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Inhibition by Guan-mu-tong (*Caulis aristolochiae manshuriensis*) of the growth of spontaneous mammary tumors in SHN virgin mice

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Multiparous SHN mice with spontaneous mammary tumors (5–10 mm in size) were given water extract of Guan-mu-tong (Gmt; *Caulis aristolochiae manshuriensis*) (0.5%) *ad libitum* as drinking water for 10 days. This treatment retarded significantly the growth of mammary tumors compared with the controls. By contrast, normal mammary gland growth, histology of adrenals and ovaries, and body weight were affected little by the Gmt treatment. Gmt appears to be the first agent inhibiting the growth of spontaneous mouse mammary tumors of palpable size by *per os* treatment.

Key words: Chinese herbal medicine, Guan-mu-tong, mammary tumor, mice, natural products.

Introduction

In a previous study, we found that Guan-mu-tong (Gmt; *Caulis aristolochiae manshuriensis*) markedly inhibited the formation of precancerous mammary hyperplastic alveolar nodules in mice.[1] In this paper, the effects of Gmt on the growth of palpable mammary tumors in mice were studied.

Materials and methods

Samples

Five hundred gram of dry matter of stalk of Gmt from the North-East area of China was extracted 10 times with 1 l of hot water (60°C), and supernatants were pooled and dried *in vacuo*. The dry matter was dissolved with tap water at a concentration of 5 g/l.

Animals

The SHN inbred strain of mice with a high mammary tumor potential[2,3] and maintained by strict brother × sister mating in this laboratory was used. Female mice were placed at 2 months of age with males and were retired after the second or third concurrent pregnancy and lactation. Each retired female mouse was checked for palpable mammary tumors every 7 days. The major two diameters of mammary tumors were measured by a caliper and their geometric mean was employed as an index of tumor size. When the sizes of the first tumors reached 5–10 mm, the mice were divided into two groups (the experimental and control groups) and these groups were given a 0.5% solution of Gmt and tap water, respectively. After 10 days of treatment, the mice were killed by decapitation under light ether anaesthesia after checking the final size of the first tumor and the total number of tumors. Blood was collected from the trunk; it was clotted for 8 h at room temperature and overnight at 4°C followed by centrifugation at 1000 ***g*** at 4°C for 20 min. Serum was stored at −20°C for assay of free fatty acid.

Mice were kept in individual Teflon cages during pregnancy and lactation, and at four to five per cage after retirement. They were maintained in a windowless animal room, which was air-conditioned (20–22°C in temperature and 60–70% in relative humidity) and artificially illuminated (15 h of light from 5.00 to 19.00 h). The mice were given a commercial diet (LAB MR Breeder; Nihon Nosan Kogyo KK, Yokohama, Japan) and water *ad libitum*.

Growth of mammary tumor

The percentage change in the size of the first mammary tumor was calculated as the index of the

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growth of tumors according to the following formula:

$$\% \text{ change of tumor size (TS)} = \frac{\text{TS at the end of exp} - \text{TS at the start of exp}}{\text{TS at the start of exp}} \times 100$$

Transforming growth factor-α (TGF-α) mRNA expression in mammary tumor

At autopsy, a portion of tumor tissue free from necrotic changes was immediately frozen and kept at −70°C. TGF-α expressed in the mammary tumors was determined by the reverse transcriptase-polymerase chain reaction (RT-PCR) method. All procedures were essentially the same as detailed elsewhere.[4]

Growth of normal and preneoplastic mammary glands

The bilaterial third thoracic glands were prepared for whole-mount evaluation and examined under 10-fold magnification. The degree of development of end-buds or lobulo-alveoli was rated from 1 to 7 in increments of 1[5] and the area bounded by the straight lines of the tops of ducts was also measured by a computerized digitizer (Model LA-535; PIAS, Tokyo, Japan) as an index of mammary duct extension. The means in the bilateral glands of the parameters were used as the values of the individual.

Furthermore, the number of preneoplastic mammary hyperplastic alvelolar nodules (HAN) in the bilateral glands were counted and the area of each HAN was also measured by the digitizer.

Serum free fatty acid level

The level of serum free fatty acid was determined by the acyl-CoA oxidase method (NEFA C-test; Wako Pure Chemical, Osaka, Japan).

Endocrine organ weights and histology

At autopsy, anterior pituitary, adrenals and ovaries were immediately removed and weighed. Adrenals and ovaries were further examined histologically.

Statistics

All values were expressed in terms of mean ± SEM and the statistical significance of difference between groups in each parameter was evaluated by Student's *t*-test.

Results

Growth of mammary tumors

At the start of the experiment, there was no significant difference in mammary tumor size between the control and the experimental groups (8.3 ± 1.2 and 7.2 ± 0.9 mm, respectively). However, the tumor size at the end of the experiment was significantly lower in the experimental group than in the control (9.8 ± 1.1 mm for Gmt treated mice versus 15.3 ± 2.3 mm for untreated controls). The percentage changes of the sizes of tumors during the experiment (10 days) were 86.6 ± 12.1 and 41.5 ± 6.9% in the control and the experimental groups, respectively, between which the difference was statistically significant (Figure 1). The number of tumors per mouse was one or two in each group.

Growth of normal and preneoplastic mammary glands (Figure 2)

Little differences were observed between groups in the rating and the area of normal glands and area of HAN. On the other hand, the number of HAN was significantly higher in the experimental group (41.9 ± 4.8) than in the control (28.9 ± 2.9).

Body weight change, weights and histology of endocrine organs and serum free fatty acid level

As shown in Table 1, body weight changes during the experiment were little and the difference from zero was statistically not significant in both groups.

Weights of anterior pituitary, adrenals and ovaries did not differ significantly between groups, and no marked difference was observed in the histological structures of adrenals and ovaries.

Little difference between groups was also seen in serum free fatty acid level.

TGF-α mRNA expression in mammary tumors

RT-PCR revealed that TGF-α mRNA expression including the different sizes of bands observed in the mammary tumors of mice[6] (Figure 3) was affected little by the treatment (Table 2).

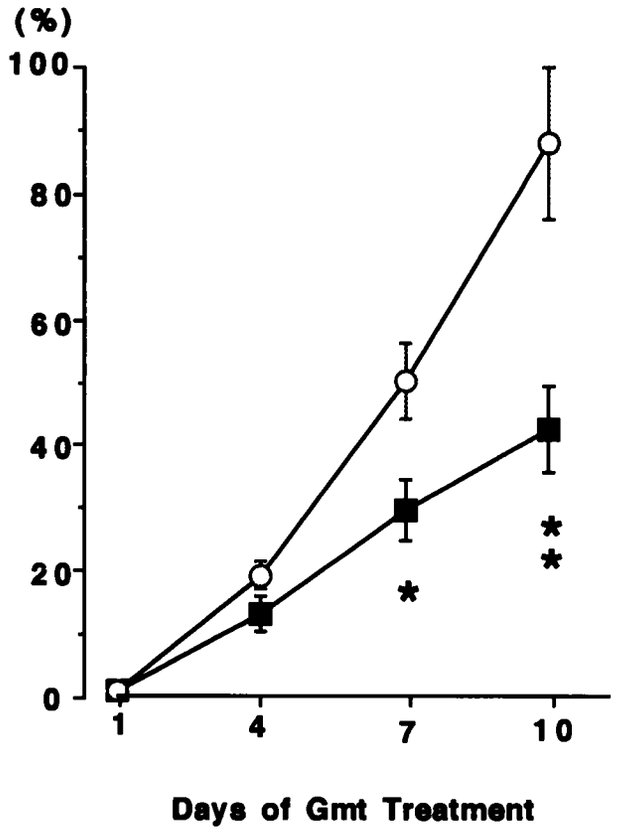

Figure 1. Percentage change of mammary tumors in the experimental (■) and the control (○) groups (mean ± SEM). Number of tumors examined was nine from eight mice for the experimental group and 11 from nine mice for the control group (one and two mice in the experimental and the control groups, respectively, had two tumors at the start of the experiment). $^{*}p < 0.05$ or $^{**}p < 0.01$.

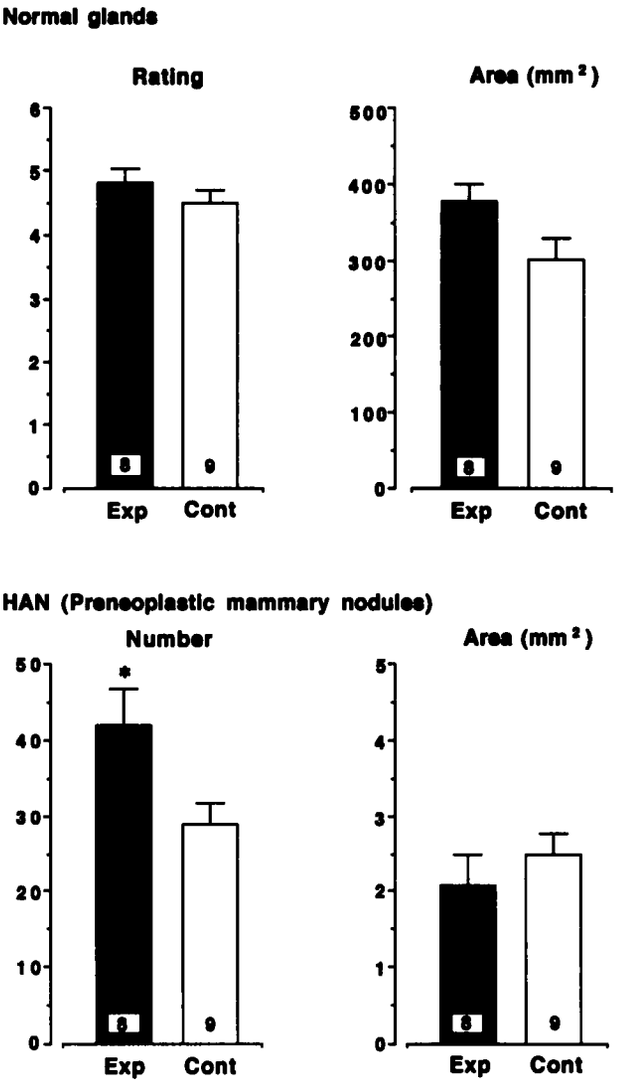

Figure 2. Normal and preneoplastic mammary gland growth in each group (mean ± SEM). Number of estimates is in the column. $^{*}p < 0.05$.

Discussion

The present study has demonstrated that *ad libitum* access of Gmt apparently inhibited the growth of spontaneous mammary tumors in mice. Gmt seems to be the first agent showing anticancer effects on this type of tumor, which is one of the most resistance to any therapeutic agents. Thus, Gmt might be a promising therapeutic agent of cancers. While the mechanism of the effects remains to be determined, the stimulation of metabolism by Gmt would contribute at least partly to this process; several specified and unspecified component levels in the urine were much higher in mice given Gmt than in the control (Wu *et al.*, unpublished).

Table 1. Body weight change, endocrine organ weights and serum free fatty acid (NEFA) level in each group (mean ± SEM)

Group and treatment	No. of mice	Body weight			Endocrine organ weights (mg/30 g body weight)			Serum NEFA (mEq/l)
		initial (g)	final (g)	change (%)	anterior pituitary	adrenals	ovaries	
Experimental	8	33.2 ± 0.8	31.8 ± 1.1	−1.6 ± 2.9	2.26 ± 0.3	10.86 ± 0.5	26.30 ± 2.5	0.101 ± 0.01
Control	10	32.8 ± 1.1	33.6 ± 1.6	2.5 ± 3.0	2.65 ± 0.2	12.44 ± 0.9	31.06 ± 3.0	0.086 ± 0.05

Table 2. TGF-α mRNA expression in the mammary tumors in each group

Group and treatment	No of tumors	No. of tumors showing	
		TGF-α mRNA expression	different sizes of bands
Experimental	6	6	5
Control	6	6	5

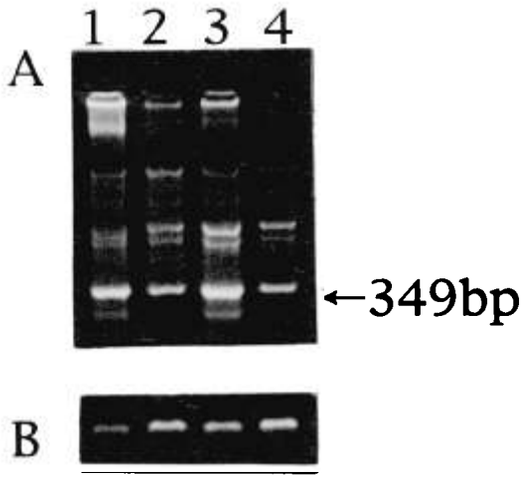

Figure 3. Agarose gel electrophoresis of RT-PCR products in TGF-α of the experimental (lanes 1 and 2) and the control (lanes 3 and 4) groups. Panels A and B indicate the results of mammary tumors and β-actin primers, respectively.

In the previous work,[1] the growth of both normal and preneoplastic mammary glands was inhibited by Gmt, but this was not seen in the present work. This would principally be due to the difference in the treatment periods (63 versus 10 days). It is curious that the number of HAN was significantly higher in the experimental group than in the control in this study. The number of HAN in the experimental group might have already been higher at the start of the Gmt treatment, but this was impossible to confirm.

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