Effects of sulindac on sporadic colorectal adenomatous polyps

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Abstract

Background—Although sulindac is known to cause regression of colorectal adenomatous polyps in familial adenomatous polyposis, less is known about the effect of sulindac on sporadic adenomas. The precise mechanisms of these effects also remain to be determined.

Aims—Sulindac was given to patients with sporadic colorectal adenomatous polyps to evaluate its effects on them, and histological analysis was performed to elucidate the mechanism of the polyp regression, as well the kind of adenomatous polyps that are susceptible to the agent.

Subjects—20 adenomatous polyps in 15 patients were studied.

Methods—Sulindac (300 mg daily) was given for four months, followed by colonoscopy with removal of the residual polyps. Polyp size, degree of atypia, inflammatory cell infiltration in the polyps, and immunostaining for mutant p53 product were evaluated before and after treatment.

Results—13 of the 20 polyps shrank or disappeared. Patient sex, polyp location, size, degree of atypia, or p53 mutation did not affect the response, but polyps in older patients were more sensitive to sulindac. The degree of atypia or inflammatory cell infiltration was not affected by the treatment. A polyp containing a focal cancer was unresponsive.

Conclusions—Sulindac can cause regression of sporadic colorectal adenomatous polyps.

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significant regression of sporadic colonic polyps.¹⁴⁻¹⁶ However, no essential differences between adenomatous polyps in FAP and sporadic polyps are known, both histologically and genetically.¹⁷ Therefore, because sulindac is known to have a regressive effect on FAP polyps, an anti-adenomatous polyp effect of sulindac in sporadic polyps might be expected. We studied the treatment of sporadic colorectal polyps with this agent in another population.

Methods

Patients

The patients (11 men age (mean (SD)), 56.9 (7.6) years old) were treated at Tokyo University Hospital. Patients in whom one or more colorectal polyps were diagnosed by barium enema studies subsequently underwent colonoscopy, and biopsy specimens of the colorectal polyps were obtained. Patients whose polyps proved to be adenomas on histological examination were included in the study. No patient had FAP or Lynch syndrome (hereditary non-polyposis colorectal cancer) that is, all patients had less than 10 polyps and no patient met the criteria for Lynch syndrome.¹⁸ Informed consent was obtained from each patient before treatment. Patients with severe liver, kidney, or cardiopulmonary diseases, past histories of gastrointestinal tract bleeding, peptic ulcer disease, or long term non-steroidal anti-inflammatory drug (NSAID) use were excluded from the study. Patients with adenomatous polyps exhibiting severe atypia were excluded from the study, because prompt polypectomy was required in such cases.

Study design

A total of 20 adenomatous polyps in 15 patients were studied. On colonoscopy, a biopsy specimen was obtained from each polyp, and to evaluate size each polyp was photographed with an open biopsy forceps (FB50Q; Olympus Corp, Tokyo) beside it. The sizes of the polyps were estimated from the photographs. Histological examination was performed by means of haematoxylin and eosin staining. After histological examination, each patient was given 150 mg of sulindac twice a day for four months, a protocol similar to previous studies.^{3-7 9-11 14-16} Compliance was assessed at monthly interviews. Histological examination was performed by two independent pathologists in a blinded fashion, and the degree of atypia was graded as mild, moderate, or severe

Keywords: sulindac, NSAID, sporadic colorectal adenomatous polyp, familial adenomatous polyposis, colorectal cancer.

The prevention of colorectal cancers in the treatment of patients with familial adenomatous polyposis (FAP) is essential. Since 1983, sulindac has been known to cause regression of adenomatous polyps in patients with FAP.¹⁻¹³ Therapeutic trials to prevent colorectal cancers in FAP patients by way of sulindac administration have been performed, and showed that the drug can reduce both the number and size of colorectal adenomas in patients with FAP.^{8 9} However, little is known about the effects of sulindac on sporadic, as opposed to familial, colorectal adenomatous polyps, and a recent study failed to show

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TABLE 1 Patient and polyp characteristics and response to sulindac

Patient	Age, sex	polyp (n)	Site*	Atypia†		Polyp size (mm)		Mononuclear cells‡		p53 staining	
				before	after	before	after	before	after	before	after
A	57, M	1	D	mil	nt‡	13	3	36 (11)	nt	—	nt
B	65, M	1	S	mod	mod	15	8	29 (2)	30 (6)	—	—
C	50, F	1	S	mil	mod	8	7	33 (2)	33 (1)	—	—
D	49, M	1	R	mil		5	0	23 (6)		—	
E	57, M	1	S	mod	mod/sev	13	15	42 (11)	33 (5)	—	focal
F	63, M	1	A	mod	mod	7	5	50 (4)	34 (3)	—	—
		2	T	mil		4	0	51 (8)		—	—
		3	D	mil		4	0	65 (7)		—	—
		4	S	mod		4	0	42 (6)		—	—
		5	S	mod		8	0	43 (2)		—	—
		6	S	mod		10	0	44 (5)		—	—
G	63, M	1	S	mod	mod	28	8	35 (14)	37 (3)	—	—
H	54, F	1	S	mod	mod	10	12	55 (14)	52 (7)	—	focal
I	64, M	1	T	mil		5	0	59 (11)		nt	±
J	48, M	1	S	mil		4	0	63 (2)		±	±
K	66, M	1	D	mod		4	0	46 (9)		±	±
L	44, F	1	R	mil	mil	14	10	48 (7)	43 (23)	+	+
M	66, F	1	S	mod	mod	12	6	48 (3)	51 (6)	+	±
N	59, M	1	D	mod	mod	15	12	44 (2)	54 (10)	±	±
O	48, M	1	R	mil	mil	5	5	nt	nt	—	—

*A; ascending, D; descending, S; sigmoid, T; transverse colon, R; rectum. †mil; mild atypia, mod; moderate atypia, sev; severe atypia. ‡nt; not tested. §Numbers of mononuclear cells counted in high power views. Data shown as mean (SEM).

atypia, with reference to goblet cell depletion, nuclear atypia, and structural disorders. The polyp size was similarly evaluated after four months of sulindac administration. Positive response was defined as a greater than 40% decrease in polyp diameter. At that time, residual polyps were polypectomised and examined pathologically. Infiltrating mononuclear cell counts were determined by counting such cells within a circle in the visual field visualised in three high power views (×1000) of the lamina propria mucosae of the polyps.

Immunohistochemical analysis for mutated p53 product was performed as described¹⁹: in brief, 5 mm thick sections of formalin fixed, paraffin wax embedded tissues were stained using the human p53 specific mouse monoclonal antibody Pab1801 (Oncogene Science, Manhasset, NY). Sections were rehydrated in graded alcohols incubated in normal rabbit serum diluted 1:5 in TRIS buffered saline (145 mmol/l NaCl, 20 mmol/l TRIS pH 7.6), and exposed to primary antibody at a dilution of 1:100 for one hour. Bound antibody was detected using biotinylated rabbit antibody to mouse immunoglobulin (Dakopatts No E354, Glostrup, Denmark) and avidin-biotin complex linked to horseradish peroxidase

(Dakopatts No 335). Staining was with diaminobenzidine (1 mg/ml) in the presence of 0.03% hydrogen peroxidase. Endogenous peroxidase was not inhibited. Normal colonic mucosa and a colon cancer tissue known to be positive for mutant p53 were used as negative and positive controls. These controls were included in each run, receiving either primary antibody or simply dilution buffer, to monitor consistency and act as controls. Sections underwent a light haematoxylin counterstain and were dehydrated in graded alcohols and xylene before mounting.

Statistical analysis
Statistical analyses were performed by Welch *t* test (patient age, polyp size, and mononuclear cell infiltration) and Fisher's exact probability test (patient sex, polyp location, histological atypia, and p53 staining) and a *p* value of less than 5% was taken to indicate significance.

Results

Response to sulindac
All patients took more than 80% of the prescribed drugs. As Table I shows, more than 40% loss in polyp diameter was observed in 13 of 20 polyps tested. The Figure shows an example of a shrunken polyp. Before treatment (A), the polyp was rather large and occupied almost half the area of the colonic lumen. After sulindac treatment (B), the polyp decreased considerably in size. Moreover, nine of the 13 sulindac sensitive polyps completely disappeared on colonoscopic examinations. Mean

Endoscopic views of a polyp (arrowheads) in a patient (patient G) before (A) and after (B) sulindac administration. In (A), the polyp was too large to be photographed in one view and the distal side of the polyp cannot be seen. The polyp occupies almost half the area of the colonic lumen. The size was determined with the aid of barium enema films (28 mm). (B) shows that the polyp size considerably decreased (8 mm) after sulindac treatment.

TABLE II Changes of polyp size and number of mononuclear cell infiltration after sulindac treatment

	Before	After	
Polyp size (mm) (n=20)	9.4 (6.0)	4.6 (5.0)*	p<0.01
Mononuclear cell infiltration (n=9)	43 (9)	41 (9)	NS

*Polyps that disappeared were calculated as 0 mm. Data shown as mean (SEM).

TABLE III Characteristics of responsive and non-responsive polyps

	Responsive	Unresponsive	
Patient age (mean SD))	59.8 (7.5)	52.0 (5.7)	p<0.05
Sex (M/F)	7/1	3/3	NS
Polyp size (mm) (mean (SEM))	8.9 (7.0)	10.3 (3.8)	NS
Site (right/left colon)	2/11	1/6	NS
Atypia (mild/moderate)	6/7	3/4	NS
Mononuclear cell infiltration (mean (SEM))	45 (13)	46 (7)	NS
p53 staining (+/-)	3/9	2/5	NS

polyp diameter decreased from 9.4 mm to 4.6 mm (Table II; $p<0.01$). In a patient with four adenomatous polyps (patient A), all polyps decreased or disappeared after sulindac administration, but three of them were excluded from the study, because biopsy samples were not obtained from them before treatment. In another patient with six polyps (patient F), five of them completely disappeared while one remained unchanged.

The mean ages of patients with responsive and non-responsive polyps were 59.8 and 52.0 years old, respectively (Table III), and patients with responsive polyps were significantly older than those with resistant polyps ($p<0.05$). As Table III shows, polyps in female patients were slightly more resistant to the treatment than male ones, but the difference was not statistically significant ($p=0.12$).

Table I shows that seven of eight polyps with a diameter of 5 mm or less responded to sulindac, while six of 12 polyps with a diameter of 6 mm or larger did not. This suggested that small polyps are more sensitive to sulindac. However, the p value for this difference was slightly above the significance level ($p=0.08$). Thus the difference was not statistically significant.

Three polyps were located in the right side colon (ascending and transverse colon) and 17 in the left side (descending and sigmoid colon, and rectum), and response did not differ significantly according to the location of the polyps (Table II).

There was also no significant difference in the mean original diameters of polyps responsive and unresponsive to sulindac treatment (8.9 and 10.3 mm, respectively; Table III).

Degree of atypia

All of the polyps tested were histologically tubular adenomas, but the degree of atypia varied: nine were adenomas with mild atypia and 11 had moderate atypia. Histological examination of the polypectomy specimen after the treatment showed that one of the polyps with moderate atypia contained a tiny focal cancer. That polyp, 13 mm in original diameter, did not regress after sulindac administration. However, six of nine polyps with mild atypia and seven of 11 polyps with moderate atypia responded to the treatment, and no significant association was noted between the polyp regression and the degree of atypia, mild or moderate (Table III). Changes in atypia were evaluated in the 10 polyps in which histological examination was available both before and after the treatment. In the three sulindac responsive polyps, the atypia did

not change after treatment. However, among the seven unresponsive polyps, two showed an increase in the degree of atypia.

Infiltrating cell numbers

Numbers of infiltrating mononuclear cells in lamina propria mucosae of adenomas slightly decreased after the treatment (mean (SEM)) (before; 43 (9) and after; 41 (9), but the difference was not statistically significant (Table II).

Pre-treatment infiltrating mononuclear cell numbers of responsive and unresponsive polyps were 45 (13) and 46 (7), respectively (Table III), and the infiltrating cell number did not have any influence on polyp regressive effect.

p53 Staining

On immunohistochemical examination for mutant p53 product, five were positive and 14 were negative at the beginning. Three of five p53 positive polyps and nine of 14 negative ones responded to the treatment, and there was no correlation between p53 positivity and sulindac responsiveness.

Discussion

Sulindac has attracted much interest because of its suppressive effect on adenomatous polyps in FAP. Anti-neoplastic effects of NSAIDs were reported as early as the 1970s.²⁰⁻²⁷ On the other hand, indomethacin has been shown to have regressive effects on human desmoid tumours since 1980.²⁸ Skin cancers in xeroderma pigmentosum²⁹ or cancers of the head and neck³⁰ are also known to be responsive to NSAIDs. The first report on a suppressive effect of sulindac on adenomatous polyps in patients with FAP appeared in 1983, in which a patient with FAP treated by sulindac for her desmoid tumour showed a pronounced regression of her colorectal polyps.¹ Thereafter, experience with FAP polyp regression by sulindac has accumulated.²⁻¹¹ Duodenal polyps in FAP patients also respond to sulindac.³¹ Indomethacin suppositories have been reported to cause regression of rectal polyps in FAP, as well.^{10 12 13} Another line of studies has suggested that NSAIDs have a preventive effect on adenoma formation,³²⁻³⁴ and NSAIDs have been suggested to decrease colorectal cancer risk.³⁵⁻³⁷ In contrast, little is known about their effects on sporadic colorectal adenomatous polyps. A recent study, for example, failed to show significant polyp regressive effects of sulindac.¹⁴ However, in that study, the number of adenomatous polyps studied was rather small (nine adenomatous polyps) and the size of the polyps tested were rather small, and the results did not completely rule out the possibility of such effects. Therefore, we examined the regressive effects of sulindac on sporadic adenomatous polyps in a different population along with histological evaluation of the treated polyps.

As described above, subsequent to treatment, 13 of 20 polyps tested had a greater than

40% loss in polyp diameter. Remarkably, nine of 13 polyps had completely disappeared on colonoscopic examination. These results show that sulindac has a regressive effect on sporadic adenomatous polyps, at least in some patients.

Certain hypotheses on the mechanism of polyp regression by sulindac have been postulated,^{15 38-41} but the precise mechanism of action is not yet known. As a polyp consists of adenoma cells and interstitial components such as infiltrating cells or connective tissues, or both, reduction in polyp size would have to be the result of a decrease in either or both of these components. Therefore, a possibility that a reduction in components other than adenoma cells, such as inflammatory cells, contributes to polyp shrinkage, has not been ruled out. This is especially noteworthy in light of the fact that sulindac is an anti-inflammatory drug. Therefore, histological changes induced by the treatment were investigated. However, no significant changes in inflammatory cell numbers could be detected. Therefore, it seems that the cause of decrease in polyp size was not inflammatory cell depletion. On the other hand, the interstitial tissues of the polyps (oedema, fibrous tissues, or haemorrhage) did not change significantly either (data not shown). Therefore, polyp regression is histologically suggested to be due to adenoma cell depletion. Histological examination failed to show any decrease in the degree of atypia in sulindac treated polyps. This implies that the anti-polyp effect was not associated with induction of differentiation.

It is possible, of course, that a certain subpopulation of polyps or polyps at a certain stage¹⁷ are susceptible to the drug. In this regard, we have investigated predisposing factors including patient age and sex, polyp location, degree of atypia, polyp size, mononuclear cell infiltration into lamina propria mucosae, and mutant p53 product via immunostaining, in relation to the responses to treatment (Table III). As described above, patient sex did not affect the responses, but polyps in older patients were more responsive to sulindac. Analyses of response to sulindac in relation to patient ages will be of relevance.

Macroscopically, the location or size of the polyps did not significantly affect the sensitivity. However, it has been reported that small duodenal polyps, 2 mm or less in size, are sensitive to sulindac in FAP patients.⁴² In this study polyps of 5 mm or less showed a higher response rate (seven of eight) than larger polyps (six of 12), although the difference was not statistically significant. However, this result does not rule out a possibility that tiny polyps (for example, 2 mm or less) are more sensitive to sulindac. Further detailed studies are necessary to elucidate this issue.

Concerning the degree of atypia, there was no statistically significant difference in responses between adenomas with mild atypia and moderate atypia, and both types of adenomas responded to the treatment. None of the polyps were judged to be adenomas with severe atypia before treatment, but in this study, the only polyp that proved to contain a

tiny focal cancer at the end of the treatment, did not respond to the treatment. Established cancers may not be sensitive to the drug. No significant difference in mononuclear cell infiltration was noted in relation to responsiveness to sulindac.

Little is known about the effects of sulindac on cancer related gene products, but an abstract reports that sulindac reduces the levels of mutant p53.⁴³ This change may be related to anti-neoplastic properties of the drug. Mutated p53 product was examined by immunohistochemical staining in this study to see whether changes in p53 expression is taking place in the treatment course, and whether p53 mutation is a predisposing factor for response to sulindac. However, it was hard to evaluate changes in mutant p53 levels induced by sulindac, because only three polyps were positive for p53 before the treatment. Both p53 positive and negative polyps responded to the drug. However, as positive staining in a colorectal adenoma is often focal,⁴⁴ as seen in several polyps tested in this study (data not shown), and specimens stored at room temperature for months may lose immunoreactivity to anti-p53 antibodies,⁴⁵ further investigations are necessary to elucidate the role of p53 in the sulindac induced polyp regression. Thus, the results of p53 analysis in this study were inconclusive and the possibility of a p53 gene alteration in sulindac induced adenoma regression cannot be ruled out.

Regression of colorectal adenomas would be of especially great benefit if such polyp regression could really lead to cancer prevention. Animal experiments⁴⁶⁻⁵⁵ as well as epidemiological studies³³⁻³⁷ suggest that NSAIDs do have a colorectal cancer preventive effect, but on the other hand, rectal cancers are reported to occur in FAP patients treated with sulindac.^{56 57} In this regard, it is noteworthy that an adenomatous polyp containing a focal cancer did not respond to sulindac, and that some of non-responsive polyps exhibited increases in atypia after sulindac therapy in this study. The degree of atypia before treatment did not affect response to sulindac in this study, but it should be noted that adenomas with severe atypia were excluded. The effect of sulindac on adenomas with severe atypia remains to be elucidated, and longer observation of FAP patients treated with sulindac is necessary.

Irrespective of the cancer preventive effect, medical reduction of colorectal polyp size by sulindac should be of benefit in reducing risks associated with polypectomy procedures. Large polyps are generally associated with higher risks of complications such as bleeding or intestinal perforation. Indeed, in this study, a polyp originally 28 mm in size in a patient could be resected very easily, because the polyp diameter decreased dramatically to 8 mm (patient G). Thus, sulindac may prove to be the drug of choice in mass reduction of adenomatous polyps prior to endoscopic polypectomy, especially in large polyps.

The optimal dose of sulindac in colorectal adenoma treatment remains to be determined.

For example, the dose of aspirin required for inhibition of platelet aggregation is known to be much lower than that for analgesic or antipyretic use. We administered 300 mg of sulindac per day, a common dose when used as an anti-inflammatory or analgesic drug, but epidemiological studies show that low doses of NSAIDs seem to be effective in colorectal cancer prevention.³²⁻³⁶ The dose of sulindac required for adenomatous polyp regression may be lower than that adopted in this and previous studies. In fact, a recent study reported a rectal polyp regressive effect with a low dose sulindac suppository (about 50 mg daily).¹² Further investigation is required to resolve this issue.

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