

A Water-soluble Extract from Culture Medium of *Ganoderma lucidum* Mycelia Suppresses the Development of Colorectal Adenomas

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ABSTRACT

A water-soluble extract from a cultured medium of *Ganoderma lucidum* mycelia (MAK) is one of the *G. lucidum* extracts that has been reported to show have exhibit cancer-preventive effects in the animal studies. To confirm cancer-preventive effects of MAK, we performed a no-treatment concurrent controlled trial on patients with colorectal adenomas. Patients who were determined to be carrying colorectal adenomas by colonoscopy were enrolled in this study. Patients in the MAK group took MAK (1.5 g/day) for 12 months. Follow-up colonoscopy was performed after 12 months, and the colonoscopists recorded the size, site and macroscopic type of all adenomas. Among 123 patients who enrolled in the MAK group, 96 eligible patients completed the trial. The 102 eligible patients in the no-treatment control group were selected randomly from our department's patients. The changes in the number of adenomas up to 12 months increased to 0.66 ± 0.10 (mean \pm SE) in the control group, while decreasing in the MAK group to -0.42 ± 0.10 ($p < 0.01$). The total size of adenomas increased to 1.73 ± 0.28 mm in the control group and decreased to -1.40 ± 0.64 mm in the MAK group ($p < 0.01$). The results suggest that MAK suppresses the development of colorectal adenomas – precancerous lesions of the large bowel.

Key words: *Ganoderma lucidum*, Colorectal adenoma, Cancer prevention, Clinical trial

Ganoderma lucidum (Fr.) Karst. (Polyporaceae) is a medicinal mushroom known to the Japanese as 'Rei-shi' or 'Mannentake', and to the Chinese as 'Lingzhi'. This mushroom is described in the Chinese medical classic *Shen Nung Ben Cao Jin* (神農本草經), and it has been widely used by Asian people in the belief that it has potent immunoenhancing effects and anti-cancer activity. Recent studies have reported on the immunomodulative^{2,3,17}, antioxidative^{15,16} and antimutagenic⁷ effects of *G. lucidum* extracts or its polysaccharides. Anti-tumor and anti-cancer effects have been reported in tumor-bearing mice¹² and in cancer patients⁸, but it has not been reported that the administration of *G. lucidum* prevented the development of colorectal adenomas in humans.

The water-soluble extract from a culture medium of *G. lucidum* mycelia (designated as MAK) that we used in this study is a typical *G. lucidum* extract. It has been reported that MAK significantly prevented the development of azoxymethane (AOM)-induced colonic aberrant crypt foci (ACF) and adenoma in F344 rats^{9,10}, N,N'-dimethylhydrazine (DMH)-induced colon tumors in ICR mice¹¹, and N-nitrosobis(2-hydroxypropyl) amine (BHP)-induced pulmonary adenocarcinoma in Wistar rats⁵.

Based on the findings from these animal studies, we deduced that MAK might provide potentially preventive effects against the development of colorectal tumor. We thus conducted a clinical intervention trial, using an established colonosco-

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py method, in order to evaluate the effects of MAK on colorectal adenomas.

MATERIAL AND METHODS

Subjects

Subjects enrolled in this study were more than 40 years old, and had been shown by colonoscopy to be carrying colorectal adenomas. Exclusion criteria were severe complications, a history of colectomy without appendectomy, and the use of non-steroidal anti-inflammatory drugs (NSAIDs), anti-cancer agents or immune enhancers including herbal formulations.

Subjects in the no-treatment control group were selected randomly from patients in our department who met the criteria and would undergo follow-up colonoscopy at 12 months.

The ethics review committee of Hiroshima University approved the MAK intervention trial, and all subjects in the MAK group provided written informed consent.

Test supplement

A water-soluble extract from a culture medium of *G. lucidum* mycelia (MAK) was prepared by Noda Shokkin-Kogyo Co., Ltd. In brief, *G. lucidum* mycelia was cultured in a solid medium composed mainly of sugar-cane bagasse for 3 months, after which the entire medium containing mycelia was extracted with hot water. The extract was filtered and spray-dried as MAK. The MAK supplement containing MAK 1.5 g per 6 capsules was prepared and supplied to us by Wakunaga Pharmaceutical Co., Ltd. The MAK supplement was taken at a dose of three capsules twice daily for 12 months.

Colonoscopy

The subjects returned for follow-up colonoscopy at 12 months after initial MAK intake. Colonoscopists recorded the size, site and macroscopic type of all colorectal adenomas. When an adenoma was removed by polypectomy, hot biopsy, endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD), the sample was examined histologically using standard techniques. We used magnifying high-resolution video-colonoscopes manufactured by Fujinon (EC-450ZH, EC-450ZW, EC-590ZW, Fujinon Corp., Omiya, Japan) and Olympus (CFQ-240Z, CF-H260AZI, Olympus Corp., Tokyo, Japan).

Statistical analysis

The primary endpoint was changes in the number and total size of adenomas within 12 months after baseline (0 months). Values for the number and total size of adenomas were added to 1 and transformed logarithmically to base 10 to approximate a normal distribution and parallel regression lines for the MAK group and the

control group. Comparison between the two groups with regard to the number or total size of adenomas was performed by analysis of covariance (ANCOVA) adjusted for baseline covariates, and difference from baseline in each group was compared by Wilcoxon paired signed rank test. The rates at which either incidence or decrease changed by at least one adenoma were compared between groups by logistic regression adjusted for baseline covariates. The baseline characteristics were compared between the two groups by Fisher's exact probability test, Mann-Whitney U test or t test depending on the scale of measurement. All tests were performed with a two-sided alpha level of 0.05, using SPSS 16.0J (SPSS Japan Inc., Tokyo, Japan).

RESULTS

We conducted the study in the Hiroshima University Hospital between June 2004 and July 2008.

Figure 1 shows the flow diagram of subjects in the MAK treatment group. A total of 123 subjects were enrolled into the study and received the MAK supplement. Of these, 18 subjects were lost to follow-up colonoscopy because of adverse events (n=6), patient request (n=4), failure to return (n=3), refusing to take the MAK supplement (n=3), and other medical events (n=2). An additional 9 patients were excluded from the efficacy analysis for protocol violations, namely, taking NSAIDs (n=5) and poor compliance (n=4). The study was completed by 96 subjects, and we set that group as the efficacy population. The 102 eligible patients who did not receive MAK were selected randomly, and were designated the control group.

Baseline characteristics of subjects are shown in Table 1. Age, sex and interval between colonoscopies were comparable between the MAK group and the control group, although the number and total size of adenomas in the MAK group were slightly larger than in the control group.

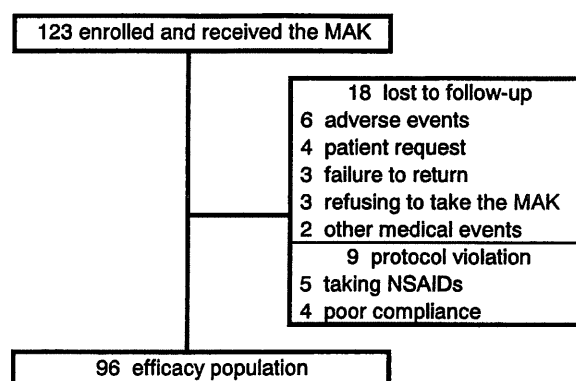


Fig. 1. Flow diagram of subjects in the MAK group

Figures 2A and 2B show changes in the number and total size of adenomas at 12 months. The mean \pm SE of adenoma number in the control group increased to 0.66 ± 0.10 ($p < 0.01$, Wilcoxon paired signed rank test), while the mean in the MAK group decreased to -0.42 ± 0.21 ($p < 0.01$). The p value for comparison between the two groups was less than 0.01 by ANCOVA with adjustment for the baseline number of adenomas (Fig. 2A). Total size of adenomas was similar to the number of adenomas; the mean increased to 1.73 ± 0.28 mm ($p < 0.01$) in the control group and decreased to -1.40 ± 0.64 mm ($p < 0.01$) in the MAK group, and the p value for comparison between the two groups was less than 0.01 (Fig. 2B).

To examine adenomatous changes in detail, we analyzed changes of adenoma number classified

by macroscopic type of adenoma and site within the large bowel. The polypoid-type adenomas increased in the control group and decreased in the MAK group, and there was a significant difference between the two groups ($p < 0.01$). The changes in superficial-type adenomas were similar to the trend seen in the polypoid-type adenomas, but with no significant difference between the MAK and control groups (Fig. 3A).

The number of adenomas occurring on the right side (cecum, ascending colon and transverse colon) and left side (descending colon and sigmoid colon) increased in the control group and decreased in the MAK group ($p < 0.01$). Changes in the rectum showed similar tendencies, but there was no significant difference between the MAK and control groups (Fig. 3B).

Table 1. Baseline characteristics of subjects

	MAK (N=96)	Control (N=102)	p Value
Age - year	64.1 ± 9.8	64.4 ± 9.8	0.81 a)
Sex Male - no. (%)	64 (67)	73 (72)	0.54 b)
Interval between colonoscopies -day	370 ± 33	373 ± 38	0.48 a)
Adenomas			
Subjects with adenomas - no. (%)	65 (68)	59 (58)	0.19 b)
Number of adenomas	2.29 ± 2.88	1.52 ± 1.88	0.05 c)
Total size of adenomas - mm	6.55 ± 8.17	4.43 ± 5.45	0.09 c)

Plus-minus values represent means \pm SD.

a) The p values were calculated by t test.

b) The p values were calculated by Fisher's exact probability test.

c) The p values were calculated by Mann-Whitney U test.

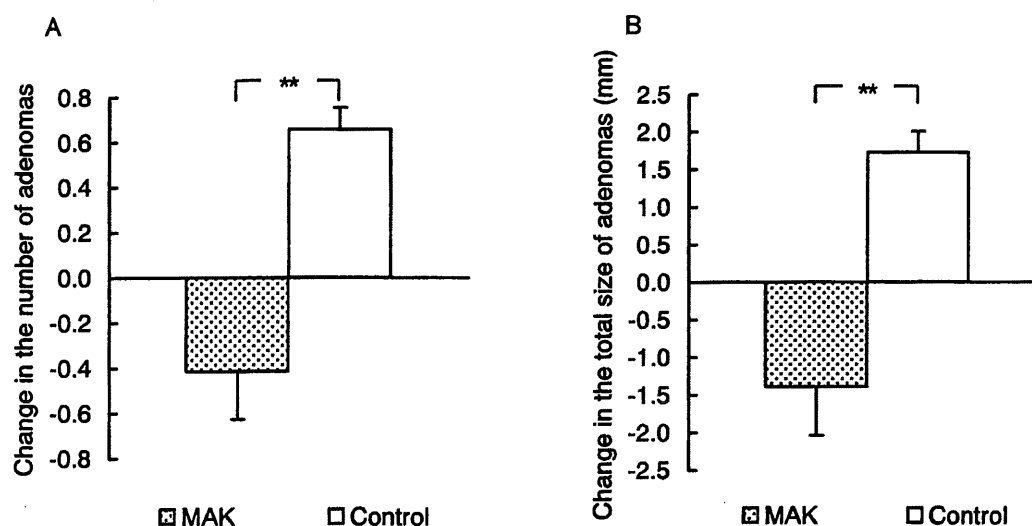


Fig. 2. Changes in the number (panel A) and total size (panel B) of colorectal adenomas at 12 months

Values represent mean \pm SE (MAK, $n=96$; Control, $n=102$). ** $p < 0.01$ by analysis of covariance with adjustment for the number or total size of adenoma at baseline.

Table 2 shows the rates of incidence and decrease by at least 1 adenoma at 12 months. These incidence rates were 42% in the control group and 11% in the MAK group, and the p value between the two groups was less than 0.01 by logistic regression with adjustment for the baseline adenomas. The rates of decrease were 2% in the control group and 52% in the MAK group ($p < 0.01$).

Adverse events were noted in 6 cases in the MAK group. Treatment with MAK was discontinued in all cases. Symptoms were diarrhea ($n=4$), stomach discomfort ($n=1$) and poor health ($n=1$).

DISCUSSION

Ganoderma lucidum is a medicinal mushroom believed to be potent in anti-cancer activity, and it has been widely used by Asian people. Clinical trials of *G. lucidum* on anti-tumor activity were mainly conducted in China; these trials are described in the review edited by Z.B. Lin⁸. For example, Qi et al conducted a Chinese medicine controlled clinical trial of *G. lucidum* spore (3 g/day for 2 months) on gastrointestinal cancer

(stomach, esophagus, liver, large bowel, and other cancer). The complete response (CR) plus partial response (PR) rate was 43% in the *G. lucidum* group ($n=100$) and 33% in the control group ($n=100$), a significant difference between the two groups. Yan et al conducted a clinical trial in lung cancer patients treated with chemotherapy, and the CR plus PR rates in the *G. lucidum* group ($n=35$, treated with a liquid preparation of *G. lucidum* for 1 month) and the control group ($n=21$, chemotherapy only) were 65.71% and 42.85% respectively ($p < 0.01$ for comparison between the two groups).

In addition, *G. lucidum* is known to have potent immunoenhancing effects. In the above clinical trial, conducted by Qi et al, the administration of *G. lucidum* spores was associated with increased percentages for CD3, CD4/CD8 T lymphocytes. Chen et al²) and Gao et al³) have reported that the use of water-soluble *G. lucidum* polysaccharides at 5.4 g/day for 12 weeks to treat advanced colorectal cancer or lung cancer patients tended to increase phytohemagglutinin-induced mitogenic reactivity, CD3, CD4, CD8 and CD56 lymphocytes counts, and plasma concentration of interleukin (IL)-2,

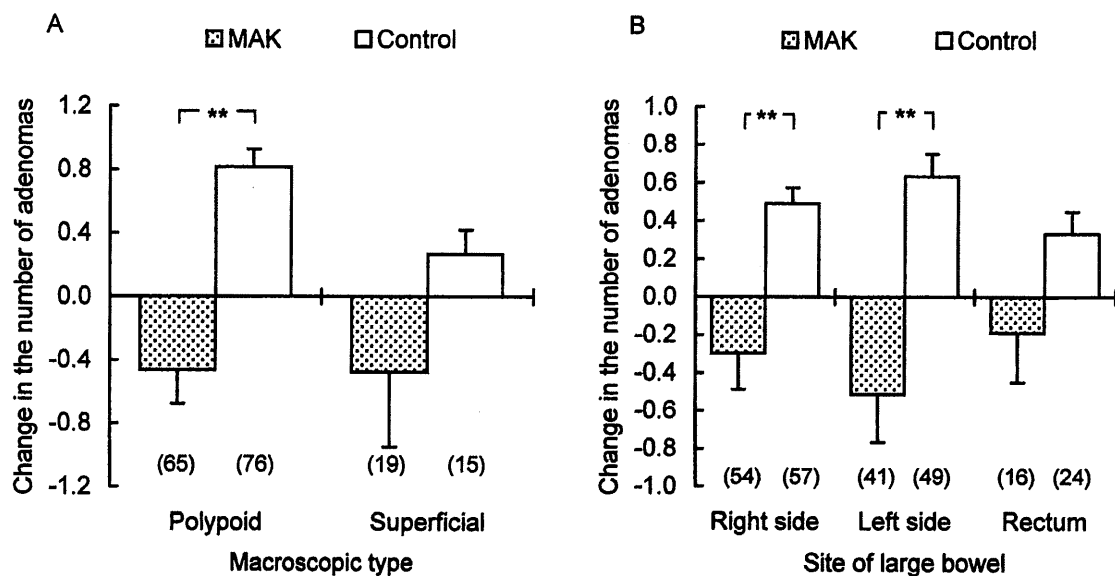


Fig. 3. Changes in the number of colorectal adenomas classified by macroscopic type (panel A) and site in the large bowel (panel B)

Values represent mean \pm SE (n). ** $p < 0.01$ by analysis of covariance with adjustment for the number of adenomas at baseline.

Table 2. Rates of incidence and decrease of at least 1 adenoma at 12 months

	MAK		Control		p value
Rate of incidence - no. / N (%)	11 / 96	(11)	43 / 102	(42)	<0.01
Rate of decrease - no. / N* (%)	34 / 65	(52)	1 / 59	(2)	<0.01

N* showed the number of subjects with one or more adenoma at the baseline.

p values were calculated by logistic regression with adjustment for the baseline adenomas.

IL-6, interferon (IFN)- γ , and natural killer (NK) activity. Their reports indicate that *G. lucidum* may have potential immunomodulating effects. One of the anti-tumor mechanisms of *G. lucidum* may involve activation of NK cells with cytotoxic activity and increment of cytokines (IL-2, IFN- γ et al) that promote T cell growth.

Yuen et al^{15,16}) tested the antioxidant activities of *G. lucidum* in an *in vitro* pre-cancerous human uroepithelial cell (HUC-PC) tumorigenic model. Results showed that the water-soluble extract of *G. lucidum* (GLw) possessed higher antioxidant capacities than the water-insoluble counterpart (GLE). However, under the challenge of carcinogenic 4-aminobiphenyl, GLw reduced the 8-hydroxy-2'-deoxyguanosine (8-OHdG) concentration in HUC-PC culture, while GLE induced the formation of hydrogen peroxide and 8-OHdG in a dose-dependent manner. The authors suggest that oxidative DNA damage may be an underlying mechanism of Lingzhi-induced apoptosis in bladder chemoprevention.

The antimutagenic activity of *G. lucidum* extract has been reported by Lakshmi et al⁷). The methanolic extract of the fruiting bodies of *G. lucidum* extract dose-dependently inhibited increase of revertants induced by several mutagens (sodium azide, N-methyl-N'-nitro-N-nitrosoguanidine, 4-nitro-phenylenediamine and benzo[*a*]pyrene (B[*a*]P)) using the Ames *Salmonella* mutagenicity test. *In vivo* antimutagenic activity of the *G. lucidum* extract was assayed by determining the mutagenicity of the urine of rats dosed with B[*a*]P, and the administration of extract markedly inhibited mutagenicity induced by B[*a*]P. In addition, the extract prevented the increase of serum hepatic enzyme (GOT, GPT and ALP) activities consequent to B[*a*]P challenge, and enhanced the levels of reduced glutathione and antioxidant-related enzymes (glutathione peroxidase, glutathione-S-transferase, superoxide dismutase and catalase) activities. These results suggest that *G. lucidum* has antioxidative and antimutagenic activities, and that it may potentially prevent DNA oxidative damage with carcinogens.

MAK is a unique water-soluble extract that is derived by culturing *G. lucidum* mycelia in a solid medium composed of sugar-cane bagasse. Immunomodulating effects of MAK have been reported by Kashimoto et al⁴). In *in vitro* C57BL/6 mouse spleen cells, MAK stimulated macrophage phagocytosis, cell growth under IL-2 addition and NK activity against YAC-1 cells, and in *in vivo* C57BL/6 mice implanted subcutaneously with Sarcoma-180 in the back, the MAK group showed decreased tumor size and increased NK activity against YAC-1 cells.

Lu et al⁹⁻¹¹) and Kashimoto et al⁵) have reported that MAK prevented the development of colonic ACF, colonic adenoma and pulmonary adenocarci-

noma in rats or mice induced by AOM, DMH or BHP as carcinogens. In those experiments, they evaluated the proliferating cell nuclear antigen (PCNA) positive cells in colonic mucosa or pulmonary tissue, and MAK dose-dependently reduced the PCNA indexes. Kubo et al⁶) has reported that MAK increased small intestine crypt survival and prolongation of average time to animal death in X-irradiated mice. These results suggest that MAK protects against DNA damage from carcinogens or radiation.

We set a primary endpoint of change in the number and total size of colorectal adenomas using a colonoscopy method that has been used in several studies^{1,13,14}), and evaluated the efficacy of MAK. Usually, we do not remove polyps of 5 mm or less, and we observe the patient's progress by follow-up colonoscopy, because these small polyps do not usually show rapid increases in size or morphological changes. The number and size of small polyps generally do not change, or they may increase slightly as seen in the control group for this study. However, on rare occasions we see patients whose polyps decrease in follow-up colonoscopy. In this study, it is worth noting that 52% of the patients in the MAK group showed a decrease of at least 1 adenoma, and the number and total size of adenomas in the MAK group significantly decreased from baseline.

In conclusion, our data suggest that MAK suppresses the development of colorectal adenomas as precancerous lesions.

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