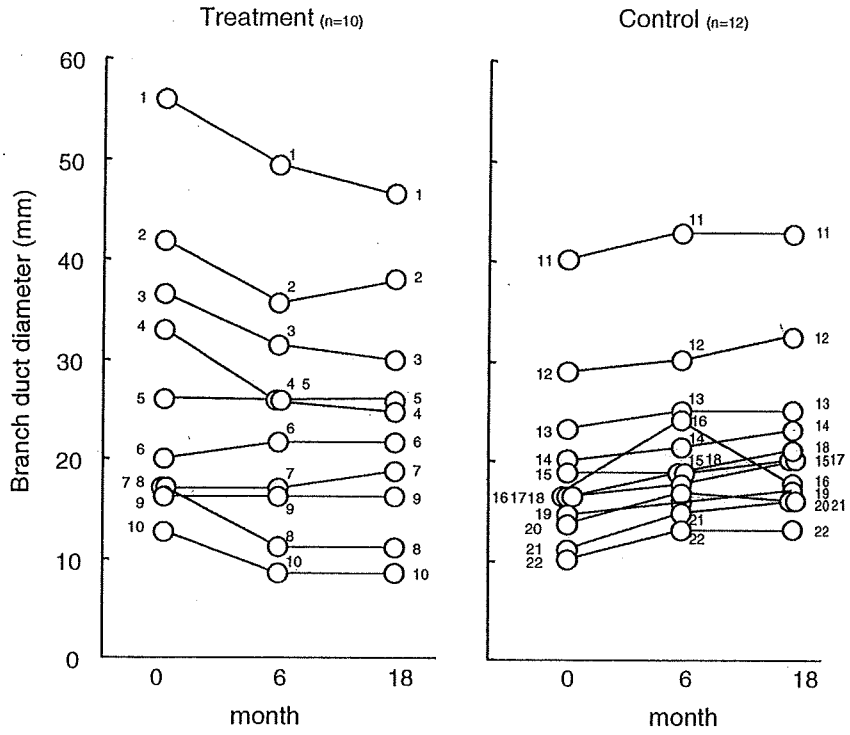

Fig. 1 Changes in branch duct diameter during the observation period. Number at each point (*circles*) represents case number. Friedman's test was used. Significant changes were observed in the treatment group ($P = 0.000055$) but not in the control group ($P = 0.12$)

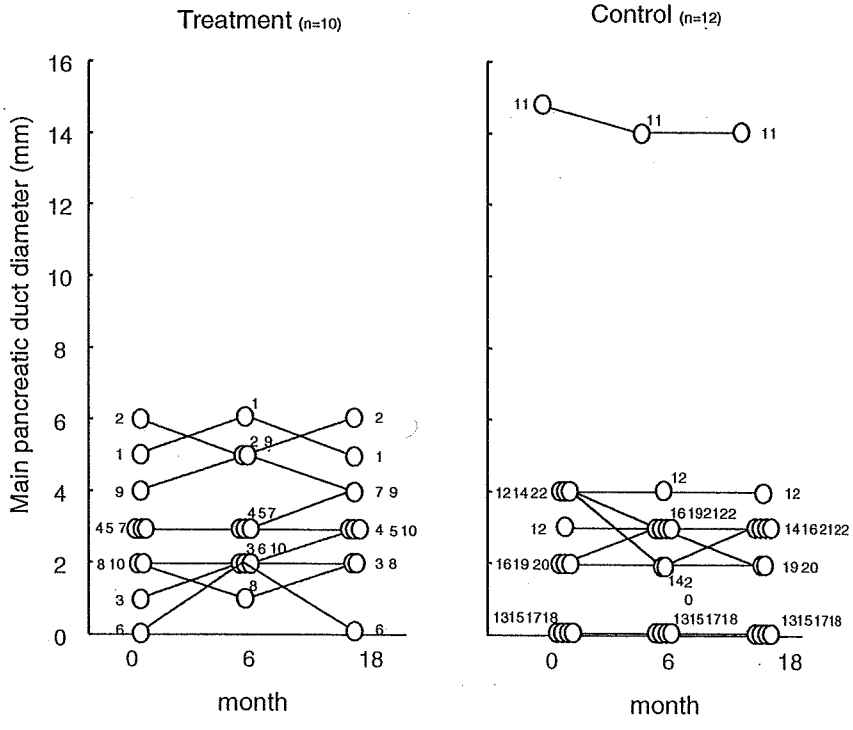

Fig. 2 Changes in diameter of the main pancreatic duct during the observation period. Number at each point (*circles*) represents case number. Friedman's test was used. Significant changes were not observed in either the treatment group ($P = 0.75$) or control group ($P = 0.66$)

decreased either by 6 or 18 months of treatment. The decrement was statistically significant by the Friedman test ($P = 0.001$). When mural nodule height was compared with branch duct diameter in each case, no correlation was observed. In cases 5, 6, and 9, who had no change in branch duct diameter, mural nodule height was clearly decreased.

In the control group, only one of 12 patients (case 11) had a detectable mural nodule. Monitoring of the lesion was performed in 8 of 12 patients which included case 11 because the other control group patients refused to undergo EUS. During 6 months of observation by EUS, mural nodule in case 11 clearly increased and only case 21 but not other cases developed a new mural nodule (Fig. 3).

Adverse effects

Among ten patients who received sulindac, none complained symptoms or suffered from apparent adverse effect related to sulindac.

Images of three representative cases (cases 1, 8, and 11)

Figures 4, 5, and 6 show impressive changes in lesions as observed by imaging modalities in three patients during the observation period.

Figure 4 shows an enlarged orifice of the ampulla of Vater (a), filling defect in the main pancreatic duct (white arrow, b), the communication of the dilated branch duct to the main pancreatic duct (white bracket, b), and grape-like multilocular cysts (white bracket, c) in case 1, which were all typical imaging features of BD-IPMNs. In this case, there were two dilated branch ducts, with diameters of 56 and 24 mm, respectively (Fig. 4c), and a nodule was present in the small branch duct. The maximum branch duct diameter decreased from 56 mm (large white bracket, Fig. 4c) to 49 mm (large white bracket, Fig. 4d) by 6 months and to 46 mm (large white bracket, Fig. 4e) by 18 months after initiation of the drug administration. The mural nodule height in the smaller branch duct was also reduced from 5 mm (white arrowhead, Fig. 4f) to 3 mm (white arrowhead, Fig. 4g) by 6 months.

Another patient (case 8) in the treatment group with a striking reduction in lesion size is shown in Fig. 5. Before the treatment, viscous fluid oozing from the ampulla of Vater (Fig. 5a), a dilated branch duct communicating to the main pancreatic duct (white bracket, Fig. 5b), and a cystic lesion in the pancreas (white bracket, Fig. 5c) were evident. Upon treatment, the diameter of the branch duct had reduced from 17 mm (white bracket, Fig. 5c) to 11 mm (white bracket, Fig. 5d) by 6 months, and remained at 11 mm (white bracket, Fig. 5e) at the 18th month. The height of the mural nodule in this case was also dramatically reduced from 7 mm (white arrowhead, Fig. 5f) to 4 mm (white arrowhead, Fig. 5g) by the 6 month of observation.

In contrast, the lesion clearly grew in size in one patient (case 11), the control group subject who rejected surgery despite meeting the criteria of the IAP and was not eligible for treatment with sulindac because of a history of allergy to NSAIDs. Figure 6 shows an enlarged orifice of Vater

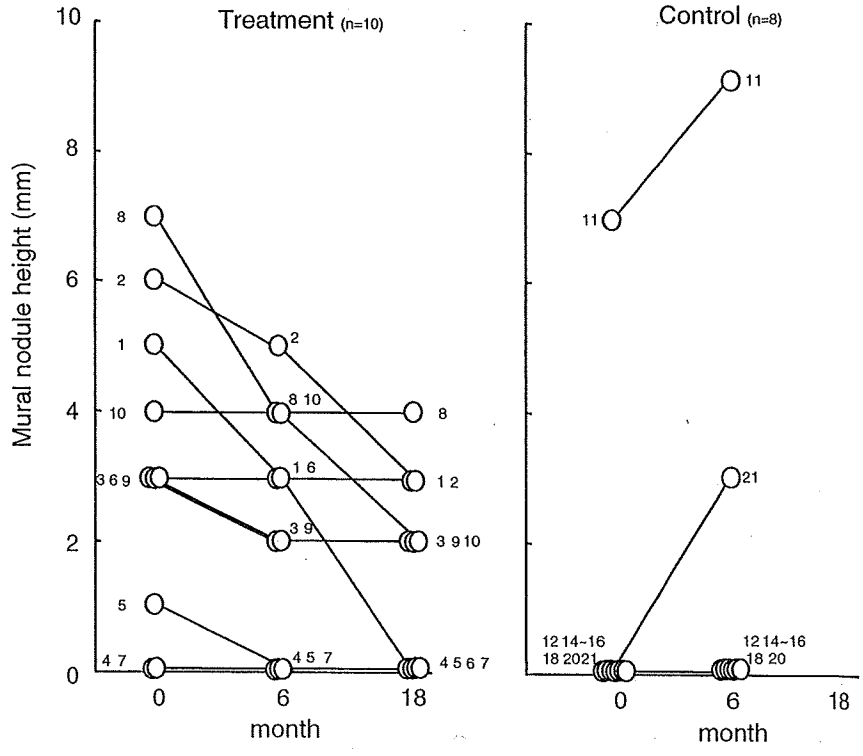

Fig. 3 Changes in mural nodule height during the observation period. Number at each point (*circles*) represents case number. Friedman's test was used. Significant changes were observed in the treatment group. ($P = 0.001$). Wilcoxon signed-rank test was performed. Significant changes were not observed in the control group ($P = 0.09$)

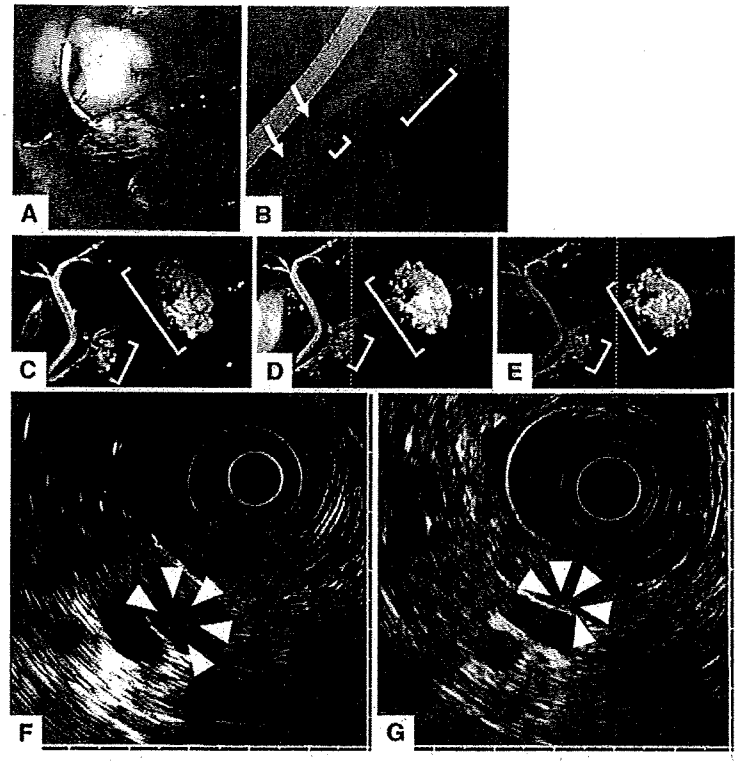

Fig. 4 Image findings in case 1. Duodenoscopic image of ampulla of Vater (**a**). ERP image of filling defect in moderately dilated main pancreatic duct and two dilated branch ducts (**b**). MRCP image of two dilated branch ducts and main pancreatic duct (**c** before drug administration, **d** 6 months after drug administration, **e**: 18 months after drug administration). EUS view of mural nodule in smaller dilated branch duct (**f** before drug administration, **g** 6 months after drug administration)

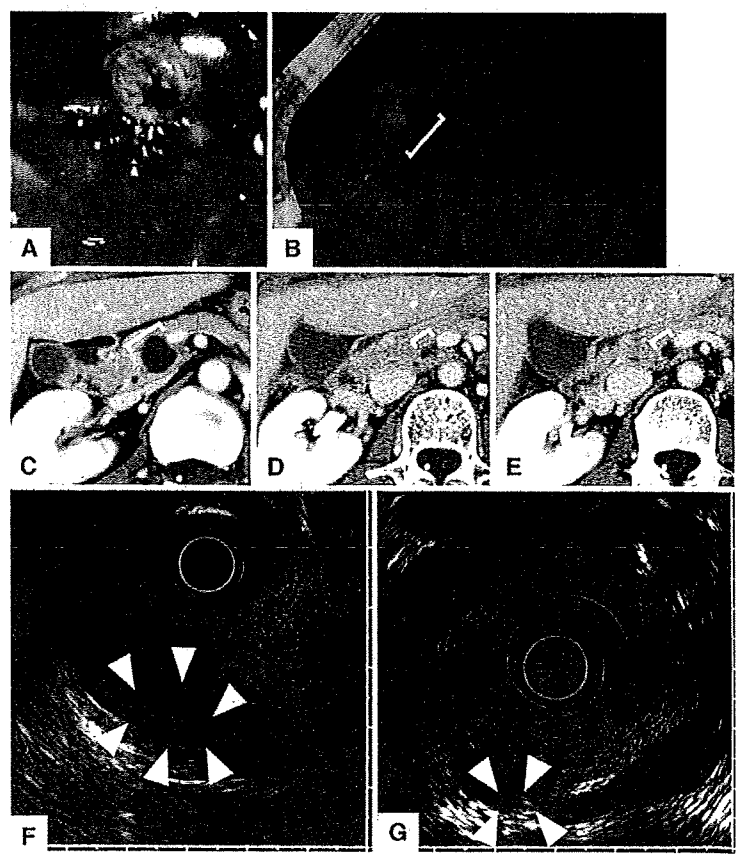

Fig. 5 Image findings in case 8. Duodenoscopic image of ampulla of Vater (**a**). ERP image of dilated branch duct communicating to main pancreatic duct (**b**). CT scans of cystic lesion of pancreas head (**c** before drug administration, **d** 6 months after drug administration, **e** 18 months after drug administration). EUS view of mural nodule in dilated branch duct (**f** before drug administration, **g** 6 months after drug administration)

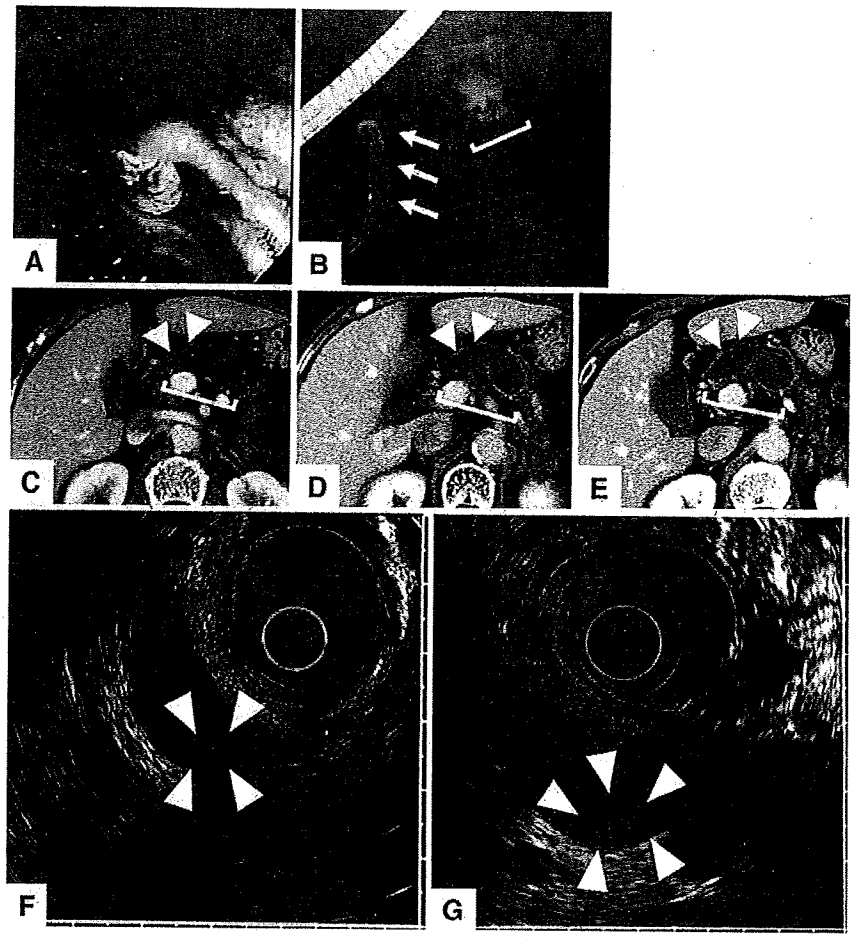

Fig. 6 Image findings in case 11. Duodenoscopic image of ampulla of Vater (**a**). ERP image of filling defect in moderately dilated main pancreatic duct and dilated branch duct (**b**). CT scans of multilocular cystic lesion of pancreas body (**c** before observation, **d** 6 months after observation, **e** 18 months after observation). EUS view of mural nodule in dilated branch duct (**f** before observation, **g** 6 months after observation)

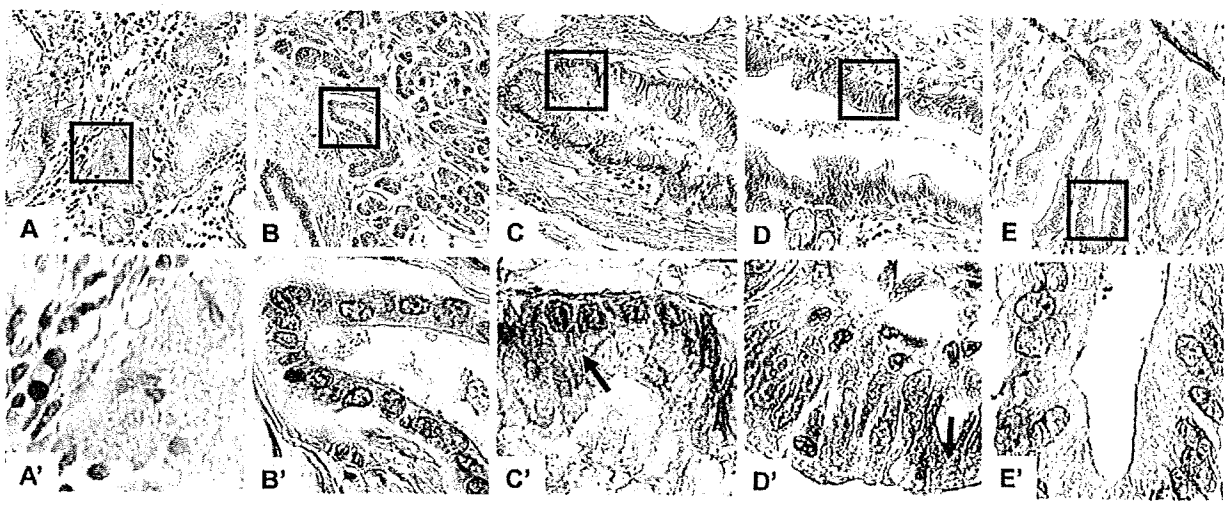

Fig. 7 Immunohistochemical staining for COX-1 in resected BD-IPMN specimens. Staining of gastric gland and mononuclear cell of gastritis mucosa for positive control (**a**). Representative specimens of normal epithelium (**b**), hyperplasia (**c**), adenoma (**d**), and carcinoma (**e**). High magnification images of the *boxed area* from **a** to **e** were shown in **a′**–**e′**. Normal epithelium showed no staining at all and was scored as 0. Perinuclear area of hyperplasia and adenoma slightly stained brownish (*arrows* in **c′** and **d′**), but because the staining was present only in a limited area, the score was 0. Carcinoma showed no staining at all and was scored as 0. ×200 in **a**–**e**, 1,000 × in **a′**–**e′**

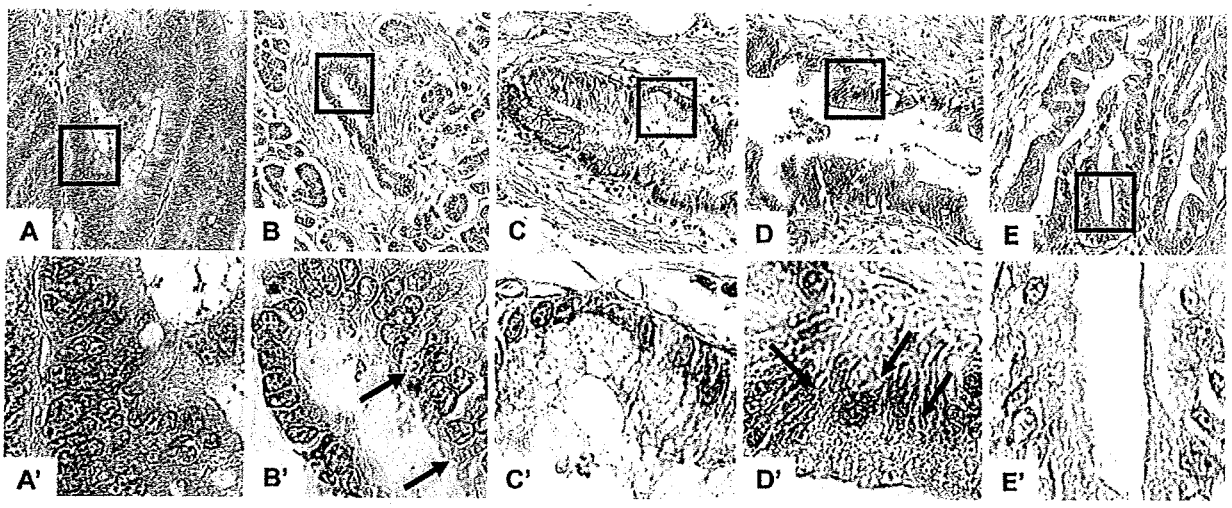

Fig. 8 Immunohistochemical staining for COX-2. Staining of colonic carcinoma tissue for positive control (**a**). Serial sections of BD-IPMN specimens in Fig. 7 (**b–e**). High magnification images of the *boxed area* from **a** to **e** were shown in **a′–e′**. The cytoplasm of normal epithelium stained light brownish (*arrows* in **b′**) only in a limited area and was scored as 0; hyperplasia did not stain at all and was scored as 0; up to half of the area of cytoplasm of adenoma stained light brownish (*arrows* in **d′**) and was scored 2; and carcinoma did not stain at all and was scored as 0. ×200 in **a–e**, ×1,000 in **a′–e′**

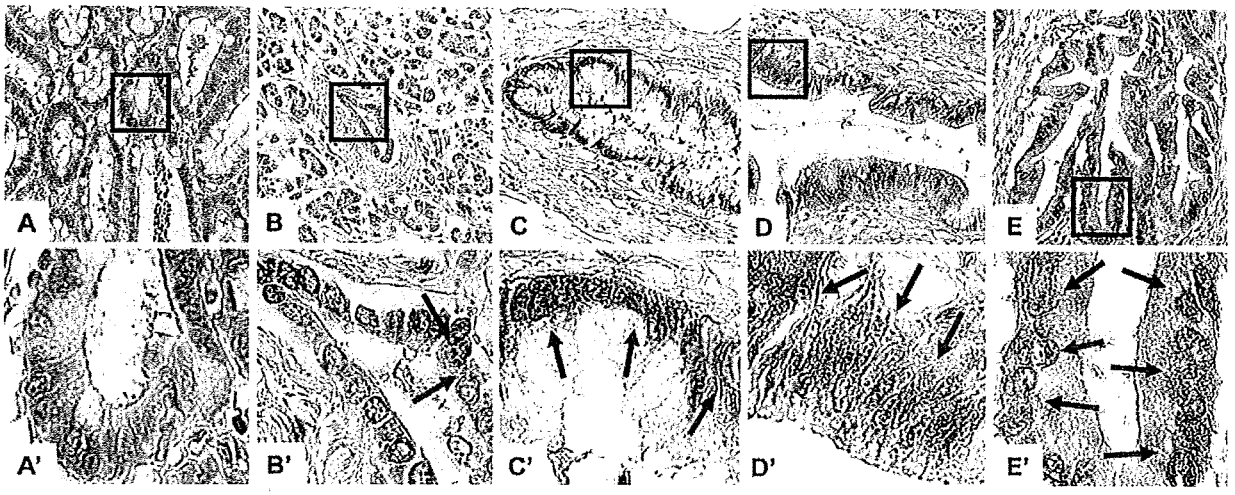

Fig. 9 Immunohistochemical staining for GST-π. Staining of colonic carcinoma tissue for positive control (**a**). Serial sections of BD-IPMN specimens in Fig. 7 (**b–e**). High magnification images of the *boxed area* from **a** to **e** were shown in **a′–e′**. The perinuclear area of normal epithelium stained light brownish (*arrows* in **b′**), but because the area of staining was limited, was scored 0; hyperplasia and adenoma were scored as 8 based on the finding that the nucleus and cytoplasm were both stained brown throughout the lesion (*arrows* in **c′** and **d′**); and carcinoma was scored as 3 because the staining intensity of nucleus and cytoplasm was low and the area showing light brown staining included more than half of the whole lesion (*arrows* in **e′**). ×200 in **a–e**, ×1,000 in **a′–e′**

(Fig. 6a), communication of the branch duct to the main pancreatic duct (white bracket, Fig. 6b), filling defect in the main pancreatic duct (white arrow, Fig. 6b), and a multilocular cyst (white bracket, Fig. 6c), consistent with imaging findings of BD-IPMNs. During the observation period, though the maximum branch duct diameter remained nearly stationary (white bracket, Fig. 6c–e), one of the branched cysts (white arrowhead, Fig. 6c–e) clearly increased in size. EUS revealed the growth of mural nodules from 7 mm (white arrowhead, Fig. 6f) to 9 mm (white arrowhead, in Fig. 6g) after 6 months.

Immunohistochemical staining of COX-1, COX-2 and GST-π in BD-IPMN tissues

In exploring the putative target molecule for sulindac, we performed immunohistochemical staining of COX-1, COX-2 and GST-π in BD-IPMN tissues from eight

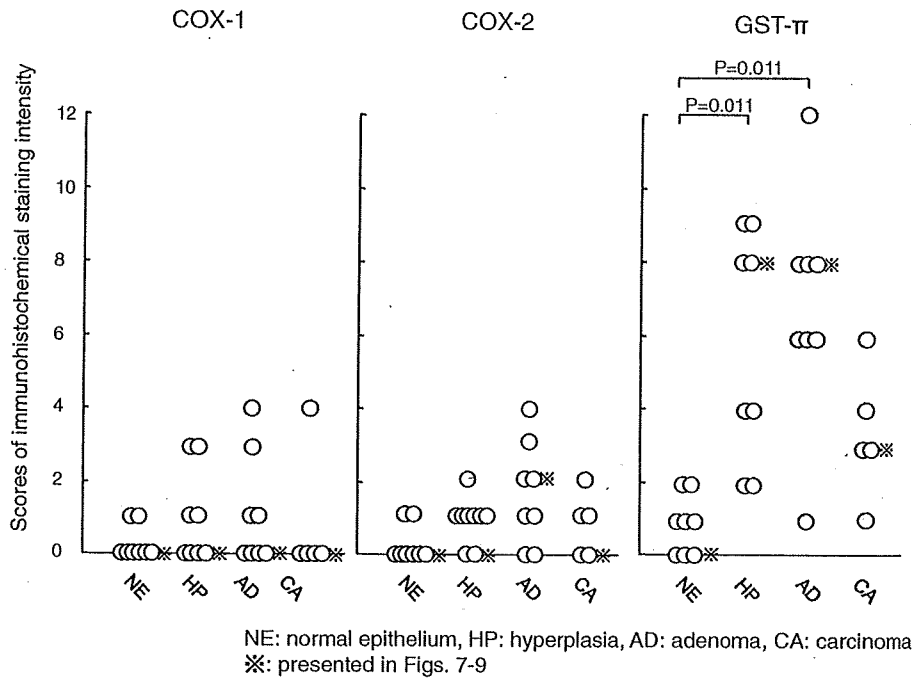

Fig. 10 Scores of immunohistochemical staining intensity for COX-1, COX-2 and GST-π in normal epithelium, hyperplasia, adenoma and carcinoma. Scoring was made according to the method of Gong et al

non-study patients who had undergone surgical resection of the pancreas because of malignant imaging signs. Of the eight resected tissues, portions of five consisted of carcinoma, adenoma and hyperplasia and portions three of adenoma and hyperplasia.

Typical immunohistochemical staining patterns for COX-1, COX-2 and GST-π in specimens from patients whose BD-IPMNs tissue was admixed with portions of carcinoma, adenoma and hyperplasia are shown in Figs. 7, 8, and 9, respectively. Staining of COX-1 (Fig. 7) and COX-2 (Fig. 8) was very faint, if at all, in specimens that included hyperplasia (c), adenoma (d) and carcinoma (e) with intensity similar to that in the normal epithelial portion (b). In contrast, staining for GST-π (Fig. 9) was evident in the specimen with hyperplasia (c), adenoma (d) and carcinoma (e) in contrast to the almost negative staining of the normal epithelium (b). When we quantified the staining intensity of all the specimens examined according to the method of Gong et al. [19] the scores of COX-1 and COX-2 in hyperplasia and adenoma were at most three with no difference compared to that of normal epithelium, while staining intensity of GST-π in hyperplasia and adenoma scored significantly higher compared to that of normal epithelium ($P = 0.011, P = 0.011$) (Fig. 10). Statistical comparison of scores for COX-1, COX-2 and GST-π in carcinoma to those of normal epithelium, hyperplasia or adenoma could not be made because carcinoma was seen in only five of the eight resected pancreases while other lesions and normal epithelial portions were present in all eight specimens.

Discussion

In forming the treatment group for the present study, for ethical reasons we assigned only those patients who rejected undergoing operation even though their lesions or symptoms met the criteria of the IAP for surgical resection. The control group was comprised of 10 patients whose lesion did not meet the IAP criteria for surgical resection and one patient (case 11) who had a history of asthma in response to NSAIDs, although her lesion met the operation criteria. Therefore, the study was neither randomized nor controlled. However, we believe that the comparison of changes between the groups was reasonable to examine the effect of sulindac since the branch duct diameter in the treatment group was generally larger than in the control group. Thus, the results showing that the branch duct diameter was significantly reduced after 18 months of treatment with sulindac compared to the change in diameter during the natural course in the control group suggested that chemoprevention held promise for treatment of BD-IPMNs.

However, when we compared the effect of the drug treatment on branch ducts with that on mural nodules in each case, we found a discrepancy. In cases 5, 6, and 9, the height of the mural nodules decreased while the branch duct diameter was unchanged during the course of treatment. The discrepancies between the mural nodule height and branch duct diameter in each case were also observed in the lesions prior to treatment (Table 1).

Those lesions having relatively large mural nodules, as in cases 1, 8 and 10, had relatively small branch ducts while lesions with no mural nodule (case 4) or with a very tiny nodule (case 5) had an obviously dilated branch duct. Such a discrepancy may be explained by the assumption that production rates of mucin are different in each mural nodule and that the draining rates of mucin to the main pancreatic duct from branch ducts are different in each case. A small mural nodule could produce a relatively large amount of mucin and the drainage of mucin from the branch duct of a lesion with a large mural nodule could be very rapid. Thus, the branch duct diameter does not necessarily reflect the mural nodule height. This is compatible with the previous notion that an increase in branch duct diameter does not necessarily indicate malignancy or tumor progression [20, 21]. In this context, data on mural nodule height are considered to be more reliable than those on branch duct diameter. Unfortunately, analysis of mural nodule height in the control group was performed by the Wilcoxon signed-rank test because data on some patients for 6 months (cases 13, 17, 19, and 22) and on all patients for 18 months were lacking. However, when we employed the Friedman test on the treatment group, the suppressive effect on nodule height was evident. Incidentally, in our study, one out of seven patients (14.3%) in control group who underwent EUS monitoring developed a new mural nodule. This ratio is apparently higher than that (4.9%) reported by Tanno et al. [20] who followed up 82 mural nodule free BD-IPMN cases for 45–148 months. This discrepancy may be due to the fact that number of our cases was relatively small as compared to that of Tanno's study.

The finding that the main pancreatic duct diameter was not affected by the treatment may be due to the fact that patients with dilated MPD (>6 mm), which is the sign for tumor invasion to the MPD, were not enrolled in this study. Nevertheless, collectively the present results on both branch duct diameter and mural nodule height of BD-IPMNs are indeed supportive of legitimate grounds for utilization of drugs to prevent carcinoma which may be derived from BD IPMNs. Further more, since it has been recently reported that BD-IPMNs are not infrequently associated with ductal carcinoma and that mucous cell hyperplasia harboring K-ras mutation which is one of the histological differentiation in the adenoma-carcinoma sequence of IPMNs might be a background for the development of ductal carcinoma [22], chemopreventive modality may also be effective on such ductal carcinoma. However, long term and larger scale follow up study should be established in future to conclude the feasibility of chemoprevention for both carcinoma derived from BD-IPMNs and ductal carcinoma.

Because we used omeprazole in addition to sulindac in the treatment group and not in the control group, we cannot single out sulindac as the sole drug causing the effect that we observed. However, previous reports have suggested that sulindac suppresses cell proliferation or induces cell apoptosis and that omeprazole rather exerts cytoprotective activity; therefore, we believe that sulindac is the principal drug that exerted the suppressive effect on the nodules [11, 12, 23].

The target molecule of sulindac is known to be COX-1 and COX-2 and hence we explored expression of those enzymes in IPMNs. To our surprise, however, unlike findings of a previous report [24], our study showed that neither COX-1 nor COX-2 was overexpressed in both the premalignant and malignant portions of BD-IPMNs. Instead, GST-π, a second class detoxification enzyme overexpressed in many precancerous lesion as well as in cancer tissue [25], was clearly stained in hyperplasia, adenoma and carcinoma associated with BD-IPMNs. Because we have recently found that GST-π knock-out mice rarely develop colonic cancer (unpublished observation) and that sulindac inhibited activity of GST-π in vitro [23], it is highly plausible that the target molecule of sulindac in IPMNs may also be GST-π. A future clinical trial using a GST-π-specific inhibitor may clarify the validity of this speculation.

In conclusion, results of the present study suggested the usefulness of chemoprevention of carcinoma derived from BD-IPMNs by sulindac, at least in those patients who refused surgery even though their lesions met the criteria for surgical resection. However, to confirm our present observation, a randomized controlled study with a larger number of patients is certainly needed.

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REVIEW

Chemoprevention of colorectal cancer -experimental and clinical aspects-

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Abstract : Colorectal cancer is a leading cause of cancer-related mortality worldwide. Therefore, an appropriate prevention strategy should be urgently established. Chemoprevention involves the use of oral agents to suppress the development of cancer. Recent progress in the molecular analysis of colorectal cancer has revealed many candidate molecules for chemoprevention. Many new agents targeting these molecules have also been developed. These agents are largely classified into three categories : 1) Signal transduction modulators including epidermal growth factor (EGF) receptor inhibitors, anti-vascular endothelial growth factor (VEGF) antibodies, and inhibitors of oncogene products. 2) Epigenetic modulators including peroxisome proliferative activated receptor (PPAR)-γ agonists, estrogen receptor (ER)-β, and histone deacetylase inhibitors. 3) Anti-inflammatory modulators including cyclooxygenase (COX)-2, EP 1-4, and NF-kB. Of these agents, some actually proceeded to human clinical trials, and have been shown to be active chemopreventive agents. J. Med. Invest. 56 : 1-5, February, 2009

Keywords **:** *colorectal cancer, chemoprevention, aberrant crypt foci*

INTRODUCTION

Colorectal cancer is a disease with a high incidence and mortality rate, and has been increasing in prevalence worldwide (1). Therefore, various prevention strategies have been investigated. Primary prevention attempts to prevent the occurrence of colorectal cancer by lifestyle modification, and secondary prevention aims to arrest the progression of colorectal cancer through early diagnosis and treatment. In addition to these, recently, chemoprevention, the use of oral drugs to prevent cancer, has attracted much attention. Many compounds have been tested to assess their inhibition of colorectal carcinogenesis in animal models, and some of them have been proceeded to clinical trials for chemoprevention.

Recent progress in the molecular analysis of colorectal carcinogenesis has revealed many candidate molecules for chemopreventive agents. In this review, we summarize new findings regarding experimental data and clinical trials for the chemoprevention of colorectal cancer.

ANIMAL MODEL OF COLORECTAL CANCER

It is very important to use an animal model for the evaluation of chemopreventive agents against colorectal carcinogenesis. There are two kinds of rodent model for colorectal cancer. One is the model of chemical carcinogenesis employing carcinogens such as azoxymethane, 1, 2-dimethylhydrazine (DMH), N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG), etc. Of these, the azoxymethane model is

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the most widely used as a model of sporadic colorectal carcinogenesis, and is reportedly very similar to human colorectal cancer in terms of the clinical symptoms, clinical course, and pathological findings (2). The other one is the genetic model harboring gene mutations such as APC, p53, etc. The Min mouse and Apc delta716 knockout mouse, both of which have APC mutations, are also used worldwide (3, 4).

In 1987, Bird reported a tiny lesion consisting of large, thick crypts in a methylene blue-stained specimen of the colon from mice treated with azoxymethane, and suggested to be a precursor lesion of colorectal cancer in the animal model (5). Then, abundant evidence was reported to support that aberrant crypt foci (ACF) are a precursor lesion of colorectal cancer. Thus, ACF are often used as a target lesion to test chemopreventive effects in animal models of colorectal carcinogenesis.

CHEMOPREVENTIVE AGENTS AND TARGET MOLECULES

Recent progress in the molecular analysis of colorectal cancer has made it possible to target a specific molecule for chemoprevention (6). Many promising target molecules have been reported so far (Table 1). These can be mainly classified into 3 categories based on the mechanism : 1) signal transduction modulation, 2) epigenetic modulation, and 3) anti-inflammatory modulation.

1) Signal transduction modulator

The signal transduction pathway has been searched for a long time as a target of chemotherapy and chemoprevention. EGF receptor inhibitors (Erlotinib, etc.), anti-EGF receptor antibody (Cetuximab), and anti-VEGF antibody (Bevacizumab) are well-known as therapeutic agents for cancer and commonly used worldwide (7). Although these agents have not yet been applied to chemoprevention, they themselves or their analogues may be put to practical use as chemopreventive agents of colorectal cancer in the future. Since mutations of K-ras and p53 are frequently observed in colorectal cancer, their oncogenic pathway is a possible target. Anti-ras agents such as Tipifarnib and perilyl alcohol, and anti-p53 agents such as CP31398 have been reported to inhibit colorectal carcinogenesis in animal models (8). Other signal transduction modulators targeting Bcl-2, ODC, GST-pi, etc., have also been examined for their chemopreventive effect on colorectal cancer.

2) Epigenetic modulation

It is well known that peroxisome proliferator-activated receptor (PPAR)-γ and -δ play a role in the

Table 1 Candidate of chemopreventive agents and target molecules for colorectal cancer

Mechanism	Target	Agents
Signal transduction modulation	EGF receptor	Cetuximab, Erlotinib
	Bcl-2	ABT-737
	Ras	Tipifarnib, Perillyl alcohol
	p53	CP31398
	Matrixmetalloproteinases	Marimistat, Prinomastat
	ODC	DFMO, NSAIDs, Retinoids
	VEGF/VEGF receptor	Bevacizumab
	GST-pi	HGBP, TLK119
Epigenetic modulation	Peroxisome proliferator activated receptor(PPAR)	Rosiglitazone, Pioglitazone
	Vitamin D	Vitamin D3 analogue
	ER-β	Resveratorol, TAS-108
	Histone deacetylase	SAHA
	Retinoic acid receptor	Retinoids
Anti-inflammation	COX-2	NSAIDs, Celecoxib, Etodorac
	EP1-4	ONO-8711
	NF-κB	Bortezomib, Curcumin, Tea polyphenols, Statins, NSAIDs

process of colorectal carcinogensis. Of these, PPAR-γ agonists such as rosiglitazone and pioglitazone reportedly inhibit the formation of colorectal cancer in animal models (9). Currently, they are being tested in human trials. There are some studies in which vitamin D inhibited the development of colorectal adenoma and cancer. Other epigenetic modulators including ER-β, histone deacetylase, and retinoic acid receptor have been reported to be potential chemopreventive agents in animal models.

3) Anti-inflammatory modulation

Cyclooxygenase-2 (COX-2) is reportedly overexpressed in colorectal adenoma and cancer of rodents and humans. It is also reported that COX-2 promotes the cell growth and inhibits apoptosis of colorectal epithelia. When an Apc delta716 knockout mouse, a model of human familial adenomatous polyposis, was crossed with a COX-2 knockout mouse, the number and size of intestinal polyps were markedly reduced (10). Moreover, there are many studies showing that selective COX-2 inhibitors suppressed colorectal adenoma and cancer. Thus, the efficacy of targeting the COX-2 molecule for chemoprevention was theoretically confirmed in animal models. There are also many other anti-inflammatory agents including EP1-4 and NF-κB currently under investigation.

CLINICAL TRIAL FOR CHEMOPREVENTION

Representative human chemopreventive trials are shown in Table 2. They are mainly classified into 3 categories according to the target lesion. The first one is a trial that targets a pre-existing polyp. Giardiello, *et al.* reported that sulindac significantly suppressed the number and size of polyps in familial adenomatous polyposis patients in 1993 (11). This study prompted investigators to conduct a trial to examine whether or not sulindac suppresses sporadic polyps. However, it did not significantly suppress the number or size of the polyps (12). This trial revealed that a pre-existing polyp is not necessarily an appropriate target for chemoprevention; a large polyp close to a cancer may not be able to respond to chemopreventive agents. Thus, chemoprevention targeting the development of a new polyp in polypectomized patients was conducted thereafter. Several randomized trials showed that aspirin inhibited the development of polyps. Since COX-2 was shown to be a good target molecule for chemoprevention in animal experiments, as noted above, two large-scale randomized clinical trials using a selective COX-2 selective inhibitor (celecoxib) were performed. Arber, *et al.* reported that celecoxib (400 and 800 mg/day) significantly reduced the new development of

Table 2 Representative chemopreventive studies for colorectal cancer

	Sporadic/FAP	Agents	Period	Results	Author
Pre-existing polyp					
	FAP	Sulindac	4 yr	No change	Giardiello, *et al.* (2002)
	FAP	Celecoxib	6 mo	30% reduction	Steinbach, *et al.* (2000)
	Sporadic	Sulindac	4 mo	No change	Ladenheim, *et al.* (1995)
	FAP	Sulindac	9 mo	65% reduction	Giardiello, *et al.* (1993)
Development of new polyp					
	Sporadic	Celecoxib	3 yr	38% reduction	Bertagnolli, *et al.* (2006)
	Sporadic	Celecoxib	3 yr	35% reduction	Arber, *et al.* (2006)
	Sporadic	Aspirin	1 yr	37% reduction	Sandler, *et al.* (2003)
	Sporadic	Aspirin	1~3 yr	17% reduction	Baron, *et al.* (2003)
	Sporadic	Calcium	4 yr	15% reduction	Baron, *et al.* (1999)
Development of cancer					
	Sporadic	Vitamin D Calcium	6 yr	32% reduction No change	Martinez, *et al.* (1996)
	Sporadic	Vitamin D Calcium	4 yr	26% reduction No change	Bostick, *et al.* (1993)
	Sporadic	Folic acid	6 yr	31% reduction	Giovannucci, *et al.* (1993)

adenoma compared to a placebo group (13). Bertagnolli, *et al.* also reported that celecoxib (400 and 800 mg/day) significantly reduced the development of adenoma in a different large-scale trial (14). However, in these trials, severe cardiovascular events including myocardial infarction and stroke occurred in about 20% of cases. Therefore, it is considered that the COX-2 inhibitor is an effective agent for the prevention of colorectal cancer, but it cannot be recommended for chemoprevention because of potential cardiovascular events.

The third one is a trial that targets the development of cancer. This kind of trial is theoretically ideal because it examines if each agent indeed suppresses the development of cancer itself. However, it takes more than 4 years, and prolongation of the trial sometimes causes severe side effects and poor compliance.

CHEMOPREVENTION TARGETING ACF

Since ACF are the earliest precursor lesions of colorectal cancer (15, 16), they would be an appropriate target for chemoprevention (Fig. 1). The advantages of using ACF as targets over a polyp and cancer are as follows : (1) short-term treatment for evaluation, (2) fewer complications caused by drugs, and (3) good compliance. Thus, we performed an open trial in which sulindac was administered for various periods to subjects positive for ACF. The results showed that the majority of ACF were eradicated after only a few months. Based on this, we next performed a randomized double-blind trial targeting ACF consisting of groups receiving sulindac, etodolac (a selective COX-2 inhibitor), or a placebo. The detailed results of this study will be clarified in the near future.

EPILOGUE

Many candidate agents for chemoprevention are currently being tested, and some of them have actually shown potential chemopreventive activity in human trials. Although the COX-2 inhibitor failed to be a major chemopreventive agent, other effective new agents will be identified in the near future.

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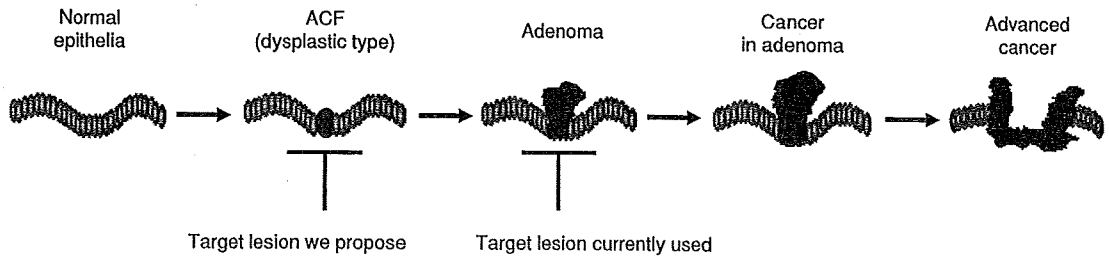

Figure 1 Colorectal carcinogenesis and target lesions for chemoprevention. In the majority of chemopreventive studies performed so far, adenoma has been used as a target lesion for evaluation. We propose the use of aberrant crypt foci (ACF), an earlier lesion, as a target. This makes it possible to evaluate the effect of a chemoprevenitve agent within a shorter period.

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Journal of Gastroenterology

Case report

Familial adenomatous polyposis complicated by chronic myelogenous leukemia: response to imatinib mesylate

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Familial adenomatous polyposis (FAP) is an autosomal dominant disorder characterized by colonic polyposis and a predisposition for developing colorectal cancer. FAP is frequently complicated by extracolonic disease, but complications of leukemia are rare. We present the first case of FAP complicated by chronic myelogenous leukemia (CML) in a 38-year-old man. The patient had numerous adenomas in the colorectum and a family history compatible with FAP. He was diagnosed as having FAP in February 2000. Two years after the diagnosis, he developed leukocytosis with the Philadelphia chromosome abnormality, indicating complication with CML. Imatinib mesylate was administered for the treatment of CML, and hematologic and cytogenetic remission of CML was achieved in 6 months. Numerous polyps, 2 to 3 mm in diameter, observed in the rectum prior to the administration of imatinib, regressed in size, but not in number, after 1 year of treatment with imatinib. Eighteen months later, however, the polyps were enlarged. In this patient, imatinib administration led to the remission of CML and might also have been responsible for the temporary regression of adenomatous polyps of FAP.

Key words: familial adenomatous polyposis, chronic myelogenous leukemia, imatinib, regression

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Reprint requests to: Y. Fujimori

Introduction

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder in which precancerous polyps grow in the colorectum. Left untreated, virtually all patients with FAP develop colon cancer in early adulthood.[1] Mutations of the adenomatous polyposis coli (*APC*) gene are thought to be responsible for the development of FAP.[2] Chronic myeloid leukemia (CML) manifests primarily as an increase in white blood cells (WBC) and is characterized by the Philadelphia chromosome translocation t(9;22)(q34;q11) resulting in the formation of the *BCR/ABL* fusion gene. Products of the *BCR/ABL* fusion gene are responsible for the development of CML.[3] Imatinib mesylate was designed to inhibit BCR/ABL tyrosine kinase of CML,[4] and the administration of imatinib effectively induces the remission of CML.[5] Imatinib mesylate is also effective against gastrointestinal stromal tumors (GIST).[6] Recently, imatinib was clinically tested for the treatment of advanced colorectal cancer (http://clinicaltrials.gov/ct/gui/show/NCT00041340?order=16) and adenomatous polyps of FAP (http://www.hereditarycc.org/cgi-bin/read.pl?i=199). We report a case with FAP complicated by CML. We administered imatinib mesylate to treat CML, and observed temporary regression of the adenomatous polyps of FAP during the administration of imatinib.

Case report

A 38-year-old man underwent screening for colon cancer after testing positive for occult blood in the stool in

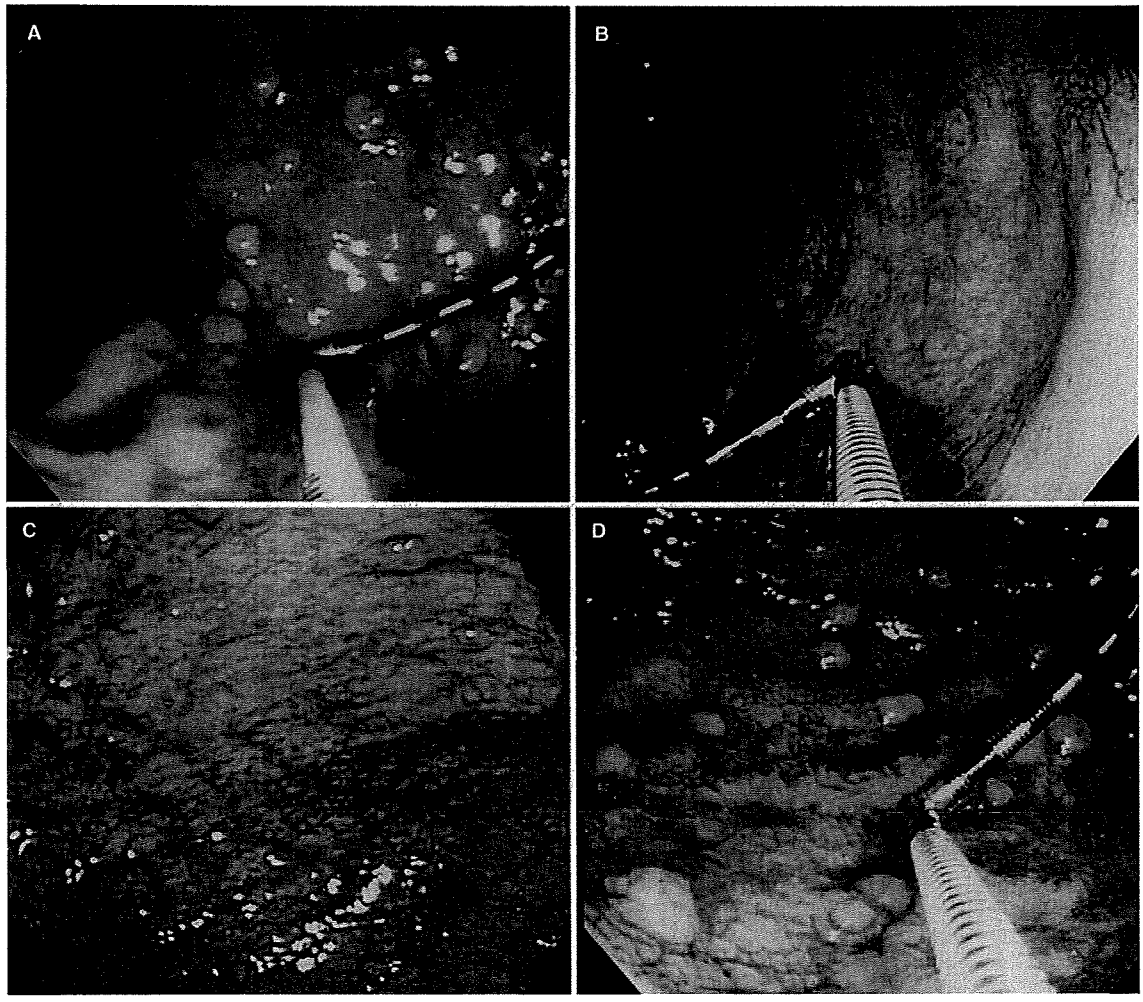

Fig. 1A–D. Endoscopic examination of the rectum. A polyp was marked to identify the same place in the rectum. *Hatched bar* on the wire indicates 2-mm intervals. **A** July 2002 (prior to the administration of imatinib); **B** and **C** after the administration of imatinib for 1 year (July 2003); **D** after administration of imatinib for 12 to 18 months (January 2004)

April 1999. Double-contrast barium enema and colonoscopy revealed numerous polyps in the colorectum. The patient was referred to the Osaka Medical Center for Cancer and Cardiovascular Disease in February 2000. His father had colorectal polyposis and had died of rectal cancer at the age of 36. Two of his father's brothers and his grandfather also had colorectal cancer. A germline mutation of the *APC* gene was not detected in this patient by a protein truncation test, which detects mutation in approximately 80% of FAP patients.[7] On the basis of the presence of colon polyposis and an autosomal dominant family history, the patient was diagnosed with FAP. He underwent prophylactic subtotal colectomy with ileorectal anastomosis in October 2000. He was under intensive colonoscopic surveillance semiannually, and polyps larger than 7 mm in diameter were removed. No colorectal cancer was detected in the removed tissues. In January 2002, his WBC count and serum lactic dehydrogenase levels increased. He was referred to the Division of Hematology-Oncology, Department of Internal Medicine, at the Hyogo College of Medicine in April 2002. Laboratory studies revealed a red blood cell count of 5×10^9/l, hemoglobin 15.7 g/d1, and a WBC count of 48.1×10^6/l, and platelet count of

222×10^6/l. Neutrophil alkaline phosphatase (NAP) activity was low (NAP rate 38%, score 82). A myelogram revealed hypercellular (nucleated cell count 987×10^6/l) and granulocyte-predominant marrow. Chromosomal analysis of bone marrow cells revealed 46XY, t(9;22)(q34;q11) in all 20 metaphases, which is known as the Philadelphia chromosome (Ph) and the critical genetic abnormality of CML. The BCR/ABL fusion transcript, which is generated as the molecular consequence of the Philadelphia chromosome, was present in 56% of bone marrow cells as detected by fluorescent in situ hybridization analysis. The patient was diagnosed with Ph(+) CML in the chronic phase. The patient was given imatinib mesylate 400 mg/day from July 2002. Owing to adverse reactions, including nausea and vomiting, the dose was reduced to 300 mg/day after 1 week. The patient achieved hematologic remission in 2 weeks based on blood count, and attained a complete cytogenetic response after 9 months of imatinib administration based on chromosome and fluorescent in situ hybridization analyses. A colonoscopy in July 2002, prior to the administration of imatinib (Fig. 1A), revealed a number of polyps 2 to 3 mm in diameter in the rectum. After 1 year of administration of imatinib (July 2003) (Fig. 1B and C), the adenomatous polyps showed significant regression in size, but not in number. Eighteen months after the beginning of imatinib administration (January 2004), the adenomatous polyps were again enlarged (Fig. 1D).

Discussion

We report a patient with FAP complicated by CML. Several cases of FAP complicated with leukemia have been reported, but the leukemia is usually the acute type.[8] To our knowledge, this is the first report of a FAP patient complicated by CML. Critical genetic changes of CML in the chronic phase are located on chromosomes 9 and 22,[3] and the *APC* gene of FAP is located on chromosome 5q;[2] thus, there seems no obvious genetic correlation between these diseases. It is possible that these two disorders occurred coincidentally at the same time in this patient.

The product of the *BCR/ABL* gene, which has tyrosine kinase activity, is constitutively produced in patients with Ph(+) CML.[3] Imatinib is a drug designed to interact with the ATP-binding site of the enzyme to inhibit intracellular signal transduction leading to apoptosis of tumor cells.[4] When administered to patients with Ph(+) CML, imatinib decreases the incidence of Ph(+) cells and *BCR/ABL* hybrid genes.[5] Furthermore, imatinib specifically inhibits the signal transduction of tyrosine kinases of c-Kit and platelet-derived growth factor receptors (PDGFR).[9] Recent reports revealed the efficacy of imatinib administration against GIST, which constitutively expresses c-Kit.[6] The present case of FAP was complicated by Ph(+) CML after prophylactic colectomy, while the patient was under careful follow-up. After administration of imatinib, the patient achieved a cytogenetically complete remission and a major molecular response of CML. Spontaneous regression of polyps of FAP is quite rare, and therefore prophylactic colectomy is recommended for the management of FAP.[1] Drugs that induce regression of polyps of FAP are limited to nonsteroidal anti-inflammatory drugs or, in a broad sense, cyclo-oxygenase-2 inhibitors.[10] As the patient did not take such drugs, imatinib mesylate was assumed to be responsible for the polyp regression. Thus, the temporary regression of colorectal adenomatous polyps might have been related to the administration of imatinib mesylate in our patient. Immunohistochemical analysis revealed no c-Kit expression in the adenomatous polyps (data not shown). Preliminary immunohistochemical analysis using currently available anti-PDGFRA antibody did not provide conclusive information on the expression of PDGFRA in adenomatous polyps. This issue and a mutation of the *PDGFR* gene in adenomatous polyps should be further analyzed to clarify the relationship between imatinib administration and polyps regression. Currently, this is the only case in which imatinib was administered to a FAP patient. Studies are currently under way in the United States to test imatinib for the treatment of colorectal cancer or FAP (http://clinicaltrials.gov/ct/gui/show/NCT00041340?order=16, http://www.hereditarycc.org/cgi-bin/read.pl?i=199). The present case might indicate the limited efficacy of imatinib for the regression of adenomatous polyps.

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Journal of Gastroenterology

Chemoprevention of colorectal cancer in Japan: a brief introduction to current clinical trials

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The rapidly increasing incidence of colorectal cancer in Japan poses a great challenge to researchers to develop preventive strategies against this disease. Thus far, several clinical trials for this purpose have been planned in Japanese subjects; some have been completed and documented while others are still ongoing. Also, the Ministry of Health, Labour and Welfare of Japan recognizes the significance of cancer prevention studies, especially against colorectal cancer, including it as one of the pillars in the "Third Research Project on General Strategies against Cancer" and funding several large-scale projects. Among them are two chemoprevention studies currently being performed: in patients with previous sporadic colorectal tumors (J-CAPP study) and in patients with familial adenomatous polyposis (J-FAPP study II). Both are double-blind randomized controlled trials with low-dose aspirin (100 mg/day), which is generally considered to be safe for long-term use. This article outlines relevant past clinical data and gives a brief introduction to these two studies.

Key words: colorectal cancer, chemoprevention, aspirin, clinical trial

Introduction

Gastric cancer used to be the most common type of cancer in Japanese. Its position is, however, rapidly being replaced by colorectal cancer. According to the 2003 statistics, colorectal cancer has become the leading cause of death in overall cancer mortality in Japanese women. With this background, studies on colorectal cancer prevention are now being performed actively in Japan. Among other strategies, cancer prevention by drugs, i.e., chemoprevention, is expected to have great potential for clinical application.

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Candidate substances and target populations

Candidate substances expected to prevent colorectal cancer are shown in Table 1.

Based on recent genetic and epigenetic analyses of colorectal cancer,[1,2] many substances are expected to be effective and have been examined. In particular, nonsteroidal antiinflammatory drugs (NSAIDs) have attracted attention and have been studied worldwide. However, none of the NSAIDs has yet been sufficiently proven to be effective for clinical application.

As for target populations, most clinical studies on chemoprevention have been conducted in high-risk groups for colorectal cancer, including patients with a previous sporadic colorectal tumor (adenoma or cancer) after endoscopic resection, familial adenomatous polyposis (FAP), and hereditary nonpolyposis colorectal cancer (HNPCC).

On the other hand, research efforts for cancer prevention in an average-risk group have focused on lifestyle modification including diet, physical exercise, smoking, and drinking.

Chemoprevention for patients with previous sporadic colorectal tumor

Background and past studies

In 2003, Baron et al.[3] reported a 3-year intervention trial in 1121 subjects with a history of colorectal adenoma. The subjects were given placebo or aspirin (81 or 325 mg/day). Although there was no difference in the development of adenoma between the groups, a significant decrease in relative risk (0.59) of advanced lesions

Table 1. Candidate substances for chemoprevention of colorectal cancer

I. NSAIDs	IV. *Dietary fiber*	VII. *Other food components*
Aspirin	Hemicellulose	S-Allylcysteine
Sulindac	Pectin	Fucoidan
Sulindac sulfone	Resistant starch	Curcumin
Indomethacin	Oligosaccharide	Epigallocatechin
Piroxicam	V. *Metals and related substances*	Lactoferrin
Celecoxib	Selenium	Chitin/chitosan
II. Vitamins	Calcium	IX. *Drugs for other diseases*
Folic acid	Phytic acid	Pioglitazone
Vitamin C	VI. *Polyunsaturated fatty acids*	Glivec
Vitamin D	Docosahexanoic acid	Statins
Vitamin E	α-Linolenic acid	α-Glucosidase inhibitor
III. 5-Aminosalycylic acid	VII. *Carotenoid*	5-Fluorouracil
Salazosulfapyridine	α-Carotene /β-Carotene	Lactic acid bacteria
5-Aminosalicylic acid	Lycopene	Ursodeoxicholic acid
		Estrogen

NSAIDs, nonsteroidal antiinflammatory drugs

was observed in the group receiving 81 mg/day aspirin. This finding was, however, not dose dependent, as the relative risk in the group receiving 325 mg/day was not considerably decreased.

At the same time, the results of another clinical trial by the same group of researchers were reported.[4] The subjects, patients with previous colorectal cancer resected surgically, received placebo or aspirin at a dose of 325 mg/day. After 12.8 months, the median intervention period, newly developed adenomas were detected in 27% of the placebo group and in 17% of the aspirin group, which rate was significantly lower in the latter group. With regard to the cumulative incidence, there was an increasing difference between the two groups during the first year, and then the two increasing curves became parallel. Therefore, the effect of long-term aspirin administration remained unclear.

Several large-scale clinical studies including the aforementioned trials have been conducted but failed to provide convincing evidence of the efficacy of aspirin. Based on these results, preventive use of aspirin or other NSAIDs against colorectal cancer in clinical settings is currently not recommended in the United States.[5]

In Japan, Takayama et al.[6] studied the effect of sulindac, another NSAID, in patients with a sporadic colorectal tumor, and reported a decrease in the number of aberrant crypt foci (ACF) in the group receiving sulindac. As for aspirin, however, no large-scale clinical trial has ever been performed in Japan.

Asians, with their generally smaller physique, may differ from Western people in their metabolism of aspirin. Therefore, clinical trials of aspirin in Japanese persons might provide different outcomes than past clinical data obtained from Americans. Based on such an expectation, planning of a multicenter research project in Japan has been initiated.

J-CAPP study

We have designed a clinical trial called the "Japan Colorectal Aspirin Polyps Prevention (J-CAPP) Study" under study director Prof. Shinkan Tokutome of Nagoya City University Medical School. The Ministry of Health, Labour and Welfare of Japan has funded this project within the framework of the Third Research Project on General Strategy against Cancer, Basic and Clinical Research on the Development of Chemopreventive Drugs (Team Leader: Dr. Wakabayashi, National Cancer Center).

The study is a double-blind trial using aspirin enteric-coated tablets (100 mg, one tablet daily) and placebo imported from Bayer HealthCare, Germany. Thirty-one tablets of the investigational drug, dosage for 1 month, were packed in one PTP sheet with a calendar printed on it (Fig. 1). This PTP sheet was coated on both sides with a waterproof aluminum layer. One box containing 30 sheets, for 30 months, was prepared for each subject.

Our study statistician Dr Suzuki was responsible for coding and sealing of boxes containing either placebo or aspirin by using a table of random digits. Then, a specially designed website for this study was set up to enable real-time random allocation by the minimization method.

Our research organization consists of specialists in colonoscopy across the country, and a study statistician as the controller. An ethics monitoring committee was also established. The functions of the data center were commissioned to Medical Research Support. Experts in colonic diseases from 23 institutions participated in this project (Table 2).

The subjects are men and women aged between 40 and 70 years, with a previous colorectal tumor, including early cancer and adenoma. All tumors should have

Table 2. Collaborators and number of cases allocated

Name	Affiliation	No. of cases
Hideki Ishikawa	Osaka Central Hospital	200
Tetsuji Takayama	Sapporo Medical Univ. School of Medicine	60
Takashi Abe	Osaka Police Hospital	36
Motowo Mizuno	Hiroshima City Hospital	30
Shozo Okamura	Toyohashi Municipal Hospital	40
Konishi Naomi	Mie Prefectural General Medical Center	5
Masato Kusunoki	Mie Univ. Graduate School of Medicine	5
Yoshihisa Saida	Toho Univ. School of Medicine	40
Masahiro Tajika	Aichi Cancer Center Hospital	40
Shin-ein Kudo	Northern Yokohama Hospital Showa Univ.	30
Keiji Hirata	Univ. of Occupational and Environmental Health	18
Shinji Tanaka	Hiroshika Univ. Hospital	30
Gondo Nobuhisa	Kimura Hospital	40
Makoto Yamamura	Kobe Ekisaikai Hospital	10
Masaki Iimuro	Higashisumiyoshi Morimoto Hospital	40
Kyowon Lee	Moriguchi Keijinkai Hospital	10
Heita Ozawa	Kitasato Univ. School of Medicine	10
Takashi Joh	Nagoya City Univ. Hospital	20
Shinji Kitamura	Sakai Municipal Hospital	30
Masahiko Tsujii	Osaka Univ. Graduate School of Medicine	10
Kenji Sugimoto	Sugimoto Kenji Clinic	20
Yasushi Sano	Sano Hospital	40
Takahisa Matsuda	National Cancer Center Hospital	40

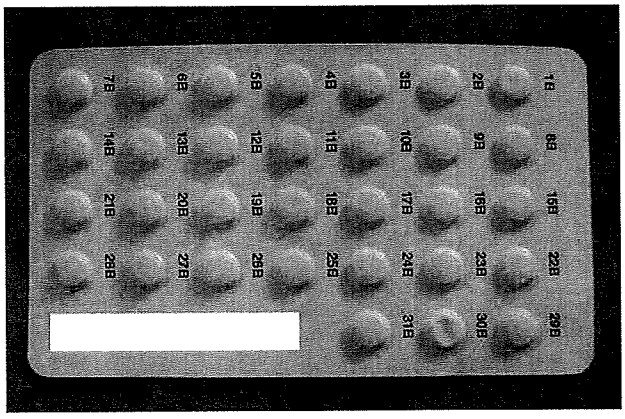

Fig. 1. Thirty-one tablets of investigational drug, for 1 month, were packed in one PTP sheet with a calendar printed on it. This PTP is coated on both sides with an aluminum layer, so it is highly waterproof. One box containing 30 sheets, for 30 months, is prepared for each subject

been resected endoscopically. The target sample size is 700, and the number of cases to be analyzed is 500. Current users of antithrombotic agents such as Bayaspirin and patients who have undergone colectomy and those with FAP or HNPCC are excluded. The duration of intervention is 2 years, followed by another 2 to 3 years of follow-up. The primary endpoint is the presence or absence of new colorectal tumors. The secondary endpoints include the number, size, and dysplasia of newly developed tumors, and frequency of adverse events.

We started registering participants in January 2007. By March 28, 2008, the 13th month of registration, 351 patients had been approached and 283 of them (81%) had consented to participate. The project is now proceeding smoothly.

Chemoprevention for patients with familial adenomatous polyposis

Background and past studies

Familial adenomatous polyposis (FAP) is an autosomal dominant inherited disease characterized by the development of numerous colorectal adenomas. As a causal gene, the *APC* gene has been identified. There are estimated to be 5000–7000 patients in Japan. Persons with this constitution are at high risk of developing colorectal cancer, which may even start in their twenties. By the age of 40, 50% of them are considered to be affected; most patients will be affected. Colectomy is generally indicated in patients diagnosed with FAP to prevent colorectal cancer. However, this procedure is associated with frequent diarrhea, resulting in a significant decrease in patients' quality of life. There is thus a strong need for alternative measures to preventive colectomy, and three options are currently being