DAMAGE AND REPAIR OF RAT LIVER DNA BY SIMULTANEOUS ORAL ADMINISTRATION OF AMINES AND NITRITE

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The effect of simultaneous oral administration of dimethylamine or aminopyrine and sodium nitrite on the damage and repair of rat liver DNA was studied by the use of centrifugation in alkaline sucrose gradient. Fragmentation of the intact DNA was observed significantly shortly after combined treatment with aminopyrine and nitrite when no liver necrosis occurred yet and the damaged DNA at the lower dosage level of aminopyrine with nitrite was found to be repaired in contrast to the increased activity of serum aminotransferase.

The lowest effective dose on DNA damage at 2 hr after administration was about 40 mg/kg of aminopyrine and 80 mg/kg of sodium nitrite. This effect was equal to that caused by single oral administration of 2 to 10 mg/kg of dimethylnitrosamine, while dimethylamine at 160 or 320 mg/kg together with 80 mg/kg of sodium nitrite produced no pronounced DNA damage.

The significant damage of rat liver DNA by aminopyrine and nitrite correlates well with the easier nitrosation of aminopyrine and the occurrence of high incidence of malignant liver tumors in rats fed aminopyrine together with nitrite already reported by Lijinsky.

Materials and Methods

Male HLA-Wistar rats (Nihon Dobutsu Co., Osaka) weighing approximately 100 g were partially hepatectomized, and liver DNA was labeled by intraperitoneal injection of thymidine [methyl-3H] (specific activity, 20 Ci/mmol, New England Nuclear, Boston, U.S.A.) during the period of regeneration until each animal received a total of 250 μCi as described by Cox et al. All the animals had free access to food and water throughout the experiment. Two weeks after thymidine incorporation, aminopyrine (dimethylaminoantipyrine, Iwaki Seiyaku Co., Tokyo), dimethylamine hydrochloride, and NaNO₂ (Wako Pure Chemical Co., Osaka) dissolved in water were orally administered by a stomach tube, either alone or in various combinations. Animals were killed by withdrawal of blood from the abdominal aorta under anesthetization with ether.

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The preparation of liver suspension and lysing of cells on the alkaline sucrose gradient were made essentially according to Cox et al.8) A suspension of liver cells was prepared by squashing 1 g of liver in a petri dish with 1.5 ml of cold 0.024 M EDTA-0.075 M NaCl buffer (pH 7.5). The suspension was centrifuged for 1 min at 300 rpm at 4° in a Tominaga Model RS-18P refrigerated centrifuge to sediment the pieces of liver tissue and 0.1 ml of the supernatant was added to 0.3 ml of a lysing solution (0.3 M NaCl-0.03 M EDTA-0.5% sodium dodecyl sulfate, pH 10) which was layered on top of 5-20% alkaline sucrose gradient (also containing 0.9 M NaCl and 0.3 M NaOH). The gradient had been formed on 1 ml of a 2.3 M sucrose cushion in a 5-ml cellulose nitrate tube. The gradients were allowed to stand at 37° for 15 min and then were centrifuged for 40 min at 40,000 rpm at 30° using an RPS-40 rotor in a Hitachi Model 65P ultracentrifuge. Fractions of 0.3 ml each were collected from the top of the gradient by a fractionator (Model 640, Instrumentation Specialties Co., Lincoln, U.S.A.), and bovine serum albumin (20 µg) was added to each fraction before precipitation with 2 ml of cold 5% trichloroacetic acid. The precipitate was collected on a Millipore nitrocellulose filter (Type HAWP) and washed twice with 2 ml of cold 5% trichloroacetic acid after cooling overnight. Radioactivity of the precipitate was assayed with a liquid scintillation spectrometer (Beckmann 133 or Packard Tri-Carb 3375) after combustion with a Packard Tri-Carb 306 auto-sample oxidizer.

Serum glutamine-alanine aminotransferase (L-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2) activity was determined by the use of a commercial diagnostic kit (STA Test Wako, Wako Pure Chemical Co., Osaka). All the experiments were replicated at least twice to ascertain their reproducibility.

**Results**

It is known that a single intraperitoneal injection of dimethylnitrosamine to rats causes breakage of single-stranded DNA.5) Dimethylnitrosamine given orally to rats was also found to induce DNA damage, as shown in Fig. 1. The lowest effective dose was 10 mg/kg under the present experimental conditions, and higher doses caused more extensive breakage of liver DNA. The percentages of radioactivity in intact DNA fractions (Nos. 13-18) of the gradients were: control, 83%;
LIVER DNA DAMAGE BY AMINES AND NITRITE

Fig. 3. Effect of aminopyrine plus nitrite on the sedimentation of rat liver DNA in alkaline sucrose gradient 2 hr after oral administration

- 40 mg/kg aminopyrine + 80 mg/kg NaNO₂
- 80 mg/kg aminopyrine + 80 mg/kg NaNO₂
- 160 mg/kg aminopyrine + 80 mg/kg NaNO₂
- 320 mg/kg aminopyrine + 80 mg/kg NaNO₂

2 mg/kg dimethylnitrosamine, 69%; 10 mg/kg, 44%; 20 mg/kg, 34%; 40 mg/kg, 22%.

On the other hand, combined administration of dimethylamine with nitrite produced no pronounced DNA damage as shown in Fig. 2. Dimethylamine at 160 or 320 mg/kg together with 80 mg/kg of NaNO₂ caused no significant fragmentation, and only a slight shift of the radioactivity to lighter fractions of the gradient was observed at 640 mg/kg of dimethylamine with nitrite.

By contrast, administration of aminopyrine in combination with nitrite obviously produced a DNA damage (Fig. 3). Even at the dosage level of 40 mg/kg of aminopyrine, an appreciable amount of the radioactivity was sedimented in the midportions of the gradient. The proportion of tritium found in lighter fractions increased with the increase of dosage up to 160 mg/kg, whereas the gradient profile at 320 mg/kg was almost similar to that of 160 mg/kg. The percentages of radioactivity in intact DNA fractions (Nos. 13~18) of the gradients were: 40 mg/kg aminopyrine + 80 mg/kg NaNO₂ 62%; 80 mg/kg + 80 mg/kg, 52%; 160 mg/kg + 80 mg/kg, 33%; 320 mg/kg + 80 mg/kg, 32%.

Forty-eight hours after administration of 160 mg/kg of aminopyrine together with 80 mg/kg of NaNO₂, the sedimentation pattern showed almost a single peak (Fig. 4). This fact may imply that most of the damaged DNA has been repaired. At the dose of 320 mg/kg of aminopyrine plus 80 mg/kg of NaNO₂, DNA damage was not recovered after 48 hr. The percentages of radioactivity...
Table I. Effect of Aminopyrine Plus Nitrite on Serum Glutamine-Alanine Aminotransferase (SGPT) Activity in Partially Hepatectomized Rats

<table>
<thead>
<tr>
<th>Compound administered (mg/kg)</th>
<th>SGPT activity (Karmen units)</th>
<th>2 hr</th>
<th>48 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminopyrine</td>
<td>NaNO₂</td>
<td></td>
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<tr>
<td>0</td>
<td>0</td>
<td>23</td>
<td>21</td>
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<tr>
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<td>21</td>
<td>56</td>
</tr>
<tr>
<td>320</td>
<td>0</td>
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</table>

Each compound was administered orally to 3 rats. The enzyme activity is expressed as a mean of 3 experiments.

in intact DNA fractions (Nos. 13~18) of the gradients were: 160 mg/kg aminopyrine + 80 mg/kg NaNO₂, 61%; 320 mg/kg + 80 mg/kg, 36%.

Animals given dimethylamine (640 mg/kg), aminopyrine (320 mg/kg), or NaNO₂ (80 mg/kg) singly showed no liver DNA damage.

Concurrently with DNA breakage described above, serum glutamine-alanine aminotransferase activities, an index of liver necrosis, were examined after simultaneous administration of aminopyrine and NaNO₂. As represented in Table I, the enzyme activity increased 2- to 3-fold 48 hr after the combined treatment, whereas, if separately given, either compound did not affect glutamine-alanine transaminase activity. Even if these compounds were dosed simultaneously, the enzyme activity was comparable to that of the control 2 hr after the treatment.

Histopathological examination of the liver 2 hr after combined oral administration of NaNO₂ with dimethylamine or aminopyrine revealed no sign of cellular damage. After 48 hr, slight necrotic areas or individual cell necrosis was found at higher doses of aminopyrine (160 and 320 mg/kg) and NaNO₂ (80 mg/kg).

**DISCUSSION**

This present study clearly showed that combined oral administration of sodium nitrite and aminopyrine induces damage of rat liver DNA shortly after the treatment when no liver necrosis occurs yet. The damaged DNA is found to be repaired in contrast to the increased activity of serum aminotransferase with the progress of liver necrosis caused by dimethylnitrosamine. Thus the DNA damage observed in this experiment is presumably not correlated with degradation of DNA in a dying cell, consistent with the results by Damjanov et al. The fact that the damaging effect on DNA by the combined administration of aminopyrine with nitrite is more significant than by dimethylamine plus nitrite apparently stems from the easier nitrosation of aminopyrine; dimethylamine is the least nitrosable compound for its strong basicity and aminopyrine gave dimethylnitrosamine in a high yield with nitrite in vitro. The lowest effective dose on DNA damage by the combined treatment with aminopyrine and nitrite is estimated to be about 40 mg/kg and 80 mg/kg, respectively, and the effect is roughly equal to that caused by a single oral administration of 2 to 10 mg/kg of dimethylnitrosamine. Therefore, yield of dimethylnitrosamine from aminopyrine and nitrite is presumably very high in the rat stomach. In accord with these facts, no carcinogenesis has been observed by feeding of dimethylamine simultaneously with nitrite, whereas occurrence of high incidence of malignant tumors was reported by aminopyrine and nitrite. As many chemicals containing dialkylamino group may react with nitrite to form dialkylnitrosamine, further studies of the DNA damage and repair by nitrosamine formed in vivo are desirable.

The authors are grateful to Dr. H. Tsuda, Nagoya City University Medical School, for carrying out the histopathological examinations. They wish to express their thanks to Miss R. Takeyasu for her skilled technical assistances.

*(Received June 25, 1976)*
REFERENCES