Induction of Sister-Chromatid Exchange by Ethylene Glycol Monomethylether and its Metabolite

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Abstract: The frequency of sister-chromatid exchange (SCE) induced by ethylene glycol monomethylether (EGME) and methoxyacetic acid (MAA), a major metabolite of EGME, was determined in human peripheral blood, and for EGME in mouse bone marrow cells. In the experiment on the human peripheral blood, the induction of SCE was observed after the addition of MAA to the culture medium, but not after the addition of EGME. However, EGME induced SCE in the mouse bone marrow cells, when administered by intraperitoneal injection. These results suggested that EGME did not induce SCE itself, but that MAA, one of the major metabolites of EGME induces SCE.

Key words: Ethylene glycol monomethylether, Methoxyacetic acid, Sister-chromatid exchange, Human peripheral blood, Mouse bone marrow cell

Introduction

Glycol ethers, which have high solubility in water, a relatively high boiling point and a relatively low vapour pressure, have been extensively used in industrial solvents. Ethylene glycol monomethylether (EGME), which has polar properties of alcohols and non polar properties of ethers, is one of the most widely used solvents among the glycol ether compounds. EGME has an especially wide range of uses as solvents for dyes, inks, paints and lacquers. However, the use of EGME has recently been limited in the USA, because it has been reported to cause toxicity in the male reproductive system, the hematopoietic system, and in embryonic development1.

Nagano et al.2,3) reported a decrease in white cell counts in animals after exposure to EGME. Miller et al.4) and Grant et al.5) also reported a decrease in white cell counts and thymus and spleen weights in rats which had been exposed to EGME. Some studies have shown that major metabolites of EGME are methoxyacetaldehyde (MALD) and methoxyacetic acid (MAA), and MAA is recognized for 75–95% in excreted urine of rats exposed to EGME6,7). It is not clear whether the toxicity of EGME is caused by one of or both of the metabolites, which are also a principal toxic metabolite of EGME8). The in vitro mutagenicity of EGME has been evaluated using the Salmonella typhimurium and unscheduled DNA synthesis. These assays showed negative results9), but MALD showed mutagenic potency with strain TA 98a at high concentration10). Also, McGregor et al.11) reported that chromosome aberrations were not found in rats exposed to EGME. The cytogenetic effect of EGME using micronucleus test and sister-chromatid exchange (SCE) has been reported. Micronucleus induction showed extreme potency12). Investigation of SCE induction has been shown to be negative for EGME, but positive for MALD with human lymphocytes13). Recently, the mutagenicity of EGME using Comet assay has been reported to be a new method for assessing DNA damage. According to Anderson et al.14), EGME caused an increase in damage in bone marrow cells of rat. Experiments have not been conducted to evaluate the carcinogenicity of glycol ethers. Teratogenicity
has been reported in some research papers: Hanley et al.\textsuperscript{15} reported that teratogenesis resulting from fetotoxic effects was not identified in an experiment on pregnant rats, rabbits and mice exposed to EGME. However, a preliminary study using monkeys, which was done by Scott et al.\textsuperscript{16}, observed teratogenicity in which MAA played a role as a possible causative factor. Other reports showed that the teratogenicity and embryotoxicity are most likely due to MALD.\textsuperscript{6,17,18}

In this study, we investigated the induction of SCE in the human peripheral blood to which EGME or MAA was added, and the bone marrow cells of mice after being exposed to EGME.

\textbf{Materials and Methods}

\textbf{Chemicals}

EGME (purity: >99\%), MAA (purity: >95\%), 5-bromo-2'-deoxyuridine (BrdU), and penicillin-streptomycin were obtained from Wako Pure Chemical Co. (Japan). Mitomycin C (MMC, biological grade), phytohemagglutinin-M (PHA) and fetal calf serum (FCS) were obtained from Gibco Laboratories (USA). RPMI Medium 1640 was obtained from Nissui Saisei-kyaku (Japan). Other chemicals used were of special reagent grade.

\textbf{Animal}

Male mice (4–5 weeks of age) were obtained from Seiwa Experimental Animals Ltd. (Japan) and kept about 2 weeks until the experiment. Animals were given a standard diet (UE-2, Clea Japan Inc.) and drinking water and kept at 25 \pm 1°C (temperature) and 55 \pm 5% (humidity) on a 12-h light/dark cycle during the study. A monthly quarantine was carried out in the experimental animal room.

\textbf{In vitro test}

The SCE test using human whole blood was performed basically according to the method of Morimoto\textsuperscript{19}. Peripheral blood samples from a healthy adult man and a healthy adult woman with no history of smoking were used. Whole blood (0.3 ml) was added to 4.7 ml of RPMI medium containing 15% FCS, 1% penicillin-streptomycin and 3% PHA. Also, the medium contained 20 \muM of BrdU. EGME and MAA sample solutions were dissolved in phosphate buffered saline solutions. Sample solutions and control solution of phosphate buffered solution were added after 24 hr from the beginning of culture. The cultures were incubated at 37°C with 5% CO₂ for 72 hr in complete darkness. Four hours before fixation, colcemid (final concentration: f.c. 2 \times 10^{-7} M) was added.

In vivo/in vitro test

This SCE test was carried out on the culture in a test tube using the bone marrow cells of animals exposed to chemicals. Therefore, we used the ddY male mice raised for approximately 7 weeks and 40 g body weight for the test, and one group had five mice. The mice were given intraperitoneal injections at a dose of 500 mg/kg, b.w., or 1,000 mg/kg, b.w.- that is approximately half of the LD₅₀\textsuperscript{20} - and then sacrificed after 24 hr. The bone marrow cells were removed from the femurs of the mice by washing with RPMI 1640 medium containing 15% FCS and 1% penicillin-streptomycin, and added to BrdU (f.c.: 20 \muM). The cells in the BrdU-containing medium were incubated at 37°C with 5% CO₂ in air for 30 hr. Three hours before fixation, colcemid (5 \times 10^{-8} M) was added.

The cells obtained from the cultures of human peripheral blood and mouse bone marrow were then collected by centrifugation (600 \times g), resuspended in 0.075 M KCl hypotonic solution, and left for 15 min in a 37°C water bath to make the chromosomes spread. They were then fixed 3 times in methanol : acetic acid solution (3:1, v/v). The concentrated suspension of cells were dropped on microslides from a height of 50 cm and then dried at 90°C. Two slides were prepared for each individual.

\textbf{Stain}

The cells were stained by a modification of Goto et al.\textsuperscript{21} The slides were stained for 18 min in a solution made up of 5 \mug Hoechst 33258 per ml in M/15 Soerensen's buffer, pH 6.8. Then they were washed, dried, mounted with a buffer solution (pH 8.0) under a coverslip, and exposed for 8 min. to 360 nm of light emitted from a fluorescent black light (National, 15 w) from the right, 2 cm above the coverslip on the slide-warming tray heated at 60°C. The coverslip was then removed and the slides were stained for 10 min in a 3% Giemsa solution.

SCEs were analyzed on 25 second-division cells for each point. The data were statistically analyzed by the Student's t-test (one-tailed). Also, cells dividing for the first (X₁), second (X₂) and third or more (X₃+) times were determined by Morimoto and Wolff report\textsuperscript{22}. The X₁, X₂ and X₃+ times were scored by percentage using two hundred metaphase cells in the culture of blood sample of adult women.

\textbf{Results}

The human peripheral blood cells obtained from the cultures exposed to EGME or MAA at various concentrations
were examined, and a summary of the SCE frequency of both compounds is shown in Table 1. The SCE frequency of EGME produced the same value as each of the control samples of the three donors. MAA from three donors, however, caused an increase in SCEs dose-dependently. SCE frequencies for a 0.1 mM concentration of MAA had the same value as for each of the control groups, and those for 1 mM and 10 mM concentrations of MAA showed a significant increase (p<0.05).

The change in the relative frequencies of $X_1$, $X_2$, and $X_3^+$ metaphases is shown in Table 2. The cultures of control fixed 72 hours contained 10% $X_1$, 28% $X_2$, and 60% $X_3^+$ metaphase. In the case of EGME, the relative frequencies of three metaphases were found about 25% $X_1$, 40% $X_2$, and 30% $X_3^+$ with three concentration levels of EGME. However, in the cultures exposed to EGME, the frequency of $X_1$ and $X_2$ cells increased, and those of $X_3^+$ cells decreased by half in comparison with those of control. This result indicates a delay in the cell cycle. In the case of MAA, the frequency of $X_1$ increased and the frequency of $X_3^+$ decreased dose-dependently, respectively. The cell cycle frequencies treated by 0.1 mM MAA caused the same as those of three concentrations of EGME, but in the case of the frequency of 10 mM MAA, $X_1$ increased more than 7 times and $X_3^+$ decreased less than a tenth in comparison with those of control. The high concentration of MAA indicated a delay in the cell cycle.

An in vivo/in vitro test for SCE frequency in the mouse bone marrow after administration of EGME by intraperitoneal injection was performed. The SCE frequency is shown in Table 3. As shown in Table 3, increasing doses of EGME caused increasing SCE frequencies. SCE frequencies induced by a dose of 500 mg/kg, b.w. or 1,000 mg/kg, b.w. of EGME were $4.00 \pm 1.54$ cell, $4.61 \pm 2.31$ cell, respectively, which were significantly higher than those of the control groups (p<0.01). Moreover, the frequency of spontaneous SCE in the mouse bone marrow as is observed here ($2.89 \pm 1.44$ cell) agrees with that reported in other studies.

### Table 1. SCE frequency obtained from human lymphocytes exposed to EGMA or MAA

<table>
<thead>
<tr>
<th>Conc. (mM)</th>
<th>Mean SCE per cell (means ± S.D.)</th>
<th>Donor A</th>
<th>Donor B</th>
<th>Donor C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.55 ± 1.64</td>
<td>4.58 ± 2.43</td>
<td>5.32 ± 1.41</td>
<td></td>
</tr>
<tr>
<td>EGME 1</td>
<td>4.90 ± 1.97</td>
<td>4.71 ± 1.73</td>
<td>4.70 ± 1.14</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4.67 ± 0.96</td>
<td>5.08 ± 1.25</td>
<td>6.10 ± 1.74</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>5.90 ± 0.97</td>
<td>5.83 ± 0.83</td>
<td>5.96 ± 1.02</td>
<td></td>
</tr>
<tr>
<td>MAA 0.1</td>
<td>4.07 ± 1.07</td>
<td>3.97 ± 1.11</td>
<td>4.50 ± 0.89</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.20 ± 2.38*</td>
<td>6.47 ± 2.61*</td>
<td>7.21 ± 2.12*</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>8.00 ± 3.28*</td>
<td>9.44 ± 1.78**</td>
<td>8.92 ± 2.28*</td>
<td></td>
</tr>
<tr>
<td>MMC 0.0005</td>
<td>9.72 ± 1.84**</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: p<0.05 compared with control. **: p<0.01 compared with control.

Donors A and B: Adult man. Donor C: Adult woman.

### Table 2. The relative frequencies of $X_1$, $X_2$, and $X_3^+$ metaphases in human lymphocytes treated with EGME or MAA in vitro for 72 hr

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>Relative frequencies (% of total metaphases)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_1$</td>
</tr>
<tr>
<td>Control</td>
<td>10.4</td>
</tr>
<tr>
<td>EGME 1</td>
<td>27.1</td>
</tr>
<tr>
<td>10</td>
<td>24.6</td>
</tr>
<tr>
<td>100</td>
<td>24.2</td>
</tr>
<tr>
<td>MAA 0.1</td>
<td>20.8</td>
</tr>
<tr>
<td>1</td>
<td>33.3</td>
</tr>
<tr>
<td>10</td>
<td>68.5</td>
</tr>
</tbody>
</table>

### Table 3. SCE frequency obtained from in vivo/in vitro test using bone marrow cell of mouse exposed to EGME

<table>
<thead>
<tr>
<th>Sample of cells analysed</th>
<th>Mean SCE/cell (means ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>500</td>
</tr>
</tbody>
</table>

*: p<0.01 compared with control between groups of total.

Discussion

The present study has examined that EGME and MAA induce SCE in human lymphocytes in vitro and in mouse bone marrow cells in vivo/in vitro, but did not use S9 (metabolic enzyme) by in vitro. Our study shows that MAA of high concentration—except concentration of 0.1 mM—but not EGME can induce a significant increase of SCE in human lymphocytes. There was no significant induction of SCE of human lymphocytes with 1–100 mM EGME. This result was in agreement with the findings of a Chiewchanwit and William report that SCE frequencies after treatment of human lymphocytes with 1–125 mM EGME ranged from...
6.0 ± 0.4/cell to 6.8 ± 0.4/cell and were negative. In contrast, mean SCE frequencies obtained from human lymphocytes ranged from 6.47 ± 2.61/cell to 9.44 ± 1.78/cell. This result was significant induction, but at a low frequency in comparison with SCE frequency (14.8 ± 2.9/cell) of human lymphocytes with 10 mM MALD\(^1\). It is difficult simply to compare those two results because of the difference in experimental design. Therefore, it is necessary to study SCE induction of EGME, MAA, and MALD under the same conditions. We investigated the distribution cell cycles from 72 hr cultures treated with EGME or MAA. The delay in cell turnover in cultured lymphocytes treated with MAA were clearly dose-dependent. These results agree with the results of SCE induction obtained from EGME or MAA by \textit{in vitro} test.

On the other hand, EGMA induction using \textit{in vivo}/\textit{in vitro} tests caused a significant increase. This SCE frequency was far less than those (16.0 ± 5.06/cell, unpublished observation) obtained by \textit{in vivo}/\textit{in vitro} tests of exposure to MMC (4 mg/kg, b.w.). This result indicates that SCE induction by EGME caused a presence of metabolic activation in the body. It is possible that SCE induction was caused by MAA according to an \textit{in vitro} test. However, it is not clear whether SCE induction causes MAA or MALD.

Our present results suggest that SCE induction by EGME is facilitated through bioactivation.

References

100.


