RELATIVE RATES AT WHICH DOMINANT-LETHAL MUTATIONS
AND HERITABLE TRANSLOCATIONS ARE INDUCED BY ALKYLATING
CHEMICALS IN POSTMEIOTIC MALE GERM CELLS OF MICE*

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ABSTRACT

There is a close relationship between the rates at which dominant lethal mutations and heritable translocations are induced by ethyl methanesulfonate (EMS) or triethylenemelamine (TEM) in male postmeiotic germ cells. This relationship does not hold for isopropyl methanesulfonate (IMS), which induced only negligible frequencies of heritable translocations at doses that induced high levels of dominant lethal mutations. Nor does IMS behave like EMS and TEM in the degree to which eggs of different stocks of females repair premutational lesions that are carried in the sperm—large differences between stocks for IMS treatment and small differences for EMS or TEM treatment. These dissimilarities between IMS and the other two alkylating chemicals are postulated to be attributable to differences in the types of lesions present at the time of repair activity and to whether or not chromosomal aberrations are already fixed prior to postfertilization pronuclear DNA synthesis.

In an earlier report, we showed that, contrary to what had been found with X rays, the formation of triethylenemelamine (TEM)-induced chromosomal aberrations following treatment of pachytene spermatocytes is delayed (Generoso et al. 1977). This phenomenon is likely to hold true for other male germ-cell stages as well. However, because of the remarkable similarities between X rays on the one hand and TEM and ethyl methanesulfonate (EMS) on the other in (1) the relative rates at which dominant lethal mutations and heritable translocations were produced in postmeiotic male germ cells, and in (2) the overall characteristics of translocation heterozygotes (Generoso et al. 1978), it was hypothesized that the bulk of the TEM- or EMS-induced aberrations following treatment of male postmeiotic germ cells were already formed prior to postfertilization pronuclear DNA synthesis, like those induced by X rays (Generoso et al. 1977). Moreover, the close association between the production of dominant lethal mutations and the production of heritable translocations at these germ-cell stages has become a widely accepted generalization. The pres-

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Recent data with isopropyl methanesulfonate (IMS) on dominant lethal mutations and heritable translocations indicate that this generalization is not correct and, together with our recent data on the repair of EMS-, TEM- and IMS-induced genetic lesions (Generoso et al. 1979), indicate that, unlike EMS- and TEM-induced aberrations, the bulk of IMS-induced chromosome aberrations are not formed prior to postfertilization pronuclear DNA synthesis.

MATERIALS AND METHODS

The relative inducibility of dominant lethal mutations and heritable translocations with IMS was determined for inseminated spermatozoa, spermatozoa treated 12 hr to 3.5 days before mating, and spermatids treated 14.5 to 17.5 days before mating. Dominant lethal effects on inseminated spermatozoa were determined as follows. Females used were (C3H × C57BL)F₁ hybrids. They were kept in a room with only five hr of daily dark period prior to their use. Males were (SEC × C57BL)F₁ hybrids. All animals were approximately 12 weeks old at the time of treatment. A four-day mating schedule was used for each batch of females. During the first three days, one or two females were caged with each male for only 20 min each morning, beginning two hr after the end of the dark period. Females were checked for the presence of vaginal plugs immediately after they were separated from males. Females that had mated were injected intraperitoneally with 50 mg of IMS/kg approximately three hr after the end of the dark period. Solutions of IMS were prepared in cold Hanks’ balanced salt solution and given in a maximum volume of 1 ml. At the time of treatment, the oocytes were at metaphase II, located in the ampulla of the oviduct, while the sperm were in the vicinity of the oocytes, but sperm entry had not yet taken place (Krishna and Generoso 1977). On the fourth day, the remaining females (those that did not mate during the first three days) were also treated approximately three hr after the end of the dark period and then caged with the males for one hr beginning 15 min after treatment. IMS is highly reactive (Lawley 1974) and unstable at physiological conditions; thus, for practical purposes, only the female genome was treated in this case. Females were checked for the presence of vaginal plugs afterwards. Because the purpose of this particular experiment was to compare the relative sensitivity of the male and female genomes, no control group was maintained. The heritable translocation study for inseminated spermatozoa was performed essentially by the same procedure, with the same mouse stocks. Because no information is available for inseminated spermatozoa of mice, parallel dominant lethal and heritable translocation experiments were performed with X rays (100 R, acute).

Dominant lethal and heritable translocation studies on spermatozoa tested 12 hr to 3.5 days before mating and spermatids treated 14.5 to 17.5 days before mating were carried out for IMS only (at 75 mg/kg dose). (101 × C3H)F₁ males (approximately 12 weeks old) and (SEC × C57BL)F₁ females were used in both studies. Females used in the dominant lethal study were approximately 12 weeks old, whereas those used in the heritable translocation study ranged in age from 12 weeks to one year. Mated females analyzed for dominant lethal effects were killed 12 to 15 days after vaginal plugs were observed. For details of the heritable translocation procedure, see Generoso et al. (1974). It should be noted that the stocks of male and female mice used in this particular heritable translocation study were the same as those used in earlier studies with EMS and TEM (Generoso et al. 1974, 1978).

RESULTS

Relative inducibility of dominant lethal mutations and heritable translocations in inseminated sperm

In females that were treated shortly after mating, both sperm and eggs were exposed to the mutagen; in females that were treated shortly before mating,
only the eggs were exposed. Thus, the net effect in the inseminated sperm can be determined by subtracting effects observed in the latter group of females from the former. Results of the dominant lethal study are shown in Table 1. Since the main purpose of this study was to separate effects on sperm from effects on eggs, a simultaneous control was not maintained. Instead, for an approximate quantitation of the incidence of induced dominant lethal mutations, the average of the number of living embryos of (C3H × C57BL)F1 females from controls of the five most recent dominant lethal experiments in our laboratory was used in the calculation. The IMS and X-ray treatments are equivalent in terms of the combined dominant lethal effects induced in sperm and eggs. However, while the two experiments with IMS clearly showed that the sperm and eggs have the same sensitivity to this compound, all three X-ray experiments showed a much higher effect on eggs than on sperm. On the average, the estimated dominant lethal frequencies induced in the sperm alone were 24 and 9% for IMS and X-ray treatments, respectively.

The frequency of dominant lethal mutations (24%) induced by 50 mg of IMS/kg in inseminated sperm was clearly higher than the frequency (9%) induced by an even higher dose of this chemical (65 mg per kg) in maturing TABLE 1

* Induction of dominant lethal mutations in inseminated sperm and metaphase II oocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment</th>
<th>Treated genome*</th>
<th>Number of mated females</th>
<th>Number of pregnant females (avg)</th>
<th>Number of implants (avg)</th>
<th>Number of living embryos (avg)</th>
<th>Dead implants (%)</th>
<th>Estimated frequency of dominant lethals (%) †</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMS, 50 mg/kg</td>
<td>A</td>
<td>male and female</td>
<td>52</td>
<td>50</td>
<td>9.6</td>
<td>5.3</td>
<td>45</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>male and female</td>
<td>46</td>
<td>44</td>
<td>9.2</td>
<td>4.4</td>
<td>52</td>
<td>58</td>
</tr>
<tr>
<td>X rays, 100 R</td>
<td>A</td>
<td>male and female</td>
<td>52</td>
<td>49</td>
<td>9.5</td>
<td>4.8</td>
<td>49</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>male and female</td>
<td>37</td>
<td>33</td>
<td>9.2</td>
<td>4.9</td>
<td>47</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>male and female</td>
<td>29</td>
<td>29</td>
<td>9.4</td>
<td>5.2</td>
<td>45</td>
<td>50</td>
</tr>
</tbody>
</table>

* Males and females used were (SEC × C57BL)F1 and (C3H × C57BL)F1 hybrids, respectively.
† Based on historical control average of 10.4 living embryos; % dominant lethals = 1 - [living embryos/pregnant female (experimental)] / [living embryos/pregnant female (control)] × 100.
sperm (Generoso et al. 1979), even though the same stock of females was used. The reason for this difference is not known, but two possibilities may be worth considering: (1) in the case of inseminated sperm, it is possible that the treatment also damaged the repair systems in the egg, since both sperm and eggs were exposed, and (2) it is possible that the difference may be a reflection of the difference in actual doses to which the respective types of sperm were exposed. It should be noted that the maturing sperm were either in the vas or in the epididymis, whereas the inseminated sperm were in the ampulla of the female at the time of treatment. Because IMS was administered intraperitoneally in the two studies, it is likely that the ampulla (but not the vas or epididymis) was directly immersed in the injected solution (maximum volume of 1 ml).

Results of the heritable translocation study on inseminated sperm and metaphase II oocytes are shown in Table 2. The comparison between IMS and X-ray treatments is based on partially sterile translocations (confirmed cytologically) alone, because cytological analysis of steriles has not been completed yet. (The picture does not change much even when the comparison is based on both partially sterile and sterile progeny, assuming that all steriles are also translocation heterozygotes). The net frequencies of partially sterile translocation heterozygotes induced in inseminated sperm are 2.79 and 0% for X-ray and IMS treatments, respectively. Thus, in the case of X rays, the low level of dominant lethal effects in inseminated sperm was associated with the production of heritable translocations, whereas in the case of IMS, the relatively higher level of dominant lethal effects was not.

**TABLE 2**

*Induction of heritable translocations*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germ cells treated</th>
<th>Number of progeny tested</th>
<th>Number of partially sterile progeny</th>
<th>Number of sterile progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>X rays, 100 R</td>
<td>inseminated sperm and metaphase II oocytes*</td>
<td>582</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>metaphase II oocytes*</td>
<td>243</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>IMS, 50 mg/kg</td>
<td>inseminated sperm and metaphase II oocytes*</td>
<td>376</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>metaphase II oocytes*</td>
<td>281</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>IMS, 75 mg/kg</td>
<td>maturing sperm†‡</td>
<td>73</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>spermatids†‡§</td>
<td>247</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Males and females used were (SEC × C57BL)F₁ and (C3H × C57BL)F₁ hybrids, respectively.
† Males were mated 0.5 to 3.5 days posttreatment.
‡ Males and females used were (101 × C3H)F₁ and (SEC × C57BL)F₁ hybrids, respectively.
§ Males were mated 14.5 to 17.5 days posttreatment.
### TABLE 3

**IMS induction of dominant lethal mutations in spermatozoa and spermatids**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Post-treatment interval (days)</th>
<th>Number of mated females</th>
<th>Number of pregnant females (avg)</th>
<th>Number of living embryos (avg)</th>
<th>Number of living implants (%)</th>
<th>Dead implants (%)</th>
<th>Dominant lethals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMS, 75 mg/kg</td>
<td>0.5–3.5</td>
<td>34</td>
<td>27</td>
<td>5.7</td>
<td>1.7</td>
<td>70</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>14.5–17.5</td>
<td>38</td>
<td>34</td>
<td>7.9</td>
<td>3.8</td>
<td>52</td>
<td>58</td>
</tr>
<tr>
<td>Control</td>
<td>0.5–3.5</td>
<td>34</td>
<td>28</td>
<td>9.5</td>
<td>9.3</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>14.5–17.5</td>
<td>38</td>
<td>36</td>
<td>9.3</td>
<td>9.1</td>
<td>3</td>
<td>—</td>
</tr>
</tbody>
</table>

* Males and females used were (101 × C3H)F₁ and (SEC × C57BL)F₁ hybrids, respectively.
† Dominant lethals = 1 – \[
\frac{\text{living embryos/pregnant female (experimental)}}{\text{living embryos/pregnant female (control)}} \times 100.
\]

**Dominant lethal and heritable translocation effects of IMS in maturing spermatozoa and spermatids**

Results of the dominant lethal experiment are shown in Table 3. A dose of 75 mg/kg produced 82% dominant lethal mutations in maturing sperm and 58% in spermatids. Despite the high frequencies of dominant lethal mutations, very low frequencies of heritable translocations were induced by IMS (Table 2). In mature sperm, one sterile and one partially sterile offspring were observed out of 73 tested, both of which were confirmed cytologically as translocation heterozygotes (2.74%). In spermatids, one sterile and one partially sterile offspring were also observed out of 247 tested, but cytological analysis of the two animals showed that only the partially sterile one was a translocation heterozygote (0.4%).

**DISCUSSION**

The processes involved from the time when initial lesions occurred in the chromosomes of germ cells to the time mutations are actually transmitted to progeny are very complex and little understood. In the case of chromosomal aberrations, no direct evidence is yet available, so that the development of concepts regarding basic mechanisms must rely upon indirect evidence that seems to explain the observations. In this context, formulation of a concept for the time of formation of chromosome breaks and rearrangements induced by alkylating chemicals in male postmeiotic germ cells is attempted here. This concept makes use of the dominant lethal and heritable translocation data given in the present report for IMS and X rays and those published earlier for EMS (Generoso et al. 1974), TEM (Generoso et al. 1978), and X rays (Generoso et al. 1978), as well as the recent data on repair in the egg of IMS-, TEM- and EMS-induced chromosomal lesions in spermatozoa and spermatids (Generoso et al. 1979).
Two striking differences exist between IMS on the one hand and EMS and TEM on the other. The first difference is in the relative rates at which dominant lethal mutations and heritable translocations were produced. In the case of EMS and TEM, these end points were positively correlated as a function of dose (Generoso et al. 1974, 1978). The frequencies of heritable translocations observed at levels of dominant lethal mutations that are more than 50% were 32.1% for EMS (early spermatozoa) (Generoso et al. 1974) and 28.9% for TEM (middle spermatids) (Generoso et al. 1978). These frequencies are similar to that induced by X rays in spermatozoa and spermatids (27.2%) at a comparable level of dominant lethal mutation (Generoso et al. 1978). These frequencies are clearly much higher than those observed in the present IMS study (0.4% in spermatids and 2.74% in spermatozoa), in which the frequencies of induced dominant lethal mutations were also more than 50%. The second difference lies in the degree to which genetic lesions carried in postmeiotic male germ cells are repaired in the egg (Generoso et al. 1979). Large repair differences between stocks of females were found in the case of IMS, but much smaller differences were found in the case of EMS and TEM. These two striking differences between IMS and the other two alkylating compounds may be explained in terms of types of chromosomal lesion present at the time of repair activity and whether or not chromosomal aberrations are already fixed at the time of postfertilization pronuclear DNA synthesis.

Evidence strongly suggesting that the bulk of aberrations induced by EMS or TEM in male postmeiotic stages were already formed prior to postfertilization pronuclear DNA synthesis was presented in detail previously (Generoso et al. 1977). It was concluded that, since EMS and TEM were remarkably similar to X rays with respect to the rates of which dominant lethal mutations and heritable translocations were induced and with respect to all characteristics of the translocation heterozygotes, the aberrations produced by EMS and TEM were of the chromosome type (as is certainly the case with X rays). Conversely, the much lower rate of induction of heritable translocations relative to dominant lethal mutations may mean that most IMS-induced aberrations were not formed prior to pronuclear DNA synthesis. It is not clear at the moment whether or not the small repair differences between stocks of females observed for EMS or TEM treatments mean that the aberrations were already fixed prior to the time of repair activity. On the other hand, it seems reasonable to conclude, on the basis of large repair differences and parallel first cleavage cytological data, that the unrepaired premutational lesions induced by IMS in spermatozoa and spermatids persisted up to the time of pronuclear DNA synthesis, whereupon they were converted primarily into chromatid and isochromatid deletions, which eventually led to dominant lethality.

It may be expected that a small proportion of the EMS- or TEM-induced premutational lesions were of the types that persisted up to the time of pronuclear DNA synthesis, since there are different sites in the chromosome where alkylation reaction can take place. This may explain why only small repair differences between stocks of females were observed for these two compounds (Genero-
Conversely, it may be expected in the case of IMS that a small proportion of induced premutational lesions were of the types that were converted into aberrations prior to pronuclear DNA synthesis. This may explain the observation that IMS induced only very low levels of heritable translocations.

Thus, it is postulated that the relative rates at which dominant lethal mutations and heritable translocations are produced from treatment of postmeiotic male germ cells with alkylating chemicals depend upon the longevity of induced premutational lesions. Heritable translocations are produced at a high rate relative to dominant lethal mutations by alkylating chemicals that primarily induce premutational lesions that are converted into breaks and interchanges prior to pronuclear DNA synthesis. Conversely, alkylating chemicals that primarily induce premutational lesions that persist up to the time of pronuclear DNA synthesis produce mainly the types of aberrations that lead to dominant lethality.

An alternative hypothesis, which states that the chromosome-breaking effect of TEM in treated postmeiotic male germ cells is mediated through pronuclear chromosome replication in the zygote (i.e., through pronuclear DNA synthesis), was put forward by Brewen and his co-workers (Brewen and Payne 1978; Luippold, Gooch and Brewen 1978) on the basis of cytological observations. However, while this alternative hypothesis involves postmeiotic male germ cells, the evidence used in its formulation was obtained from entirely different germ cell stages, i.e., maturing dictyate oocytes (Brewen and Payne 1978) and differentiating spermatogonia (Luippold, Gooch and Brewen 1978). It was found that the aberrations observed were exclusively of the chromatid type. Not surprisingly, the TEM cytological results of Brewen and co-workers on treated differentiating spermatogonia and maturing dictyate oocytes differ substantially from the cytological findings of Burki and Sheridan (1978) on early cleavage division, in which postmeiotic male germ cells of mice were treated with TEM. They found that, while up to 48% of first cleavage metaphases in the experimental group had chromosome-type aberrations, no chromatid fragments or chromatid-type exchanges were observed. However, the frequencies of first cleavage metaphases with aberrations were lower than those expected on the basis of the rates of dominant lethal mutations produced by the TEM treatments. Using the data of Hitotsumachi and Kichuchi (1977), which were obtained from cytological analysis of four-day-old embryos (morulae/blastulae), Burki and Sheridan (1978) speculated that presumably part of the aberrations that were not expressed in first cleavage metaphase were expressed in later divisions, thus accounting for all dominant lethality. In other words, it was interpreted that the expression of part of the premutational lesions was delayed. Seemingly in keeping with this interpretation are the findings of Matter and Jäger (1975). They analyzed cytologically embryos fixed about 60 hours after mating of normal females with TEM-treated males (postmeiotic germ cells were involved) and observed the frequent occurrence of chromatid-type aberrations in second and third cleavage divisions, together with chromosome-type aberrations. Furthermore, they observed premature chromosome condensation of par-
ticular chromosome regions. The appearance of both premature chromosome condensation and chromatid-type aberrations in these embryos was taken by these investigators as evidence for the existence of long-lasting TEM-induced lesions.

As we have repeatedly stated, certain types of lesions may persist up to the time of the first round of pronuclear DNA synthesis and may lead to chromatid-type aberrations in the first cleavage division. However, the observation of chromatid-type aberrations in later cleavage divisions, particularly in the third and later, is considerably more difficult to explain. For this reason, information on the mechanics of replication of alkylated DNA strand is needed. Unfortunately, it was not possible to determine from the data of Matter and Jaeger (1975) whether or not embryos that contained one blastomere with chromatid-type aberrations also had other blastomeres with the same or different types of aberrations. This is important in view of their own reservation that "some of the chromatid type fragments may be artifacts, i.e., acentric chromosome fragments may split apart during chromosome preparation." Finally, the usefulness of premature chromosome condensation as an indicator of the presence of long-lasting TEM-induced lesions (Matter and Jaeger 1975) appears to have been placed in doubt by the failure of Burki and Sheridan (1978) to observe this phenomenon on similar cytological materials.

It should be obvious that the hypothesis presented here does not state that the production of chromosome- and chromatid-type aberrations are mutually exclusive. On the contrary, it allows both types of aberrations to be produced at rates that may differ from one mutagen to another, depending upon the types of pre-mutational lesions produced. However, it requires that the production of heritable translocations must result from exchanges that were already fixed prior to pronuclear chromosome replication. The well-known storage effect in Drosophila may also be explained by this hypothesis.

LITERATURE CITED


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