Short communication

A Preliminary Study of the Effect of *Plantago Ovata* Forsk on the Development of 7, 12-Dimethylbenz[a]anthracene-initiated Rat Mammary Tumors under the Influence of Hypercholesterolemia

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Abstract: As a preliminary study to elucidate whether *Plantago ovata* forsk (PO) exhibits inhibiting effects on mammary carcinogenesis under a condition of hypercholesterolemia, 50 female Sprague-Dawley rats were first given a single intragastric dose of 200 mg/kg body weight of 7, 12-dimethylbenz[a]anthracene (DMBA). Ten of 50 rats died of acute toxicity of DMBA within one week. From one week later, the survived animals were divided into 4 groups: 13 rats in group 1 and 7 in group 2 received high cholesterol diets (HC) with and without 5% PO supplementation for 26 weeks, respectively. Eleven rats in group 3 and 9 in group 4 received basal diet (BD) with and without 5% PO supplementation for the same period, respectively. One to three rats in groups 1 to 4 died of mammary tumors, lymphomas and/or adrenal impairment attributable to DMBA treatment during the treatment period. Group 1 showed a tendency to decrease in the number of palpable mammary masses as compared with group 2, whereas group 3 showed a tendency to higher values compared with group 4. At the termination of the study, the serum levels of total cholesterol in group 1 were significantly lower than those in group 2 and the number of mammary masses was significantly decreased. Histopathologically, this decrease was mainly due to the decreased incidences of mammary adenocarcinomas, while no significant difference in the multiplicity of mammary tumor was observed between BD+PO and BD alone groups. The results of the present study suggest a possibility that PO exerts inhibiting effects on mammary carcinogenesis by decreasing circulating cholesterol levels in a rat two-stage mammary carcinogenesis model. (*J Toxicol Pathol* 1999 ; 12 : 141~145)

Key words: Mammary carcinogenesis, High cholesterol diet, *Plantago ovata* forsk, 7, 12-Dimethylbenz[a]anthracene, Rat

Introduction

Breast cancer has become a major cause of tumor death in women with a dramatic increase since the 1960s¹. The disease occurs more frequently and with a higher mortality rate in countries where people have a high intake of dietary fat². Because of the epidemiologic association of coronary heart disease with cancers of the breast and bowel, it has been suggested that cholesterol may be a risk factor in the pathogenesis of these neoplasms³. Indeed, there is good evidence that high dietary cholesterol intake may be involved in mammary carcinogenesis in humans⁴, although the results of animal studies regarding promoting effects of serum cholesterol have not been consistent^{5,6}.

Plantago ovata forsk (PO), powdered husk of the seed of *Plantago ovata*, also called as ispaghul or psyllium mucilloid, has been widely used as a stool bulking agent for the treatment of constipation^{7,8}. Since PO contains a high proportion of cellulose, hemicellulose and soluble polysaccharide, it has been also documented to exert mucosal-protective

effects⁹, stimulate fecal bile acid excretion^{9,10}, and lower total and low-density-lipoprotein cholesterol in man^{11,12}.

It is therefore conceivable that mammary carcinogenesis enhanced by the ingestion of high-fat diet could be inhibited by the ingestion of sufficient PO through a circulating cholesterol-lowering effect. The present preliminary study was performed to clarify whether relatively high amounts of PO exhibit modifying effects on mammary carcinogenesis under hypercholesterolemic conditions in a rat two-stage mammary carcinogenesis model after initiation with 7, 12-dimethylbenz[a]anthracene.

Materials and Methods

Chemicals

Plantago ovata forsk (PO) was yellow to tan powdered, water-soluble fiber (purity: 99% or more) obtained from the seed of *Plantago ovata*, and was kindly provided by Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). 7, 12-Dimethylbenz[a]anthracene (DMBA) was purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, U.S.A.).

Animals

Female Sprague-Dawley rats, 6 weeks old, were purchased from Charles River Japan Inc. (Atsugi, Japan) and housed at a maximum of 5 to a polycarbonated cage with

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white chips as bedding in an air-conditioned animal room (room temperature, $23 \pm 2^\circ\text{C}$; relative humidity, $55 \pm 5\%$; lighting cycle, 12 hr. light/12 hr. dark cycle). Animals without any abnormal findings after a 1-week acclimatization period were selected for the present study. They were allowed free access to a high cholesterol diet (Oriental CRF-1 containing 2.0% cholesterol and 0.3% cholic acid, Oriental Yeast Co., Ltd., Tokyo, Japan) or a basal diet (Oriental CRF-1) throughout the study. Tap water was also available *ad libitum*.

Experimental design

After a 1-week acclimatization period, 50 rats were given a single intragastric dose of 200 mg/kg body weight of DMBA dissolved in corn oil according to the modified method of Huggins *et al.*¹³. Within one week, 10 died of DMBA acute toxicity. The number of rats in groups 1 and 3 were increased as compared to groups 2 and 4, because there might be a possibility that the mortality would increase by the supplementation of PO. Therefore, the remaining animals were randomly divided into groups 1 to 4 consisting of 13, 7, 11 and 9 rats, respectively (Fig. 1). Groups 1 and 2 received a high cholesterol diet (HC) with and without 5% PO for 26 weeks, respectively. Groups 3 and 4 were the equivalents receiving the basal diet (BD). After 11 weeks, first palpable mammary masses appeared. Their numbers and incidences were then recorded on a weekly basis. At the end of the scheduled treatment period, blood samples were

taken from the abdominal artery under light ethyl ether anesthesia from all the surviving rats to determine levels of total cholesterol and low-density lipoprotein (LDL). LDL was separated according to the method of Hatch and Lees¹⁴, and serum cholesterol and LDL concentrations were measured with enzymatic calorimetric method (Monotest cholesterol, Chod-PAP, method, Boehringer Mannheim GmbH, Mannheim, Germany). Then, all the surviving animals were autopsied and mammary tumor masses were dissected, fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4–5 μm , and stained with hematoxylin and eosin for microscopic examination.

Statistical analysis

Data for final body weights, serum cholesterol and LDL levels, numbers and diameters of mammary tumor masses, and final multiplicity of mammary tumors were used to generate mean and standard deviation values, and intergroup differences were then analyzed with the Student's *t* test. The incidences of mammary tumor masses were compared using the Fisher's exact probability test.

Results and Discussion

During the treatment period from week 1 to 27, 2 of 13 rats in the HC+PO group, 3 of 7 in the HC alone group, 2 of 11 in the BD+PO group and 3 of 9 in the BD alone group died or were killed in moribund condition because of the aggravation of general condition due to the rapid growth of mammary tumors, adreno-cortical impairment and/or lymphomas that were attributable to DMBA treatment.

The final body weights of rats in the HC+PO, HC alone, BD+PO and BD alone groups at week 27 were 357.73 ± 72.47 , 335.50 ± 57.53 , 357.90 ± 57.72 and 319.40 ± 31.52 g, respectively, non-significant tendencies for increase being observed with PO.

In the time-course observation by palpation, the mammary masses were observed in 1/13, 4/7, 2/11 and 1/9 in the HC+PO, HC alone, BD+PO and BD alone groups, respectively, from week 12. Significantly decreased incidences of mammary masses were seen in the HC+PO as compared to the HC alone group at weeks 12 and 13, this continuing as a tendency until the end of the experiment. Incidence data (%) for BD+PO and BD alone groups showed no significant difference (Fig. 2).

Fig. 1. Experimental design

* : Significantly different from the HC alone group value at $P < 0.05$.

Fig. 2. The time-course of palpable mammary mass development in rats treated with and without HC and PO after DMBA treatment

Fig. 3. Numbers of palpable mammary masses per rat treated with and without HC and PO with time after DMBA treatment

Fig. 4. Values for serum cholesterol and low-density-lipoprotein in rats treated with PO for 26 weeks after DMBA treatment

Fig. 5. Microscopic characteristics of mammary fibroadenoma in a rat treated with HC+PO after DMBA initiation treatment H.E stain $\times 220$

The HC+PO group also exhibited a tendency for decrease in the number of palpable mammary masses/rat, as compared to the HC alone group, with significant differences at weeks 18 and 19. On the contrary, the BD+PO group showed a tendency to increase as compared to the BD alone group, with significant differences at weeks 20 and 23 (Fig. 3).

At the terminal examination, the serum levels of total cholesterol were significantly decreased in the HC+PO group as compared to the HC alone group, and showed a tendency for decrease in BD+PO group as compared to the BD alone group. Serum levels of low-density-lipoprotein also tended to decrease in HC+PO and BD+PO groups as compared to HC and BD alone groups, respectively (Fig. 4). There were no significant inter-group differences in the size, mammary (Table 1). The multiplicity of mammary masses in the HC+PO group was significantly lower than that in the HC alone group (Table 2). Histopathologically, the mammary tumors were fibroadenomas (Fig. 5), adenomas (Fig. 6) and adenocarcinomas (Fig. 7), the numbers of the latter in the

HC+PO group being significantly decreased as compared to the HC alone group value (Table 3). There was no significant difference in the numbers of mammary tumors between BD+PO and BD alone groups.

In the present study, 10 of 50 rats died of the acute toxicity of DMBA used as an initiation treatment and some animals in each group became moribund because of the occurrence of mammary tumors, lymphomas and adrenal impairment that are attributable to DMBA treatment. Such an increased mortality in each group after the DMBA treatment indicates that the dosage of DMBA was too high to examine the modifying effect of PO on mammary carcinomas. However, the present results suggest a possibility that PO exerts suppressive effects on DMBA-induced mammary carcinogenesis in female SD rats fed a high cholesterol diet, possibly linked to reduction in serum cholesterol. Histopathological examination further revealed a decreased

Table 1. Sizes of Mammary Masses in Rats Treated with and without HC and PO for 26 Weeks after DMBA Initiation and Killed at Termination

PO supplement	Group			
	HC		BD	
	No. of rats examined	Diameter of tumor masses (mm)	No. of rats examined	Diameter of tumor masses (mm)
Yes	11	16.3±13.8 ^{a)}	10	14.6± 8.7
No	4	16.0±11.1	6	14.8±10.9

^{a)}: Mean value (mm)±S.D.

Table 2. Incidences and Multiplicities of Mammary Masses in Rats Treated with and without HC and PO for 26 Weeks after DMBA Initiation and Killed at Termination

PO supplement	Group					
	HC			BD		
	No. of rats examined	No. of rats with mammary masses	No. of tumors/rat	No. of rats examined	No. of rats with mammary masses	No. of tumors/rat
Yes	11	8	3.00±1.84 ^{a)} *	10	9	6.60±6.10
No	4	4	9.00±3.56	6	5	2.17±2.93

^{a)}: Mean values±S.D.

*: Significantly different from the value of HC alone group at $P<0.05$.

Fig. 6. Microscopic characteristics of mammary adenoma in a rat treated with HC+PO after DMBA initiation treatment H.E stain ×220

Fig. 7. Microscopic characteristics of mammary adenocarcinoma in a rat treated with HC after DMBA treatment H.E stain ×220

number of mammary tumors in the HC+PO group in accordance with the gross findings, with a significant difference in adenocarcinomas. On the contrary, it is speculated that the actual intake of HC in the HC+PO group is lower than that in the HC alone group, since the test diet containing 5% of fibers consisting of PO was given to the HC+PO group. Therefore, we must also take into account the possibility that such a decreased intake of HC probably inhibits the mammary carcinogenesis in the HC+PO group.

Psyllium, a water-soluble, gel-forming fiber derived from the husks of blonde psyllium seeds, belongs to a group of fibrous materials, which include gums, pectins, mucilages, and certain hemicelluloses, that show cholesterol-lowering effects when added to patient's diets¹⁵. While the mechanism by which PO influences cholesterol is currently un-

known¹⁶, the available information suggests one or more of the following may be operative. First, certain soluble fibers are known to increase the fecal excretion of bile acids by preventing their normal reabsorption^{7,17}. A second possible mechanism is the fermentation of soluble fiber into short-chain fatty acids by colonic bacteria, which may secondarily decrease hepatic cholesterol synthesis¹⁸. Thirdly, soluble fibers that bind acids may interfere with micelle formation in the proximal small bowel, resulting in alterations in the quantity of cholesterol or fatty acids absorbed by the intestine¹⁹. It has been reported that treatment with hydroxyethylcellulose or konjac mannan results in low circulating cholesterol levels by this mechanism²⁰.

It has been proposed that the carcinogenic process can be efficiently modulated by changing food consumption, such

Table 3. Incidence and Multiplicities of Mammary Tumors/Animal in Rats Treated with and without HC and PO for 26 Weeks after DMBA Initiation and Killed at Termination

Group	No. of rats examined	No. of rats with mammary tumors	Fibroadenoma	Adenoma	Adenocarcinoma
HC+PO	11	8	0.27±0.47 ^{a)}	0.27±0.47	2.36±1.43*
HC	4	4	0.50±0.58	1.25±0.96	7.00±2.71
BD+PO	10	9	0.50±0.97	0.90±1.20	4.60±4.97
BD	6	5	0	0.17±0.41	2.00±3.03

^{a)}: Mean values ±S.D.

*: Significantly different from the value of HC alone group at $P < 0.05$.

as cutting down intake of cholesterol- and lipid-rich food stuff. With colon carcinogenesis, it has been documented that high lipid diet enhances colon carcinogenesis, while a fiber-rich diet suppresses²¹. The present results are probably in line with the literature, suggesting that high cholesterol could act as a promoter, whilst foods with high fibers, like PO, could act as inhibitors by depressing serum cholesterol.

In the experiment of female rats fed basal diets, PO showed a tendency to enhance the number of mammary tumors. The BD+PO group ingested more dietary fibers than BD alone group because the main component of PO was composed of fibers such as cellulose and hemicellulose. Thus, dietary intakes in the BD+PO group are speculated to be greater than those in the BD alone group because the mean body weight in the BD+PO group was higher than that in the BD alone group. Consequently the amount of energy intake would have been greater in the BD+PO group than in the BD alone group. This may explain a tendency of increased mammary tumors caused by BD+PO treatment, although the effect of PO on mammary carcinogenesis in female rats fed basal diet cannot be clarified completely. This question requires further study, since the effective numbers of animals was reduced because relatively many rats died of the high dose of DMBA initiation treatment. A new investigation with a different protocol might be warranted.

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