Relationship between chromosome aberrations at the first cleavage metaphases and postimplantation loss in dominant lethal mutations induced by isopropyl methanesulfonate

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ABSTRACT

A high dose (200 mg/kg) of isopropyl methanesulfonate (iPMS) induced a high frequency of postimplantation loss in postmeiotic male germ cell stages, but the frequency of eggs with chromosome aberrations observed at the first cleavage metaphases was very low. Therefore, we suggest that the postimplantation loss in dominant lethal mutations induced by iPMS is mainly attributable to the alteration of subchromosome material at the first cleavage metaphases.

1. INTRODUCTION

In general, the principal cause of dominant lethal mutations in mammalian spermatogenesis is considered to be chromosome aberrations at the first cleavage metaphase (Brewen et al. 1975; Bürki and Sheridan 1978; Generoso et al. 1979; Katoh and Tanaka 1980; Katoh et al. 1981; Katoh et al. 1982), because the stage and frequency of the maximum sensitivity for the induction of chromosome aberrations in the first cleavage metaphases (in paternal chromosome sets) agrees very well with those for induced dominant lethal mutations. Dominant lethal mutations cause pre- and post-implantation loss of eggs, and the relation between pre- and post-implantation loss is generally dose-dependent; postimplantation loss decreases with increasing dose whereas the preimplantation loss increases (Ehling 1977). However, a high dose of iPMS induces dominant lethal mutations with a high frequency of postimplantation loss in postmeiotic male germ cells (Ehling et al. 1972). This is a characteristic feature of iPMS. iPMS-induced chromosome aberrations in postmeiotic stages might therefore be different from those of other chemicals.

In the present experiments, comparative studies through cytological examinations of the first cleavage metaphase and dominant lethal tests were conducted in order to determine whether postimplantation loss induced by iPMS is attributable to eggs with chromosome aberrations.
2. MATERIALS AND METHODS

Nine-week-old BDF1 (C57BL/6 × DBA/2, purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Japan) male mice were injected intraperitoneally within 15 min with 200 mg/kg of iPMS (obtained from Eastman Kodak Co.) in cold Hanks' balanced salts solution at a volume of 20 ml/kg. The control males received the same volume of Hanks' solution. The relative times between treatment and mating were determined on the bases of data reported by Oakberg (1956) and Oakberg and Diminno (1960). In chromosome experiments and dominant lethal tests, each male was sequentially mated with one virgin female of the same strain per week during a 5-week period after treatment and females were checked daily for the presence of a vaginal plug after the end of the dark period. The ovulation times of mated females were partially synchronized by maintaining them under reversed dark-light conditions (dark period, 10:00–18:00) for at least 3 weeks before matings. The time of ovulation was near the end of the dark period under the present conditions.

Chromosome analysis of the first cleavage metaphase

Females with vaginal plug were injected intraperitoneally with 4 mg/kg colchicine at 15 h after ovulation, and the eggs were collected from the ampulla 7 h later. Chromosome preparations were made by using the method of Katoh and Tanaka (1980). Chromosome analysis was done on the paternal chromosome sets. An egg with the second meiotic metaphase chromosomes was considered to be unfertilized.

Dominant lethal test

On the 13th day after detection of a vaginal plug, females were dissected and the numbers of living and dead embryos were scored. The frequency of dominant lethal mutations was estimated as follows.

(a) pre- and post-implantation loss (%) =

\[
100 \times \left(1 - \frac{\text{living embryos/female with vaginal plug (iPMS)}}{\text{living embryos/female with vaginal plug (control)}}\right)
\]

(b) preimplantation loss (%) =

\[
100 \times \left(1 - \frac{\text{implants/female with vaginal plug (iPMS)}}{\text{implants/female with vaginal plug (control)}}\right)
\]

(c) postimplantation loss (%) = (a) − (b)
Analysis of cleavages stages

Females mated during 7–10 days after iPMS treatment of male mice were sacrificed at 72 h after ovulation. Embryos collected from the uterus were prepared in the same way as chromosome preparations and scored for blastomere nuclei.

3. RESULTS

Chromosome analysis of the first cleavage metaphases

A pronounced increase of the frequency of chromosome aberrations was not observed in any spermatogenic stage. A low yield of chromosome aberrations was uniformly detected in spermatozoa, spermatids and late spermatocytes. The mean frequency of eggs with one or two chromosome aberrations was only 7.8% in the mating interval of 0–26 days posttreatment. Fifty-two chromosome aberrations sampled up to 26 days after treatment exhibited the following types of aberrations: 11 chromatid-type (21.1% of total chromosome aberrations) and 41 chromosome-type aberrations (78.9% of total chromosome aberrations); there were 9 chromatid-type and 12 chromosome-type gaps and breaks, 27 acentric fragments, 1 triradial and 1 quadriradial translocation figures. Only 2 chromosome-type exchanges (monocentric or dicentric) were induced in spermatids. No stage-specific differences of chromosome aberrations were observed.

After 23 days, the frequency of unfertilized eggs increased dramatically until the mating interval of 31–35 days.

Dominant lethal test

The mean number of living embryos per female was clearly reduced and a high yield of dominant lethal mutations was consistently induced in all spermatogenic stages. The mean frequency of pre- and post-implantation loss based on formula (a) was 65.3% and postimplantation death was found to be 45.6% according to formula (c) in the period of 0–18 days after treatment of paternal postmeiotic germ cells. Thus, the majority of iPMS-induced dominant lethal mutations in spermatozoa and spermatids resulted in postimplantation loss (mean 69.8% of pre- and post-implantation losses). On the other hand, those induced in spermatocytes were preimplantation losses due to unfertilized eggs.

Analysis of cleavage stages

Most of the blastomeres in the control had more than 31 cells at 72 h after ovulation. However, the iPMS group showed a slight retardation of embryonic development (Table 3).
In comparing the result for chromosome aberrations with that for dominant lethal mutations, the frequency of eggs with chromosome aberrations did not coincide with that of dominant lethal mutations. Despite the high frequency of dominant lethal mutations, a very low frequency of chromosome aberrations was consistently induced by a dose of 200 mg/kg iPMS in the postmeiotic stage (Table 1, 2). This is an interesting phenomenon. The mechanism of dominant lethal mutations induced with iPMS may be quite different from that of the mutations induced with other agents, such as methyl methanesulfonate, triethylenemelamine, mitomycin C, cyclophosphamide and X-ray irradiation (Katoh and Tanaka 1980; Bürki and Sheridan 1978; Katoh et al. 1981; Katoh et al. 1982; Katoh et al. 1980). In the mating interval of 7-10 days after treatment, iPMS induced only 8.8% eggs with chromosome aberrations (Table 1), 19.4% preimplantation loss and 48.0% postimplantation loss (Table 2). Most of the preimplantation loss seems to be due to eggs with chromosome aberrations. However, the 48.0% postimplantation loss does not seem to be due to eggs with chromosome aberrations. On the other hand, as shown in Table 3, pronounced retardation of embryonic development was observed after iPMS treatment of male mice. Most of the embryos with 1-15 cells, which comprise 12.7% of total embryos examined, may have chromosome aberrations such that development will stop at an early stage, resulting in preimplantation loss. The 58.2% underdeveloped embryos of 16-30 cells probably represent postimplantation loss, while the 29.1% embryos of more than 31 cells should develop to normal implantation embryos. This hypothesis can conveniently explain the disagreement between the induction of dominant lethal mutations and chromosome aberrations with iPMS.

Thus, we suggest that the large differences between pre- and postimplantation loss may be correlated with the induction of chromosome aberrations at the first cleavage metaphase. However, the mechanism of the induction of postimplantation loss is quite unknown. iPMS-induced postimplantation loss may be attributable to eggs with DNA lesions (for example, minute deletion, point mutation and other types of DNA lesions) rather than visible chromosome aberrations at the first cleavage metaphases. It may also be expected that the postimplantation loss is caused by the failure of DNA repair of premutational lesions induced by iPMS. Although iPMS has been studied with regard to the induction of specific locus mutations, iPMS did not induce these in postmeiotic male germ cells (Ehling and Russel 1969). Generally, specific locus mutations do not induce death of embryos. Also, the cause of postimplantation loss by iPMS is probably not exchanges which will produce unbalanced genotypes after the first cleavage division, because iPMS induced few exchanges as shown in Table 1. As one possible explanation, we suggest
Table 1. Cytogenetic analysis at the first cleavage metaphase after iPMS treatment of male mice

<table>
<thead>
<tr>
<th>Matings post-Injection (days)</th>
<th>Number of eggs examined</th>
<th>Number of eggs with chromosome aberrations (% of eggs examined)</th>
<th>Number of chromosome aberrations</th>
<th>Frequencies and types of chromosome aberrations</th>
<th>Per cent of unfertilized eggs (% of eggs collected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0- 2</td>
<td>84</td>
<td>3 (3.6)</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3- 6</td>
<td>66</td>
<td>5 (7.6)</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>7-10</td>
<td>113</td>
<td>10 (8.8)</td>
<td>12</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>11-14</td>
<td>119</td>
<td>10 (8.4)</td>
<td>10</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>15-18</td>
<td>94</td>
<td>8 (8.5)</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>19-22</td>
<td>75</td>
<td>6 (8.0)</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>23-26</td>
<td>78</td>
<td>7 (9.0)</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>27-30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31-35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (%)</td>
<td>629</td>
<td>49 (7.8)</td>
<td>52</td>
<td>9</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 2. Dominant lethal test after iPMS treatment of male mice

<table>
<thead>
<tr>
<th>Matings post-injection (days)</th>
<th>Females with vaginal plug</th>
<th>Females with implants (%)</th>
<th>Implants per female with vaginal plug</th>
<th>Living embryos per female with vaginal plug</th>
<th>Dead implants per female with vaginal plug</th>
<th>Dominant lethal mutation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0-35)</td>
<td>75</td>
<td>64 (85.3)</td>
<td>9.8</td>
<td>9.5</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>0-2</td>
<td>9</td>
<td>8 (88.9)</td>
<td>8.0</td>
<td>3.0</td>
<td>5.0</td>
<td>68.4</td>
</tr>
<tr>
<td>3-6</td>
<td>8</td>
<td>8 (100.0)</td>
<td>7.8</td>
<td>3.1</td>
<td>4.7</td>
<td>67.4</td>
</tr>
<tr>
<td>7-10</td>
<td>10</td>
<td>10 (100.0)</td>
<td>7.9</td>
<td>3.1</td>
<td>4.8</td>
<td>67.4</td>
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<tr>
<td>11-14</td>
<td>8</td>
<td>8 (100.0)</td>
<td>7.9</td>
<td>3.6</td>
<td>4.3</td>
<td>62.1</td>
</tr>
<tr>
<td>15-18</td>
<td>16</td>
<td>16 (100.0)</td>
<td>7.8</td>
<td>3.7</td>
<td>4.1</td>
<td>61.2</td>
</tr>
<tr>
<td>19-22</td>
<td>11</td>
<td>10 (90.9)</td>
<td>7.3</td>
<td>3.5</td>
<td>3.8</td>
<td>63.2</td>
</tr>
<tr>
<td>23-26</td>
<td>16</td>
<td>12 (75.0)</td>
<td>4.6</td>
<td>2.2</td>
<td>2.3</td>
<td>76.9</td>
</tr>
<tr>
<td>27-35</td>
<td>23</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

(a) Pre- and post-implantation loss (%) = \( \left(1 - \frac{\text{living embryos/female with vaginal plug (iPMS)}}{\text{living embryos/female with vaginal plug (control)}} \right) \times 100 \\

(b) Pre-implantation loss (%) = \( \left(1 - \frac{\text{implants/female with vaginal plug (iPMS)}}{\text{implants/female with vaginal plug (control)}} \right) \times 100 \\

(c) Post-implantation loss (%) = (a) - (b)
that DNA lesions leading to postimplantation loss induced by iPMS are mainly due to the alteration of subchromosome material at the first cleavage metaphases; such DNA lesions might develop to visible chromosome aberrations (for example, deletion) after the first cleavage division.

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REFERENCES


