【Short Communication】

Inhibitory Effect of Chaga (*Inonotus obliquus*) on Tumor Promotion in Two-Stage Mouse Skin Carcinogenesis

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[ABSTRACT]
Chaga (the sclerotia of *Inonotus obliquus*) has been widely used as a folk medicine in the treatment of cancer, cardiovascular disease and diabetes in Russia, Poland, and several Baltic countries. More recently, this herb has been assessed for its cancer-preventing activity. Using a mouse model of skin cancer, oral administration of Chaga was found to inhibit tumor promotion by 12-O-tetradecanoylphorbol-13-acetate following initiation with 7,12-dimethylenz[a]anthracene in mouse skin.

[Key words]
Chaga (*Inonotus obliquus*), cancer prevention, two-stage carcinogenesis

1. INTRODUCTION

In the prevention of cancer, complementary and alternative medicine (CAM) plays an important role. Cancer prevention strategies used in CAM involve the use of several types of mushrooms, as mushrooms have been reported to possess anti-tumor activities. Our previous studies have illustrated that the edible mushrooms, buna-shimeji and buna-haritake, and the supplemental and medicinal mushrooms, reishi, meshimakobu (hardwood trunk rot), and poria inhibited tumor promotion in two-stage mouse skin carcinogenesis.

Chaga (the sclerotia of *Inonotus obliquus*) is a medicinal mushroom that is widely distributed in Russia, Europe, and Japan. It has been used as an effective agent to treat various diseases such as tuberculosis, cardiovascular disease, cancer and diabetes mellitus. Chaga has also been shown to exhibit a variety of biological activities, including anti-HIV-1, immuno-stimulatory, anti-oxidant, and anti-tumor effects.

In this paper, we report that oral administration of a diet containing Chaga was found to inhibit tumor promotion in a two-stage mouse skin carcinogenesis test, using 7,12-dimethylbenz[a]anthracene (DMBA) as an initiator and 12-O-tetradecanoylphorbol-13-acetate (TPA) as a promoter.

2. MATERIALS AND METHODS

2.1 Materials
Chaga (the sclerotia of *Inonotus obliquus* (Pers. Fr.) Pil.) was obtained from Kinokuniya-Kanyakkyoku Co. Ltd. (Tokyo, Japan). The herb was authenticated by one (K.Y.) of the authors, and a voucher specimen (SM-0703) has been deposited at the Research Unit of Selfmedication, School of...
The dried powder of the sclerotia of Chaga was combined with a basal diet (FR-2; Oriental Yeast Co. Ltd.) at 0.2% and 1.0%, respectively.

2.2 Chemicals

Chemicals were purchased as follows: DMBA from Sigma Chemical Co. St. Louis, MO, USA, TPA from Chemicals for Cancer Research, Inc. Chicago, IL, USA, acetone from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

2.3 Animals

Experiments were performed in accordance with the Guidelines of the Institutional Animal Care and Use Committee of the School of Pharmacy, Nihon University, Chiba, Japan. Female ICR mice (7 weeks old) were purchased from Japan SLC Inc. (Shizuoka, Japan) and housed in an air-conditioned specific pathogen-free room (22–23°C) illuminated from 08:00–20:00. Food and water were available ad libitum.

2.4 Two-stage mouse skin carcinogenesis model

The dorsal surfaces of mice were shaved with electric clippers. The skin of the back of mice were initiated with 50 µg DMBA, and from 1 day later the mice were given a diet containing either 0.2% or 1.0% of Chaga (approximately 10 or 50 mg/mouse/day), respectively. Promotion with 1 µg TPA, applied twice weekly, was begun 1 week after initiation. DMBA and TPA were dissolved in acetone, and applied to the shaved area in a volume of 100 µl, using a micropipette. The back of each animal was shaved once a week to remove hair. The numbers of skin tumors were measured every week, and the experiment was continued for 20 weeks. The experimental and control groups each consisted of 15 mice.

2.5 Statistical analysis

Differences between experimental groups were determined using one-way analysis of variance (ANOVA) followed by post hoc Tukey’s multiple test and Mann-Whitney U exact test.

3. RESULTS

Chaga moderately inhibited tumor promotion by TPA following initiation with DMBA. Fig. 1A shows the time course of skin tumor formation in the group treated with DMBA plus TPA, with or without Chaga. In the group treated with DMBA plus TPA, the first tumor appeared at week 5 and all 15 mice presented with tumors at week 12. In the groups treated with DMBA plus TPA and 0.2% and 1.0% Chaga, the first tumor appeared at weeks 6 and 7, respectively, and all 15 mice had tumors at weeks 16 and 17, respectively. Fig. 1B shows the average number of tumors per mouse at week 20. The group treated with DMBA plus TPA produced 23.7 tumors per mouse, whereas the DMBA plus TPA and 0.2% and 1.0% Chaga groups had 18.5 and 12.3 tumors per mouse, respectively. Thus, treatment with 0.2% and 1.0% Chaga resulted in a 23% and 49% reduction in the average number of tumors per mouse at week 20, respectively. There were no differences in body weight between the control and treatment groups during

![Fig. 1](image-url) Inhibitory effect of diet containing of Chaga on the tumor promotion of skin papillomas by TPA in DMBA-initiated mice. A: Percentage of mice bearing tumors; B: average number of papillomas per mouse. ●, + TPA with basic diet; ○, TPA with diet containing 1.0% Chaga, □, TPA with diet containing 0.2% Chaga. n=15. *p<0.05 (A: Mann-Whitney U exact test; B: one-way ANOVA followed by Tukey’s test).
the experiment. In addition, the intake of the bait did not have the difference between the control group and two administrated groups (data not shown).

4. DISCUSSION

Oral administration of Chaga inhibited tumor promotion by TPA following initiation with DMBA in mouse skin. Polyphenolics, triterpenoids and polysaccharides have already been reported from the sclerotium of Chaga. Polyphenolics found in Chaga have been reported to exhibit antioxidant activity. Additionally, many lanostane-type triterpenoids have been isolated from Chaga; inotodiol\(^{15}\) and 3β-hydroxylanosta-8,24-dien-21- \(\beta\)-hydroxylanosta-8,24-dien-21-ol\(^{16}\) suppressed tumor promotion in two-stage mouse skin carcinogenesis. In particular, polysaccharides have been reported to have many physiological activities, including cancer prevention\(^{17}\).

Mushrooms are valued as both an edible and a medicinal resource, and anti-tumor substances have been identified in several mushroom species. Polysaccharides are the best known and most potent mushroom-derived substances with antitumor and immunomodulating properties. In particular, it has been reported that aqueous extracts of Chaga inhibited cell growth in a dose-dependent manner, which was accompanied by G\(_0\)/G\(_1\) arrest and apoptotic cell death\(^{18}\). Additionally, aqueous extracts of Chaga prevented the inhibition of gap junctional intercellular communication through the inactivation of ERK1/2 and p38 MAP kinase\(^{19}\). Furthermore, a methanol extract of Chaga was found to significantly inhibit the production of nitric oxide (NO), prostaglandin E\(_2\) (PGE\(_2\)) and tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\))\(^{20}\).

In an effort to screen for new cancer inhibitors that can be used as chemopreventive agents, we have intentionally chosen non-toxic compounds. In the present study, Chaga did not exhibit toxicity by oral administration. Furthermore, we have demonstrated that Chaga acts as a moderate inhibitor of tumor promotion. Therefore, Chaga is regarded as a material that has potential in CAM for the prevention of cancer.

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REFERENCES

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チャーガ（カバノアナタケ）のマウス皮膚二段階発癌実験の腫瘍抑制効果

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チャーガ（Inonotus obliquus の菌核）は，ロシア，ポーランド，パルト海の数ヶ国で癌，心血管疾患，糖尿病の治療に民間療法として広く使われてきた．本研究では，チャーガの内服が，7,12-dimethylbenz[a]anthracene でイニシエーションをし 12-O-tetradecanoylphorbol-13-acetate でプロモートするマウス皮膚二段階発癌を抑制することが明らかになった．

キーワード：チャーガ（Inonotus obliquus），癌予防，二段階発癌