The Effect of Metformin in Treatment of Adenomas in Patients with Familial Adenomatous Polyposis

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ABSTRACT

Familial adenomatous polyposis (FAP) is a hereditary disease characterized by the development of numerous colorectal adenomas in young adults. Metformin, an oral diabetic drug, has been shown to have antineoplastic effects and a favorable safety profile. We performed a randomized, double-blind, controlled trial to evaluate the efficacy of metformin on the regression of colorectal and duodenal adenoma in patients with FAP. Thirty-four FAP patients were randomly assigned in a 1:2:2 ratio to receive placebo, 500 mg metformin, or 1,500 mg metformin per day orally for 7 months. The number and size of polyps and the global polyp burden were evaluated before and after the intervention. This study was terminated early based on the results of the interim analysis. No significant differences were determined in the percentage change of colorectal and duodenal polyp number over the course of treatment among the three treatment arms ($P = 0.627$ and $P = 1.000$, respectively). We found no significant differences in the percentage change of colorectal or duodenal polyp size among the three groups ($P = 0.214$ and $P = 0.803$, respectively). The overall polyp burdens of the colorectum and duodenum were not significantly changed by metformin treatment at either dosage. Colon polyps removed from the metformin-treated patients showed significantly lower mTOR signal (p-S6) expression than those from patients in the placebo arm. In conclusion, 7 months of treatment with 500 mg or 1,500 mg metformin did not reduce the mean number or size of polyps in the colorectum or duodenum in FAP patients (ClinicalTrials.gov ID: NCT01725490).

Prevention Relevance: A 7-month metformin treatment (500 mg or 1,500 mg) did not reduce the number or size of polyps in the colorectum or duodenum of FAP patients as compared to placebo. These results do not support the use of metformin to promote regression of intestinal adenomas in FAP patients.

Introduction

Familial adenomatous polyposis (FAP) is a rare hereditary disease characterized by the development of hundreds of adenomatous polyps in the colon and rectum. FAP is caused by germline mutations in the tumor suppressor gene, adenomatous polyposis coli (*APC*), located on chromosome 5q21-q22 (1), and its estimated prevalence rate is 3–5 per 100,000 (2, 3). If left untreated, the rate of progression to colorectal cancer is nearly 100% in individuals with FAP, with an average age of 39 years at cancer diagnosis. Moreover, duodenal adenomas occur in 45% to 90% of FAP patients (4). Once the extent of colorectal polyposis is no longer endoscopically manageable, prophylactic colectomy becomes the standard of care.

Although colectomy prevents the development of colorectal cancer, it carries appreciable short- and long-term complications and can negatively affect patients' quality of life. Accordingly, there has always been an understandable desire to delay

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or prevent colectomy through the use of medications. In addition, drugs showing an effect on chemoprevention have the potential to effectively delay or prevent colon surgery when applied in combination with endoscopic polyp removal. Regarding endoscopic management of FAP patients, recent guideline, although derived on the basis of the low quality of evidence, suggests that, in individuals with FAP who do not need immediate colectomy and are manageable by endoscopy, all polyps >5 mm be removed (5). Moreover, colonoscopy surveillance is recommended every 1–2 years, depending on the polyp burden (5).

Multiple studies have shown that nonsteroidal anti-inflammatory drugs (NSAID) and COX-2 inhibitors, including sulindac (6–8) and celecoxib (9, 10), significantly inhibit colorectal adenomatous polyps in FAP patients. Combined therapy using sulindac and an EGFR inhibitor, such as erlotinib, has recently been shown to cause significant regression of colorectal adenomas in FAP patients (11). However, the use of these drugs is currently limited owing to adverse effects that have been identified during trials or concerns regarding potential issues such as gastrointestinal damage or cardiovascular events, as a result of long-term use.

Metformin, an oral hypoglycemic agent in the biguanide class, is the most widely used drug of its class for the treatment of type II diabetes mellitus. Metformin has recently received attention as a potential chemopreventive drug. One meta-analysis showed that metformin use is associated with a reduced risk of colorectal adenoma in patients with diabetes mellitus (12). Metformin suppresses aberrant colorectal crypt foci, which are tiny lesions that develop in the early stages of colorectal carcinogenesis in both mice (13) and nondiabetic humans (14). Moreover, a recent randomized trial demonstrated that low-dose metformin reduces the prevalence and number of metachronous adenomas in nondiabetic patients (15). Given the aforementioned chemopreventive effect and the favorable safety profile of metformin, this drug may be a potential agent for delaying or preventing bowel resective surgery in FAP patients. Accordingly, we performed the first double-blind, randomized, controlled trial to evaluate the safety and efficacy of metformin on colorectal and duodenal adenoma regression in FAP patients.

Methods

Subjects and study design

This multicenter study was conducted at several hospitals in Korea using prospective, double-blind, placebo-controlled, randomized trials. The trial is registered at clinicaltrials.gov (NCT01725490). FAP patients aged 19 to 65 years were recruited from July 2013 through December 2018, including those who had previously undergone total colectomy with ileorectal anastomosis or total proctocolectomy with ileal pouch-anal anastomosis. The eligible patients had five or more endoscopically assessable polyps with a ≥2-mm diameter at the time of colonoscopy, sigmoidoscopy, or duodenoscopy (9, 16, 17). All participants provided written informed consent before participating in this trial. The study was performed in accordance with the Declaration of Helsinki statement for medical research involving human subjects. Ethics approval for the study was obtained from the institutional review board (IRB) of Severance Hospital (IRB no.: 4–2012–0491).

Patients were excluded on the basis of the following criteria: having undergone colectomy within the previous 12 months, needing to undergo colectomy within 8 months after the trial initiation, diagnosis of a malignant disease such as colorectal cancer, NSAID, or aspirin use three or more times per week within the 6 months preceding the trial, a diagnosis of DM, pregnancy or breastfeeding, abnormal results from serum laboratory tests (renal and liver function tests), or significant infectious or respiratory diseases.

Patients were randomly assigned in a 1:2:2 ratio to orally receive the placebo, 500 mg metformin, or 1,500 mg metformin, respectively, for 7 months. Patients in the placebo arm received three placebo tablets; those in the metformin 500 mg arm received one 500-mg metformin tablet and two placebos; and those in the metformin 1,500 mg arm received three 500-mg metformin tablets (**Fig. 1**). All tablets were identical in appearance and were taken individually three times per day. The patients were assigned to each of the three arms using permuted-block randomization with a block size of five based on the presence of colorectal polyps only, the coexistence of colorectal and duodenal polyps, or the presence of only duodenal polyps after total proctocolectomy. The double-blind method was performed according to our research IP (internet protocol): IP codes were assigned to each bottle, and placebo and test drugs were assigned to each code so that neither researchers nor participants were aware of the treatment regimen. Protocol assignments were randomly allocated sequentially according to each patient's order of enrollment. An independent statistician performed this random allocation, a research nurse enrolled the participants, and a research coordinator assigned them.

The enrolled patients were adapted to the treatment protocol with a 1-month period of dose escalation in all three treatment arms. In the absence of adverse events with a dose escalation of one tablet per 2 weeks, the patients took the assigned medication three times per day from the second month for 6 months. The total medication period, including the adaptation period, was 7 months. The metformin and placebo were generously supplied by Daewoong Pharm. Co.

Endoscopic procedure and measurements

An India ink tattoo was placed in the ascending colon, rectum, and duodenum during the baseline colonoscopy and upper endoscopy. Sigmoidoscopy was performed in patients with a retained rectum after colectomy with ileorectal anastomosis. All polyps larger than 1 cm in size were resected endoscopically during baseline endoscopy examination, and if the removed polyps showed the pathology of high-grade dysplasia or adenocarcinoma, we did not enroll patients and

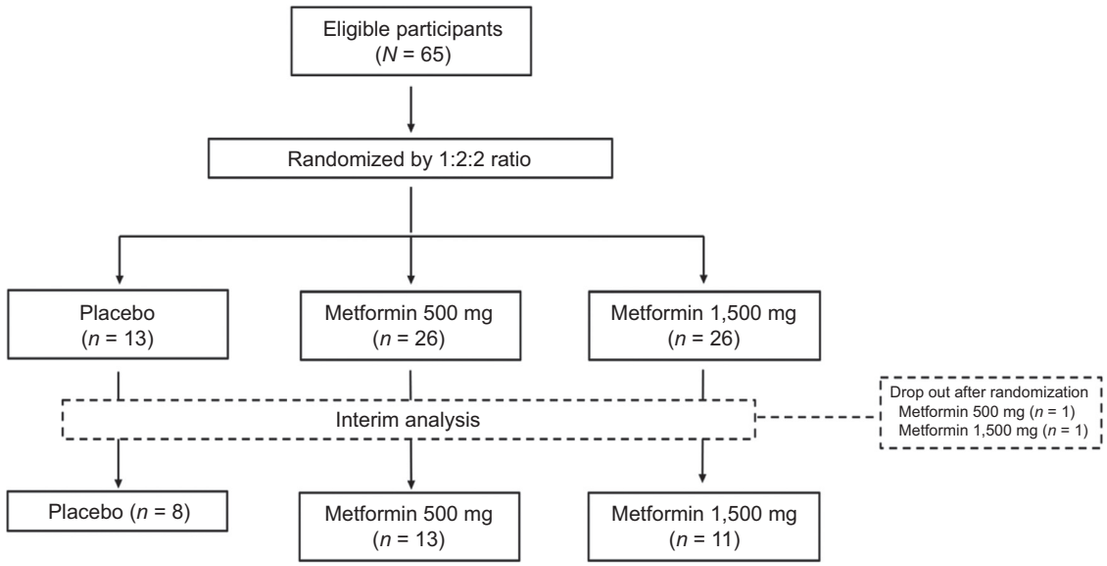

Figure 1.
Study flow diagram.

recommended surgical treatment for FAP. After the 7-month treatment period, colonoscopy/sigmoidoscopy and upper gastrointestinal endoscopy were performed. The baseline and posttreatment endoscopic examinations were recorded by video clips, and photographs were taken of the tattoo-marked area and used to measure the number and sizes of polyps. The diameter of each polyp was measured using biopsy forceps included in the photographic field; only distinct polyps at least 2 mm in diameter were counted. Photographs from the baseline and posttreatment endoscopies of the tattooed area with visible biopsy forceps were selected by two independent reviewers (B.C. Kim and J.J. Park) to compare the same areas of the colorectum and duodenum before and after treatment.

The number of polyps in the same marked area of the selected photographs was measured by two independent observers (B.C. Kim and J.J. Park), who were blinded to treatment allocations. Individual polyp diameters were measured using the biopsy forceps, which were measured with a ruler, and using a magnification correction factor derived from the known size of the forceps (16). We also used recorded video clips of the baseline and posttreatment endoscopies to evaluate the overall polyp burden status, including sizes and shapes, to classify polyp burden as decreased, no change, or increased.

Primary and secondary outcomes

The primary outcome was the mean percentage change of the number and size of polyps in the colorectum and/or duodenum between the baseline and posttreatment endoscopies. The secondary outcome was the interval change of overall polyp burden between the baseline and posttreatment endoscopies in the three treatment arms.

Evaluation of adverse events

Adverse events were monitored by interview, questionnaire, and physical examination at the 1-, 2-, and 4-month visits; compliance and safety were assessed via telephone surveys that were conducted every 2 weeks. A complete blood cell count was obtained, and the levels of glucose, serum urea nitrogen, serum creatinine, serum electrolytes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and bilirubin were measured at 0, 1, 2, 4, and 7 months. Adverse events were graded in accordance with the Common Toxicity Criteria for Adverse Events (CTCAE v4.0; ref. 18). On this scale, grade 0 indicates no adverse event and grade 5 indicates death related to an adverse event.

IHC for molecular markers

IHC staining for β-catenin, Ki-67, and p-S6 (phospho-S6 ribosomal protein Ser235/236) was performed on 4-μm sections of formalin-fixed, paraffin-embedded tissue blocks of polypectomized colonic polyp tissues. The details of IHC staining methods are described elsewhere (19). The primary antibodies used in IHC are as follows: anti-β-catenin antibody (Santa Cruz Biotechnology), anti-Ki67 antibody (Abcam), and anti-pS6 antibody (Cell Signaling Technology). IHC scores were evaluated on the basis of methods presented in previous report using ImageJ program (20).

Statistical analysis

The sample size was calculated to provide the study with 80% power to detect a difference (−4.5, −11.9, and −28) of 24 SDs in the percentage of change in polyp number (%) between groups using two-sided α values of 0.05 by one-way ANOVA, according to a previous study (9). The sample sizes of 13, 26, and 26 were obtained from the three groups (placebo,

metformin 500 mg, and metformin 1,500 mg, respectively) based on the above calculation and the assignment of a 1:2:2 ratio.

The patient population in this study carries a rare hereditary disease, and substantial polyp reduction is needed to obtain meaningful clinical results for FAP patients (21). Therefore, an interim analysis for futility and harm was planned when the colorectal and duodenal patient enrollments each reached 33% to assess whether the trial should continue or be terminated early. The plan stipulated that if statistical significance was not determined upon interim analysis and trial harm or futility were shown to outweigh treatment benefits, early termination would be considered through discussion with the Data and Safety Monitoring Board (DSMB).

The categorical variables were analyzed by a χ^2 test or Fisher exact test, and the continuous variables were analyzed by ANOVA or Kruskal–Wallis test for differences among the three groups. Changes in the number, size, and IHC score of polyps from baseline were analyzed by a paired *t* test. A two-sided *P* value of less than 0.05 was considered to indicate a statistically significant result. All statistical analyses were performed using the statistical software package SPSS 18.0 for Windows (SPSS Inc.).

Results

Baseline characteristics of subjects

Thirty-four eligible participants were randomly assigned to the three treatment arms. Among the initial enrollees, one patient in the metformin 500-mg group and one patient in the metformin 1,500-mg group withdrew owing to adverse events. The baseline demographic data are listed in **Table 1**. Most demographic characteristics did not differ among the three groups; however, gender ratios were not evenly distributed despite random assignment.

Efficacy

We performed only per-protocol analysis because posttreatment endoscopy was not performed in the two withdrawn patients. The number and mean size of polyps in the marked areas of the colorectum and duodenum were measured during the baseline and posttreatment examinations (**Figs. 2** and **3**; Supplementary Table S1). We found no significant differences in the mean number or size of polyps in the target area of the colorectum between the baseline and posttreatment examinations in any of the three treatment groups (**Figs. 2** and **3**; Supplementary Table S1). In addition, changes in polyp number and size in the target area of the colorectum ($P = 0.531$ and $P = 0.199$, respectively) and the percentage of change ($P = 0.627$, and $P = 0.214$, respectively) did not significantly differ among the three treatment arms (**Table 2**). The change in overall polyp burden, estimated by videotape evaluation and considered a secondary outcome, was not significantly different among the three groups (Supplementary Table S2). To evaluate the differences in treatment response according to location in the colorectum, separate analyses were performed in the ascending colon and rectum. However, no significant differences in polyp number or size were observed in either the ascending colon or the rectum between the baseline and posttreatment examinations in any of the three groups. Furthermore, we found no differences in the percentage of changes in polyp number or size, or in the overall polyp burden response among the three groups (Supplementary Tables S1 and S2; Supplementary Figs. S1 and S2). In addition, no significant differences were noted between pre- and posttreatment duodenum polyp number or size in any of the groups (**Figs. 2** and **3**; Supplementary Table S1). The percentage of change in polyp number and size, and the response in overall polyp burden did not significantly differ among the treatment arms (**Table 2**; Supplementary Table S2).

On the basis of these interim analysis results and through discussions with the DSMB, it was determined that there was

Table 1. Baseline demographic characteristics.

Characteristic	Placebo (*n* = 8)	Metformin 500 mg (*n* = 14)	Metformin 1,500 mg (*n* = 12)
Age (y; mean ± SD)	33.4 ± 11.1	38.4 ± 13.7	38.1 ± 13.3
Sex			
Male, *n* (%)	4 (50.0)	2 (14.3)	6 (50.0)
Female, *n* (%)	4 (50.0)	12 (85.7)	6 (50.0)
BMI (kg/m^2, mean ± SD)	22.6 ± 3.9	21.2 ± 2.4	22.4 ± 3.9
Smoking			
Ex-smokers, *n* (%)	2 (25.0)	1 (7.1)	2 (16.7)
Current smokers, *n* (%)	2 (25.0)	0 (0)	3 (25.0)
Target organ with measurable polyps			
Colon or rectum	7	11	8
Duodenum	5	8	7
Surgical status			
TPC/TC with IPAA/IRA	1	6	7
No surgical status	7	8	5

Abbreviations: BMI, body mass index; IPAA, ileal pouch-anal anastomosis; IRA, ieorectal anastomosis; TC, total colectomy; TPC, total proctocolectomy.

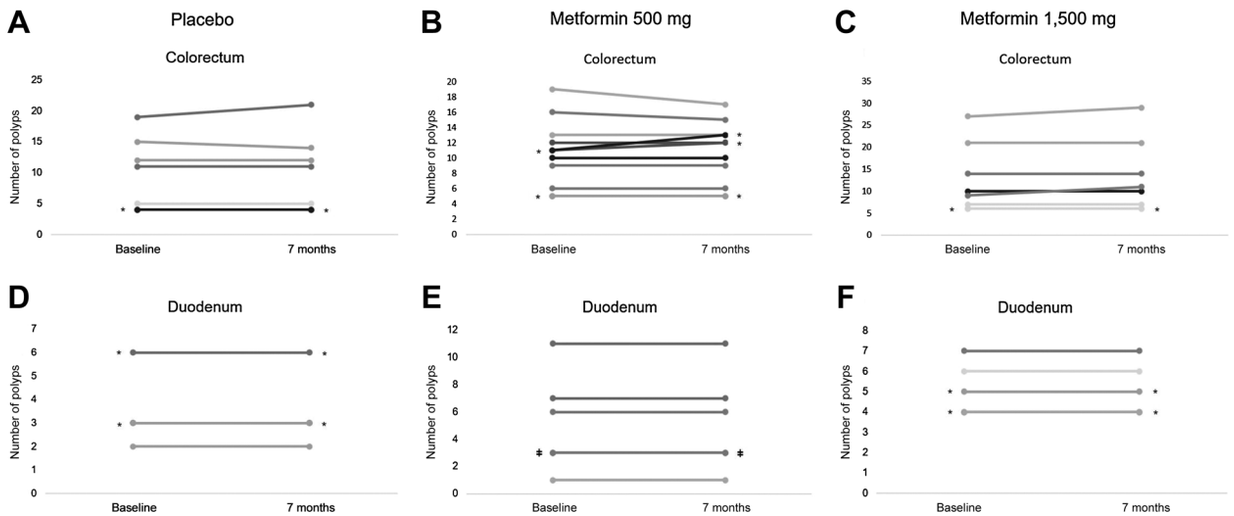

Figure 2.

The number of polyps detected in the colorectum and duodenum at the baseline and posttreatment examinations in the placebo and metformin groups. * represents two cases; ǂ represents three cases.

no benefit in continuing the study because the use of metformin at doses of 500 mg and 1,500 mg tended to increase the percentage of change in polyp number and size compared with the placebo.

IHC for molecular markers

To evaluate the molecular markers associated with FAP, the effects of metformin, and tumor growth, we performed IHC staining for β-catenin, p-S6, and Ki67 in the colonic polyp tissues. Three patients from the placebo group, four from the metformin 500-mg group, and four from the metformin 1,500-mg group were selected on the basis of the size similarity of the polyps removed from these patients at the baseline and posttreatment colonoscopies. **Figure 4** shows the representative IHC staining of polyp tissues for p-S6, β-catenin, and Ki-67 at the baseline and posttreatment examinations in each patient group. The IHC score of p-S6 was significantly reduced (mean: 1.54 vs. 1.35, $P = 0.015$) in both groups of patients who received metformin, but no change was observed in the placebo group (**Fig. 5A**). In addition, no significant differences in IHC scores were found between the baseline and posttreatment examinations for β-catenin or Ki-67 in either the placebo or the metformin groups (**Fig. 5B** and **C**).

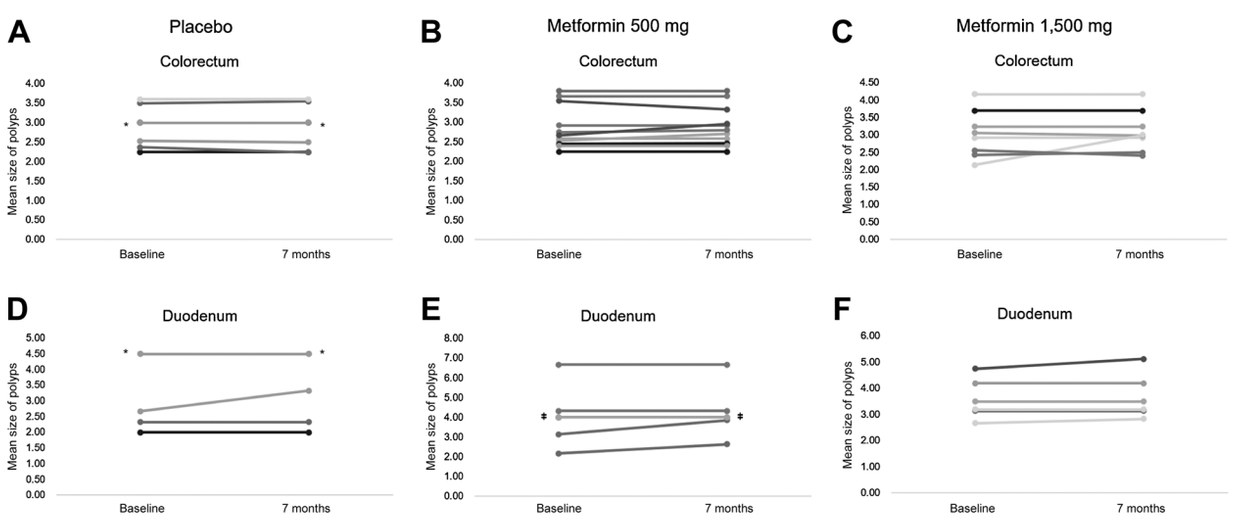

Figure 3.

The size of polyps in the colorectum and duodenum at the baseline and posttreatment examinations in the placebo and metformin groups. * represents two cases; ǂ represents three cases.

Table 2. The change in number and size of polyps from baseline to post-treatment examinations in the placebo and metformin treatment groups.

		Placebo	Metformin 500 mg	Metformin 1,500 mg	*P*
Colorectum	Change of polyp number (mean ± SD)	0.14 ± 0.90	0.00 ± 1.00	0.50 ± 0.93	0.531
	Percentage of change in polyp number (%) (mean ± SD)	0.55 ± 5.05	0.95 ± 7.44	3.70 ± 7.92	0.627
	Change of polyp size (mean ± SD)	−0.07 ± 1.48	0.50 ± 2.09	1.81 ± 2.36	0.199
	Percentage of change in polyp size (%) (mean ± SD)	−0.31 ± 3.72	2.12 ± 6.77	7.88 ± 13.98	0.214
Duodenum	Change of polyp number (mean ± SD)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.000
	Percentage of change in polyp number (%) (mean ± SD)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.000
	Change of polyp size (mean ± SD)	0.40 ± 0.89	1.25 ± 2.31	0.42 ± 0.67	0.555
	Percentage of change in polyp size (%) (mean ± SD)	5.00 ± 11.18	5.45 ± 10.09	2.36 ± 3.69	0.803

Note: Change in polyp number = number of polyps after treatment – number of polyps at baseline. Change in polyp size = size of polyps after treatment –size of polyps at baseline. Percentage of change in polyp number or size = change of polyp number or size/polyp number or size at baseline × 100

Compliance and adverse events

The mean rate of compliance with treatment did not significantly differ between the placebo group [96.7%; 95% confidence interval (CI), 94.4–98.9], the metformin 500-mg group (90.9%; 95% CI, 83.8–98.1), and the metformin 1,500-mg group (85.5%; 95% CI, 78.1–92.8). Adverse symptoms were observed in both metformin groups, but not in the placebo group; these symptoms included nausea, epigastric discomfort, diarrhea, constipation, general weakness, headache, and myalgia (**Table 3**). Five subjects (14.7%) reported at least one drug-related adverse event during treatment. Two subjects withdrew from the study owing to grade 2 adverse events (general weakness and myalgia; **Table 3**). Other symptoms were mild (grade 1), tolerable, and disappeared over time. None of the three treatment arms showed significant changes in hematologic or blood chemistry profiles, including complete blood cell count, glucose, renal and liver function values, and electrolyte values between the baseline and posttreatment evaluations. No significant changes in weight or waist circumference occurred between baseline and posttreatment in any of the treatment arms.

Discussion

To date, many clinical trials have been conducted to assess potential chemopreventive agents with favorable safety profiles that are capable of preventing or delaying gastrointestinal adenomas in patients with FAP. However, because the side effects of some effective agents have limited prolonged use, the development of such drugs is a crucial unmet need in the management of FAP patients. Metformin is a classic biguanide drug that has been used as first-line therapy for type II diabetes mellitus (22). It is known to be a relatively safe drug, has been used widely for more than 50 years, and has recently attracted attention owing to its antitumor effects, in addition to its antidiabetic efficacy.

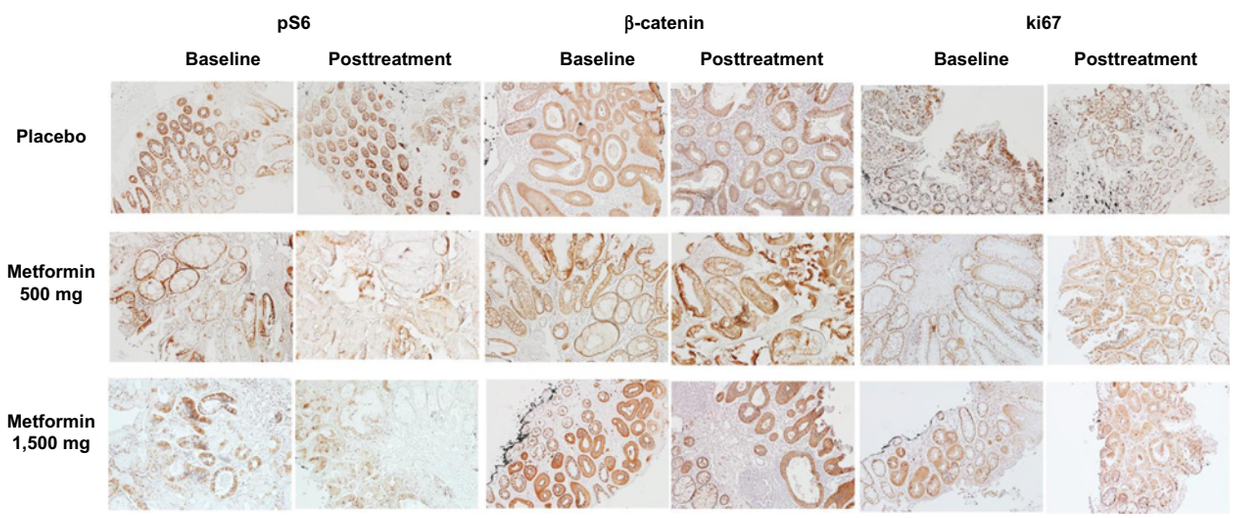

Figure 4.
Representative images of IHC staining for pS6, β-catenin, and Ki-67 in isolated colonic polyp tissue at the baseline and posttreatment examinations in the placebo and metformin groups (×100 magnification).

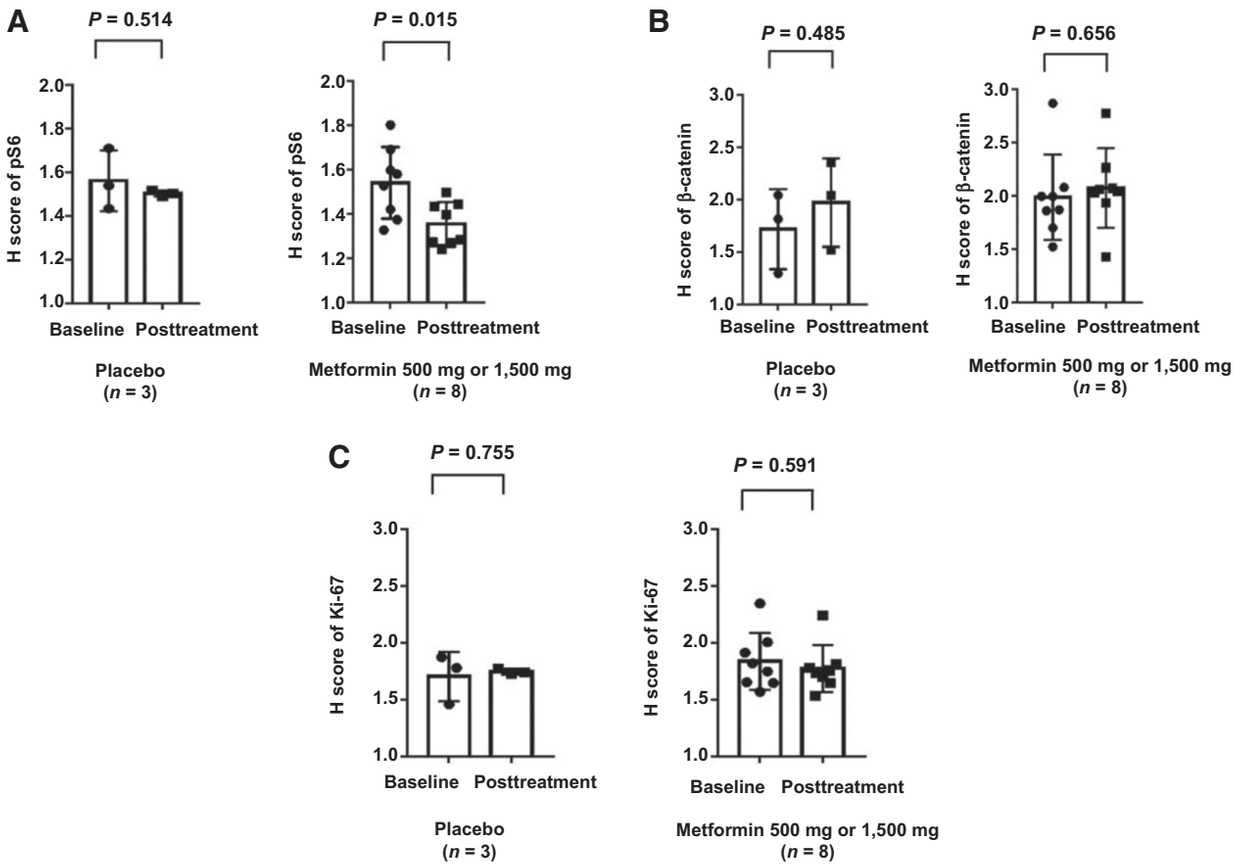

Figure 5.

IHC scores for pS6, β-catenin, and Ki-67 on colonic polyps were compared between the baseline and posttreatment examinations in FAP patients treated with placebo and metformin (paired *t* test).

Previous meta-analyses have reported that patients with type II diabetes mellitus who were treated with metformin had a lower risk of colorectal cancer than patients not taking the drug (23). Another meta-analysis reported that metformin use is associated with a reduced risk of colorectal adenoma (12). Although the exact mechanisms underlying the antineoplastic effects of metformin have not been entirely elucidated, an AMPK-dependent effect has been suggested as the mechanism involved in metformin-related tumor suppression (24–26). Metformin activates AMPK, which inhibits the mTOR pathway (27). The mTOR pathway plays an essential role in protein translational machinery and cell proliferation, both of which may be potentially related to tumorigenesis (28). Metformin may also promote antineoplastic effect via an AMPK-independent mechanism, such as through the Rag GTPase-dependent suppression of mTORC1 (29), or through the inhibition of cell-cycle progression, antiapoptotic signaling molecules, and angiogenesis, which would eventually result in decreased cellular proliferation and tumor progression (30–33). In addition, the administration of metformin (250 mg/kg), although it did not prevent polyp formation, suppressed polyp growth in the animal experiment of ApcMin^{+} mice (34).

On the basis of the evidence mentioned above, we conducted this prospective study to demonstrate the chemopreventive

Table 3. Incidence and severity of adverse events.

	Placebo (*n* = 8) *n* (%)	Metformin 500 mg (*n* = 14) *n* (%)	Metformin 1,500 mg (*n* = 12) *n* (%)
Nausea	0	1[a] (7.1)	1[b] (8.3)
Epigastric discomfort	0	0	1[b] (8.3)
Diarrhea	0	1[c] (7.1)	0
Constipation	0	0	1[d] (8.3)
General weakness	0	0	1[b] (8.3)
Headache	0	1[c] (7.1)	0
Myalgia	0	1[e] (7.1)	0

Note: All adverse events observed were grade 1 or grade 2.
[a]One patient experienced nausea (grade 1).
[b]One patient experienced nausea (grade 1), epigastric discomfort (grade 1), and general weakness (grade 2); this patient was withdrawn.
[c]One patient experienced headache and diarrhea (grade 1).
[d]One patient experienced constipation (grade 1).
[e]One patient experienced myalgia of (grade 2); this patient was withdrawn.

efficacy of metformin in FAP patients. This is the first randomized, controlled trial to evaluate the efficacy of metformin on the regression of the number and size of polyps in FAP patients. In this double-blind, placebo-controlled, randomized trial, we found no significant differences in changes of either the mean number or the size of colorectal or duodenal polyps between patients who received metformin at doses of 500 mg/day or 1,500 mg/day and those received placebos for 7 months.

The chemopreventive efficacy of metformin was considered in a recent randomized trial, which showed that a low dose (250 mg) reduced sporadic adenoma recurrence in nondiabetic patients (15). However, unlike the sporadic adenoma that occurs in the general population, the adenoma burdens harbored by FAP patients are substantial. Therefore, the suppressive effect of metformin on the recurrence of sporadic adenoma in the general population may not translate into the regression of adenomas in FAP patients. Unlike FAP-associated polyps, sporadic adenoma is influenced by various environmental factors and molecular mechanisms. Although the recruited subjects in the above-mentioned low-dose metformin trial were patients with non-diabetes mellitus, the efficacy of metformin on adenoma recurrence was dependent on its effect on HOMA-IR (insulin resistance; ref. 15). This suggests that the underlying metabolic alteration was influenced by additional factors, such as older age, as the study was performed in an elderly population. Therefore, the association between metabolic changes and the occurrence of adenoma may have been accentuated in that study, thus amplifying the effect of metformin on sporadic adenoma beyond what we detected in our trial with FAP patients.

Several other explanations can be posited regarding the lack of efficacy of metformin in promoting intestinal adenoma regression in FAP patients. First, drug dosage and duration must be considered. However, in the previous retrospective study (12) that showed the efficacy of metformin on adenoma reduction, the metformin dosage was similar to that used in our study. Furthermore, a prospective study on sporadic adenoma showed efficacy at a lower dose of metformin (250 mg; ref. 15). In addition, patient compliance was fairly high in our study. Therefore, we suggest that drug dosage is not likely a reason for the negative results of our study.

The relatively short duration of this trial could be a principle factor in the lack of adenoma regression in FAP patients. Although the study periods of the majority of randomized trials investigating colonic and duodenal (8, 35–37) polyp regression in FAP patients were 6 months or less, the 7-month trial period in our study may be not sufficient to allow the observation of the chemopreventive efficacy of metformin. In the above-mentioned study on sporadic adenoma (15), in which a low dose of metformin was administered, the study period was 1 year; thus, if the indirect metabolic effect of metformin is a primary mechanism of adenoma prevention, a longer administration period may be required to observe treatment-related changes.

Second, the small number of patients enrolled in our trial could be a possible factor contributing to the negative result. This study was terminated early based on the results of the interim analysis, as mentioned in Statistical Analysis of Methods. Because the modest decrease in polyp number and size in patients with FAP is of limited clinical impact (21), we incorporated interim analysis into our study model, allowing early termination to prevent unnecessary exposure to ineffective drugs. Although the number of patients in the control group of our study was relatively small compared with those in other studies, the number of patients in the intervention groups (metformin 500 mg and 1,500 mg) was 18 in total for colorectal adenoma, which is comparable with the patient numbers in other studies. Combined analysis of all treated patients did not reveal a regression effect.

Third, factors related to the antitumor molecular mechanisms of metformin could account for our negative results. In this study, IHC staining was performed on polyps from patients in each group to investigate the molecular effects of metformin on intestinal adenomas. It was confirmed that the expression of mTOR was decreased in the metformin-treated group, suggesting a potential antitumor effect on intestinal adenoma in FAP patients. However, there was no significant change in either β-catenin or Ki-67 levels. These results suggest that the inhibition of mTOR alone may not be enough to impel adenoma regression in FAP patients and that metformin can be expected to promote regression when combined with drugs that directly target other major pathways, such as the WNT and RAS/ERK pathways. In addition, considering that metformin was more effective on sporadic adenoma in patients who showed a HOMA-IR response (15), its efficacy may be lower in young FAP patients.

Furthermore, it is important to consider biomarkers that are related to the effects of metformin. First, metabolic differences in tumors, which are related to their energy sources, could play a role in differential tumor responses to metformin. We identified tumor glucose or glutamine dependency as a potential factor related to the effect of metformin (19). Second, responses to metformin are known to exhibit significant interindividual variations through the localization and expression of membrane transporters, such as organic cation transporters (OCTs), especially OCT1 and OCT2, and multidrug toxin extrusion proteins in normal and tumor tissues (38–41). In addition, various genetic and epigenetic factors may affect transporter expression and activity (41). Therefore, the different activities of glutamine transporters, glutaminase, and membrane transporters in colorectal tumor should be considered possible factors related to differential effects of metformin.

There are several limitations to this study. First, although treatment group was randomly assigned, the gender distribution was significantly different among three groups, and the mean age in the placebo group was relatively younger than metformin groups. These factors can affect the results; thus

caution may be needed when interpreting the results. Second, as for the design of the clinical trial, the most reliable endpoint of a chemoprevention trial for FAP patients should be a significant delay of disease progression, including the development of advanced adenoma and colorectal cancer, which could be defined as the need for major surgery and endoscopic removal of advanced neoplasia. However, our study focused only on the change in size and number of polyps. In future clinical trials, the primary outcome should be more robust and reliable outcome like disease progression with assessment by the need for surgery and development of advanced neoplasia, and for this purpose, the clinical study might have a longer period of trial and enough number of patients.

In conclusion, our randomized, double-blinded trial of patients with FAP showed no significant reduction in the mean number or size of colorectal or duodenal adenomas in patients who received metformin at two different dosages for 7 months, compared with patients who received placebo. Our results do not support the use of metformin as a chemopreventive agent for the regression of intestinal adenomas in FAP patients. Future clinical trials will need to take into consideration the possible causes underlying the negative results obtained in our study, perhaps by incorporating a longer duration, targeting additional molecular pathways, and determining biomarkers that can distinguish responders from nonresponders.

Authors' Disclosures

No disclosures were reported.

Authors' Contributions

J.J. Park: Conceptualization, data curation, formal analysis, investigation, writing–original draft, writing–review and editing. **B.C. Kim:** Conceptualization, data curation, formal analysis, investigation, writing–original draft, writing–review and editing. **S.P. Hong:** Data curation, writing–review and editing. **Y. Seo:** Data curation, writing–review and editing. **H.S. Lee:** Formal analysis, validation, writing–review and editing. **Y.S. Park:** Data curation, writing–review and editing. **S.-Y. Na:** Data curation, writing–review and editing. **S.C. Park:** Data curation, writing–review and editing. **J. Park:** Data curation, writing–review and editing. **J.H. Kim:** Data curation, writing–review and editing. **C.M. Moon:** Data curation, writing–review and editing. **K.C. Huh:** Data curation, writing–review and editing. **S.J. Park:** Data curation, writing–review and editing. **J.H. Cheon:** Data curation, writing–review and editing. **W.H. Kim:** Data curation, writing–review and editing. **T.I. Kim:** Conceptualization, resources, data curation, supervision, funding acquisition, validation, investigation, visualization, methodology, project administration, writing–review and editing.

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