

Inhibitory Effect of Lignin-Related Pine Cone Extract on Cell Proliferating Enzyme Activity of Spontaneous Mammary Tumours in Mice

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Abstract. A lignin-related cone extract of pine (*Pinus parviflora* Sieb et Zucc) (FrVI) or a synthetic lignin (DHP-FA) (175 µg/0.1 ml 0.9% NaCl solution) was injected intravenously to SHN mice bearing spontaneous mammary tumours three cycles each with consecutive 3 days of treatment and 4 days of interruption. Activities of both thymidylate synthetase (TS) and thymidine kinase (TK), i.e., DNA synthesizing enzymes in de novo and salvage pathways of pyrimidine metabolism, respectively, were apparently decreased in mammary tumours of FrVI-treated mice compared to those of the control mice bearing tumours without treatment. While the percent change of mammary tumour size during the experiment differed little among groups, both FrVI and DHP-FA prevented tumours from ulceration. Furthermore, the development and growth of preneoplastic mammary hyperplastic alveolar nodules were decreased by the treatments of both agents. They showed no toxicity. All results suggest that these lignin-related compounds, especially FrVI, may be useful as chemopreventive agents, with some improvement of administration method, and/or for employment in combination with any other agents.

Lignin-related fractions of hot water- or NaOH-extract of cone from pine (*Pinus parviflora* Sieb et Zucc), which has been used as a herbal medicine for the treatment of some types of tumours including gastric cancer, have been reported to show antimicrobial (1), antiviral (2) and antimitogenic (3) activities. Furthermore, they elongated the life-span of mice bearing Sarcoma 180 and inhibited the growth of Meth A fibrosarcoma (4). These reports were recently reviewed by Sakagami *et al* (5).

Activities of both thymidylate synthetase (TS) and thymidine kinase (TK), i.e., DNA-synthesizing enzymes in *de novo*

and salvage pathways of pyrimidine metabolism, respectively, are elegant indicators for estimating the proliferating activity of the cells (6,7).

In this paper, we examined the effects of FrVI and DHP-FA on DNA-synthesizing enzyme activities in spontaneous mammary tumours of mice as a possible step to evaluate the antitumour activity of these agents.

Materials and Methods

Samples. FrVI and DHP-FA were prepared as previously detailed (2, 4). They were donated by Prof. Kawazoe and Dr. Sakagami and were dissolved with 0.9% NaCl solution at the concentration of 1.75 mg/ml.

Animals and treatments. Virgin and retired female SHN mice maintained in our laboratory were used. They were checked for palpable mammary tumours every 7 days and mice bearing tumours of 6-8 mm in size were divided into 3 groups. The 1st group received the vehicle only and served as the control. The 2nd and the 3rd groups were injected with 0.1 ml FrVI or DHP-FA daily into the tail vein under light ether anaesthesia for 3 cycles, each with 3 consecutive days of treatment and 4 days of interruption. Mice were killed on the morning after the last injection and the size of the first tumours and the total number of tumours were recorded. The first tumours were immediately kept at -80° C.

Mice whose tumours were ulcerated during the experiment were discarded.

Mammary tumour growth. Percent change of mammary tumour size expressed in terms of the geometric mean of the major two diameters was used as an index of tumour growth.

Normal and preneoplastic mammary gland growth. Immediately after killing, bilateral third thoracic glands were prepared for the wholemount evaluation and examined under 10-fold magnification. The degree of end-bud formation was rated from 1 to 7 in increments of 1. The number of preneoplastic mammary hyperplastic alveolar nodules (HAN) was counted and their sizes were also measured by the digitizer.

Endocrine organ weights. At autopsy, anterior pituitary, adrenals and ovaries were removed and weighed.

TS and TK activities. The mammary tumour specimens were pulverized with an autopulverizer in liquid nitrogen and homogenized at 0° C with 10 volumes of a 5 mM Tris-HCl buffer (pH 7.5) containing 0.1 mM EDTA, 1 mM mercaptoethanol and 0.25 M sucrose at the final concentrations. The homogenates were centrifuged for 1 hr at 4° C at 105,000 xg and the supernatants were used as the enzyme preparations. TS and TK activities

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