Liver Injury Due to 3-Amino-1-methyl-5H-pyrido [4,3-b] indole (Trp-P-2) and Its Prevention by Miso

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Trp-P-2(3-amino-1-methyl-5H-pyrido [4,3-b] indole) ingestion for 42 d by C3H/HeJcl mice caused elevation of serum alanine transaminase (ALT) activity and several signs of liver injury. These alterations were not observed in mice fed the diet supplemented with 10% miso. This suggests a preventive effect of miso as to Trp-P-2 induced liver injury.

Key words: 3-amino-1-methyl-5H-pyrido [4,3-b] indole (Trp-P-2); miso; liver injury

Miso is a traditional Japanese fermented soybean food. It contains various physiologically functional ingredients, such as soy protein and isoflavones, that improve hypercholesterolemia and prevent atherosclerosis,¹,² and melanoidin, which reduces reactive oxygen species.³ It also inhibits the occurrence of gastric and liver tumors in rats.⁴–⁶ During our studies of the anti-carcinogenic effects of miso in mice, we found several signs of liver injury in animals administrated 3-amino-1-methyl-5H-pyrido [4,3-b] indole (Trp-P-2). Trp-P-2 is produced by pyrolysis of tryptophan,⁷ as in the roasting of proteinous food.⁸ Trp-P-2 has been identified as a mutagen by the Ames test, and as a rodent carcinogen that induces a high incidence of hepatoma.⁹–¹¹ Although heterocyclic amines produced in cooking and food processing are known as mutagen/carcinogens,¹² there have been few reports on toxicity, besides carcinogenicity and mutagenicity, with the exceptions of cardiomyopathy and hypertriglyceridemia caused by 2-amino-1-methyl-6-phenylimidazo[4,3-b]pyridine (PhIP) in rats.¹³,¹⁴ Here we report liver injury due to Trp-P-2 in mice and protection against it by feeding of miso.

All reagents were obtained from Wako Pure Chemical Industries (Osaka, Japan), except for Trp-P-2, from the NARD Institute (Amagasaki, Japan). Commercial miso (rice-koji miso) was purchased from a local market in Niigata, Japan. All animal experiments were conducted in accordance with Niigata Women’s College’s institutional guidelines and the Japanese Physiological Society’s guidelines for animal use. Six-week-old male C3H/HeJcl mice were obtained from CLEA Japan (Tokyo), and were housed separately in polycarbonate cages under a 12-h light-dark cycle. The mice were divided to three groups consisting five or six mice, of the control group, the Trp-P-2 (TP2) group, and the Trp-P-2 + miso (TP2M) group. Each group was fed a standard commercial laboratory rodent diet (MF; Oriental Yeast, Tokyo) supplemented 10% (w/w) miso (the TP2M group) or a miso-replaced mixture (the control group and the TP2 group). The components of the miso-replaced mixture were as follows: 1.03% soy protein, 0.52% soybean oil, 2.34% β-starch, 1.24% NaCl, and 4.87% water. All diets and water were fed ad libitum. The mice of TP2 group and the TP2M group ingested Trp-P-2 (30 mg/kg of body weight) per os every other day. The dosage of Trp-P-2 was determined according to an experiment on DNA adduct formation.¹⁵ On the 42nd day, all the mice were anesthetized with diethylether and sacrificed. Blood was obtained from the abdominal aorta, and separated serum samples were subjected to determination of albumin contents and alanine transaminase (ALT) and gamma-glutamyl transpeptidase (γ-GTP) activities. Albumin and serum enzyme analysis were entrusted to BML (Tokyo). The livers were removed and subjected to histological examination. Significant differences between values were analyzed by one-way ANOVA with Bonferroni post hoc tests using StatView 5.0 (SAS Institute, Cary, NC).

The effects of administration of Trp-P-2 and miso over 42 d are shown in Table 1. During the experimental period, body weight gain in both the TP2 group and the

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Abbreviations: Trp-P-2, 3-amino-1-methyl-5H-pyrido [4,3-b] indole; ALT, alanine transaminase; γ-GTP, gamma-glutamyl transpeptidase; N-OH-Trp-P-2, 3-hydroxyamino-1-methyl-5H-pyrido[4,3-b]indole
Table 1. Effects of Miso on Growth, Serum Parameters, and Liver Injury in Mice Ingesting Trp-P-2

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>TP2</th>
<th>TP2M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>20.5 ± 0.3</td>
<td>20.9 ± 0.4</td>
<td>21.3 ± 0.4</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>32.1 ± 0.2</td>
<td>29.3 ± 0.7*</td>
<td>29.4 ± 0.6*</td>
</tr>
<tr>
<td>Average food intake (g/2d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 1–10</td>
<td>13.0 ± 0.4</td>
<td>11.2 ± 0.2***</td>
<td>12.3 ± 0.4</td>
</tr>
<tr>
<td>Days 11–20</td>
<td>12.1 ± 0.6</td>
<td>12.4 ± 0.3</td>
<td>12.0 ± 0.3</td>
</tr>
<tr>
<td>Days 21–30</td>
<td>12.0 ± 0.2</td>
<td>10.7 ± 0.3***</td>
<td>10.9 ± 0.6*</td>
</tr>
<tr>
<td>Days 31–40</td>
<td>12.5 ± 0.2</td>
<td>10.4 ± 0.3***</td>
<td>10.4 ± 0.4***</td>
</tr>
<tr>
<td>Relative liver weight (mg/g of body weight)</td>
<td>47.3 ± 1.6</td>
<td>40.1 ± 0.7***</td>
<td>40.3 ± 0.9***</td>
</tr>
<tr>
<td>Serum parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>1.66 ± 0.03</td>
<td>1.68 ± 0.03</td>
<td>1.62 ± 0.04</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>25 ± 1.8</td>
<td>56 ± 8.7**</td>
<td>30 ± 5.4</td>
</tr>
<tr>
<td>γ-GTP (IU/l)</td>
<td>1.2 ± 0.2</td>
<td>1.7 ± 0.4</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Signs of liver injury</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatopathic petechial lesion</td>
<td>0/5</td>
<td>2/6</td>
<td>0/5</td>
</tr>
<tr>
<td>Necrosis, vacuolar degeneration, or neutrophil invasion</td>
<td>0/5</td>
<td>3/6</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Values are given as means ± S.E. (control and TP2M; n = 5; TP2; n = 6)
Asterisks represent significant differences from the control group. *p < 0.05, **p < 0.01, ***p < 0.001

TP2M group were significantly reduced as compared to the control group. The average food intake of both groups also decreased after day 21, so this reduction might reflect body and liver weight loss. The serum ALT activity of the TP2 group increased more than 2-fold, as compared with the control mice. γ-GTP levels were not statistically different between the TP2 and control mice, although there did appear to be a trend to higher levels in the TP2 mice. In some of the TP2 mice, γ-GTP levels were 2 to 3-fold higher than in the control mice. No difference was observed in serum albumin levels among the three groups. In addition, Trp-P-2 ingestion caused several histological signs of liver injury, hepatopathic petechial lesion, necrosis, vacuolar degeneration, and invasion of neutrophils (Fig. 1). These changes were considered to be drug-induced liver injury caused by Trp-P-2. Elevation of serum ALT activity is a critical marker of drug-induced liver injury, but we did not find any tumors in the livers of the Trp-P-2 fed mice. Matsukura et al. reported that a period of more than 1 year was needed to express carcinogenicity in mice fed a 0.02% Trp-P-2 diet. The estimated daily dosage of Trp-P-2 in their experiment was nearly same as in our study. Hence, it is probable that the Trp-P-2 administration period in our experiment was not enough long to cause cancer. In the mice fed the diet with miso, no alteration of serum parameters or signs of liver injury were found. These results indicate a preventive effect of dietary miso as to liver injury induced by Trp-P-2. Trp-P-2 incorporated into the liver is catalyzed by cytochrome p450 and transformed to the active metabolite, 3-hydroxyamino-1-methyl-5H-pyrido[4,3-b]indole (N-OH-Trp-P-2). It causes DNA adduct followed by mutation of DNA, increasing the risk of cancer. On the other hand, it is possible that active oxygen species are produced during spontaneous degradation of N-OH-Trp-P-2, and that they cause cleavage of DNA strands in FM3A cells. Continuous severe damage to cellular DNA often brings cell death and necrosis of tissues. Hence, some miso components prevented the development of this injury process. Since no preventive effect as to liver injury against Trp-P-2 was observed in the mice fed the diet with the miso-replaced mixture containing...
soy protein and soybean oil, the effective substrate might lie in other components. One of the possible candidates is isoflavone, which shows anti-inflammatory effects on the lipopolysaccharide-stimulated liver in rodents and has a protective effect against d-galactosamin-induced oxidative damage in the rat liver.\(^9,^{20}\) Furthermore, it has also been reported that phytic acid (PA) and lysophosphatidylcholine (LPC), which are found in the soybean, have protective effects against liver injury caused by CCl\(_4\) and lipopolysaccharide respectively in rodents.\(^{21,22}\) These functional substances, including isoflavones, might show similar effect also in Trp-P-2 induced liver injury. Our data provide the first evidence of a preventive effect of miso as to drug-induced liver injury in mice that ingest Trp-P-2. Further investigation is necessary to determine the protective component as to liver injury in miso. In addition, since observation of liver injury is possible within a relatively short period, it might be a useful marker in the evaluation of the anti-carcinogenic activity of various foods \textit{in vivo}.

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References


