The Effects of Commercial whole Milk on the Prostate Carcinogenesis in Rats with or without Induction by 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine

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Prostate cancer has become the most common male cancer in Western countries and the incidence of this disease is increasing steadily in developing countries including China. The aim of the present study was to determine whether milk consumption promotes the development of prostate carcinogenesis in rats since milk consumption is considered to be a risk factor in some epidemiological studies. In the present study, we compared the prostate carcinogenesis in rats fed on commercial whole milk and artificial milk with or without induction by amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). The atrophic changes became more serious in the ventral prostate in the milk groups with or without PhIP administration than in the corresponding artificial milk groups. In the ventral prostate, the incidence of prostatic intraepithelial neoplasia (PIN) also was significantly higher in the milk group than in the artificial milk group irrespective of PhIP administration. The rats given PhIP, either fed on milk or artificial milk, showed significantly higher incidences of PIN and adenocarcinoma in the ventral prostate, and dysplasia in the seminal vesicle. The present study suggests that commercial whole milk is likely to promote the development of prostate, especially ventral prostate, carcinogenesis in rats.

Key words —— prostate carcinogenesis, milk, amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine

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INTRODUCTION

Prostate cancer is the third most common cancer in men worldwide, and the most common cancer in men in Europe, North America, and some parts of Africa.[1)] The incidence of prostate cancer is increasing steadily in almost all countries.[2)] However, we know little about what causes this disease. In a Danish study, this increase was not caused by attempted early detection or changes in diagnostic strategy.[3)] Results from ecological studies suggest that prostate cancer is associated with Western lifestyle and, in particular, a diet that includes a high intake of fat, meat and dairy products. Calculating the relationship between the incidence rate of prostate cancer and dietary practice in 42 countries, milk was the most closely correlated (r = 0.771) with incidence.[4)] In our meta-analysis study, the combined odds ratio of prostate cancer to milk consumption was 1.68 (95% confidence interval 1.34–2.12) in the 11 published case-control studies.[5)]

Some chemical carcinogens have been found to induced prostate carcinoma in rats and these rodent models are valuable for analysis of individual factors (for example, dietary factors).[6)] Of these chemical compounds, 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine (PhIP) is a carcinogen in the rat colon, prostate and mammary glands, which are among the sites of the most prevalent human cancers.[7)] In the present study, we assessed the effects of commercial whole milk on prostate carcinogenesis directly or induced by PhIP in rats.

MATERIALS AND METHODS

Male F344/NSIc (5-week old) were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan) and housed individually in stainless steel wire-bottomed cages in an air-conditioned room (22 ± 2°C, 55 ± 10% relative humidity) with a 12 : 12 hr light : dark cycle. The care and use of laboratory animals followed the Guidelines for Animal Experiments, Medical University of Yamanashi.

Table 1. The Components of the Basal Diet and Artificial Milk

	Basal diet (g)	Artificial milk (g)
Choline bitartrate	3.3×10^{-2}	
KC fiber	0.63	
Xanthan gum	0.19	
Vitamin A	3.83×10^{-4}	9.5×10^{-6}
Vitamin D3	1.25×10^{-6}	
Vitamin E	1.84×10^{-3}	2.5×10^{-5}
Vitamin mixture	3.37×10^{-3}	
Mineral mixture	0.45	
Glutein	2.65	0.98
Lysine	0.14	4.78×10^{-2}
Valine	5.98×10^{-2}	1.65×10^{-2}
Threonine	2.98×10^{-2}	9×10^{-3}
DL-methionine	4.64×10^{-2}	1.48×10^{-2}
Ethyl linoleate	0.28	
Corn oil	0.33	
Olive oil	1.11	
Coconut oil		0.8
Dextrin Maltose	9.32	1.13
$CaCO_3$		7.13×10^{-2}
Water		21.9
Total	15.3	25

Milk diet: 15.3 g basal diet + 25 g whole milk. Artificial milk: 15.3 g basal diet + 25 g artificial milk.

A basal diet was prepared by modifying the diet described by Lieber and DeCarli (Table 1).[8] Casein was replaced with gluten as milk is the origin of commercial casein. Protein, fat and carbohydrate accounted for 15, 25, and 60% of the total calories in the basal diet, respectively. Commercial, nationally available whole milk, the fat content of which was 3.6%, was purchased locally every day. Artificial milk, which was made as a control, included protein, fat, carbohydrate, calcium and sodium equalized to the whole milk we used. Vitamins were added to the artificial milk according to the Standard Table of Food Composition in Japan.[9] Artificial milk excluding water was prepared once a month and stored at 4°C. Then, water was added to make artificial milk directly before use. Finally, the artificial milk diet for one rat per day contained 15.3 g basal diet and 25 g artificial milk (Table 1). The milk diet contained 15.3 g basal diet and 25 g whole milk. The energy in both diets was about 80 kcal. The mean milk consumption (25 g) per day was based on the data from our preliminary experiment.

After one-week adaptation to our animal room with a commercial powder diet (CE-2, Nippon Clea, Tokyo, Japan), 80 rats at 6-weeks old were divided randomly into four groups with different diet regimens: artificial milk diet (AM) group; milk diet (M) group; artificial milk diet with PhIP administration (AM + PhIP) group; milk diet with PhIP administration (M + PhIP) group. The method of PhIP administration will be described later. The diet was mixed and replenished daily at 16:00 in a glass container and removed at 10:00 the next morning. The rats drank water ad libitum. Body weight and dietary consumption obtained by subtracting the uneaten food were recorded weekly throughout the experiment.

PhIP (HCl-Salt) was obtained from the Nard Research Institute (Osaka, Japan). PhIP was ground with corn oil in a mortar to a concentration of 20 mg/ml. After a one-week adaptation, it was administered to the rats belong to the AM + PhIP and M + PhIP groups at 100 mg PhIP/kg body weight between 14:00 and 15:00 by intragastric intubation. Throughout the process of gavage, PhIP was mixed with a stirrer in a beaker. The rats in the AM and M groups were given corn oil at 0.5 ml/100 g body weight. They were gavaged once a week for 15 weeks.

If a rat was in a moribund condition, it was decapitated early. Sixty weeks after the beginning of PhIP administration, all remaining rats were decapitated between 14:00 and 16:00. The dorsolateral prostate, ventral prostate, anterior prostate (coagulating gland) with seminal vesicle were excised, freed from fat tissue and quickly weighed. These organs, including those from rats killed prior to 60 weeks, were fixed and stored in 10% buffered formalin and embedded in paraffin. Sections were cut at 3 mm and stained with hematoxylin and eosin for histological examination by a histologist who was blind to the groups.

Data were subjected to analysis of variance (ANOVA) using StatView 5.0 (SAS Institute Inc., Cary, NC, U.S.A.). Tumor incidence was analyzed by Fisher's exact probability test. $p < 0.05$ were considered significant.

RESULTS

At week 43 after the beginning of PhIP administration, one rat in the AM + PhIP group was decapitated because of large bowel obstruction. At week 55, two rats in the M + PhIP group were decapitated because of continuous bleeding from the skin.

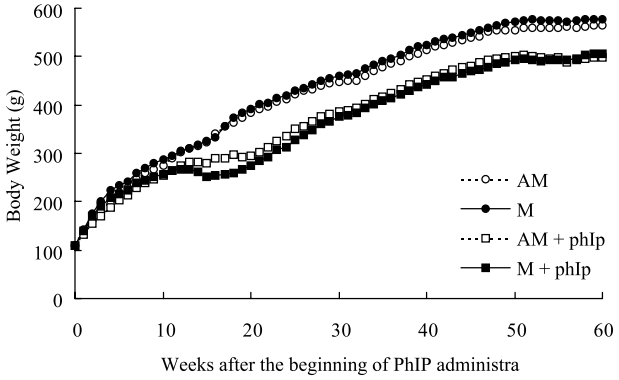

Fig. 1. Body Weights of Rats in the Four Groups during the Experimental Period

Table 2. Prostate and Seminal Vesicle Weight (mg, mean ± S.D.)

Group	N	Dorso-lateral	Ventral	Anterior and seminal vesicle
AM	20	308 ± 51	398 ± 57	1237 ± 192
M	20	270 ± 69	342 ± 84	1208 ± 169
AM + PhIP	20	278 ± 42	236 ± 78*,**	1150 ± 293
M + PhIP	20	249 ± 49	206 ± 49*,**	1065 ± 298

*$p < 0.01$: Significantly different from the AM group. **$p < 0.01$: Significantly different from the M group.

During week 14–20, the energy intake was markedly lower in the AM + PhIP and M + PhIP groups than in the AM and M groups, probably due to the toxicity of PhIP. Finally, the average energy intake was 63.8 ± 4.7, 62.8 ± 5.0, 58.8 ± 5.5 and 58.5 ± 6.9 kcal/day for the groups of AM, M, AM + PhIP and M + PhIP, respectively.

In general, rats not given PhIP (the AM and M groups) were heavier than those given PhIP (the AM + PhIP and M + PhIP groups) (Fig. 1). During weeks 14–23, the body weight of rats was significantly lower in the M + PhIP group than in the AM + PhIP group.

There were no significant differences in the prostate and seminal vesicle weights between the AM and M groups, or between the AM + PhIP and M + PhIP groups. In general, the prostate and seminal vesicle weights were heavier in the former than in the latter. Of these, the ventral prostate weights were significantly higher in the AM and M groups than in the AM + PhIP and M + PhIP groups (Table 2). However, this significance disappeared when considering the body weight (the ratio of ventral prostate weight to body weight).

All rats had atrophic changes in the ventral prostate at decapitation from 90% (18/20) low atrophic changes in the AM group to 75% (15/20) high atrophic changes in the M + PhIP group (Table 3). The atrophic changes were serious in the M and M + PhIP groups, when compared with the corresponding artificial consumption groups (AM group and AM + PhIP group, respectively). The seriousness of atrophic changes showed the following order: M + PhIP group > AM + PhIP group > M group > AM group.

Table 3. Atrophic Changes in the Ventral Prostate

	Low	Moderate	High
AM	18	1	1
M*	10	7	3
AM + PhIP*,**	3	9	8
M + PhIP*,**,†	0	5	15

*$p < 0.05$: significantly different from the AM group. **$p < 0.05$: significantly different from the M group. †$p < 0.05$: significantly different from the AM + PhIP group.

Prostatic intraepithelial neoplasia (PIN) was found in almost all parts of the prostate in the four groups (Table 4). The PIN incidence in the ventral prostate was significantly higher in the M group than in the AM group, and in the M + PhIP group than in the AM + PhIP group. Considering the PhIP effect (AM + PhIP group *vs*. AM group), the AM + PhIP group showed significantly high incidences of PIN

Table 4. Carcinogenesis in the Prostate and Seminal Vesicle

Group	N	Dorso-lateral		Ventral		Anterior		Seminal Vesicle	
		PIN	AdC	PIN	AdC	PIN	AdC	Dysplasia	Adenoma
AM	20	3	0	0	1	1	0	0	0
M	20	2	0	7*	1	2	0	2	0
AM + PhIP	20	2	0	13*	15*,**	8*,**	0	15*,**	0
M + PhIP	20	4	0	19*,**,†	16*,**	8*,**	0	16*,**	0

PIN: Prostatic Intraepithelial Neoplasia; AdC: Adenocarcinoma. *$p < 0.05$: significantly different from the AM group. **$p < 0.05$: significantly different from the M group. †$p < 0.05$: significantly different from the AM + PhIP group.

in the ventral and anterior prostate and dysplasia in the seminal vesicle. When comparing the M + PhIP group with the AM or M group, the differences in incidence also included the ventral, anterior prostate and seminal vesicle. The relationships of multiplicity were similar with those of PIN incidence (data not shown). No differences in PIN incidence among the four groups were found in the dorso-lateral prostate.

Adenocarcinoma was found only in the ventral prostate (Table 4). The incidences of adenocarcinoma were significantly higher in the AM + PhIP and M + PhIP groups than in the AM and M groups.

DISCUSSION

The effects of milk on prostate carcinogenesis were evaluated through a comparison between control (AM) and milk (M) groups. However, spontaneous prostate cancer incidence in most rat strains is between 0.05 and 0.3% at 2.5–3 years of age.[10] Therefore, we used PhIP as a carcinogen to induce prostate carcinogenesis in the AM + PhIP and M + PhIP groups. Also, a comparison of the AM and AM + PhIP groups can explain the effect of PhIP on prostate carcinogenesis. However, the differences in body and prostate weights between these two groups should be noted. The PhIP administration in the present study inhibited rat growth in the AM + PhIP and M + PhIP groups. Near the end of PhIP administration (at week 14), the rats consuming milk (M + PhIP group) stopped gaining body weight, resulting in a significant difference from the rats that consumed artificial milk (AM + PhIP group), although the same PhIP was administered. Until 8 weeks after the end of PhIP administration, the body weight in the M + PhIP group approached that in the AM + PhIP group. Contrary to the popular belief that milk is a food that is beneficial to development, our finding suggests that milk consumption augments an inhibitory action on body weight with PhIP administration.

The positive association between milk and prostate cancer is one of the most consistent dietary predictors for prostate cancer in epidemiological studies.[11–16] However, there were few animal experiments to verify this association. The present study found that commercial whole milk is likely to promote the development of prostate carcinogenesis because milk without or with PhIP administration (M group and M + PhIP groups, respectively) induced a significantly higher incidence of PIN, a preinvasive stage of adenocarcinoma,[17] in the ventral prostate, when compared with the corresponding artificial milk groups. Compared with other parts of the prostate gland, milk plays a toxic role mainly in the ventral prostate. On the other hand, atrophic changes also occurred in the ventral prostate with different degrees in the four groups, which is consistent with their weights. In the present study, milk consumption and/or PhIP administration led to more serious atrophic changes. Our study found that PIN incidence in the ventral prostate increased with the seriousness of atrophic changes, which is consistent with the conclusion drawn from human observation that there was a significant correlation between atrophy and carcinoma.[18] It is plausible because old men, whose prostate glands are characterized by atrophic changes, are prone to prostate cancer.

Similar to the report of Shirai *et al.*,[19] PhIP-induced adenocarcinoma was limited to the ventral prostate in the present study. Because PhIP-induced adenocarcinoma incidences in the ventral prostate were as high as 75% (15/20) in the AM + PhIP group and 80% (16/20) in the M + PhIP group, we cannot conclude that there is no effect due to milk on the PhIP-induced carcinogenesis in the ventral prostate. This limitation also existed when observing the results from the seminal vesicle.

Summarizing the possible risk factors in milk, special attention has been given to the fat and cal-

cium content. However, few of the more recent studies presented evidence of a positive association between milk fat and prostate cancer.[20] Some studies also doubted the conclusion that calcium intake might increase prostate cancer.[21] Therefore, some other risk factor(s) in milk are probably related to prostate cancer in addition to fat and calcium. Recently, we hypothesized that estrogen in commercial milk may be responsible for prostate cancer.[22,23] Since commercial milk is mainly produced by pregnant cows in developed countries, it contains considerable amounts of estrogens.[23,24] Some studies in humans also demonstrated that milk consumption increased the estrogen level in the circulation.[25,26] Since increasing evidence has implicated 17β-estradiol, an estrogen, as a carcinogen for prostate cancer, estrogen in milk should not be ignored when considering milk as a risk factor for prostate cancer.[5,17,22,23] Interestingly, a study has demonstrated that estradiol given to rats resulted in notable atrophy of the ventral prostate,[27] which supports our hypotheses because rats that consumed milk (M and M + PhIP groups) showed serious atrophic changes compared with the corresponding artificial milk groups in the present study. In our previous study, commercial low-fat milk promoted the development of 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced mammary tumors, another hormone-dependent cancer, in rats. The high levels of estrogen were considered to be responsible for this promotional effect.[28] Recently, PhIP was detected in the milk of healthy women.[29] If it exists in cows' milk, the adverse effect of this milk on humans is obvious because of the definitive carcinogenesis of PhIP.

PhIP is a carcinogenic heterocyclic amine produced during high temperature cooking of foods such as meat and fish, and it was estimated that dietary PhIP intake is 0.6 ng/kg per day in Japanese population.[30] In the present study, the highest incidence of PIN after PhIP administration was found in the ventral prostate, where milk alone induced the PIN incidence to some extent. Moreover, adenocarcinoma was induced only in the ventral prostate by PhIP administration. It has been demonstrated that ventral prostate is particularly sensitive to milk and PhIP in rats. Considering the inhibitory action of milk on the body weight increment of rats given PhIP, it is also suggested that milk consumption maybe play a synergetic role with PhIP-induced carcinogenesis. Unfortunately, milk consumption after PhIP administration did not show significant increase in adenocarcinoma incidence in the ventral prostate because of the relatively high incidence recorded in both groups. Therefore, the interaction of consumption of "very well-done" meat, and milk, two major Western diet habits, should be investigated further.

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