Effects of Dietary Polyamines on the Promotion of Mammary Tumor in Rats

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The effects of dietary polyamines have been investigated on 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP)- induced carcinogenesis of the breast in rats by feeding spermidine (Spd) at three different concentrations. The cumulative incidence of mammary tumor in the group treated with PhIP plus Spd was 92% (low spermidine diet) and 68% (high spermidine diet), compared to 50% in the PhIP group with a control diet. These results suggest that dietary polyamines may enhance the promotion of PhIP- induced mammary carcinogenesis. In addition, however, a low spermidine diet can promote the development of tumor in rats, but on the other hand a high spermidine diet may suppress the mammary carcinogenesis.

Key words —— carcinogenesis, 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine, polyamine, spermidine

INTRODUCTION

Polyamines are widely known to have various functions. They promote nucleic acid and protein synthesis, and stabilize cell membranes *in vivo*. While polyamines promote cancer, they are also reported to have functions of DNA stabilization and antioxidative properties which are related to cancer control.[1–6] We are especially interested in polyamines taken from food in terms of their antioxidative properties and cancer.

We reported that polyamines are mainly included in soybeans, green tea and fermented foods.[7] These foods are traditionally ingested by the Japanese. From these facts, the Japanese are thought intake a large quantity of polyamines from their traditional diet. The physiological effect of polyamines found in food, however, is unclear. Epidemiological study has shown that the incidence of cancer is lowered by ingesting food which contains a high proportion of polyamines. Identification of the components effective in the cancer control, such as polyphenols and flavonoids have also been studied. There is no detailed study, however, examining whether polyamines included in the food suppress cancer. In this context, we examined the effect of polyamine ingestion as a food ingredient on carcinogenesis of the breast in rats.

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MATERIALS AND METHODS

Male Sprague-Dawley rats, 6 weeks old, weighing166 ± 1.57 g, were assigned to groups of 28 rats. 2-Amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP) was used as a carcinogen. It is a heterocyclicamine compound with known mammary tumor induction. PhIP was administered orally at a dose of 85 mg/kg body weight, 8 times for 40 weeks. The breeding plan is shown in Fig. 1. Spermidine hydrochloride (Spd: Sigma Chemical Co., U.S.A.) was used for the experiment. The Spd dosage included in the experimental diet was referenced by the content of Spd in foods or the intake of polyamines in foods. It was calculated on the basis of the calculation standard shown Table 1. The standard is the amount of 1-day intake of Spd. The experimental compositions are shown in Table 2. Diets of polyamine-free (control population: C), 0.035% Spd addition (low-feed group: L), and 0.175% Spd addition (high-feed group: H) were used. All food components, except for the polyamine itself, were polyamine free. The diets included 20 g of food daily. Body weight and oncogenesis number by palpation was measured during the experi-

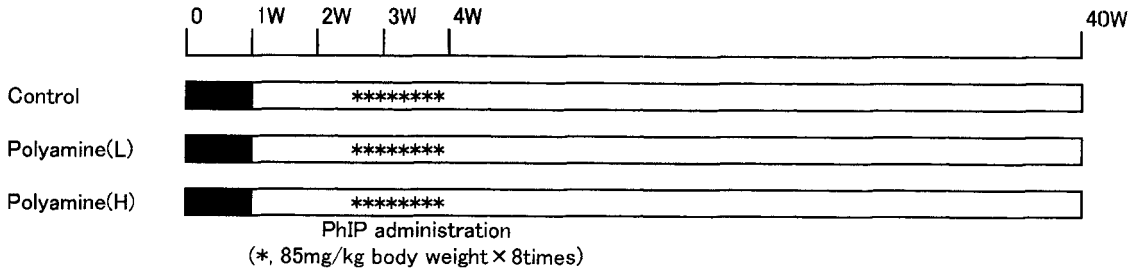

Fig. 1. Plan of the Animal Experiments
Control: polyamine free diet. Polyamine (L): Supplied 0.035% Spd in the diet. Polyamine (H): Supplied 0.175% Spd in the diet.

Table 1. Calculation Standard of the Polyamine Dosage*

Daily uptake (as spermidine) in Human

Food	Spd (nmol/g)	Food intake (g)	Spd intake (μmol)
Rice	30	500	15
Soybean	2400	100	240
Animal meat	400	200	80

Total: about 330 μmol /day /adult

↓

Rat: about 0.55 μmol (80 μg Spd) /day /rat

50 times of daily uptake: 4 mg Spd /day /rat = 7 mg Spd·3HCl /day /rat

7 mg Spd·3HCl /20 g diet = 0.035%

250 times of daily uptake: 20 mg Spd /day /rat = 35 mg Spd·3HCl /day /rat

35 mg Spd·3HCl /20 g diet = 0.175%

*In the normal rat solid feed, including Spd 20 μg/day /rat. This is correspondent to about 36 times a human intake.

Table 2. Diet Ingredients

Ingredient	Control	Polyamine (L)	Polyamine (H)
		%	
Casein	20.0	20.0	20.0
Wheat starch	50.3	50.3	50.1
Olive oil	20.0	20.0	20.0
Cellulose	5.0	5.0	5.0
Mineral mixture (AIN-76)	3.5	3.5	3.5
Vitamin mixture (AIN-76)	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2
Spermidine·3HCl	0.0	0.035	0.175

mental period, and the accumulation oncogenesis rate was calculated. In addition, liver, spleen and tumor weight were measured.

The significance of intergroup differences was tested by one-way ANOVA using SPSS (SPSS Japan, Inc., Japan), and then Duncan's multiple range test was performed for parametric analysis at $p < 0.05$. In the case of heteroscedasticity, the Kruskal-Wallis test was used for non-parametric analysis at $p < 0.05$.

RESULTS AND DISCUSSION

Body weight gain, liver and spleen weight in breeding for 40 weeks, by giving each diet to the PhIP administration rat, are shown in Table 3. The accumulative oncogenesis rate is shown in Fig. 2. Mammary tumor development is shown in Table 4. Though the body weight gain was higher in the L and H groups than in the C group, there was no significant difference. A similar tendency was observed

Table 3. Body Weight Gain, Liver and Spleen Weight

Group	Body weight gain (g/40 weeks)	Liver (g/100 g body weight)	Spleen (mg/100 g body weight)
C	180±24.9	3.47±0.71	206± 91.2
L	194±21.1	3.70±0.80	288±125
H	190±22.8	3.34±0.75	241± 78.5

Values are mean ± S.D. ($n = 28$).

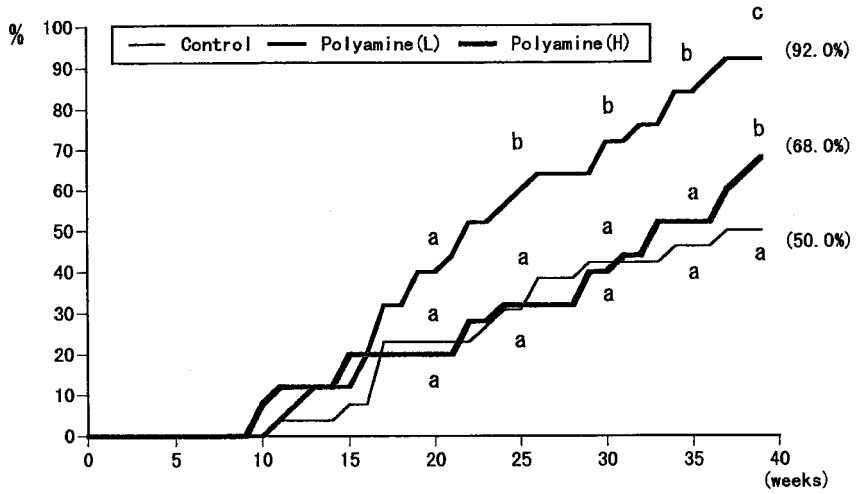

Fig. 2. Accumulation Oncogenesis Rate of Mammary Tumor
Values are mean ± S.D. (n = 28). Values with different superscript letters are significantly different ($p < 0.05$).

Table 4. The Status of Mammary Tumor

Group	accumulation oncogenesis rate (%)	tumor weight (g/Rat)
C	50.0	2.30 ± 0.37^a
L	92.0	3.32 ± 0.37^b
H	68.0	2.52 ± 0.38^a

Values of the tumor weight are mean ± S.D. ($n = 28$). Values with different superscript letters are significantly different ($p < 0.05$).

for liver and spleen weight. The accumulation oncogenesis rate was higher in the L and H groups than in the C group, proving that the incidence of breast cancer by PhIP was promoted by the oral administration of Spd. In addition, the accumulation oncogenesis rate was greater in the L group than in the H group. Namely, carcinogenesis was rather suppressed when the polyamine concentration was higher. It should be noted here that though the tumor weight increased by the polyamine feeding, the weight of the H group was less than that of the L group.

The tumor was suppressed when a polyamine deficient diet and an inhibitor of ornithine decarboxylase (ODC) which is a polyamine synthetase, were administered to the mouse transplanted with Lewis lung cancer cell.[8] The administration of PhIP employed in this study was at a dose which caused 100% mammary cancer with an incidence of 100% within 30 weeks, and the oncogenesis rate in giving the polyamine- free diet for 40 weeks was 50%. The polyamine level of normal solid feed (MF: Oriental Yeast Co., Ltd., Japan), which was measured in this study, corresponds to the L group. These results demonstrated that the decrease in dietary polyamine level suppressed carcinogenesis by PhIP, and that dietary polyamine is involved in carcinogenesis and growth stimulation of the tumor. It was also observed that the polyamine concentration influenced the promotion of mammary tumor by PhIP. Though this is presumed to be due to the DNA stabilizing and antioxidative effect of polyamine, more studies are required for greater detail.

Components previously identified as cancer suppressants include isoflavonoids in soybean,[9] isothiocyanate in broccoli,[10] allyl hydrosulphide in garlic,[11] β-glucan in mushroom[12] and catechin in green tea and powdered green tea.[13] The polyamine

Table 5. The Polyamine Content in the Food

Food	Putrescine	Spermidine	Spermine
		nmol/g	
Corn[7]	980	240	N.D.
Broccoli*	94	427	24
Garlic*	50	370	40
Shiitake[7]	29	890	N.D.
Honshimeji[7]	210	480	68
Enokitake[7]	15	600	< 5
Soybean, dried[7]	470	1430	340
Soybean hypocotyl, dried*	320	4280	760
Green tea, dried leaf[7]	547	851	524
Powdered green tea*	400	560	640

*The new data by the same method according to Ref. 7.

levels of food with known effects of cancer depression are shown in Table 5. Spd was the main component in the polyamine content ratio, except for powdered green tea, and it was also the main component in soybean, soybean hypocotyl, shiitake mushroom and green tea. These foods abounded with polyamine content.

Carcinogenesis by PhIP is suppressed when the Spd concentration is high, and the effect of coexistent components with antioxidative properties is also considered. The foods in Table 5 also have such antioxidative components as carotenoids, vitamin E, vitamin C, flavonoids and polyphenol. In terms of the effect of polyamines in food on carcinogenesis and cancer growth, it is necessary to examine their interaction with coexisting antioxidative components.

Little is known about the intestinal absorption and intake of ingested polyamines.[14] Recently, however, it has been shown that putrescine is decomposed in the intestine, and that 70–80% of spermine and Spd are absorbed, not decomposed.[15] It has also been reported that polyamine production of the enterobacterium is stimulated by the intake of dietary fiber, that intestinal cells are proliferated by ingested polyamine,[16] and that spermine induces the maturation of gut immune function of the newborn mouse.[17] In addition, the growth of rats fed an Spd supplemented diet is promoted, the nitrogen balance becomes positive, and amino acid metabolism is affected.[18] On the other hand, there is a new report showing the toxicity of polyamines when the ingested quantity is changed.[19]

Accordingly, more study may be necessary to clarify the effect of polyamine intake in the prevention of disease and improvement of health.

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