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Inhibitory Effects of Biochanin A on Mouse Lung Tumor Induced by Benzo(a)pyrene

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Biochanin A, an isoflavone compound, is reported to have an inhibitory effect on benzo(a)pyrene [B(a)P] metabolism. We examined the modifying effect of biochanin A on in vivo carcinogenesis using a mouse lung tumor model. As carcinogens, a single subcutaneous injection of 0.5mg of B(a)P was given within 24 hours after birth. The test groups were injected with 0.125mg of biochanin A in 0.1ml DMSO by i.p. 3 times a week for 6 weeks after weaning. All mice were sacrificed at week 9 and the incidence and multiplicity of lung tumors were examined. Concomitant administration of biochanin A showed a significant inhibitory effect on the incidence of tumor-bearing mice (12.5%, $P<0.01$), as well as the mean number of tumors (0.13, $P<0.001$), compared with the group treated with B(a)P alone in which the incidence was 57.1% and the mean number was 1.0. These results suggest that biochanin A has inhibitory potential on the development of mouse lung tumor induced by B(a)P.

Key Words: *Biochanin A, Mouse lung tumor, Benzo(a)pyrene, Carcinogenesis*

INTRODUCTION

Flavonoids constitute an integral part of the human diet. They are ubiquitously distributed in the leaves and stems of vascular plants. The estimated human daily consumption of flavonoids is approximately 0.02g/kg/day (Singleton, 1981; IARC, 1983). Some interesting data have appeared on the relationship between certain flavonoids and carcinogenesis. Several flavonoids, such as apigenin and quercetin, have been shown to possess anticarcinogenic activity (Wattenberg, 1985; Verma et al., 1988; Kato et al., 1983). Huang et al. (1983) examined 28 flavonoids for their effect on mutagenicity and found that some flavonoids had significant antimutagenic activity.

Biochanin A is one of the flavonoids isolated and identified in red clover, cabbage bark, and alfalfa. Cassady et al. (1988; 1990) had screened over 70 species and varieties of plants and vegetables comprising 27 families using mammalian cell culture B(a)P metabolism assay. Biochanin A was one of the plant extracts demonstrated to produce reproducible inhibition of B(a)P metabolism in the hamster cell culture assay. Based upon inhibition of B(a)P metabolism, the crude red clover extract was fractionated, and a pure active compound, biochanin A, was isolated, which produced an inhibition of B(a)P metabolism. After pure active compounds are isolated, they will then be further tested using in vivo bioassays to determine their effect on tumor induction by various classes of carcinogens, but until now in vivo anticarcinogenesis studies have not been reported on this compound.

The objective of the present study was to evaluate the effect of biochanin A on the development of mouse lung tumor induced by B(a)P.

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MATERIALS AND METHODS

Experimental animals:

Noninbred Swiss-Webster mice were obtained from

NCI (National Cancer Institute, U.S.A., [NIH]) and bred at random *inter se*. The five experimental groups were as followed: B(a)P alone,B(a)P combined with biochanin A, B(a)P combined with dimethylsulfoxide (DMSO), biochanin A alone, and the untreated control group. All mice were housed in a controlled room. Food and water were given *ad libitum*. Food was made in solid pellets based on an NIH-7-open formula.

Treatment:

Newborn mice less than 24 hr old were injected subcutaneously in the scapular region with 0.02ml of a suspension, containing 0.5mg of benzo(a)pyrene (Sigma Chemical Co., St. Louis, MO, U.S.A.) in 1% aqueous gelatin. The carcinogen was used within 1 hr after emulsification in the experiment. Biochanin A (5,7-dihydoxy-4'-methoxy isoflavone, Sigma Chemical Co., St. Louis, MO, U.S.A.) was dissolved in DMSO and used at a concentration of 0.125mg/0.1ml DMSO 3 times per week intraperitoneally. Biochanin A was administered for 6 weeks after weaning. The LD_{50} value of biochanin A was determined using 30 mice of 3-week-old female mice divided into 5 groups.

Scoring of lung tumors:

All mice were sacrificed at 9 weeks after birth. Their various organs lungs, heart, spleen, and kidneys were inspected and except for the lungs, fixed in 10% buffered formalin. To determine the number of lung adenoma, the lungs were fixed in Tellyesniczky's solution. Some tumors were embedded in paraffin, then cut and stained with hematoxylin-eosin. To obtain an index of tumor incidence, the percentage of tumor-bearing mice per total number of micr in each group was calculated. Tumor multiplicity was defined as the average number of tumors per mice, obtained by dividing the total number of tumors by the total number of mice per group, including nontumor-bearing animals.

Statistical comparisons were made using the Chi-square test for tumor incidence and Student's t-test for multiplicity and organ weight. A null hypothesis was rejected whenever a P value of 0.05 or less was found.

RESULTS

Survival and body weight:

The LD_{50} value was 63mg/kg, calculated by the Lichfield Wilcoxon method. Four out of 20 mice died in the biochanin A-treated groups. The mean body weight showed a significant decrease in the biochanin A-treated groups ($P<0.001$), but relative lung weight did not show any significant difference between groups (Table 1).

Incidence and multiplicity of lung tumor:

The incidence and mean number of lung tumors are shown in Table 2. The incidence of lung adenomas was 57.1% in mice given B(a)P alone. However, in the mice given B(a)P combined with biochanin A, the tumor incidence was 12.5%. The mean number of lung adenomas was 1.0 in the mice given B(a)P alone. The adenoma number in the mice given B(a)P combined with biochanin A was 0.13. Therefore, administration of biochanin A after B(a)P injections resulted in a significant decrease in the frequency of tumor-bearing mice ($P<0.01$), as well as the mean number of tumors ($P<0.001$), compared with the B(a)P alone group.

DISCUSSION

The present results clearly demonstrated that biochanin A had inhibitory potential on the development of mouse lung tumor induced by B(a)P. Even though the biochanin A dosage of 0.125mg was much less than the LD_{10} level, the total dosage of biochanin A

Table 1. Body and relative lung weights

Groups and Treatment	Number of Mice	Body Weight (g)	Relative Lung Weight (mg/g b.w.)
1. B(a)P	35	27.4 ± 2.7	8.4 ± 2.3
2. B(a)P + biochanin A	16	22.1 ± 2.3[a]	8.3 ± 1.3
3. B(a)P + DMSO	19	25.9 ± 2.6	7.4 ± 0.7
4. Biochanin A	16	24.6 ± 2.7[a]	7.3 ± 0.9
5. Control	35	27.3 ± 2.3	8.9 ± 1.8

B(a)P: Benzo(a)pyrene; DMSO: Dimethylsulfoxide
Values represent mean ± SD.
Significantly different from the corresponding control group at [a]$p<0.001$

Table 2. Incidence and multiplicity of lung adenomas

Groups and Treatment	Number of mice	Incidence (%)	Multiplicity
1. B(a)P	35	20 (57.1)	1.00 ± 1.26
2. B(a)P + biochanin A	16	2 (12.5)[a]	0.13 ± 0.34[b]
3. B(a)P + DMSO	19	11 (57.9)	1.07 ± 1.42
4. Biochanin A	16	0	0
5. Control	35	0	0

Values represent mean ± SD.
Significantly different from the group treated with B(a)P alone at [a]$P < 0.01$, [b]$P < 0.001$

(0.125mg per day 3 times a week for 6 weeks) seemed to be toxic to mice because four out of 20 mice died during the treatment and the body weights were significantly decreased compared with untreated control ($P < 0.001$) after treatment. The newborn mouse lung tumor system used in this study appears to be an appropriate experimental model for assessing possible antipromoting effects towards B(a)P. Our previous data revealed the validity of this model system (Jang et al., 1989; Yun et al., 1987a; 1987b).

Flavonoids are benzo-γ-pyrone derivatives which are widespread among food plants including vegetables and fruits. These natural compounds have been reported to have a pleiotropic biological effect, including binding macromolecules such as vital enzymes and divalent ions of heavy metals (Wiltrout and Hornung, 1988). Various flavonoids have previously been demonstrated to have anticarcinogenic activity (Wattenberg, 1985). Van Duuren et al. (1976) reported that the flavonoids, rutin, morin, and quercetin inhibited tumorigenesis by B(a)P in mouse skin (Slaga et al., 1978). Mukhtar et al. (1988) have investigated the effects of a number of plant phenols on carcinogen metabolism and tumorigenesis in mouse skin. The two flavonoids tested, quercetin and myricetin, showed similar activity in inhibiting B(a)P metabolism and B(a)P DNA adduct formation in SENCAR mouse epidermis (Das et al., 1987). Both flavones also demonstrated activity in inhibiting complete carcinogenesis by hydrocarbons such as B(a)P and 3-methylcholanthrene, and also by the direct-acting carcinogen N-methyl-N-nitrosourea (Mukhtar et al., 1988). Some flavonoids have also been shown to inhibit tumor promotion (Fujiki et al., 1986; Kato et al., 1983; Nishino et al., 1984). Quercetin inhibited tumor promotion by 12-0-tetradecanoylphorbol-13-acetate in a mouse skin assay. One mechanism by which plant flavonoids can inhibit the activity of ultimate carcinogenic metabolites is direct reaction with these compounds resulting in their detoxification (Huang et al., 1983). The ultimate carcinogenic metabolite of B(a)P -B(a)P-7, 8-diol-9, 10 epoxide- was shown to undergo rapid reaction with the plant phenol ellagic acid.

It was reported that biochanin A, an isoflavone, significantly inhibited the metabolism of B(a)P and decreased the level of binding of B(a)P to DNA in hamster embryo cell culture (Cassady et al., 1988; 1990). Biochanin A is considered to affect at least 2 metabolic pathways in hamster embryo cells: the oxidation of B(a)P by cytochrome P450 to form compounds such as the dihydrodiols, and the conjugation of B(a)P phenols to glucuronides. In studies of the effect of biochanin A on the binding of B(a)P to DNA, it was demonstrated that biochanin A treatment resulted in a decrease in the total level of binding at all time points tested between 24 and 120 hrs. This decrease resulted from a decrease in formation of both the (+)-*anti*-B(a)P diol-epoxide and the *syn*-B(a)P diol-epoxide. This isomer of the diol-epoxide is the one with the highest carcinogenic activity in rodent bioassays.

In conclusion biochanin A strongly inhibits B(a)P-induced carcinogenesis not only in an in vitro system but also in an in vivo mouse pulmonary system. However elucidation of this antipromotion mechanism would require further study.

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