Carcinogenicity of Triethanolamine in Mice and Its Mutagenicity after Reaction with Sodium Nitrite in Bacteria

Hiroshi Hoshino and Hiroshi Tanooka

ABSTRACT

Mice fed a diet containing 0.3 or 0.03% triethanolamine developed malignant tumors. Females showed a high incidence of tumors in lymphoid tissues, while this type was absent in males. Tumors in other tissues were produced at a considerable rate in both sexes, but no hepatoma was found. Triethanolamine was not mutagenic to Bacillus subtilis by itself, but it became mutagenic after reacting with sodium nitrite under acidic conditions or when the mixture was heated. Although N-nitrosodiethanolamine, a known carcinogen and mutagen, was detected in the reaction mixture by thin-layer chromatography, it may not be the main mutagenic product, because the product was a stable and direct mutagen and its mutagenic activity was destroyed by liver enzymes, unlike N-nitrosodiethanolamine. The lethal and mutagenic DNA damages produced by this unidentified product were susceptible to some extent to the repair functions of the bacteria.

INTRODUCTION

TREA has been suspected to be a carcinogen, since it can be converted to NDEA by reaction with nitrous acid under acidic conditions (4), and NDEA is known to be a carcinogen that produces hepatomas in rats when given with drinking water (2). An appreciable amount of TREA is contained in a widely used synthetic cutting fluid (3) and in commercial cosmetics. TREA in cutting fluid can be converted to NDEA under gastric conditions (11), and the presence of NDEA in the cutting fluid has been directly confirmed (3).

We report here the carcinogenicity of TREA in mice and its mutagenicity in bacteria after reacting with sodium nitrite. Our results suggest that some product other than NDEA is responsible for the carcinogenicity and mutagenic activity of TREA.

MATERIALS AND METHODS

Chemicals. TREA hydrochloride (analytical grade) was a product of Merck, Darmstadt, Germany. Sodium nitrite was a product of Wako Pure Chemicals, Tokyo, Japan. NDEA was synthesized and kindly supplied by Dr. M. Okada, Tokyo Biochemical Research Institute, Tokyo, Japan.

Mouse Experiments. Male and female ICR-JCL mice used were 6 weeks old at the start of the experiment. Control diet was Mouse Diet CE-2 (Clea Japan, Inc., Tokyo, Japan). TREA-containing diet was prepared by Clea Japan, Inc. by adding 0.3 or 0.03% (w/w) TREA to the powdered diet and by heating for 40 min at 100°. Almost 100% of the TREA ingredient was recovered from the TREA diet 2 years and 3 months after its preparation, according to measurement by A. Tanamura and A. Sakai (personal communication). Mice were fed these 2 types of diet throughout their life span. After death or sacrifice at an appropriate period, each mouse was examined by autopsy, and the types of tumors were histologically determined.

RESULTS

Tumor Induction in Mice. Tumors produced in mice fed TREA-containing diet were histologically examined, and the...
results are shown in Table 1. Tumors are grossly classified into 2 groups, those produced in lymphoid tissues and others. No single mouse carried 2 or more types of tumors. The number of tumors and tumor incidence rates are also shown in Table 2 with statistical evaluations.

In females the total incidence of malignant tumors in 0.03% and 0.3% TREA-fed mice was significantly higher than that in the controls (32%; p < 0.01). The dose-response relationship seemed to exist in tumor incidence rates in the control (2.8%), 0.03% TREA (27%), and 0.3% TREA (36%) groups, but the difference between the latter 2 values was statistically not significant. The higher tumor incidence in females was due to lymphoma formation (22%; p < 0.05), which was absent in males. Various types of malignant tumors were also produced with TREA in other tissues and seemed to be independent of sex. Their incidence, when summed up for both sexes, was significantly higher than that in untreated mice (8.2%; p < 0.05).

Mice fed TREA-containing diet survived as long as the controls did. The survival rate was 50% at 85 weeks in females and at 65 weeks in males.

**Mutation Induction in Bacteria.** Mutagenicity of TREA was tested with an excision repair-deficient B. subtilis strain TKJ5211 (Chart 1). TREA alone did not induce His+ mutants, with or without activation by liver homogenates. When TREA was incubated with sodium nitrite at pH 3.5, a directly acting mutagen was produced, but its mutagenic activity was destroyed by the S-9 liver homogenate. TREA alone kept in acid showed no mutagenicity. Sodium nitrite itself exhibited some mutagenicity, but it was not high enough to explain the mutagenic activity of the reaction mixture.

The mutagenic activity was more pronounced with direct heating of TREA:sodium nitrite. Chart 2 shows the lethal and mutagenic effects of the heated mixture on B. subtilis cells carrying different DNA repair capacities. Both effects were larger in the excision repair-deficient strain than in the wild type and were more pronounced in the strain further lacking the polA function. The mutagenic activity of the heated mixture was not lost after standing for 5 days at room temperature (Chart 2b) and was detectable even after

![Chart 1. Mutant yields in B. subtilis strain TKJ5211(uvr-)](chart1.png)

**Table 1**

<table>
<thead>
<tr>
<th>Sex</th>
<th>TREA in diet (%)</th>
<th>No. of mice</th>
<th>Effective no.</th>
<th>No.</th>
<th>Thymic lymphoma</th>
<th>Nonthymic lymphoma</th>
<th>No. of malignant tumors</th>
<th>Tumor incidence (B/A) x 100 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>0</td>
<td>40</td>
<td>36</td>
<td>1</td>
<td></td>
<td>(90)</td>
<td>0</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>40</td>
<td>37</td>
<td>7</td>
<td>LC (36)</td>
<td></td>
<td>4</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>40</td>
<td>36</td>
<td>9</td>
<td>LC (69, 114)</td>
<td></td>
<td>13</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0</td>
<td>40</td>
<td>35</td>
<td>1</td>
<td></td>
<td>(46)</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>40</td>
<td>33</td>
<td>0</td>
<td></td>
<td></td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>40</td>
<td>28</td>
<td>0</td>
<td></td>
<td></td>
<td>1</td>
<td>3.6</td>
</tr>
</tbody>
</table>

- a Number of mice at the first tumor appearance (36 weeks).
- b Adenomas were eliminated.
- c Statistical evaluations: p(f, g) < 0.01, p(f, h) < 0.01, p(g, h) > 0.05, p(i, j) > 0.05, p(i, k) > 0.05.
- d LC, lymphocytic; LB, lymphoblastic; RC, reticulum cell type; PC, plasmacytic.
- e Numbers in parentheses, age of mice at sacrifice or death (weeks).
concentration are the same as above. D. mutagenic activity of TREA:sodium TKJ6321 (•), and TKJ5211 treated with autoclaved TREA (O) or sodium excision-repair-deficient TKJ5211 (A), excision and polymerase l-deficient and washed before plating, a, surviving fractions of wild-type HA101 (•), 3920

5 months. Heating of TREA or sodium nitrite alone did not yield any lethal (Chart 2a) or mutagenic activity (data not shown). Synthetic NDEA without S-9 activation induced no lethal (Chart 2b) or mutagenic activity (data not shown). Heating of TREA or sodium nitrite alone did not yield any lethal (Chart 2a) or mutagenic activity (data not shown). Synthetic NDEA without S-9 activation induced no lethal (Chart 2b) or mutagenic activity (data not shown). Heating of TREA or sodium nitrite alone did not yield any lethal (Chart 2a) or mutagenic activity (data not shown).

Product Analysis by Thin-Layer Chromatography. The reaction mixtures of TREA and sodium nitrite prepared under various conditions were analyzed by thin-layer chromatography. Authentic NDEA was found at Rf 0.22. At this position, a coincident spot was found in the TREA:sodium nitrite mixture incubated for 8 hr at 37° and pH 3.5 or autoclaved for 20 min at 120°, indicating the formation of a nitroso compound. Heating of TREA alone did not yield any detectable spot of a nitroso compound.

DISCUSSION

Feeding of mice on TREA-containing diet produced various types of tumors (Table 1). Formation of the secondary nitrosamine, NDEA, is immediately suggested, since TREA might be converted to NDEA under gastric conditions (4) or during preparation of TREA diet. However, tumor types in mice were quite different from those found by Druckrey et al. (2) in rats given NDEA in drinking water, which were mostly hepatomas. This discrepancy may be due to the difference between mouse and rat or to different methods of administration, but another strong indication is the production of a carcinogen other than NDEA from TREA. Decomposition of TREA is unlikely ("Materials and Methods"). TREA must be converted to a stable carcinogen through some unknown mechanisms. This possibility was further examined with B. subtilis by assuming the parallelism between the carcinogenicity and mutagenicity of the chemical.

In bacterial experiments TREA alone was not converted to a mutagen either by incubation at acidic conditions or by heating. The mutagenic activity was found after these treatments in the presence of sodium nitrite. A nitroso compound corresponding to NDEA was also found in these reaction mixtures. However, mutagenic action of the mixture was quite different from that of NDEA. The product was a stable and direct mutagen and lost activity in the presence of liver enzymes, while NDEA is rather unstable and did not induce mutation in B. subtilis by itself. According to the experiments of M. Okada and E. Suzuki (personal communication), NDEA requires activation by liver enzymes to become mutagenic to Salmonella typhimurium TA1535, as do other nitrosamines (10). Therefore, the mutagenic product found in the present experiment seems to be different from NDEA.

An interesting mutagenic product was found in the reaction of polyamines with nitrous acid (P. E. Hartman, personal communication; Ref. 9). The product is a short-lived and direct mutagen, and induction of the dose-response mutation in S. typhimurium is a 2-hit type. This reaction is suggestive for our case. However, the mutagenic product in the TREA:sodium nitrite reaction mixture was stable, the dose-response curve for mutation frequency in the wild type was a 1-hit type, and furthermore its action was little dependent on the excision repair function of the treated cells and to some extent on the polA function (Chart 2). In these respects the action of the product resembles that of ionizing radiation.

At present, the chemical nature of the mutagenic product in TREA:sodium nitrite is unidentified. Furthermore, whether this product really produced tumors in mice given the TREA diet is still uncertain. It might be formed under gastric conditions or during preparation of the TREA diet. These questions are left for further investigation. At least, NDEA is not the sole suspect.

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REFERENCES

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