DIFFERENCE IN THE RATIO OF DOMINANT-LETHAL MUTATIONS TO HERITABLE TRANSLOCATIONS PRODUCED IN MOUSE SPERMATIDS AND FULLY MATURE SPERM AFTER TREATMENT WITH TRIETHYLENEMELAMINE (TEM)*


Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

Manuscript received November 16, 1981
Revised copy accepted January 23, 1982

ABSTRACT

The relative induction of dominant-lethal mutations and heritable translocations in triethylenemelamine-treated male postmeiotic germ cells of mice was determined depending on the stage treated. Males were mated either 11.5-14.5 days after treatment (middle spermatids) or less than 2.5 hours after treatment (fully mature sperm). Results clearly showed that, even though similar levels of dominant-lethal mutations were induced in fully mature sperm and in middle spermatids, the frequency of heritable translocations induced in mature sperm was markedly lower than that induced in middle spermatids. This observation was used, together with earlier ones, to suggest a mechanism by which dominant-lethal mutations and heritable translocations are produced following chemical treatment of male postmeiotic germ cells.

RECENTLY, we showed that there is a lack of association between induction of dominant-lethal mutations and heritable translocations in certain postmeiotic male germ cells following treatment with isopropyl methanesulfonate (GENEROSO, HUFF and CAIN 1979) or benzo[a]pyrene (GENEROSO et al. 1981a) in contrast to the results with X rays, ethyl methansulfonate (EMS) and triethylenemelamine (TEM). To explain this unusual observation, we postulated that the relative rates at which dominant-lethal mutations and heritable translocations are produced from chemical treatment of postmeiotic male germ cells depend upon the longevity of induced premutational lesions. High rates of heritable translocations relative to dominant-lethal mutations are expected when the corresponding premutational lesions are converted into interchanges prior to pronuclear chromosome replication, whereas for chemicals whose induced premutational lesions persist to the time of pronuclear chromosome replication or possibly even to subsequent early cleavage divisions, the types of aberrations


By acceptance of this article, the publisher or recipient acknowledges the U.S. Government's right to retain a non-exclusive, royalty-free license in and to any copyright covering the article.

Genetics 100: 633-640 April, 1982.
expected are mainly those that lead to dominant lethality. In an attempt to find support for this hypothesis, it was assumed that altering the time between treatment and fertilization, and hence the time between treatment and pronuclear DNA synthesis, could change the relative frequencies of dominant-lethal mutations and heritable translocations as a result of increase in the transformation of premutational lesions into some intermediate form.

The compound TEM was chosen to test this assumption. It is an effective inducer of dominant-lethal mutations in all postmeiotic germ cell stages of male mice, and has been shown to be highly efficient in inducing heritable translocations in all postmeiotic stages studied so far (Bateman 1960; Cattanach 1957, 1959; Generoso, Cain and Huff 1978). Furthermore, a positive dose response correlation between the two end points has been demonstrated for TEM-treated spermatids (Generoso, Cain and Huff 1978; Matter and Generoso 1974). Thus, we performed a study with TEM comparing the relative yields of dominant-lethal mutations and heritable translocations in males that were mated several days after treatment and in males that were mated within 2.5 hr after treatment.

MATERIALS AND METHODS

Studies on the effects of TEM on fully mature sperm were carried out using a single intraperitoneal injection with 4.0 mg/kg dose. A preliminary experiment indicated that this dose would induce a similar level of dominant-lethal mutations to the dose of 0.2 mg/kg given to middle spermatids. It should be noted that the spermatids are contained in the seminiferous tubules, where they are embedded in Sertoli cell cytoplasm, while the fully mature sperm are in the vas deferens. This difference in location could account for the difference in the dosage needed to produce the same level of dominant-lethal effects. TEM (Lederle Laboratories) was dissolved in Hanks' Balanced Salt Solution and given in a maximum volume of 1 ml. Each control mouse was given 0.8 ml of the carrier solution. A new batch of males were injected each morning beginning around 8:30. Injection of all experimental males was completed within 15 min. One hour after the last experimental male was injected, each male was caged with two virgin females. One and one-half hr later, females were examined for presence of vaginal plugs. We found earlier that under this condition practically all matings occur within 20 min after the males and females were put together (Krishna and Generoso 1977). Mated females were separated and the males were discarded. This treatment and mating procedure was carried out for four consecutive days using a freshly prepared solution each day and using the still unmated females along with new ones that were added to replace those that were mated the previous morning. A corresponding study on middle spermatids was carried out on males that were mated 11.5-14.5 days after single intraperitoneal injection with 0.2 mg/kg TEM dose.

The males used in both dominant-lethal and heritable translocation studies were of (SEC x C57BL)F₁ stock. The females, on the other hand, came from different stocks, (SEC x C57BL)F₁ for the dominant-lethal study and (C3H x C57BL)F₁ for the heritable translocation study. It was found earlier that the yields of TEM-induced dominant-lethal mutations were the same when treated males from one stock were mated to untreated females from these two stocks (Generoso et al. 1979). All mice were around 12 weeks old at the time they were used. Females used in the dominant-lethal study were killed for uterine analysis 11-13 days after observation of the vaginal plug. In the heritable translocation study, litter size of first generation progeny was recorded. All male progeny were weaned and tested for translocation heterozygosity by use of the sequential procedure employed routinely in our laboratory (Generoso 1978). Suspect animals were cytologically examined.
STAGE DIFFERENCE IN ABERRATION TYPES

Table 1

Rates of dominant-lethal mutations induced in spermatids or in fully mature sperm*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment to fertilization interval</th>
<th>Number of mated females</th>
<th>Number of pregnant females</th>
<th>Number of implants per pregnant female</th>
<th>Number of living embryos per pregnant female</th>
<th>Dead implants %</th>
<th>Dominant-lethal mutations (%)*</th>
<th>Among fertile matings</th>
<th>Among all mated females</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEM (4.0 mg/kg)</td>
<td>1 - 2.5 hours</td>
<td>53</td>
<td>37</td>
<td>8.3</td>
<td>3.5</td>
<td>58</td>
<td>63</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Control (1 - 2.5 hours)</td>
<td>55</td>
<td>48</td>
<td>10.1</td>
<td>9.5</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEM (0.2 mg/kg)</td>
<td>11.5-14.5 days</td>
<td>91</td>
<td>69</td>
<td>8.7</td>
<td>4.1</td>
<td>53</td>
<td>56</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Control (11.5-14.5 days)</td>
<td>68</td>
<td>55</td>
<td>9.8</td>
<td>9.3</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Males and females were of (SEC × C57BL) F1, stock.
† Percentage of induced dominant-lethal mutations was calculated as: % dominant-lethal mutations = \[ \frac{\text{average living embryos in experimental group}}{\text{average living embryos in control group}} \times 100. \]

RESULTS

Results of the dominant-lethal studies are shown in Table 1. As expected, the level of dominant-lethal mutations induced by 4.0 mg/kg TEM dose in fully mature sperm is similar to that induced in middle spermatids by 0.2 mg/kg dose. The dominant-lethal frequencies are slightly higher when the calculation is based on all mated females (assuming that in some females dominant-lethal effects in all conceptuses were expressed prior to implantation) than when calculation was based on pregnant females only. However, the apparently lower proportion of pregnancies among mated females in both experimental groups was not statistically significant. The similarity in dominant-lethal effect induced in the two stages can also be seen in Table 2 which shows that the average litter size among first generation progeny (males were subsequently tested for translocation heterozygosity) is identical for the two experimental groups.

The incidences of male progeny carrying reciprocal translocations are shown in Table 3. The frequency of translocation carriers observed for spermatids treated with 0.2 mg/kg TEM dose closely resemble that observed earlier (GENE-

Table 2

Litters sired by TEM-treated male parents*

<table>
<thead>
<tr>
<th>TEM dose (mg/kg)</th>
<th>Treatment to fertilization interval</th>
<th>Number of litters born</th>
<th>Litter size (average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>1.0-2.5 hours</td>
<td>112</td>
<td>3.8</td>
</tr>
<tr>
<td>0.2</td>
<td>11.5-14.5 days</td>
<td>161</td>
<td>3.8</td>
</tr>
</tbody>
</table>

* These males were parents of male progeny that were tested for translocation heterozygosity (see Table 3).
TABLE 3

Rates of heritable translocations induced in spermatids or fully mature sperm*

<table>
<thead>
<tr>
<th>TEM dose (mg/kg)</th>
<th>Treatment to fertilization interval</th>
<th>Number of progeny tested</th>
<th>Number of partially sterile progeny</th>
<th>Number of sterile progeny</th>
<th>Frequency of translocation carriers %</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>1.0- 2.5 hours</td>
<td>206</td>
<td>9</td>
<td>7</td>
<td>7.8</td>
</tr>
<tr>
<td>0.2</td>
<td>11.5-14.5 days</td>
<td>282</td>
<td>71</td>
<td>27</td>
<td>34.8</td>
</tr>
</tbody>
</table>

*Male (SEC × C57BL)F, mice were given single intraperitoneal injection of TEM and mated with untreated (C3H × C57BL)F, females.

The key finding of the present study is that TEM induced considerably fewer heritable translocations relative to dominant-lethal mutations in males that were mated within 2.5 hr after treatment than in males that were mated 11.5–14.5 days after treatment. This observation may be explained in one of three ways: (1) in terms of processes that may or may not take place depending upon the length of the interval between treatment of postmeiotic male germ cells and fertilization, (2) in terms of relative amounts of alkyl derivatives in treated chromosomes of the two germ cell stages, or (3) in terms of recovery of symmetrical reciprocal translocations. With respect to the first explanation, the observation may indicate that the amount of time from the reaction of TEM with the chromosomes of fully mature sperm until pronuclear chromosome replication was not sufficiently long for the conversion of the bulk of alkylation products into intermediate lesions to take place. Without this transformation, the persisting alkylation product may result mainly in chromatid-type aberrations that lead to dominant lethality, i.e., aberration formation is dependent upon pronuclear chromosome replication. The observation is similar to the well known storage effect in Drosophila where the frequency of whole body translocations induced by certain alkylating chemicals, including EMS and TEM, increases with storage time. A study by Lee, Skinner and Janca (1979) with Drosophila sperm showed that there was progressive loss of ethyl groups with storage time and that the rate of this loss was in accordance with the expected rate of apurinic site formation due to hydrolysis. Accumulation of apurinic sites from hydrolysis has, in turn, been thought of as the basis for the increase in translocations with storage time. The explanation is also consistent with our earlier findings that the formation of breaks and interchanges in EMS- or TEM-
treated spermatocytes, spermatids, or early spermatozoa is delayed (Generoso et al. 1977, 1981b) and that the lesions induced by EMS in early spermatozoa and late spermatids are converted into reciprocal translocations after the sperm has entered the egg. Further support for this explanation comes from the study of Sega and Owens (1978) and Sega (1980) with EMS- or methyl methanesulfonate (MMS)-treated mouse spermatids and spermatids whereby they showed that the degree of DNA alkylation decreased with time presumably as a result of loss of alkyl groups through hydrolysis.

The second explanation, i.e., difference in the proportion of alkyl derivatives, must be entertained inasmuch as no comparative study for the two stages involved has been done. However, it has been concluded that the proportion of each alkyl derivative is generally very similar whether DNA is reacted in test tubes, in cells, or in tissues of treated animals (Lawley 1980; Singer 1979).

The third explanation assumes that the higher dose used in the experiment with fully mature sperms (4.0 mg/kg) may have produced correspondingly higher incidence of premutational lesions than the lower dose in spermatids (0.2 mg/kg). This could result in an increase, among translocation-containing conceptions, of those that also possess aberrations that lead to dominant-lethality. Thus, the lower frequency of heritable translocations for fully mature sperm may be assumed to be the result of reduced recovery. This explanation does not have much support. On the contrary, there is evidence against it. First, there is no difference in the frequency of dominant-lethal mutations between the two stages. Although data in Table 1 show slightly higher calculated frequency of dominant-lethal mutations for mature sperm than for spermatids, the difference is well within normal range of variation. More importantly, the two groups of litters from which the males tested for translocations were obtained are of the same size (Table 2). And second, there is evidence which clearly indicates that the frequency of heritable translocation does not decrease with increasing frequency of dominant-lethal mutations (Generoso et al. 1974, 1981; Generoso, Cain and Huff 1978; Generoso 1982). This is true even at very high levels of induced dominant-lethal mutations. Perhaps this is what should be expected if, indeed, induced dominant-lethal mutational events are randomly distributed among the treated cell population.

We postulated earlier a mechanism by which dominant-lethal mutations and heritable translocations are formed following chemical treatment of male post-meiotic germ cells (Generoso, Huff and Cain 1979; Generoso et al. 1981a). The present result not only adds support to this proposed mechanism but it also helps improve on the details. Thus, we may restate the mechanism as follows. The kinds of chromosomal aberrations resulting from chemical treatment of post-meiotic male germ cells depend upon the longevity of the reaction products in question and upon the length of the interval between the formation of some of these products and the first postfertilization chromosome division. The production of chemically induced heritable translocations may require a transformation of the initial reaction products into suitable intermediate lesions either before or shortly after sperm enters the egg, whereupon it is converted into
chromosome exchange before pronuclear chromosome replication (chromosome-type exchanges). Conversely, when the reaction products remain unchanged and persist up to the time of first postfertilization chromosomal division, the main types of aberrations produced are those that lead to dominant lethality (i.e., chromatid deletions and chromatid symmetrical and asymmetrical exchanges). It is inherent in this hypothesis that aberrations that lead to dominant lethality can also arise from the intermediate lesions. Furthermore, it allows that both chromosome- and chromatid-type aberrations be produced at rates that may differ from one mutagen to another, depending upon the proportion of various alkylated derivatives.

Cytological data on early cleavage metaphases are generally in agreement with this concept. BREWEN et al. (1975), GENEROSO et al. (1979), and KATOH, TANAKA and IWASHARA (1981) attributed the induction of dominant-lethal mutations in male postmeiotic germ cells by MMS, isopropyl methanesulfonate or mitomycin C to the formation of chromatid-type aberrations. MATTER and JAEGGER (1975), BURKI and SHERIDAN (1978), and HITOTSUMACHI and KIRUCHI (1977) related the TEM-induced aberrations found in embryos at the four or more blastomere stage, which correlated with dominant-lethal mutations induced in postmeiotic male germ cells, to delayed formation of aberrations and to formation of chromatid-type aberrations in first-cleavage metaphase. With respect to induction of heritable translocations, BURKI and SHERIDAN (1978) and TANAKA, KATOH and IWASHARA (1981) found, as expected, that MMS or TEM induced chromosome-type aberrations were observed in first cleavage metaphase.

In short-term cultures of somatic cells exposed to alkylating agents the aberrations observed in the first post-treatment mitosis are mainly of the chromatid-type. Recently, EVANS and VIJAYALAXMI (1980) found that storage prior to stimulation with phytohemagglutinin of mitomycin C-treated human lymphocytes markedly increased the incidence of chromosome-type aberrations. The reason for this observation was thought to be a time-dependent alteration in the induced lesions following storage. The similarity between this observation in somatic cells, the storage effect in Drosophila and the present one in mice germ cells may be the result of similar molecular transformations of induced premutation lesions, the rate of which increases with post-treatment time.

The authors acknowledge E. F. OAKBERG, R. J. PRESTON and G. A. SEGA for reviewing this paper.

LITERATURE CITED

BATEMAN, A. J., 1960 Induction of dominant-lethal mutations in rats and mice with triethyl-

BREWEN, J. G., H. S. PAYNE, K. P. JONES and R. J. PRESTON, 1975 Studies on chemically in-


GENEROSO, W. M., S. W. HUFF and K. T. CAI N, 1979 Relative rates at which dominant-lethal mutations and heritable translocations were induced by alkylating chemicals in postmeiotic male germ cells of mice. Genetics 93: 163–171.


Corresponding editor: S. Wolff