

Suppression by a Pine Cone Extract of *Pinus parviflora* Sieb et Zucc of Mammary Tumour Virus in Milk of Mice

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Abstract. The intravenous or the oral administration of a pine cone extract of *Pinus parviflora* Sieb et Zucc (Fr VI) and the related synthetic agent (DHP-FA) to lactating SHN mice prevented an increase of milk levels of mouse mammary tumour virus (MMTV) from day 7 to day 14 of lactation. Furthermore, Fr VI decreased the MMTV level at the 2nd lactation compared to the 1st lactation. This is the first report on the inhibition of milk MMTV of mice in the *in vivo* system.

The hot water-extract of the cones of pine trees, especially *Pinus parviflora* Sieb et Zucc, is known traditionally as a herbal medicine effective against some types of cancers.

Sakagami and his colleagues have found that some fractions of hot water- or NaOH-extract of pine cone of this species has immunopotentiating activity (1-3). Furthermore, some fractions have potent antiviral activity against human immunodeficiency virus (4), herpes simplex virus (5) and influenza virus (6). It has also been reported that the synthetically polymerized phenylpropenoids have a similar antiviral activity (7). These were recently reviewed by Sakagami *et al* (8).

It is well known that mouse mammary tumour virus (MMTV) transmitted through milk is a major factor in spontaneous mammary tumour development of this species.

In this paper, we have examined the anti-MMTV activity of the most potent extract fraction of pine cone of *Pinus parviflora* Sieb et Zucc (Fr VI) and the related synthetic agent (DHP-FA).

Materials and Methods

Samples. Fr VI and DHP-FA were prepared as detailed previously (Refs

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1 and 7, respectively). Each agent was dissolved with tap water or physiologic saline for oral (*po*) or intravenous (*iv*) administration. The concentrations of DHP-FA and Fr VI were 10 and 1 mg/liter for *po* or 1.75 and 0.175 mg/0.1 ml for *iv* respectively.

Animals and treatment. The mice used were a high mammary tumour strain of SHN (9, 10) maintained by the authors' laboratory by strict brother x sister mating. At 2 months of age, 3 to 4 females were placed with males and pregnant animals were kept individually. They were placed again with males near parturition in order to induce concurrent pregnancy, and only animals that achieved concurrent pregnancy and lactation were used in this study. On the day of parturition (day 0 of lactation), the number of pups was adjusted to 7-8 and pups were normally nursed until day 20 when they were weaned.

The administration schedule of each agent is illustrated in Figure 1. In *po* treatment groups, the agents were given as drinking water throughout the experiment beginning on day 0 of lactation. In *iv* treatment groups, the agents (0.1 ml) were injected into the tail veins for 3 consecutive days beginning on days 0, 7 and 14 of lactation under light ether anaesthesia in both the 1st and the 2nd lactations.

Milking. Each lactating mouse was milked by the method of Nagasawa (11) on the morning of days 7 and 14 of each lactation after 18 hours separation from pups by wire netting and milk was immediately stored at -80 C for assay of MMTV.

MMTV assay. MMTV-gp52 antigen was estimated by radioimmunoassay as detailed by Imai *et al* (12).

Mammary tumourigenesis in offspring. Female offspring of mothers in each group were checked for palpable mammary tumours every 7 days beginning at 3 months of age.

Statistics. Statistical significance of difference in MMTV antigen levels between the experimental groups and the corresponding controls was evaluated by the Student's *t*-test, and for within individual difference of the parameter the paired-sample test (13) was used.

Results

The results of the milk MMTV levels are presented in Figures 2 and 3. In the control, the level was increased on day 14 of lactation compared to day 7. On the other hand, there was no significant difference in MMTV levels between days 7 and 14 in all experimental groups (Figure 2).

Little difference was observed in milk MMTV level be-