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Inhibitory effects of propolis granular A. P. C on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in A/J mice

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Abstract

We examined the effect of propolis granular A. P. C on lung tumorigenesis in female A/J mice. Lung tumors were induced by the tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) administered in drinking water for 7 weeks in mice maintained on an AIN-76A semi-synthetic diet. Propolis granular A. P. C (100 mg/kg body wt.) was administered orally daily for 6 days/week from 1 week before NNK administration and throughout the experiment. Sixteen weeks after the NNK treatment, the mice were killed and the number of surface lung tumors was measured. The number of lung tumors in mice treated with NNK alone for 7 weeks (9.4 mg/mouse) was significantly more than in that observed in control mice. Propolis granular A. P. C significantly decreased the number of lung tumors induced by NNK. These results indicate that propolis granular A. P. C is effective in suppressing NNK-induced lung tumorigenesis in mice.

Keywords: Propolis; Artepillin C; 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone

1. Introduction

Propolis is a resinous material collected by honeybees from the buds and bark of certain plants and trees, and this compound is thought to serve as a defense substance for their hives [1]. The characteristic components of propolis include many kinds of flavonoids and cinnamic acid derivatives, and some of which have been known to show antitumor effects [2–6]. Matsuno et al. [7] reported that artepillin C (3,5-diprenyl-4-hydroxycinnamic acid) is one of the active components of propolis, and Kimoto et al. [8,9] showed that artepillin C is capable of reducing tumor burden in animal models. Lung cancer, thought to be caused by smoking, is the leading cause of cancer death [10]. Thus, it is worthwhile to study whether propolis extract containing a large quantity of

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artepillin C could act as a useful chemopreventive agent against lung cancer. Recently, propolis granular A. P. C has been developed by Yamada Apiculture Center, Inc. (Okayama, Japan) and Fujisaki Institute, Hayashibara Biochemical Laboratories Inc. (Okayama, Japan). The propolis granular A. P. C contained more than 35.8 μg artepillin C/1 g. It is well known that 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is the most potent carcinogen among the tobacco-specific nitrosamines, and this compound is thought to be an etiological factor in tobacco-related human cancers. In rodents also, NNK is reported to show a potent carcinogenic activity, and the lung is the major target organ in NNK-induced tumorigenesis in mice [11]. In the present study we investigated the inhibitory effect of propolis granular A. P. C on NNK-induced lung tumorigenesis in A/J mice.

2. Materials and methods

2.1. Animals

Female A/J strain mice, 6 weeks old, were purchased from Japan SLC, Inc. (Shizuoka, Japan) and acclimated 1 week before the start of treatments. They were housed in groups of five in solid bottom polycarbonate cages with conventional woodchip bedding, and maintained in an air-conditioned room with a temperature of 24 ± 2 °C, relative humidity of 55 ± 15%, and an alternating 12:12-h light–dark cycle. All procedures involving animals were conducted in accordance with the guidelines of the Animal Care and Use Committee, Faculty of Pharmaceutical Sciences, Okayama University.

2.2. Experimental protocols

The animals were randomly divided into four groups and fed ad libitum (AIN-76A diet, Oriental Yeast, Tokyo, Japan). All mice were weighed once a week. Stock solution of NNK (Toronto Research Inc., Toronto, Canada) were prepared in distilled water (11 mg/ml) and diluted in tap water. The initial concentration of NNK was 62.4 μg/ml and thereafter the concentration of NNK was adjusted for each cage according to water consumption. Consumption of NNK solution was monitored twice a week for 7 weeks. The propolis used in this preparation was harvested in Minas Gerais, Brazil. The propolis used in the experiments was slightly soluble in water, therefore we used some dissolved substances including maltose. Propolis granular A. P. C was suspended in distilled water (20 mg/ml) and given orally to the mice daily 6 days/week from 1 week before NNK administration and throughout the experiment (week 0–24). Details of the treatment with NNK, propolis granular A. P. C and its vehicle are shown in Fig. 1. The mice in group 1 received tap water ad libitum. The mice in groups 2–4 received NNK in drinking water for 7 weeks (weeks 1–7) and were retained for an additional 16 weeks for determining the production of lung tumors. The mice in group 4 were treated with propolis granular A. P. C (100 mg/kg body wt., p.o.) daily 6 days/week, starting 1 week before NNK administration began and continuing throughout the study (weeks 0–24). The mice in group 3 were treated with vehicle of propolis granular A. P. C (maltose water solution) in the same way as propolis granular A. P. C (weeks 0–24). All animals were killed under ether anesthesia 16 weeks after the end of NNK treatment. At the time of autopsy, the number of surface lung tumors was counted by gross observation. The number of tumors was counted independently by two observers. The difference between the two observer counts was less than 5% of the number of tumors.

2.3. Statistical analysis

All data are represented as means ± S.E.M. Statistical analysis was performed using Wilcoxon's rank sum test. A probability value of less than 0.01 was considered statistically significant.

3. Results

In this study, NNK was given in drinking water from week 1 to week 7 for 7 weeks. Total doses of NNK averaged 9.4 mg (45.5 μmol)/mouse, and NNK consumption was 224 μg/mouse per day on average. Water consumption was not significantly different among the four groups (data not shown). Body weights of NNK-treated mice (25.0 ± 0.7 g) were

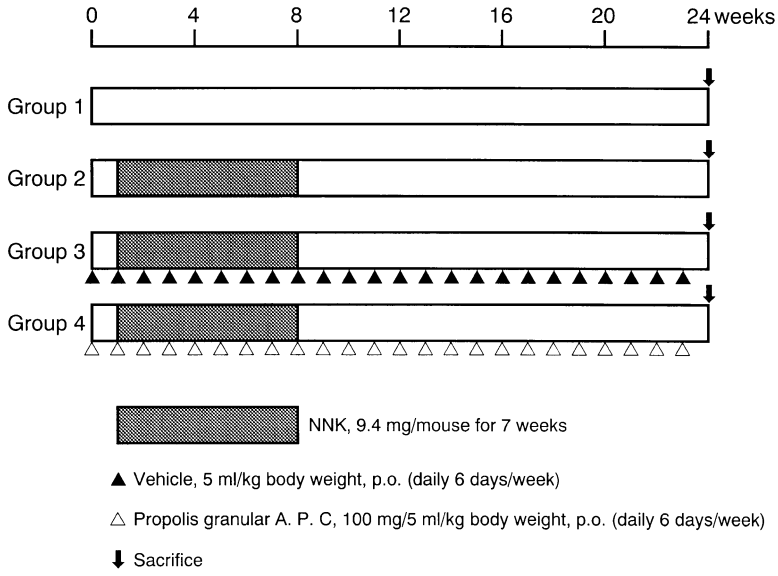

Fig. 1. Experimental protocol for carcinogenesis induced by NNK in female A/J mice. NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

slightly decreased compared with that of control mice (25.3 ± 0.6 g) at the time of killing. In addition, no statistical difference was observed with body weights of mice among the four groups. As shown in Table 1, the lung tumor incidence was 21% in the control mice (group 1), whereas the tumor incidence of NNK-treated mice was 100% (group 2). The multiplicity of lung tumors in control mice was similar to published values [12–14]. NNK induced 12.3 ± 2.7 tumors/mouse, and this value was also similar to that previously reported using a similar protocol [12–14]. The lung tumor incidence was not reduced by propolis granular A. P. C. However, the compound reduced lung tumor multiplicity by 72% (9.1 ± 3.0 in NNK + vehicle; 3.5 ± 0.6 in NNK + propolis granular A. P. C), and this effect was statistically significant ($P < 0.01$).

4. Discussion

Hecht et al. [15] have reported that animals maintained on commercially available normal diets developed fewer neoplasms than animals maintained

Table 1
Effects of oral administration of propolis granular A. P. C on NNK-induced lung tumorigenesis in female A/J mice

Treatment group	Total number of mice	Total number of mice with tumors	Lung tumor incidence[a]	Numbers of lung tumors per mouse[b]
1. Control	14	3	21	0.2 ± 0.1
2. NNK alone	14	14	100	12.3 ± 2.7[c]
3. NNK + vehicle	14	14	100	9.1 ± 3.0[c]
4. NNK + propolis granular A. P. C	14	13	93	3.5 ± 0.6[d]

NNK was given in drinking water from week 1 to 7 for 7 weeks. Propolis granular A. P. C (100 mg/kg body wt. in water suspension, p.o.) and its vehicle were given daily 6 days/week from week 0 to killing. Sixteen weeks after the NNK treatment, mice were killed and the number of tumors on the lung surface were counted.

[a] Tumor-bearing animals per total number of animals in group (percentage).

[b] Values are given as means ± S.E.M.

[c] Significantly different ($P < 0.01$) from control animals by Wilcoxon's rank sum test.

[d] Significantly different ($P < 0.01$) from 'NNK + vehicle' treated animals by Wilcoxon's rank sum test.

on semisynthetic diets (AIN-76A). Commercially available normal diets consist of many plant-derived materials, and it is well known that plant-derived materials contain several inhibitors of NNK-induced tumorigenesis. Therefore, we used AIN-76A based on these observations, because propolis consists of plant-derived materials. As shown in this study, propolis granular A. P. C has a protective effect against NNK-induced lung tumorigenesis in A/J mice. It has been reported that the mechanism of inhibition by several compounds of NNK-induced lung tumorigenesis is due at least partly to their antioxidant properties [16–18]. Propolis granular A. P. C contains many kinds of flavonoids [1], and these compounds are powerful antioxidants, capable of scavenging H_2O_2 and superoxide anion [19,20]. Therefore, it is reasonable to presume that the inhibitory activity of propolis granular A. P. C on NNK-induced lung tumorigenesis may be due to the antioxidant activity of flavonoids.

Artepillin C, an active component of cinnamic acid in Brazilian propolis, which was isolated after ethanol extraction, has been shown to have antibacterial and antiviral activities [21]. In addition, artepillin C is also reported to inhibit the growth of transplanted solid human and mouse tumors [22]. The cytotoxicity of artepillin C has been shown in human tumor cell lines [7], and the antitumor activity of artepillin C has been demonstrated in mouse renal [8] and pulmonary [9] carcinogenesis induced by ferric nitrilotriacetate. Moreover, Kimoto et al. [23] reported that artepillin C has anti-leukemic effects induced by direct apoptosis of leukemia cells. Therefore, it seems likely that artepillin C contained in propolis may play an important role in the anti-carcinogenesis activity of propolis granular A. P. C to some extent. In the present study, the body weight gain was not significantly different between intact group and NNK + propolis granular A. P. C treated group (data not shown). This result indicated that propolis extract containing artepillin C exerted no adverse effects on feeding or drinking. Therefore, propolis granular A. P. C may be less toxic when it was used clinically. This is the first observation showing that propolis granular A. P. C effectively inhibited NNK-induced lung tumorigenesis. However, it remains unclear which components of propolis granular A. P. C were responsible.

From the above finding, it was concluded that propolis granular A. P. C may be effective as a chemopreventive agent against lung cancer in humans.

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