Hypertriglyceridemia in Rats Induced by Consumption of a Food-derived Carcinogen, 2-Amino-1-methyl-phenylimidazo[4,5b]pyridine (PhIP)

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2-Amino-1-methyl-phenylimidazo[4,5b]pyridine (PhIP), the most abundant mutagenic heterocyclic amine produced in cooked meat and fish, is known to be a carcinogen for rats and mice. This study provides the first evidence for hypertriglyceridemia in rats exposed to PhIP, suggesting its potential risk to induce not only carcinogenesis, but also atherosclerosis, and highlighting the potential importance of PhIP for humans.

Key words: carcinogen; 2-amino-1-methyl-phenylimidazo[4,5b]pyridine (PhIP); hypertriglyceridemia; rats

In recent years, a number of heterocyclic amines present in charred parts of broiled fish and meat have been demonstrated to have tumorigenic potential in a variety of organs in either mice or rats.1-3 They are therefore considered as strong candidates for human carcinogens.1-3 One in particular, 2-amino-1-methyl-phenylimidazo[4,5b]pyridine (PhIP), was identified as the most abundant mutagenic heterocyclic amine produced in cooked meat and fish.4-6 PhIP has also been found in beer and wine.5 It has been shown to induce colon, mammary glands, and prostate carcinomas in rats.6-8 During our studies on the development of several tumors by PhIP, we often obtained white and turbid serum samples from the rats chronically exposed to it (Ishimura et al., unpublished observation). This observation led us to examine the concentrations of serum lipids in rats receiving PhIP. Here we report the evidence for elevations in serum triglyceride and phospholipids in rats by PhIP administration, which may cause white and turbid serum samples in the rats received it.

Male F344/DuCry rats, weighing 340-400 g (21 wk of age) at commencement, were purchased from Charles River Japan Inc., Hino, Japan and housed in polycarbonate cages under constant conditions of temperature (24 ± 2°C) and humidity (55 ± 10%), with a 12:12-hour light-dark cycle (lights on, 07:00-19:00). The animals were maintained according to the “Guide for the Care and Use of Laboratory Animals” established by Hiroshima University. All rats were fed a commercial diet (MF, Oriental Yeast, Tokyo, Japan). All rats had free access to tap water and the food. A total of 16 rats were divided into two groups. They were ingested PhIP (75 mg/kg) every other day for three weeks (nine times in total). The animals were killed 12 months after the first PhIP administration. After the experimental period, the rats were lightly anesthetized by diethylether and killed between 9:00 h and 11:00 h. Blood was collected from the abdominal aorta, and samples were left to clot on ice. Serum samples were obtained by centrifugation. Liver and epididymal adipose tissue were immediately removed and weighed. Serum concentrations of total cholesterol, HDL-cholesterol, triglyceride, free fatty acid, and glucose were measured by kits (Cholesterol C-Test Wako, HDL-Cholesterol Test Wako, Triglyceride G-Test Wako, NEFA C-Test Wako and Glucose C-II Test Wako, respectively, Wako Pure Chemicals, Osaka, Japan). Total liver lipids were extracted by the method of Folch et al.,9 and the contents of triglyceride, cholesterol, and phospholipids in the lipids were measured as reported previously.10 Serum activity of glutamic-oxaloacetic transaminase (GOT) was measured by a reagent (Iatro LQ GOT Rate J), Iatron Laboratories, Inc., Tokyo, Japan). Each result is expressed as the mean ± SE, and the data were analyzed by Student’s t-test.

Although a reduction in the body weight by PhIP was observed when the rats were receiving PhIP (Fig. 1), the final body weight was unaffected by the treatment (Table 1). Relative weight of liver and epididymal adipose tissue was unaffected by PhIP. Concentration of serum triglyceride was 72% higher in the PhIP group than in the control group (P < 0.05). Concentrations of serum total cholesterol and HDL-cholesterol and the ratio of HDL-cholesterol versus total cholesterol were unaffected by PhIP. Concentration of serum phospholipids was 26% higher in the PhIP group than in the control group (P < 0.05). Concentrations of serum free fatty acid and glucose were unaffected by the treatment. Serum activity of GOT (indicator of hepatic damage) was also unaffected by PhIP (data not shown). Liver concentrations of triglyceride, cholesterol, and phos-

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Abbreviations: PhIP, 2-Amino-1-methyl-phenylimidazo[4,5b]pyridine; GOT, glutamic-oxaloacetic transaminase
PhIP-induced Rat Hypertriglyceridemia

Fig. 1. Changes in Body Weight of Rats with or without Receiving 2-Amino-1-methyl-phenylimidazo[4,5-b]pyridine (PhIP).

Values are means±SE (N=8). *Significantly different from the control group (P<0.05).

Table 1. Effects of Administration of 2-Amino-1-methyl-phenylimidazo[4,5-b]pyridine (PhIP) on Tissue Weight and Serum and Liver Lipids in Rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PhIP</th>
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</thead>
<tbody>
<tr>
<td>Final body wt (g)</td>
<td>397±14</td>
<td>406±13</td>
</tr>
<tr>
<td>Liver wt (% of body wt)</td>
<td>2.66±0.19</td>
<td>2.59±0.08</td>
</tr>
<tr>
<td>Epididymal fat pad wt (% of body wt)</td>
<td>2.63±0.24</td>
<td>2.68±0.10</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>0.85±0.14</td>
<td>1.46±0.12*</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l) (a)</td>
<td>2.28±0.23</td>
<td>2.56±0.10</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l) (b)</td>
<td>1.60±0.23</td>
<td>1.77±0.10</td>
</tr>
<tr>
<td>Ratio of (a) versus (b)</td>
<td>0.69±0.03</td>
<td>0.69±0.02</td>
</tr>
<tr>
<td>Phospholipids (mmol/l)</td>
<td>1.98±0.16</td>
<td>2.48±0.08*</td>
</tr>
<tr>
<td>Free fatty acid (mmol/l)</td>
<td>0.35±0.01</td>
<td>0.34±0.02</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>8.10±0.28</td>
<td>9.10±0.33</td>
</tr>
<tr>
<td>Liver</td>
<td>Triglyceride (μmol/g of liver)</td>
<td>30.6±3.1</td>
</tr>
<tr>
<td></td>
<td>Cholesterol (μmol/g of liver)</td>
<td>10.4±0.8</td>
</tr>
<tr>
<td></td>
<td>Phospholipids (μmol/g of liver)</td>
<td>41.7±1.0</td>
</tr>
</tbody>
</table>

Mean±SE (n=8).
* Significantly different from control group (P<0.05).

pholipids were unaffected by PhIP treatment. Since hepatoma-bearing rats have been shown to be associated with hyperlipidemia characterized by rises in serum triglyceride and cholesterol, it was carefully examined whether the rats received PhIP had hepatocarcinoma. However, we failed to observe any carcinomas in the livers of rats fed PhIP. We further examined other organs histopathologically. Among the PhIP group, one animal had prostate adenoma, and three animals had interstitial cell adenoma in the testis. Among the control group, one animal had a pheochromocytoma in abdominal cavity, and three animals had interstitial cell adenoma in the testis. All these histopathological data were not associated with the alterations in serum lipids. Since no difference in food intake between the two groups was observed during the final several days (data not shown), it is unlikely that the elevation in serum lipids by PhIP is due to higher food intake.

The discovery of PhIP carcinogenicity in the rat colon, mammary glands, and prostate attracted much attention because of the fact that PhIP is relatively abundant in cooked meat and fish, and human may therefore be exposed to appreciable amounts on a daily basis. This study provided further evidence for hypertriglyceridemia by PhIP administration, which may lead to atherosclerosis or coronary heart disease. We have found increased thrombosis in Donryu rats received PhIP (Watanabe et al., unpublished observation). PhIP has also been reported to induce cardiac damage through generation of mitochondrial DNA adducts in rats. Colon, mammary, and prostate carcinomas are all common malignancies in Western countries, along with coronary heart disease. Thus, the finding of hyperlipidemia by consumption of PhIP is of great importance. Our study suggests that PhIP may play a role in development of atherosclerosis in Western countries. Further studies on the underlying mechanism of the hypertriglyceridemia by PhIP and on the time-course effect of PhIP on serum triglyceride are in progress in our laboratory.

In conclusion, our data provide the first evidence of hyperlipidemia in rats after ingestion of PhIP. This environmentally significant compound may be extremely important with regard to not only human neoplasia, but also atherosclerosis. Minimizing the consumption of well-cooked meat and fish is recommended as a preventive strategy. Further evaluation is necessary for the potential risk of PhIP as a factor leading to atherosclerosis or coronary heart disease.

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References


