2 weeks and fewer than three of the six subjects in each dose level had experienced grade 2 or worse toxicity according to the Common Toxicity Criteria. The occurrence of grade 2 or worse toxicity in three or more patients in a given dose level was considered unacceptably toxic, defining the maximum safe dose as the next lower dose level.

During the initial week of therapy, participants were contacted daily on weekdays by a study nurse to assess adverse events, record concomitant medications, and assess drug compliance. These contacts were repeated weekly for the first 8 weeks and monthly thereafter. Overnight fasting blood draws for a serum biochemistry profile, complete blood count with differential, and bleeding time were performed at weeks 1 and 2 and monthly thereafter. Urinalyses were performed at weeks 1 and 4 and then monthly. All participants underwent physical examination, Simplate bleeding time determination, and sigmoidoscopy after 1, 4, and 6 months of treatment.

On the first day of treatment, patients were administered a single dose of exisulind after an overnight fast, and detailed pharmacokinetic studies were performed. Thereafter, patients were dosed twice daily for 6 months. On the final day of treatment, the morning dose was taken after an overnight fast, and detailed pharmacokinetics were again performed; the second dose of exisulind was withheld on the final day of treatment to allow prolonged sampling for pharmacokinetic studies. For the detailed pharmacokinetic studies performed after the initial dose of exisulind, blood samples were drawn at time 0 (pre-dose) and again 0.25, 0.50, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 22.0, and 24.0 h postdose. A 24-h urine collection for pharmacokinetic studies and creatinine clearance was collected during this time. Patients were served a breakfast similar to one that they eat normally after the 1-h sample was drawn. A similar schedule was followed for the detailed pharmacokinetic studies performed after the final dose of exisulind, except that additional blood samples were drawn 36.0 and 48.0 h after the final dose. In addition, "trough" plasma samples and 24-h urine collections were assayed for exisulind prior to the morning dose after 1, 2, and 4 weeks of therapy and monthly thereafter.

Upper endoscopy was performed prior to study entry and after the completion of 6 months of therapy. All participants underwent physical examination and sigmoidoscopy prior to the initiation of therapy and after 1, 4, and 6 months of treatment. At

each sigmoidoscopy, the polyps in each rectal segment were counted. At the time of each sigmoidoscopy, four biopsies were taken from normal-appearing mucosa from each rectal segment. In addition, multiple polyps were biopsied at the time of each sigmoidoscopic procedure. All biopsies of polyp and mucosal tissue were fixed in neutral-buffered formalin and examined histologically after H&E staining. In addition, all biopsy specimens were analyzed with respect to proliferation index, as determined by Ki-67 expression, and apoptotic index, as determined by cell morphology and TUNEL assay.

Analytic Methodology for Assaying Exisulind

The concentration of exisulind in plasma and urine was determined by high-pressure liquid chromatography using a methodology described previously (15). The analyte exisulind and the added internal standard, indomethacin, were extracted using methylene chloride. After separation and evaporation of the methylene chloride, the residue was dissolved in mobile phase and analyzed by reversed phase high-pressure liquid chromatography. A 5- μm , 15 \times 0.46-cm Octyl column was used to provide excellent chromatographic separation with high sensitivity for the analyte. The analyte and internal standard were measured by UV absorbance detection at 329 nm. The standard curve used the peak height ratio of the analyte to that of the internal standard. Samples were analyzed in duplicate, and duplicate values were averaged. If the difference of the duplicate values relative to their mean was >10%, the sample was reanalyzed. The limit of detection in plasma was 1 ng/ml, and the standard curve was linear up to 10,000 ng/ml. Validation studies were performed to determine the accuracy and reproducibility of the assay. In addition, plasma samples containing known concentrations of exisulind were shipped on dry ice from the Ohio State University to the Cleveland Clinic and back, stored for 3 months, and subsequently assayed to determine the stability of exisulind handled under the same conditions as the clinical specimens. In other experiments, samples were assayed after being maintained at refrigerator temperature (2°C) for 2–6 days or at room temperature (~25°C) for 1 day to examine potential sources of error in the clinical specimens.

The assay was validated in plasma prior to the initiation of the study. The within-day coefficients of variation were 2.3, 1.9, and 1.7% at concentrations of 0.081, 12.0, and 24.1 μM , respectively, with corresponding accuracy values of 113, 102, and 101% ($n = 8$). The corresponding between-day coefficients of variation were 7.0, 3.8, and 2.0%. The quality control standard indicated <3% variation when assayed after 3 months as described above.

Pharmacokinetic and Pharmacodynamic Analyses

The plasma concentration-time profile after the first dose was analyzed using the WinNonlin pharmacokinetic modeling software package (16). The noncompartmental analysis was performed on each individual profile after the first dose. This analysis was selected because the multiple peaks that were observed in several patients, apparently because of enterohepatic recirculation, precluded compartmental analysis. In addition, average plasma concentration profiles, which did not reflect the multiple peaks observed in the individual profiles, were analyzed using a two-compartment model with first-order ab-

sorption and a lag time before absorption began. The weighting function was Y^{-2} . The mean and SD of each mean was calculated using a standard spreadsheet software program (17). The influences of gender and body size were examined using the Multiple General Linear Hypothesis-General Linear Model section of the SYSTAT statistical analysis program (18). The relationship between toxicity, dose, and pharmacokinetic parameters was examined using the LogXact statistical analysis program for exact logistic regression (19).

Proliferation Index (Ki-67 Expression)

Ki-67 Staining. The 4- μm sections on slides were deparaffinized, and antigen retrieval was obtained by microwaving the slides in 0.01 M citrate buffer (pH 6) for 10 min. Endogenous peroxidase was blocked with 3% hydrogen peroxide for 15 min. Specific binding was blocked by incubating the slides with 1% nonfat dried milk for 30 min. The slides were incubated with a 1:100 dilution of MIB1 primary antibody (Immunotech, Marseille, France) for 1 h in a humidified chamber at room temperature. Normal mouse immunoglobulin G (Vector Laboratories, Burlington, CA) was used as a secondary antibody in a 1:100 dilution for 30 min. Immunoperoxidase detection was achieved with the avidin-biotin conjugate method (Vector Laboratories), followed by the diaminobenzidine (Vector Laboratories) enhancement reaction. Counter staining was performed with Harris hematoxylin. A negative control was prepared on each slide by omission of the primary antibody.

Imaging. The percentage of Ki-67-positive nuclear area per total nuclear area was determined on one slide of normal tissue and three slides, if available, of dysplastic tissue from each patient. In the dysplastic tissue, Ki-67 levels were determined in ~10 normal-appearing glands and 10 dysplastic glands. In normal tissue, Ki-67 levels were determined in up to 10 normal-appearing glands. The Roche image analysis software (Roche, Elon College, NC) was used for quantification of Ki-67 levels, which were obtained by plotting the Ki-67-positive nuclear area against the total nuclear area and expressing the positive Ki-67 area as a percentage of the total. Stained slides were viewed under a bright-field Zeiss Axioskop microscope with a $\times 20$ objective. The level of negative and positive staining was determined by grayscale threshold.

Apoptotic Index (TUNEL Assay)

The apoptotic indices of adenomas and normal-appearing mucosa were determined in slides adjacent to the slides in which proliferative indices were determined. Apoptotic cells in adenomas and normal-appearing mucosa were identified by TUNEL labeling using an adaptation of the methodology described by Gavrieli *et al.* (20). The staining protocol used with the adenomas and normal mucosa was found to give results similar to a method previously reported for the detection for apoptotic cells (21, 22). Formalin-fixed tissue sections were cut on to Probe-On slides (Fisher), deparaffinized, and rehydrated in graded alcohol. Sections were treated for 30 min with 0.5% pepsin (1:2500 strength; Sigma Chemical Co., St. Louis, MO) in 0.1 N HCl to digest protein, and then washed in H_2O (4×2 min), treated with 2% H_2O_2 in PBS to quench endogenous peroxidase, and washed (2×5 min) in PBS. Samples were then covered with

Table 1 Baseline characteristics of the 18 evaluable participants

	All doses	200 mg bid	300 mg bid	400 mg bid
Race				
White	18	6	6	6
Gender				
Female	9	2	4	3
Male	9	4	2	3
Median no. of polyps per patient at baseline (range)	28 (7–104)	34 (21–104)	22 (10–89)	33 (7–81)
Median age in years (range)	36 (18–45)	30 (18–42)	39 (31–45)	33 (22–44)
Median weight in kg (range)	77 (58–132)	68 (63–87)	92 (74–132)	80 (58–100)
Median body surface area in m ² (range)	1.88 (1.65–2.35)	1.83 (1.65–2.15)	2.10 (1.81–2.35)	1.92 (1.66–2.06)

freshly prepared reaction buffer (126 ml equilibrium buffer, 8.6 ml CoCl₂ solution (0.037 g of CoCl₂ in 10 ml of H₂O), 5.7 ml dUTP (dUTP solution, 6 parts stock:100 parts H₂O; Boehringer-Mannheim, Indianapolis IN), 59 ml of PBS (Sigma), and 1.4 ml of TdT enzyme for a final concentration of 0.1 enzyme unit/ml (TdT from Life Technologies, Inc., Gaithersburg, MD). Slides were placed in a humid box inside a 37°C oven for 60 min and then transferred to stop wash (300 mM NaCl and 30 mM sodium citrate) in 37°C agitating water bath for 30 min. After washing with PBS (3 × 5 min), the sections were covered with 1:20 diluted anti-digoxigenin peroxidase (Boehringer-Mannheim) for 30 min. Samples were washed with PBS (3 × 5 min) and then placed in an ambient temperature diaminobenzidine solution of one tablet Sigma DABfast/H₂O₂ in 150 ml of PBS (Sigma) for 9 min to develop the reaction product, washed under running tap water, counterstained with hematoxylin, rinsed in tap water, dehydrated through graded alcohols, xylene, and mounted with Permount. For dysplastic tissue, the section was divided into equal-size quadrants, and 250 epithelial cells/quadrant were counted (or all of the cells, if <250 were present). For normal-appearing mucosa, 10 crypts (20 crypt columns) were counted, with all epithelial cells from the crypt base to five nuclei past the top of the column crypt being tallied. Cells were scored as apoptotic only if they displayed morphological features of apoptosis (*i.e.*, condensed or segmented nuclei), regardless of whether they labeled positive with the TUNEL method. The apoptotic index was calculated by dividing the total number of apoptotic cells by the number of epithelial cells counted and expressing the quotient as a percentage. Adenoma specimens were confirmed for dysplasia by histology.

RESULTS

Subject Characteristics. Twenty patients were entered into this trial between August 8, 1995 and July 22, 1996. Six patients began treatment at 200 mg p.o. bid, and six began treatment at 400 mg p.o. bid. Two patients, entered at the 300-mg p.o. bid dose level, discontinued therapy early. One of these cases was discontinued after a single dose of exisulind because of poor venous access. The second patient discontinued therapy after ~1 month of treatment when surgery was necessitated by the diagnosis of high-grade dysplasia of the ampulla of Vater, based upon evaluation of the pretreatment upper endoscopy specimens. The remaining 18 participants completed the prescribed 6 months of therapy. Six additional subjects were screened but were found to be ineligible. Table 1 summarizes the baseline characteristics of the 18 fully evaluable participants.

Compliance. Compliance with the prescribed treatment was assessed by examining trough levels over time and by pill counts. Participants were considered to be compliant if their use of the study drug was 80–120% of the amount prescribed. Two patients in the 200-mg p.o. bid dosing group (drug use, 60 and 79% of prescribed) and one in the 400-mg p.o. bid group (drug use, 72% of prescribed) were considered to be noncompliant; all patients in the 300-mg p.o. bid dose group were compliant. Subject 1001, who was 60% compliant on the basis of pill counts, was also suspected of being noncompliant on the basis of lower than expected or undetectable trough blood levels on several occasions.

Safety and Toxicity. Table 2 summarizes the most frequently reported adverse events. Exisulind was generally well tolerated, and only one symptomatic adverse event considered to be “severe” (elevated alanine aminotransferase) was reported. Four of six patients in each dose level experienced headaches. Gastrointestinal toxicities were reported by all patients treated at the 300- and 400-mg bid dose levels and by two patients treated at the 200-mg bid dose level. These complaints consisted of nausea or vomiting, diarrhea, changes in the frequency or consistency of bowel movements, abdominal pain, or dyspepsia. These complaints were generally mild in degree and did not require cessation of the study drug. Dose-limiting hepatic toxicity was noted at a dose of exisulind of 400 mg p.o. bid (see Table 2). When dose-limiting hepatic toxicity was noted at the 400-mg bid dose level, all patients at that dose level were dose reduced. Dose re-escalation was attempted in patients not suffering toxicity. Fig. 1 summarizes the relationship between the liver function tests and the temporal course, severity, and the administered dose for the six evaluable patients treated at the 400-mg bid dose level. As can be seen, liver function abnormalities reversed rapidly after treatment with exisulind was withheld or the dose reduced. Reinstitution of therapy at a lower dose was well tolerated, and all evaluable patients were able to complete 6 months of therapy with exisulind.

Pharmacokinetics. The mean plasma concentration-time profiles of exisulind after the first dose and after the final, 6-month dose are shown in Fig. 2. Because of dose modifications for toxicity, only two of the six patients starting therapy at 400 mg p.o. bid were receiving the intended dose after 6 months of therapy; the curve for the 6-month time point reflects the mean of these two patients. The overall plasma concentration-time profiles of exisulind at 6 months are higher than those after the first dose, indicating some accumulation of the drug. Be-

Table 2 Most commonly reported adverse events, graded according to the Common Toxicity Criteria of the National Cancer Institute

The occurrence of grade 2 or worse toxicity in three or more patients in a dose level defined an unacceptably toxic dose level. Except as otherwise specified, six patients were entered into each dose level.

	200 mg p.o. bid		300 mg p.o. bid		400 mg p.o. bid		
	Grade 1	Grade 2	Grade 1	Grade 2	Grade 1	Grade 2	Grade 3
Nausea/Vomiting	1		2	1	2	1	
Indigestion/Heartburn	1		4		3		
Pain/Cramps	1	1	1	1	2	1	
Decreased appetite	1		1		1		
Change in bowel habits			5			1	
Overall GI (any of the above)	1	1	6 ^a	1	3	3	
Headache	4		4		3	1	
Elevated ALT, AST, or bilirubin	1	1 ^a		2	1	2	1

^a Elevated liver functions felt secondary to cholelithiasis; $n = 7$ because subject treated for 1 month before withdrawal is included.

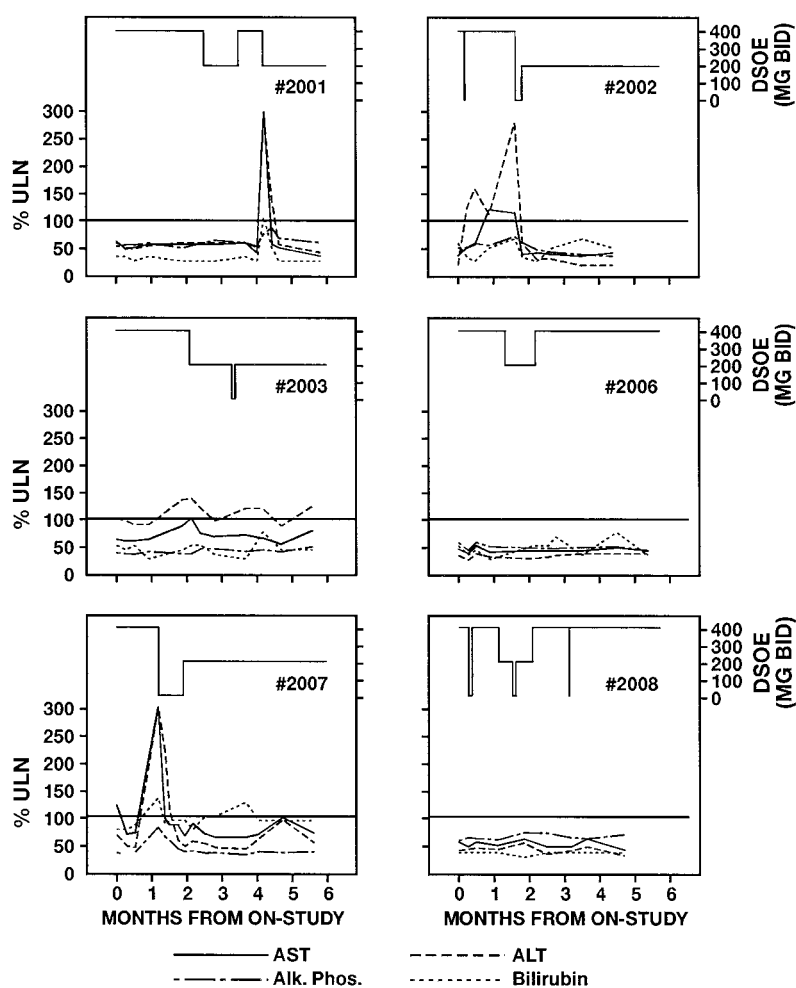


Fig. 1 Changes in liver function and dose over time in patients treated with sulindac sulfone 400 mg p.o. bid.

cause exisulind has a half-life that is < 8 h in most patients, ~ 1.5 maintenance doses would accumulate at steady state with a dosing interval of 12 h. The log-linear phases (generally 10 h and beyond) appeared parallel, consistent with a lack of change in the elimination of exisulind during the course of the study. There was a systematic trend toward a decrease in clearance and volume of distribution after 6 months (Table 3).

Table 3 summarizes the pharmacokinetic parameters for each dose level, using a model-independent analysis of the plasma concentration-time profiles. The T_{max} values (time of peak concentration) were ~ 2 h after drug administration and were similar for the three dosage levels studied. The C_{max} values (maximum plasma concentration) increased proportionally with dose for the 200- and 400-mg doses, but the C_{max} value for the

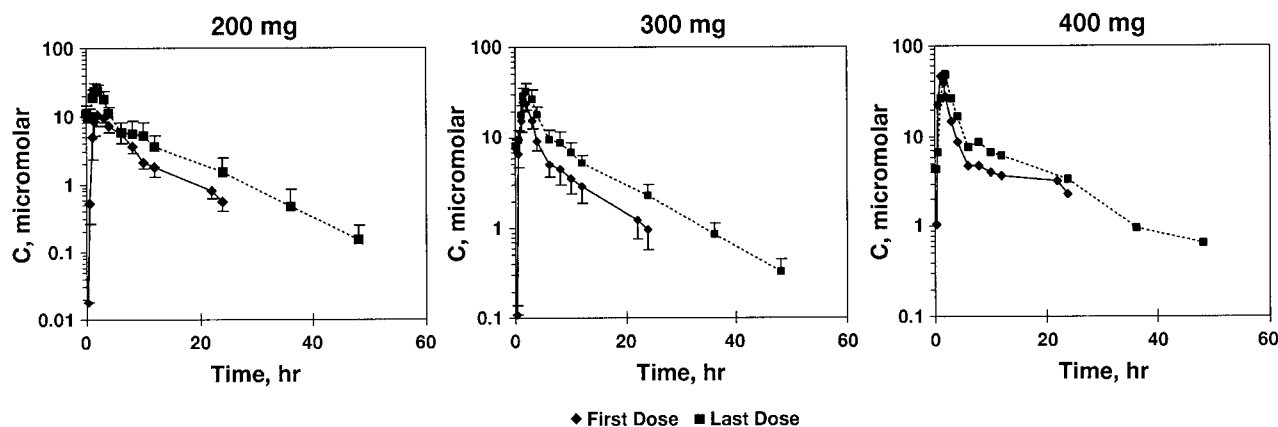


Fig. 2 Plasma concentration-time profile of sulindac sulfone after the first dose (▲) and after 6 months (■); bars, SE. 200 mg: each point represents the mean of six patients; 300 mg: each point represents the mean of seven patients after the first dose and six patients after 6 months; 400 mg: each point represents the mean of two patients, and SEs are not shown.

Table 3 Sulindac sulfone pharmacokinetic parameters after the first dose

Parameter values determined using WinNonlin software and noncompartmental analysis.	T_{\max} h	C_{\max} mg/l	$T_{1/2}$ h	AUC mg \times h/L	V_{β}/F^b liter	CL_s/F^b l/h	MRT h	V_{ss}/F^b liter
200-mg dose								
Mean	2.25	4.91	6.29	27.96	76.84	8.22	8.17	69.26
SD	0.612	2.16	1.42	10.2	41.8	3.67	1.66	40.4
300-mg dose								
Mean	1.93	11.08	6.81	49.21	72.00	8.18	7.91	58.80
SD	0.5	4.18	2.17	30.1	35.1	4.5	3.0	36.8
400-mg dose ^a								
Mean	2.50	9.85	4.88	48.7	65.5	10.1	6.65	60.1
SD	2.00	2.66	1.40	27.4	21.2	4.58	3.21	24.8

^a Values from one subject with outlying results were omitted from the mean and SD calculations at the 400-mg bid dose level.

^b F is the fraction of the oral dose that reaches the systemic circulation. Because the dose was administered orally, the value of F was unknown and potentially <1.00 . This uncertainty in F was transmitted to the clearance and volume of distribution values, *i.e.*, if $F < 1$, then the clearance and volume of distribution values are larger than the true values. For this reason, these parameters were shown divided by F ; F was omitted when the symbols were used in the text.

300-mg dose was higher than expected. The $t_{1/2}$ was ~ 6 –7 h for the two lower dosages but was somewhat shorter for the 400-mg dose, although considerable interpatient variability was noted. The AUC also showed considerable interpatient variability. As with the C_{\max} values, the AUC values seemed to display a nonlinear relationship to dose (Fig. 2). The apparent volume of distribution (V_{β} and V_{ss}) was slightly less than 1 l/kg, consistent with distribution throughout total body water. The total body clearance (CL_s) was 8–10 l/h. This relatively small value compared with blood flow to the organs of elimination does not suggest a significant first pass elimination of exisulind. There was no dose dependence in the CL_s , V_{β} , and V_{ss} values after the first dose, and all of these parameters showed considerable variability among patients. This variability was not significantly reduced by normalizing the parameter values to body weight, lean body weight, or to body surface area (data not shown).

A two-compartment model analysis of the average plasma concentration-time profiles was performed at baseline and after 6 months (Table 4). The parameters that are common to those in Table 3 (the noncompartmental analysis) have similar values. The absorption half-life ($t_{1/2, \text{abs}}$) was ~ 0.7 h. The lag time, the

period after dose administration during which there is no absorption, was ~ 20 –40 min. These values are consistent with a lag time attributable to gastric emptying and capsule disintegration, followed by rapid absorption.

The trough plasma concentrations over time are summarized for the 200- and 300-mg dose levels in Table 5; the results for the 400-mg dose level are not shown because the actual administered dose varied from patient to patient and over time as a result of dose modifications for toxicity at that dose level. The trough plasma concentrations observed over the 6-month course of the study were relatively constant over time for each dose level. Substantial interpatient variability in these values was present, however. Barely detectable levels were found on several occasions for patient 1001, consistent with the 60% compliance documented by capsule counts. Overall, the observed trough concentrations were consistent with those expected based upon the first-dose pharmacokinetics, *i.e.*, the expected average concentration would be dose rate divided by total body clearance.

The recovery of sulindac sulfone in the 24-h urine samples collected during the study was 20–30% of the daily dose in most

Table 4 Parameter values from two-compartment model analysis of sulindac sulfone plasma concentration-time profiles, after the first dose and after the 6-month dose

The average concentration for each dosage was analyzed. The last two rows represent the same data, expressed in differing units.

Parameter	Units	200 mg		300 mg		400 mg		
		baseline ^a	6 mo	baseline	6 mo	baseline ^a	baseline ^b	6 mo ^b
V_1/F^c	liter	32.6	11.3	13.9	15.1	35.7	10.3	9.44
V_{ss}/F^c	liter	58.7	36.4	49.4	42.9	55.1	92.8	54.2
$t_{1/2}$ abs	h	0.63	0.62	0.62	0.84	0.57	0.53	0.52
T_{lag}	h	0.655	0.369	0.377	0.420	0.211	0.242	0.827
CL/F^c	l/h	6.78	4.57	6.68	4.93	8.46	4.45	6.16
AUC	$\mu\text{M} \times \text{h}$	78.9	118	121	164	127	241	174
$T_{1/2}$	h	9.40	7.77	7.90	8.54	6.03	21	10.9
T_{max}	h	2.28	1.41	1.37	1.81	1.69	1.06	1.61
C_{max}	mg/l	3.75	8.62	8.52	10.00	6.77	14.8	17.9
C_{max}	μM	10.1	23.1	22.9	26.9	18.2	39.7	48.1

^a All six patients were analyzed for the first dose baseline.

^b Only two patients ended the 6-month study still taking the 400-mg bid dosage; these base line and 6-month parameter sets were an analysis of the average C versus t profiles from these two patients: 2006 and 2008.

^c F is the fraction of the oral dose that reaches the systemic circulation. Because the dose was administered orally, the value of F was unknown and potentially <1.00 . This uncertainty in F was transmitted to the clearance and volume of distribution values, *i.e.*, if $F < 1$, then the clearance and volume of distribution values are larger than the true values. For this reason, these parameters were shown divided by F ; F was omitted when the symbols were used in the text.

Table 5 Trough plasma concentrations of sulindac sulfone at various times during 6 months administration of sulindac sulfone twice a day, orally

The 400-mg p.o. bid dose level is not shown because subjects received varying doses over time at that dose level.

	Trough plasma concentrations, $\mu\text{g}/\text{ml}$								
	12 h	1 week	2 week	1 month	2 month	3 month	4 month	5 month	6 month
200 mg									
Mean	0.666	2.75	2.96	2.49	1.97	3.24	2.06	1.22	1.37
SD	0.455	1.21	1.66	1.29	0.71	2.91	1.33	0.83	1.41
300 mg									
Mean	1.08	3.73	2.89	2.74	3.75	4.56	3.57	3.38	1.94
SD	1.01	3.68	2.35	1.52	2.72	3.48	4.25	2.34	1.19

patients (data not shown). Sulindac sulfone, an acid, has been shown to form a glucuronide conjugate (15). The urine samples were subjected to a hydrolysis step, which involved treatment of the urine with β -glucuronidase at pH 5.0 with acetate buffer at 37°C; therefore, the sulindac sulfone values represent the total (both unchanged and conjugated) drug. Pilot studies indicated that most of the sulindac sulfone found in the urine was in the conjugated form.

Pharmacodynamics. Adjusting for dose, the occurrence of hepatic toxicity was unrelated to any of the pharmacokinetic parameters summarized in Table 3 ($P \geq 0.28$ for all parameters).

Polyp Numbers and Morphology. Fig. 3 summarizes the mean number of polyps observed over time, accounting for polyps that were excised. Data from one patient in the 400-mg p.o. bid dose group were incomplete and are not included. Polyp counts were not statistically different between any of the dose groups at baseline or after 1 month of therapy. However, polyp numbers rose significantly after 4 and 6 months of therapy at the 200-mg p.o. bid dose level ($P < 0.001$). Polyp numbers were not significantly different from at baseline after 4 and 6 months of therapy at the 300- and 400-mg p.o. bid dose levels (also

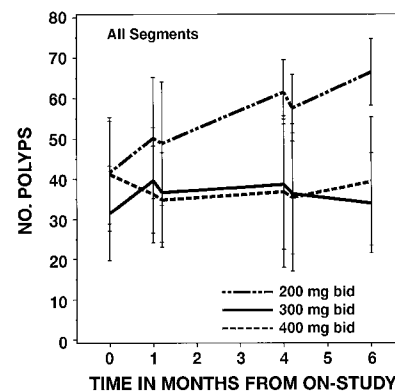
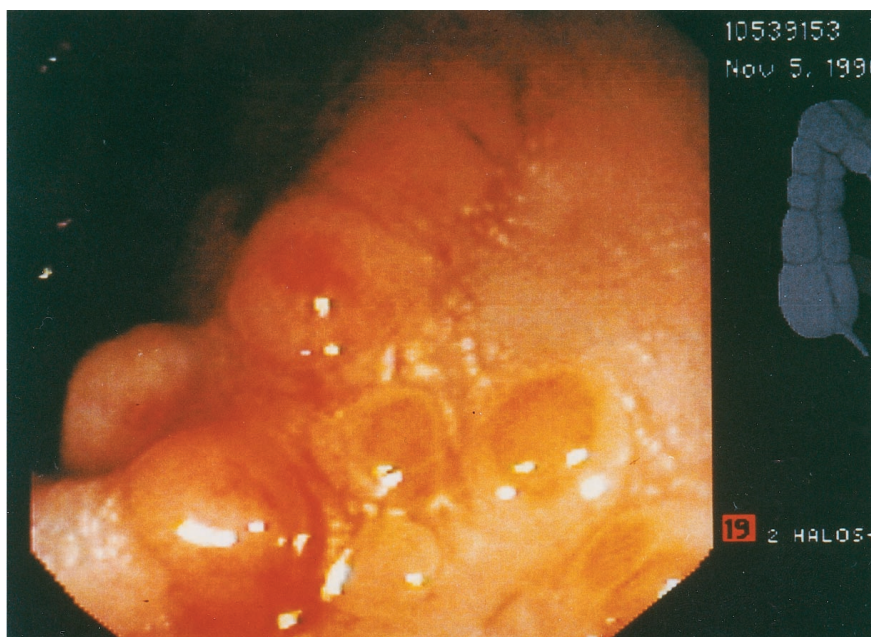


Fig. 3 Changes in polyp number over time by dose level. The brief decreases in polyp number reflect diagnostic biopsies.

referred to as the 400/200-mg dose level because all patients were dose reduced during the course of therapy).

During therapy, we noticed that some polyps exhibited an unusual morphology. These polyps consisted of a central raised,

Fig. 4 Endoscopic appearance of “halo” polyps noted during therapy with exisulind.



erythematous lesion surrounded by a flattened, white area (Fig. 4). We have used the term “halo polyps” to describe these lesions. Because these polyps were an unanticipated finding, they were not counted prospectively during the course of the trial. Halo polyps were occasionally seen prior to therapy with exisulind but were more common after therapy. These polyps have been found to represent adenomas when biopsied.

Proliferative Index of Intestinal Crypt Cells in Normal-appearing Mucosa and Adenomatous Polyps. The proliferative index, as determined by Ki-67 expression, of the intestinal crypt cells in normal-appearing mucosa and in adenomatous polyps was determined. The proliferative index was higher in adenomatous polyps than in normal-appearing mucosa. However, no significant changes in proliferation were noted over time in either normal-appearing mucosa or in adenomas at any dose level (data not shown).

Apoptotic Index of Intestinal Crypt Cells in Normal-appearing Mucosa and Adenomatous Polyps. Fig. 5 summarizes the apoptotic index, as determined by the TUNEL assay, of the intestinal crypt cells in normal-appearing mucosa and in adenomatous polyps. Both prior to and after therapy, the apoptotic index was greater in polyps than in normal-appearing mucosa ($P < 0.001$ at both baseline and month 6, Wilcoxon Signed Rank Test). Overall, the apoptotic index was not significantly different after 6 months of therapy than at baseline in either normal-appearing mucosa or in adenomas, although there appeared to be a trend for the apoptotic index of the polyps to increase after therapy ($P = 0.09$, Wilcoxon-Signed Rank Test). This appeared particularly true at the 300-mg bid dose level, but the number of patients treated at each dose level was too small to allow any conclusion to be drawn with respect to dose and response, and there was no statistically significant dose effect ($P = 0.14$, Jonckheere-Terpstra Test).

Histological Appearance of Adenomatous Polyps. All polyp and normal-appearing mucosal specimens were examined

for histological features. A morphological change in the histological features of the adenomatous polyps was noted. The posttreatment specimens displayed greater amounts of mucinous material, a change that we have termed “mucinous differentiation.” An example of this change is presented in Fig. 6B. The degree of this mucinous differentiation was subjectively graded as “none,” “mild,” “moderate,” or “extensive.” Overall, mucinous differentiation was noted in the polyps of only 4 of 15 patients with evaluable polyps at baseline, and the degree of this differentiation was termed “mild” in all cases. After 6 months of therapy, some degree of mucinous differentiation was observed in 16 of 17 patients from whom polyps were available. The degree of this change was termed “mild” in 4 cases, “moderate” in 7 cases, and “extensive” in 5 patients.

DISCUSSION

The nonsteroidal anti-inflammatory drug sulindac can reduce the incidence of gastrointestinal adenomas in the *Min* mouse model of familial adenomatous polyposis (10). This effect is associated with an increase in apoptosis in the luminal portion of the crypt-villus of *Min* mice, a reduction in mucosal cyclooxygenase-2 protein levels, and a reduction in prostaglandin E2 levels in normal-appearing small bowel mucosa (10). In patients with FAP, treatment with sulindac can induce the regression of polyps, although disease progression resumes when treatment is discontinued (8). These effects have been attributed to the ability of the sulfide metabolite of sulindac to inhibit cyclooxygenase-2 (10). APC^{Δ23} knockout mice are a murine model of FAP in which a truncating mutation of the APC gene results in gastrointestinal polyp formation (23). In APC^{Δ23} mice in whom COX-2 has also been knocked-out, the rate of adenoma formation is greatly attenuated, further suggesting that COX-2 is important to the process of adenoma formation (24).

In other colon cancer model systems, however, inhibition

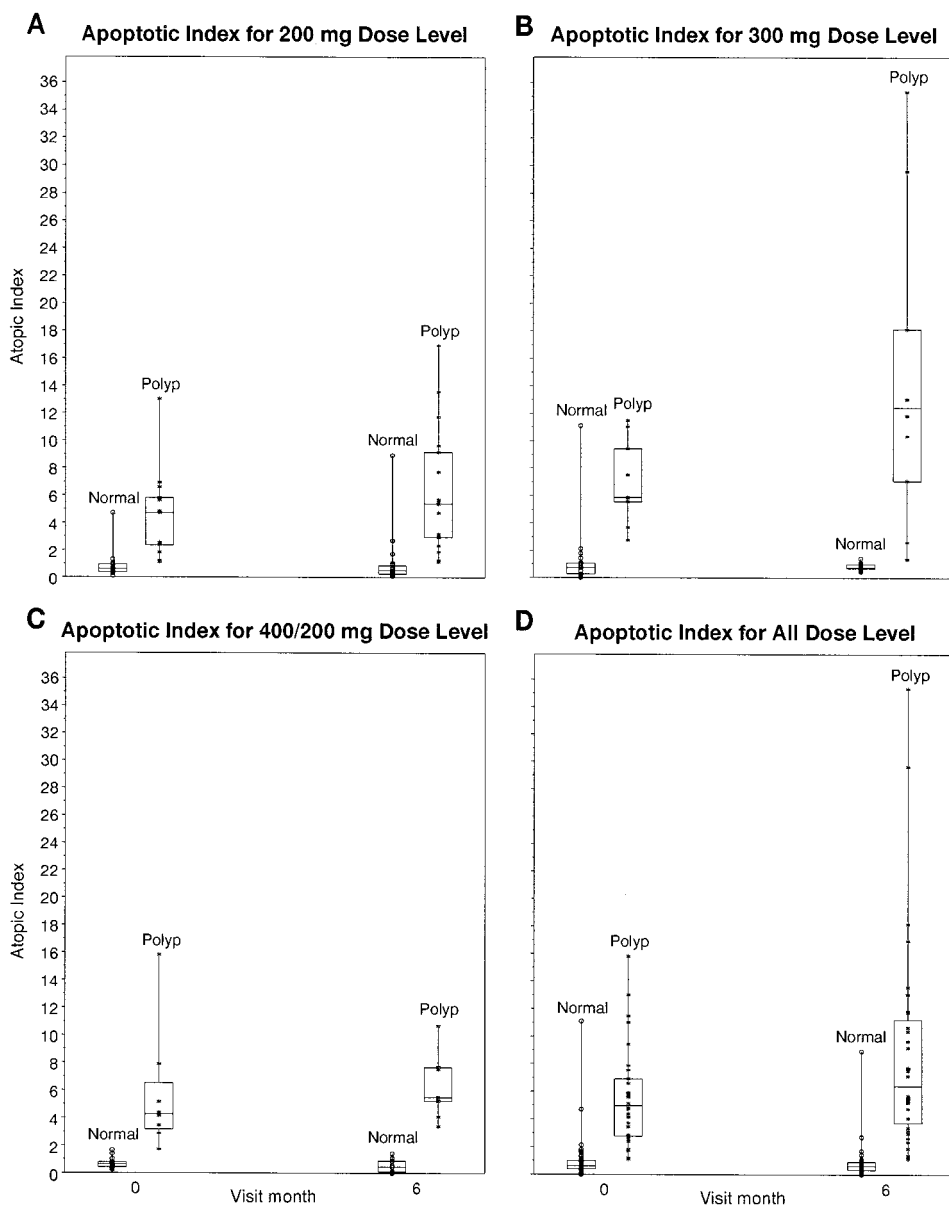


Fig. 5 Apoptotic index in normal-appearing mucosa and in polyps according to dose level (A, 200 mg; B, 300 mg; C, 400 mg) and over all dose levels (D).

of COX-2 has not been found to be the mechanism of action of NSAIDs and related drugs. The COX-2 inhibitor NS-398 induced apoptosis in both the HT29 colon carcinoma cell line, in which COX-2 is expressed constitutively, and in the S/KS cell line, in which COX-2 protein is undetectable (25). Chiu *et al.* (11) did not find COX-2 to be overexpressed in the normal-appearing mucosa of *Min/+* mice and dietary supplementation with arachidonic acid did not increase tumor formation. The observation that the sulfone metabolite of sulindac, exisulind, does not inhibit COX-2 (12, 13) but inhibits azoxymethane-induced colon carcinogenesis (14) has been interpreted as consistent with the hypothesis that exisulind, and perhaps NSAIDs, have a chemopreventive mechanism independent of COX-2 inhibition. *In vitro* studies have indicated that exisulind could induce apoptosis independent of COX-2 inhibition, and that this

effect was not reversed by the prostaglandin analogue dimethyl-prostaglandin E2 (13). The current trial was undertaken on the basis of the hypothesis that exisulind treatment would induce apoptosis in rectal polyps of patients with FAP and result in a reduction in polyp numbers.

This trial demonstrates that the dose-limiting toxicity of exisulind in patients with FAP is reversible hepatic dysfunction. The MTD of exisulind that can be given to this patient population is 300 mg p.o. bid, or a total daily dose of 600 mg/day. Over the 6-month treatment course, no long-term toxicities were noted. Patients completing the current study have been entered on a longer term extension trial to further evaluate safety and efficacy. Preliminary data from this trial suggest that long-term therapy at the MTD can be administered with acceptable safety, although the study remains ongoing. Clinical trials in patients

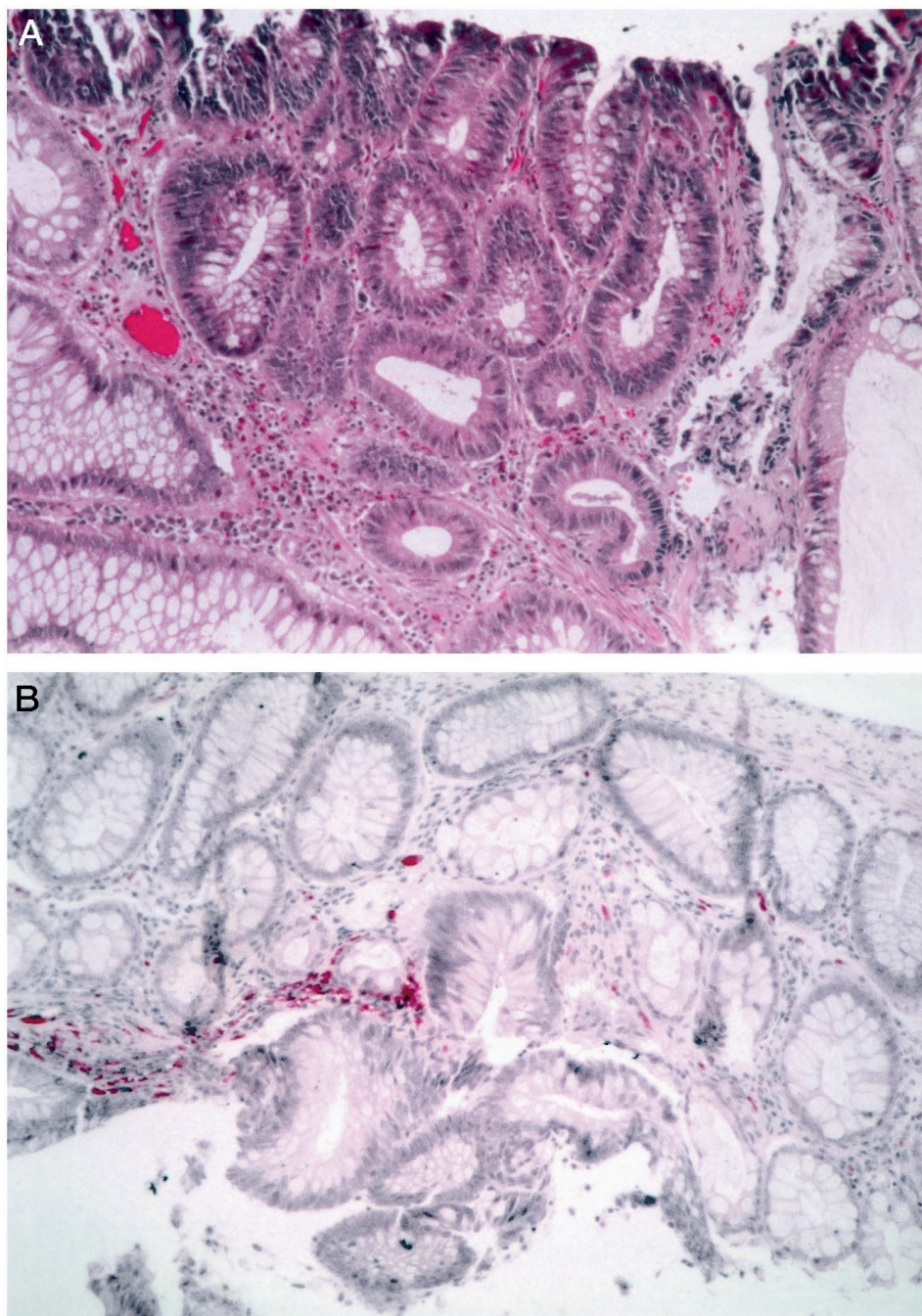


Fig. 6 Histological appearance of polyps. A polyp removed prior to therapy (A) shows mild to severe dysplastic changes and little mucin production. A polyp removed after 6 months of therapy with exisulind (B) shows mild dysplasia and increased mucin production.

with sporadic polyps or other lower risk populations might be considered after long-term safety has been demonstrated in patients with FAP.

The pharmacokinetics of exisulind in patients with FAP who have undergone colectomy are nonlinear over the dosage range of 200–400 mg p.o. bid. The pharmacokinetics are not time dependent over the 6-month course of the study, and no gender-associated differences are apparent. The volume of distribution of exisulind is consistent with distribution throughout total body water, with clearance studies suggesting low extrac-

tion. Pharmacodynamic studies show no clear relationship between pharmacokinetic parameters and hepatic toxicity, with dose being the most important predictor of toxicity. Pharmacokinetic studies indicate that exisulind is absorbed rapidly and, likely, completely. Although large interpatient differences are noted, in general, the half-life is in the range of 6–9 h in FAP patients.

These pharmacological findings should be related to those reported for normal volunteers with intact colons and to the concentrations of sulindac sulfone used in preclinical studies. At

the 200- and 300-mg single doses of exisulind, major pharmacokinetic parameters of exisulind are similar in these patients of both sexes with FAP who have undergone colectomy and ileo-rectal anastomosis and those reported previously for normal healthy male volunteers (Ref. 15; $C_{\max 200} = 4.91$ mg/l for FAP patients *versus* 6.67 in normal healthy male volunteers; $AUC_{200} = 27.96$ mg \times h/l in FAP patients *versus* 32.25 in normal healthy male volunteers; $C_{\max 300} = 11.08$ mg/l in FAP patients *versus* 7.85 in normal healthy male volunteers; $AUC_{300} = 49.21$ mg \times h/l in FAP patients *versus* 40.49 in normal healthy male volunteers). At the 400-mg dose level, however, the C_{\max} in patients with FAP was lower than in normal male volunteers (9.85 *versus* 13.14 mg/l), as was the AUC (48.7 *versus* 85.04 mg \times h/l). Future studies in patients with FAP who have undergone subtotal colectomy should investigate a daily dose of exisulind of 600 mg/day. The increased reabsorption of exisulind and the resultant prolonged mean residence time in patients with intact colons may necessitate a reduction in the total daily dose in patients with intact colons (15).

At the maximum safe dose of 300 mg p.o. bid, steady-state plasma concentrations in the range of 1–5 mg/l (2.7–13.4 μ M) and peak plasma concentrations of 8.5–11.1 mg/l (23–30 μ M) were achieved. These concentrations are lower than the IC_{50} of exisulind for various cell lines (90–200 μ M; Refs. 26 and 27) or the concentration of exisulind reported to induce apoptosis in HT-29 colon or MCF-7 breast carcinoma cells (240 μ M; Refs. 26 and 27). The achieved plasma concentrations of exisulind are, however, in the range of the concentration that has been reported to increase the expression of APC mRNA in malignant colonic epithelial cells (10 μ M; Ref. 28) and to inhibit 7,12-dimethylbenz(a)anthracene-induced mammary lesions in organ culture studies (10 μ M; Ref. 27).

At no dose did we observe a reduction in overall polyp numbers, although the numbers of polyps remained stable during the 6-month treatment period for patients treated at the MTD of 300 mg p.o. bid. This might represent a chance selection of patients with relatively indolent disease at this dose level or might represent a perturbation of the generally progressive natural history of the disease. Treatment with exisulind did not affect cellular proliferation in either normal-appearing mucosa or in polyps, consistent with preclinical observations. No statistically significant change in apoptotic rate was noted, but a nonsignificant trend toward an increased apoptotic index after 6 months of therapy as compared with baseline was noted. The most interesting observations were those related to the gross and microscopic morphology of polyps in patients treated with exisulind. There was an increase in the number of polyps with a "halo" appearance and an increase in mucinous differentiation of polyps after therapy. Polyps showing these changes, however, remained adenomatous.

These observations suggest that exisulind may have biological effects in patients with FAP, but that these effects are insufficient to produce a reduction in polyp numbers. These findings are similar to those reported for sulindac sulfone in preclinical studies in FAP model systems. Exisulind has proven inactive in two studies in the *Min/+* mouse model of FAP (29, 30), despite showing activity in the azoxymethane rat colon carcinogenesis system. Thus, although reversible hepatic toxicity prevented the achievement of plasma concentrations of ex-

isulind in the range found to be biologically active in the majority of the preclinical investigations of this agent, further explication of the biological effects of exisulind and its congeners may result in the development of clinically useful chemopreventive and therapeutic approaches.

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Phase I Trial of Exisulind (Sulindac Sulfone, FGN-1) as a Chemopreventive Agent in Patients with Familial Adenomatous Polyposis

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