Inhibitory Effect of a Combined Treatment of Glycyrrhizin and Caffeine on Tumor Promotion by 12-*O*-Tetradecanoylphorbol-13-Acetate in Two-Stage Carcinogenesis in Mouse Skin

Ken Yasukawa*

School of Pharmacy, Nihon University, Chiba, Japan

Abstract: Cancer prevention is an important problem in the field of public health. Glycyrrhizin, a natural sweetening agent, is one of the components of licorice and caffeine in coffee and tea. Glycyrrhizin and caffeine were found to inhibit tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in two-stage carcinogenesis in mouse skin. Furthermore, the combined treatment of glycyrrhizin and caffeine is more effective than their single treatment on tumor promotion by TPA in mice following initiation with 7,12-dimethylbenz[*a*]anthracene.

Keywords: Glycyrrhizin, caffeine, combination therapy, antitumor promotion, two-stage carcinogenesis.

1. INTRODUCTION

Prevention of cancer is very important and chemotherapy is used to avoid recurrence of cancer. Our earlier studies demonstrated that some extracts from edible plants, edible mushrooms, and crude drugs inhibited 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammatory ear edema, and active compounds were separated from their extracts [1]. In a previous study [2], we showed that glycyrrhizin and caffeine (Figure **1**) can inhibit the tumor-promoting activity of TPA in two-stage carcinogenesis in mouse skin. Caffeine is commonly consumed by humans in infusions extracted from the seeds of the coffee plant and leaves of the tea bush, as well as in various foods and drinks that contain products derived from the kola nut. Other sources include yerba mate, guarana berries, guayusa, and yaupon holly. Thus, caffeine is found in many popular foods and drinks. On the other hand, glycyrrhizin is added to food as a sweetener. Thus, these compounds can be consumed simultaneously. In this study, effects of a combined glycyrrhizin and caffeine treatment on tumor promotion by TPA in two-stage carcinogenesis in mouse skin were compared with those of individual treatment with glycyrrhizin and caffeine.

2. MATERIALS AND METHODS

2.1. Chemicals

The chemicals were purchased from the following suppliers: 7,12-dimethylbenz[*a*]anthracene (DMBA) and caffeine from Sigma Chemical Co., St. Louis, MO, USA, TPA from Chemicals for Cancer Research, Inc., Minnesota, Mo., USA, and acetone from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Glycyrrhizin was provided by Minophargen Co., Tokyo, Japan.

2.2. Animals

The 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 (6 weeks old) were purchased from Japan SLC Inc. (Shizuoka, Japan) and housed in an air-conditioned specific pathogen-free room (24 ± 1 °C) that was illuminated from 08:00−20:00. Food and water were available *ad libitum*.

2.3. Two-Stage Carcinogenesis Experiment

The back of mice (7 weeks old) were shaved using electric clippers. Tumors were initiated by a single topical application of 50 μg DMBA in acetone (100 μl) and they were promoted using 1 μg TPA in acetone (100 μl), which was applied twice weekly from 1 week after initiation. Glycyrrhizin (0.5 mg/mouse), caffeine (1.0 mg/mouse), glycyrrhizin plus caffeine (0.25 mg plus 0.5 mg/mouse) or the vehicle acetone-dimethyl sulfoxide (9:1; 100 μl) were topically applied to the shaved area using a micropipette 30 min before each TPA treatment. The back of each animal was shaved once each week to remove fur. The number and diameter of the skin tumors were recorded every other week and the experiment lasted 20 weeks. The experiment and appropriate control groups each comprised 15 mice each.

2.4. Data Analysis

Statistical differences were tested by one-way analysis of variance followed by correction with Tukey–Kramer test and by Mann-Whitney *U* exact test.

*Address corresponding to this author at the School of Pharmacy, Nihon University, Funabashi, Chiba, Japan; Tel/Fax: +81 47 465 1107;
E-mail: yasukawa.ken@nihon-u.ac.jp, yasukawa.ken@nihon-u.ne.jp

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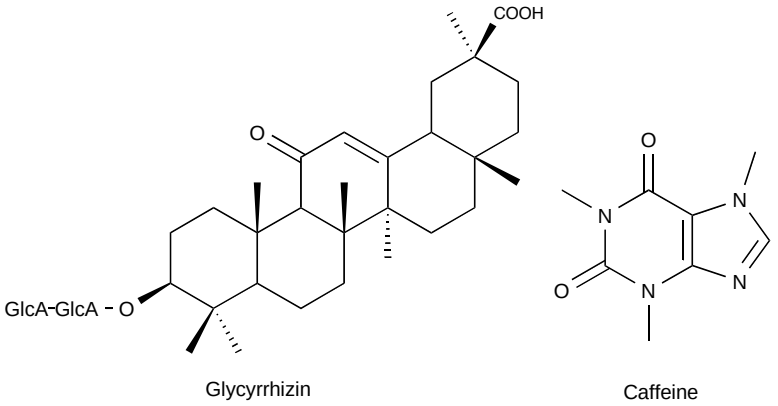

Figure 1: The chemical structures of glycyrrhizin and caffeine.

3. RESULTS

Figure **2A** shows the time course of skin tumor formation in the groups treated with DMBA plus TPA with or without glycyrrhizin, caffeine, and glycyrrhizin plus caffeine. The first tumor appeared at week 5 in the group treated with DMBA plus TPA, and all mice had tumors at week 12. In the groups treated with DMBA plus TPA and glycyrrhizin and with DMBA plus TPA and caffeine, the first tumor appeared at weeks 5. The percentage of tumor-bearing mice in the group treated with DMBA plus TPA was 100% at week 20, whereas the percentages in the groups treated with DMBA plus TPA and glycyrrhizin, caffeine, and glycyrrhizin plus caffeine were 40%, 40% and 20%, respectively. Figure **2B** shows the average number of tumor per mouse. The group treated with DMBA plus TPA had 11.2 tumors per mouse at week 20, whereas the groups treated with DMBA plus TPA and glycyrrhizin and with DMBA plus TPA and caffeine had 5.2 and 5.7 tumors per mouse, respectively. The group treated with DMBA plus TPA and glycyrrhizin plus caffeine had 1.5 tumors per mouse at week 20. Thus, the treatment with glycyrrhizin, caffeine, and glycyrrhizin plus caffeine led to a 54%, 50% and 87% reduction, respectively, in the average number of tumors per mouse at week 20. There were no differences in body weights between the control group and the three treatment groups throughout the duration of the experiment.

4. DISCUSSION

Cancer prevention is currently one of the most urgent priorities in the field of public health throughout the world. It would be particularly useful if a method of prevention is found that acts at the carcinogenesis

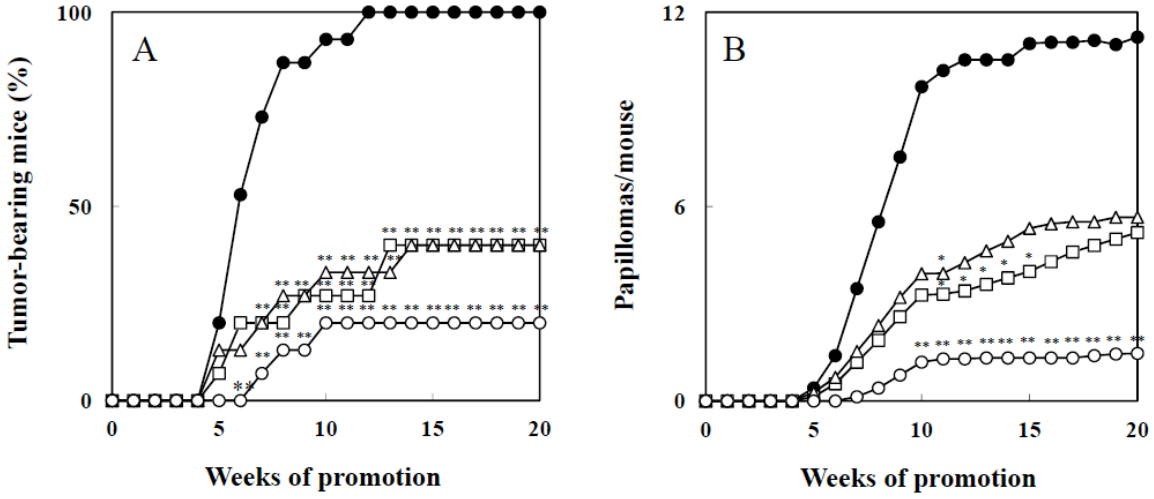

Figure 2: Inhibitory effect of combined treatment of glycyrrhizin and caffeine on the promotion of skin papillomas by TPA in DMBA-initiated mice. From 1 week after initiation by a single topical application of 50 µg of DMBA, 1 µg of TPA was applied twice weekly. Topical application of glycyrrhizin and caffeine and vehicle was performed 30 min before each TPA treatment. Data are expressed as percentage of mice bearing papillomas (**A**), and as average number of papillomas per mouse (**B**). ● = +TPA with vehicle alone; □ =+TPA with glycyrrhizin (0.5 mg); Δ =+TPA with caffeine (1.0 mg); ○ =+TPA with glycyrrhizin (0.25 mg) and caffeine (0.5 mg). The treatment groups were statistically different from the control group (* $p < 0.05$, ** $p < 0.01$).

promotion stage, because such a method would be applicable even after exposure to tumor-initiating agents, which in many cases, appears to be unavoidable in our daily lives.

Glycyrrhizin, a sweetening agent, and caffeine, found in tea and coffee, are used as food additives throughout the world. These compounds have been found to inhibit tumor promotion by TPA following tumor initiation with DMBA in mouse skin [2]. In the present study, the combined treatment with glycyrrhizin and caffeine showed greater inhibitory effects than individual treatment with these compounds. This suggests that it may be better to consume moderate amounts of multiple food types rather than eating considerable amounts of a single type. Thus, cancer may be prevented by a balanced diet.

Glycyrrhetinic acid, an aglycone of glycyrrhizin, has also been shown to suppress the promoting effect of teleocidin B on skin tumor formation in mice induced by DMBA [3,4]. Glycyrrhetinic acid has been demonstrated to inhibit PKC activity (90% inhibition at 1 mM) [5]. The potent antagonistic activity against tumor promotion in mouse skin by glycyrrhetinic acid may be a consequence of both its binding interactions with steroid receptors and its inhibition of PKC [5].

Glycyrrhetinic acid was investigated to determine its effects on the release of nitric oxide (NO) and on the level of inducible NO synthase (iNOS) gene expression in mouse macrophages [6]. Glycyrrhetinic acid elicited a dose-dependent increase in NO production and in the level of iNOS mRNA. Transient expression assays using nucrea factor κB (NF-κB)-binding sites linked to the luciferase gene showed that the increased level of iNOS mRNA induced by glycyrrhetinic acid was mediated by the NF-κB transcription factor complex. Thus, glycyrrhetinic acid stimulates NO production and can upregulate iNOS expression through NF-κB transactivation in macrophages.

Furthermore, glycyrrhizin has been demonstrated to have inhibitory effects on liver carcinogenesis induced by *N*-nitroethylamine (DEN) [7]. Oral administration of glycyrrhetinic acid and glycyrrhizin suppressed DMBA- and TPA-induced carcinogenesis in mouse skin [8,9].

The extract of edible plants and mushrooms inhibited TPA-induced tumor promotion in two-stage carcinogenesis in mouse skin [1]. In addition, plant constituents such as sterols, triterpenes [10,11], sesquiterpenes, flavonoids, and azaphilones inhibited tumor promotion by TPA in DMBA-initiated mice [1]. These compounds are found in food additives and crude drugs. We consume glycyrrhizin and caffeine every day in foods and drinks such as coffee and tea. These results demonstrate the importance of a balanced diet for cancer prevention.

Indomethacin, an anti-inflammatory drug and an inhibitor of tumor promotion [12], has been studied for its role in the clinical prevention of recurrence of stomach cancer in Japan. Thus, glycyrrhizin and caffeine may be used to prevent the recurrence of cancer in a similar way to indomethacin.

CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

FINANCIAL DISCLOSURE

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ABBREVIATIONS

DEN = Diethylnitrosamine

DMBA = 7,12-Dimethylbenz[*a*]anthracene

iNOS = Inducible NO synthase

NF-κB = Nucrea factor κB

NO = Nitric oxide

PKC = Protein kinase C

TPA = 12-*O*-Tetradecanoylphorbol-13-acetate

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