Increased Risk of Mammary Carcinoma Development following Transplacental and Trans-Breast Milk Exposure to a Food-derived Carcinogen, 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), in Sprague-Dawley Rats

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ABSTRACT

Effects of transplacental and trans-breast milk exposure to a food-derived mammary and colon carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), were investigated in rats. Female Sprague-Dawley rats were administered PhIP in the diet (100 ppm) for 4 weeks before mating with nontreated males and also during gestation and lactation. As controls, additional females were maintained on the basal diet without PhIP and mated as with the treated animals. The offspring of both groups were subdivided for each sex at weaning into three dietary groups receiving 100, 25, and 0 ppm and were killed at 47 weeks of age. Effects of the transplacental and neonatal exposure to PhIP on mammary carcinogenesis were most evident in females administered 25 ppm PhIP after weaning; the incidence and multiplicity of adenocarcinomas in offspring from the PhIP fed dams (42.9%, 0.62/rat) was significantly higher than the value for offspring from nontreated dams (4.8%, 0.05/rat). Furthermore, in the basal diet groups, the incidence of adenocarcinomas in females was higher, albeit not significantly, in offspring of the PhIP-treated than the nontreated dams (16.7%, 0.22/rat as compared with 3.3%, 0.07/rat). Although the highest incidence of mammary adenocarcinomas was found in the female progeny given 100 ppm PhIP from PhIP-treated dams (70.0%, 1.55/rat), this was only slightly higher than the 61.9% and 0.90/rat of the same dose group from the nontreated dams. In males, no apparent effects of transplacental and neonatal exposures were evident. In a separate experiment, excretion of PhIP into breast milk and transfer of PhIP to fetuses and neonates with resultant hepatic PhIP-DNA adduct formation were demonstrated. Thus, maternal exposure to this food-derived carcinogen may be a critical risk factor for generation of mammary carcinomas.

INTRODUCTION

Epidemiological studies have indicated that most human cancers are caused by exposure to environmental carcinogenic agents, partly as a reflection of individual lifestyle (1). Diet is considered to be a major factor determining human cancer risk (2), and one group of dietary compounds that may constitute a causative factor in the etiology of human cancer is that comprising the heterocyclic amines (3–5). These compounds are formed when protein-rich foods such as beef, fish, and fowl are cooked under typical household conditions (6, 7) and many have proved to be rodent carcinogens inducing tumors in the liver, lung, breast, small and large intestines, and other organs (8, 9).

Among the heterocyclic amines, PhIP¹ is considered to be generally the most abundant (10, 11). Layton et al. (12) recently estimated that human exposure to PhIP was the highest among the 5 heterocyclic amines investigated, based on a dietary survey of the United States population, and concluded that nearly one-half of the incremental cancer risk due to heterocyclic amines was because of ingestion of PhIP. Human exposure to PhIP and other heterocyclic amines was actually demonstrated by their identification in the urine of volunteers on a normal diet (13). In addition to the exposure evidence in humans, the results of carcinogenicity studies in rats are very convincing; PhIP induces mammary tumors in females and colon carcinomas in both sexes of F344 rats (14, 15) and mammary tumors in Sprague-Dawley rats (16, 17). It is of great importance that both types of tumors are common in the Western world where the intake of fried ground beef and fowl is relatively high (18–20). The available findings thus strongly suggest that this mutagen might be of importance as an environmental factor in the production of human cancers.

Breast cancer incidence rates have historically been 4–7 times higher in the United States than in China or Japan. Ziegler et al. (21) have conducted a population-based case-control study among women of Chinese, Japanese, and Filipino ethnicities in California and Hawaii and reported that breast cancer risk rises over several generations to approach that among United States whites. They concluded that exposure to Western lifestyles has a substantial impact on breast cancer risk in Asian migrants to the United States during their lifetime with no direct evidence of an especially susceptible period during either menarche or early reproductive life. Their observations indicate a possible importance of dietary factors in the early period of the life through transplacental and neonatal exposure. Indeed, it has recently been demonstrated that PhIP is excreted into the breast milk in rats and mice treated with PhIP (22–24), with adducts being formed in liver DNA after both transplacental (22) and neonatal (23) exposure.

In response to these findings, we conducted a long-term carcinogenicity study to analyze the effects of a two-generation dietary exposure to PhIP, from the time of conception to 1 year after birth, using the Sprague-Dawley strain of rats, which is susceptible to mammary carcinogenicity (16, 17, 25). The protocol was designed to reflect the human lifestyle, except for the dose of PhIP.

MATERIALS AND METHODS

Chemicals and Animals. PhIP (PhIP hydrochloride) was obtained from the Nard Institute (Osaka, Japan). Male and female 6-week-old Sprague-Dawley rats were purchased from Charles River Japan, Inc. (Atsugi, Japan) and housed 3/cage, or 1 dam/cage with its offspring during nursing, on wood-chip bedding in an air-conditioned animal room at 23 ± 2°C and 50 ± 10% humidity. PhIP was incorporated into basal diet (Oriental MF powdered diet, Oriental Yeast Co., Tokyo, Japan) at doses of 100 or 25 ppm. Corn oil was added to all diets, including the control basal diet, at a concentration of 2% to moisten the diet and prevent air-borne exposure to PhIP.

Chronic Experiment. The experimental protocol is shown in Fig. 1. A total of 59 virgin female rats were divided into two groups. The rats in group 1 received PhIP at a dose of 100 ppm in the diet from 7 weeks of age, mated with nontreated males at about 11 weeks of age, and maintained on the PhIP diet until weaning. Group 2 rats were mated with nontreated males as in group...
The offspring were nursed by their own dams for throughout the period of gestation and lactation.

A diet containing PhIP before conception and other main organs were histologically examined. All s.c. nodules and tumors and other main organs were histologically examined.

The offspring were killed at 47 weeks of age, and all s.c. nodules and tumors and other main organs were histologically examined. Additions, mammary tissues in the right thoracic region were sampled for one paraffin block from each animal. The small and large intestines were inflated with the fixative, and Swiss rolls of tissue were made for histopathological examination. Pituitaries were examined grossly. The tissue samples were stained with hematoxylin and eosin and routinely examined under a microscope.

HPLC Analysis of PhIP in Breast Milk. In a separate experiment, concentrations of PhIP in breast milk were analyzed. Milk samples were collected on day 12 after birth from the stomachs of pups from dams fed on diet containing PhIP at the concentration of 100 ppm for 1 week before sacrifice.

As controls, stomach contents were similarly obtained from pups without maternal exposure to PhIP. Stomach contents from 5 or 6 siblings were combined, and 2 test samples were made for each dam. The samples were kept in a deep freezer at −80°C until analysis.

Fifty-μg samples of the stomach milk contents were mixed with 450 μL of 50% methanol. The mixtures were centrifuged at 14,000 rpm for 2 min, and aliquots of the supernatant were injected into an analytical ODS column (5 μm particle size, 4.6 × 250 mm, Shiseido, Tokyo, Japan). The mobile phase of 20% CH3CN in 25 mM H3PO4-Na2HPO4 (pH 2.0) was pumped in at a flow rate of 1.0 ml/min. PhIP was detected by its fluorescence with excitation and emission wavelengths of 345 and 395 nm, respectively. All chromatographic procedures were performed at 25°C. The contents of PhIP in the specimen were estimated from the standard curve obtained from various doses of the authentic compound. The minimum amount of PhIP detectable was 0.001 ng.

Table 1 Weight data and macroscopic findings in offspring at 47 weeks of age

<table>
<thead>
<tr>
<th>Sex</th>
<th>Group</th>
<th>Dams</th>
<th>Offspring (ppm)</th>
<th>No. of rats</th>
<th>Weight (g)</th>
<th>Incidence (%)</th>
<th>Multiplicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
<td>Final (%)</td>
<td>Body</td>
<td>Liver</td>
<td>Mammary tumors</td>
</tr>
<tr>
<td>Female</td>
<td>1-a</td>
<td>+</td>
<td>100</td>
<td>21</td>
<td>14 (67)²</td>
<td>328 ± 37</td>
<td>10.7 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>1-b</td>
<td>+</td>
<td>25</td>
<td>21 (100)</td>
<td>349 ± 36</td>
<td>10.8 ± 1.3</td>
<td>9 (42.9)³</td>
</tr>
<tr>
<td></td>
<td>1-c</td>
<td>+</td>
<td>26</td>
<td>36 (97)</td>
<td>353 ± 43</td>
<td>10.4 ± 1.7</td>
<td>6 (16.7)²</td>
</tr>
<tr>
<td></td>
<td>2-a</td>
<td>-</td>
<td>100</td>
<td>21 (76)²</td>
<td>338 ± 40²</td>
<td>9.6 ± 1.6²</td>
<td>13 (61.9)²</td>
</tr>
<tr>
<td></td>
<td>2-b</td>
<td>-</td>
<td>25</td>
<td>21 (100)</td>
<td>370 ± 44</td>
<td>10.9 ± 1.6</td>
<td>1 (4.8)³</td>
</tr>
<tr>
<td></td>
<td>2-c</td>
<td>-</td>
<td>0</td>
<td>30 (97)</td>
<td>370 ± 48</td>
<td>11.1 ± 1.6</td>
<td>2 (6.7)²</td>
</tr>
<tr>
<td>Male</td>
<td>1-a</td>
<td>+</td>
<td>100</td>
<td>21</td>
<td>16 (76)²</td>
<td>634 ± 68²</td>
<td>18.7 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>1-b</td>
<td>+</td>
<td>25</td>
<td>20 (100)</td>
<td>666 ± 66²</td>
<td>19.2 ± 2.9²</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1-c</td>
<td>+</td>
<td>36</td>
<td>35 (97)</td>
<td>673 ± 70</td>
<td>19.6 ± 2.8</td>
<td>1 (2.8)²</td>
</tr>
<tr>
<td></td>
<td>2-a</td>
<td>-</td>
<td>100</td>
<td>21</td>
<td>19 (95)</td>
<td>658 ± 85</td>
<td>19.8 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>2-b</td>
<td>-</td>
<td>25</td>
<td>21 (100)</td>
<td>718 ± 75</td>
<td>21.6 ± 3.8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2-c</td>
<td>-</td>
<td>0</td>
<td>30 (90)</td>
<td>700 ± 106</td>
<td>21.1 ± 3.9</td>
<td>0</td>
</tr>
</tbody>
</table>

* Significantly different from the respective 0 ppm group at P < 0.01.
* Significantly different from the respective 0 ppm group at P < 0.001.
* Significantly different from the respective group 2 at P < 0.01.
* Significantly different from the respective group 2 at P < 0.05.
RESULTS

Chronic Experiment. Several animals died or were killed upon becoming moribund before the termination of the experiment, mostly due to mammary tumor development. A complete autopsy was performed on these animals, and any gross lesions were histologically examined. Final body weights of female and male rats at 47 weeks of age are given in Table 1. Dose-dependent lowering of body and liver weights was apparent, especially in the 100 ppm PhIP dose group. Body weights of rats from PhIP-treated dams were lower than those from PhIP-nontreated dams for both sexes in all 3 dose groups. Significantly lower body and liver weights were evident in the 25 ppm treated males. Kidney weights were not affected by the treatments.

The time courses of tumor development are illustrated in Fig. 2. In females, the first s.c. tumor was identified at week 16 in groups 1-a and 2-c, and the incidences were highest in group 1-a throughout the experiment, followed by the 100 ppm group from the nontreated dams (group 2-a). Although the incidence for the 25 ppm group from the nontreated dams (group 2-b) was almost the same as the control value (group 2-c), palpable tumors in the 25 ppm group from PhIP-treated dams (group 1-c) were observed from week 20 and were constantly more frequent than in group 2-c. In males, palpable tumors were first found at week 24 in the 100 ppm PhIP group from nontreated dams, and no differences were observed in tumor development between groups 1 and 2.

Data for final incidence and multiplicity of palpable s.c. tumors at week 47 are shown in Table 1. The incidence of s.c. tumors in females was highest (76.2%: 16 of 21 rats) in the offspring from the dams that received 100 ppm PhIP (group 1-a). The value was significantly higher than the 16.7% (6 of 36 rats) for the basal diet group (2.9%), but no difference was observed in tumor development between the progenies from the PhIP-treated and nontreated dams, and values were only slightly higher than for the basal diet group.

In female offspring, a few palpable tumors were induced in the 100 ppm and basal diet groups. However, no significant difference was observed between the progenies from the PhIP-treated and nontreated dams, and values were only slightly higher than for the basal diet animals.

The incidence and multiplicities of histopathologically confirmed mammary adenocarcinomas are shown in Table 2. Most palpable tumors were adenocarcinomas. Their mean diameters were usually greater in the high dose groups. In females, a dose-dependent increase of adenocarcinomas was evident, especially those from PhIP-treated dams. Higher values for offspring from the treated dams (42.9%) than nontreated dams (4.8%) were apparent in the 25 ppm dose groups and also in the basal diet groups (16.7% and 3.3%), although in the later case the difference was not statistically significant. The incidences of adenocarcinomas in the 100 ppm dose groups were almost the same for offspring from PhIP-treated (70.0%) and nontreated (61.9%) dams. Similar trends were observed for adenocarcinoma multiplicity. A few palpable tumors were identified as adenomas or fibroadenomas. Additionally, microscopic tumors such as adenomas and fibroadenomas were observed in the right thoracic region, generally with a dose-dependent manner except for females in group 1.

In males, both incidence and multiplicity of adenocarcinomas in the 100 ppm dose groups from PhIP-treated dams (15.0%) were slightly higher than those of the basal diet group (2.9%), but no difference was evident in the groups given the same dose of PhIP after weaning between the treated and nontreated dam cases. Few microadenomas were also observed.

to day 12 (for 1 week), and then both dams and pups were killed and their livers were removed. In both fetus and neonate cases, livers from 5 or 6 siblings were combined to make 1 sample for each dam. The livers removed were frozen in liquid nitrogen and kept in a deep freezer at —80°C until analysis.

PhIP-DNA adduct levels were analyzed by 32P postlabeling under standard and adduct intensification conditions with modifications as reported previously (26). The DNA, isolated by phenol-chloroform extraction, was digested with micrococcal nuclease and spleen phosphodiesterase (Worthington Biochemical Co., Freehold, NJ). A sample of 0.17 μg of the digest was labeled with 32P by T4 polynucleotide kinase (Takara Shuzo Co., Ltd., Kyoto, Japan) with [γ-32P]ATP (930 Ci/mmol, 50 μM, ICN Biomedicals, Irvine, CA) under standard conditions, and 5 μg of the DNA digest with [γ-32P]ATP (7000 Ci/mmol, 2.3 μM) under adduct-intensification conditions, at 37°C for 1 h. Two-μl aliquots of 15 μl of these reaction mixtures after kination were used for total nucleotide analysis after treatment with apyrase (Sigma Chemical Co., St. Louis, MO). The remaining 13 μl of each mixture was further treated with nuclease P1 (Yama- sa Shoyu Co., Choshi, Japan) and phosphodiesterase I (Worthington Biochemical Co.). Then each aliquot was spotted on a polyethyleneimine-cellulose TLC sheet (Polygram Cell 300 polyethyleneimine, Machery-Nagel, Duren, Germany) and developed under the same conditions as used in previous studies (26). The TLC sheets were exposed to Fuji imaging plates (Fuji Photo Film Co., Tokyo, Japan), and the radioactivities of adducts were analyzed with a Bio Imaging Analyzer (BAS 2000, Fuji Photo Film Co.). Under these conditions, PhIP-DNA adducts are detected as a single spot of 5′-dG-C8-PhIP (26).

Statistical Analysis. Statistical analysis was performed using the Fisher’s exact probability test for incidence data and the Student’s t test or Welch’s t test for mean data after analysis by the F-test for equal variance between any two groups for means.
In the large intestines, a few adenomas were histopathologically identified in 3 rats; 1 male and 1 female in group 1-a and 1 male in group 1-b. No other tumors were observed, and no gross lesions were found in the kidneys or pituitary in any group.

**PhIP in Breast Milk.** As shown in Table 3, PhIP excretion into the breast milk was confirmed. The concentration of PhIP in the milk obtained from the pup stomachs was $3.22 \pm 2.51$ ng/g of milk, as compared to nondetectable levels in the nontreated groups.

**PhIP-DNA Adduct Formation.** A single adduct spot, corresponding to authentic 5'-'pdG-C8-PhIP (26), was detected under modified adduct intensification conditions in all positive liver samples from fetuses, neonates, and dams (Fig. 3). The levels of the PhIP-DNA adducts are summarized in Table 4. With both transplacental and neonatal exposures, the adduct levels were lower in fetuses and neonates than in their dams with ratios to values of mothers of about 1:3 for fetuses and 1:10 for neonates. The adduct level was slightly higher in dams used for the neonatal exposure analysis than in dams used for the transplacental exposure, although the exposure period was shorter in the former case.

**DISCUSSION**

We reported previously dose-dependent colon and mammary carcinogenicity for PhIP in F344 rats administered this heterocyclic amine in the diet at doses of 400 ppm for 52 weeks (14) and 100 and 25 ppm for 104 weeks (15). This is a very significant finding because the chemical is relatively abundant in cooked meat and fish (6–8), and these tumor types are commonly observed in Western countries (18–20) where broiled or fried meat consumption is relatively high. Organ specific carcinogenic action of PhIP could also be demonstrated using two stage carcinogenesis models; promoting effects were observed in the large and small intestines in a dose-dependent manner (27) with no effects in the liver (28). Mammary carcinogenicity was also demonstrated in female Sprague-Dawley rats in a dose-dependent manner.

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**Table 2** Histopathological analysis of mammary tumors

<table>
<thead>
<tr>
<th>Sex</th>
<th>Group</th>
<th>No. of rats examined</th>
<th>Microadenoma&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Incidence (%)</th>
<th>Multiplicity (no./rat)</th>
<th>Adenoma/Fibroadenoma</th>
<th>Incidence (%)</th>
<th>Multiplicity (no./rat)</th>
<th>Adenocarcinoma</th>
<th>Incidence (%)</th>
<th>Multiplicity (no./rat)</th>
<th>Mean diameter&lt;sup&gt;b&lt;/sup&gt; (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>1-a</td>
<td>20</td>
<td>1 (5.0)</td>
<td>0.05 ± 0.22</td>
<td>2 (10.0)</td>
<td>0.20 ± 0.70</td>
<td>14 (70.0)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.55 ± 1.85&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.81 ± 2.32&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1 (4.8)</td>
<td>0.05 ± 0.22</td>
<td>9 (42.9)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1-b</td>
<td>21</td>
<td>1 (4.8)</td>
<td>0.05 ± 0.22</td>
<td>1 (4.8)</td>
<td>0.05 ± 0.22</td>
<td>6 (16.7)</td>
<td>0.22 ± 0.54</td>
<td>1.81 ± 1.19&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2 (9.5)</td>
<td>0.05 ± 0.22</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td></td>
<td>1-c</td>
<td>36</td>
<td>4 (11.1)</td>
<td>0.14 ± 0.42</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>2-a</td>
<td>21</td>
<td>6 (28.6)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.29 ± 0.46</td>
<td>3 (14.3)</td>
<td>0.19 ± 0.51</td>
<td>13 (61.9)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.90 ± 1.14&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.09 ± 2.33&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2 (b)</td>
<td>0.05 ± 0.22</td>
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<tr>
<td></td>
<td>2-b</td>
<td>21</td>
<td>2 (9.5)</td>
<td>0.14 ± 0.48</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>2-c</td>
<td>30</td>
<td>1 (3.3)</td>
<td>0.07 ± 0.37</td>
<td>1 (3.3)</td>
<td>0.03 ± 0.18</td>
<td>1 (3.3)</td>
<td>0.07 ± 0.25</td>
<td>7 (1)</td>
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<tr>
<td>Male</td>
<td>1-a</td>
<td>20</td>
<td>3 (15.0)</td>
<td>0.15 ± 0.37</td>
<td>0</td>
<td>0</td>
<td>3 (15.0)</td>
<td>0.15 ± 0.37</td>
<td>4.17 ± 1.76&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>1-b</td>
<td>20</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>1-c</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>2-a</td>
<td>21</td>
<td>1 (4.8)</td>
<td>0.05 ± 0.22</td>
<td>0</td>
<td>0</td>
<td>3 (14.3)</td>
<td>0.14 ± 0.36</td>
<td>5.67 ± 3.06&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0</td>
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<tr>
<td></td>
<td>2-c</td>
<td>30</td>
<td>0</td>
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</tbody>
</table>

<sup>a</sup> Mammary lesions in the right thoracic region.
<sup>b</sup> Numbers in parentheses are number of adenocarcinomas.
<sup>c</sup> Significantly different from respective group 2 at $P < 0.05$.
<sup>d</sup> Significantly different from the respective 0 ppm group at $P < 0.001$.
<sup>e</sup> Significantly different from the respective 0 ppm group at $P < 0.01$.
<sup>f</sup> Significantly different from the respective group 2 at $P < 0.01$.

**Table 3** Concentration of PhIP in breast milk from rats fed 100 ppm PhIP

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of samples&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PhIP (ng/g milk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhIP</td>
<td>4</td>
<td>3.22 ± 2.51</td>
</tr>
<tr>
<td>Basal</td>
<td>2</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Stomach contents from 5 to 6 pups were combined for each dam.
<sup>b</sup> ND, not detected.
fashion; incidence of mammary adenocarcinomas was 72% with 200 ppm, and 5-15% of rats bore carcinomas in the 25 and 12.5 ppm dose groups after 48 weeks (17).

In the present study, an increased mammary tumor development due to two-generation exposure to PhIP was clearly demonstrated. Even the 25 ppm dose level of PhIP, which caused less than a 15% incidence of mammary adenocarcinomas in our previous study (17) and only 4.8% in the present study after about 1 year using female Sprague-Dawley rats, resulted in development of adenocarcinomas in 42.9% of animals whose dams had been exposed to 100 ppm PhIP during gestation and lactation. A similar trend was evident for the basal diet groups, although the difference was not statistically significant. When the progeny were exposed to the highest dose of PhIP (100 ppm), significant effects of transplacental and neonatal exposure to PhIP were not observed, indicating a relative weakness as compared to long-term dietary exposure to 100 ppm PhIP. However, it is noteworthy that offspring exposed to PhIP only during the period of gestation and lactation showed a higher incidence of mammary carcinomas than the background incidence after 1 year.

These results are in line with the fact that PhIP is excreted into breast milk after a single oral dose of 10 mg/kg in female F344 rats (22, 23) and after a single i.p. injection at doses of 5.2 mg/kg in C57BL/6 mice (24). This could be confirmed in the present study after dietary feeding of 100 ppm PhIP to the dams. Similarly, the transplacental transfer of PhIP to fetuses after i.p. injection at doses 4.7-5.2 mg/kg was reported in mice by using radioactive PhIP (24) and was also found in the present study. On the basis of the adduct levels in the liver, it was suggested that the efficiency of PhIP transfer is higher with transplacental than with trans-breast milk exposure. In the case of mice, the highest fetal levels were observed at late gestation (24), and from the present experiment, exposure from the time of conception to weaning would appear to be very critical. This can be partly explained by the fact that cell proliferation is very high in the whole body during this period. However, the organotropism of PhIP carcinogenicity did not shift from the mammary gland to other organs in the present study. It is of great interest concerning organ specificity in PhIP carcinogenesis that metabolic activation of PhIP by the mammary tissue of neonates is more efficient than for other heterocyclic amines (29). Although enzyme activities in neonates have not been examined, the present results suggest that, either systematically or locally, they are similar in this respect to the dams. It has been demonstrated in male mice that neonates are more sensitive than elder animals to the carcinogenic action of representative heterocyclic amines, including PhIP, when they are injected i.p. (30).

Because PhIP is the most abundant heterocyclic amine (6, 8, 12), the present findings add weight to the conclusion that it is a major environmental causative agent for human breast cancer. However, epidemiological examinations have demonstrated that, in addition to heterocyclic amines, a high-fat and high-protein diet, as well as estrogenic replacement therapy, obesity, and smoking, are risk factors for mammary carcinogenesis (1, 18, 31). Khan et al. (32) reported mammary ductal epithelial hyperproliferation and hyperplasia due to a Western-style diet in mice, and increased mammary carcinogenesis was reported with a high-fat diet in combination with PhIP in Sprague-Dawley rats (16). Thus, a westernized diet, including heterocyclic amines, may be a main reason for the geographical bias in mammary tumor development.

The importance of PhIP and possibly other heterocyclic amines, which are produced during ordinary cooking processes, for human cancer was again emphasized by the present results. Although the doses of heterocyclic amines used in animal carcinogenicity studies are very high as compared to the amounts ordinarily consumed in daily life (12), the fact that two-generation exposure actually increases the cancer risk may be extremely important with regard to the situation in man. Furthermore, it must be taken into consideration that several carcinogenic substances (33), including heterocyclic amines, are generally simultaneously produced in cooked food (3-12), and they may demonstrate true synergism or isoaddivitivily in terms of their carcinogenicity (34-36).

Although inhibitory effects of chlorophyllin (37) and some antioxidants (38), which are contained in our daily food, have been demonstrated against PhIP-induced mammary carcinogenesis, and caloric restriction reduces mammary tumor development induced by chemical carcinogens (39, 40), it is strongly recommended that women avoid possible heterocyclic amine-containing foodstuffs, especially during the period of gestation and nursing with their own breast milk.

REFERENCES

Increased Risk of Mammary Carcinoma Development following Transplacental and Trans-Breast Milk Exposure to a Food-derived Carcinogen, 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), in Sprague-Dawley Rats

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