The Type of Chromosomal Damage Caused by Methylazoxy-
methanol Acetate. A Comparison between chromosome
breakage and SCE

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Several studies have established the mutagenicity and carcinogenicity of me-
thylazoxymethanol acetate (MAM AC) (Laqueur 1964, Teas et al. 1965, Smith 1966,
Teas and Dyson 1967). It methylates nucleic acids at the N-7 position of guanine
(Shank and Magee 1967, Natarajan et al. 1976). An increased frequency of chro-
mosomal breaks was observed by Teas et al. (1965) in onion root-tip cells when they
were exposed to the glucoside, cycasin, from which MAM is derived naturally.
Zedeck et al. (1974), Zedeck and Sternberg (1975) have observed mitotic abnormali-
ties in rat liver cells exposed to MAM AC.

We have shown that MAM AC causes an increased incidence of sister chromatid
exchange (SCE) in human short-term leucocyte cultures derived from nine normal
male individuals (Evans et al. 1977). A similar effect on SCE was also shown by Abe
and Sasaki (1977) in Chinese hamster cell cultures.

In comparison to chromosomal breakage (CB) studies, SCE is considered a more
sensitive indicator of chromosomal damage (Latt 1974, Beek and Obe 1975, Perry
and Evans 1975, Bayer and Bauknecht 1977, Nevstad 1978). However, there is
evidence which shows that SCE and CB may have the same sensitivity (Kucerova
et al. 1979). On the other hand some substances have been shown to increase chro-
mosomal aberrations without affecting the incidence of SCE (Kato 1974, Basler
et al. 1979).

The present study addresses the question of whether or not MAM AC causes
both CB and SCE in short-term human leucocyte cultures derived from the same
individuals and exposed to the same concentrations of MAM AC. The answer to
this question provides additional basic knowledge on the profile of MAM AC.

Materials and methods

Short-term phytohemagglutinin (PHA)-stimulated peripheral blood cultures
were derived from five karyotypically normal male individuals (Table 1, 1–5). Each
of three culture series (Table 1, A-C) was inoculated with blood from a different
individual. Eight identical cultures were inoculated per variable. Half of the cul-
tures contained 20 μM 5-bromodeoxyuridine (BrdU) during the last 24 hours of
incubation. The other half did not. The latter half were evaluated for the fre-

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velopmental Disabilities.
quency of CB. Thus each experiment was composed of 24 cultures. Equivalent volumes (0.05 ml) of sterile distilled water were added to all control cultures (A series) to account for equivalent additions of MAM AC to treated cultures (B and C). Table 1 shows the two concentrations (B and C) of MAM AC that were used in the blood cultures from each individual.

The complete culture medium contained RPMI 1640, 15% fetal bovine serum, 1 mM glutamine, 100 U (or µg)/ml of Pen-strep (GIBCO), 20 U/ml heparin (Scientific Products) and 0.2 ml % PHA-P (DIFCO). All cultures were incubated at 37°C for four days. MAM AC was present continuously. Colchicine (0.2 µg/ml) was added during the last 2½ hours of incubation.

For CB analysis, slides were stained with Gurr's Giemsa. Each intact metaphase was analyzed by determining the number of chromatid breaks and exchange figures. A break was defined as a visual discontinuity along the longitudinal axis of the chromosome (Jenkins 1970).

Differential sister chromatid staining was carried out according to the method of Evans and Jenkins (1975). Reciprocally stained areas along the linear axis of the chromatids were scored as SCE. Since two types of cultures were processed, one with and one without BrdU, a total of 160 cells were analyzed per variable. All slides were read blindly. Significant differences between groups were determined by the t test. The SCE values in Table 1 were statistically analyzed with both t and Mann-Whitney U tests (Conover 1971).

Results

SCE analysis resulted in the evaluation of 1020 metaphases. Table 1 shows a

<table>
<thead>
<tr>
<th>Experiment no., Culture series A–C</th>
<th>Concentration of MAM AC in µM</th>
<th>Number of metaphases analyzed</th>
<th>Mean and S. D. No. of chromosome breaks in 20 cells</th>
<th>Mean and S. D. No. of SCE per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 A</td>
<td>0</td>
<td>80/80^a</td>
<td>1.75 ± 1.50</td>
<td>3.68 ± 0.70</td>
</tr>
<tr>
<td>B</td>
<td>6.8</td>
<td>80/80</td>
<td>1.25 ± 0.50</td>
<td>3.73 ± 0.91</td>
</tr>
<tr>
<td>C</td>
<td>34.0</td>
<td>80/80</td>
<td>0.50 ± 0.58</td>
<td>5.31 ± 1.10</td>
</tr>
<tr>
<td>2 A</td>
<td>0</td>
<td>80/80</td>
<td>0.50 ± 0.58</td>
<td>5.39 ± 1.00</td>
</tr>
<tr>
<td>B</td>
<td>68</td>
<td>80/80</td>
<td>1.00 ± 1.15</td>
<td>8.50 ± 2.19</td>
</tr>
<tr>
<td>C</td>
<td>170</td>
<td>80/80</td>
<td>0.75 ± 0.96</td>
<td>9.36 ± 2.29</td>
</tr>
<tr>
<td>3 A</td>
<td>0</td>
<td>80/60</td>
<td>0</td>
<td>5.23 ± 0.67</td>
</tr>
<tr>
<td>B</td>
<td>68</td>
<td>80/80</td>
<td>0</td>
<td>13.65 ± 2.18</td>
</tr>
<tr>
<td>C</td>
<td>170</td>
<td>48^b</td>
<td>0.75 ± 1.50</td>
<td>6.00 ± 1.25</td>
</tr>
<tr>
<td>4 A</td>
<td>0</td>
<td>80/80</td>
<td>0.25 ± 0.50</td>
<td>10.73 ± 1.21</td>
</tr>
<tr>
<td>B</td>
<td>34</td>
<td>80/60</td>
<td>0.75 ± 0.50</td>
<td>10.20 ± 1.37</td>
</tr>
<tr>
<td>C</td>
<td>85</td>
<td>80/80</td>
<td>0.25 ± 0.50</td>
<td>10.72 ± 0.83</td>
</tr>
<tr>
<td>5 A</td>
<td>0</td>
<td>80/80</td>
<td>1.00 ± 0.82</td>
<td>5.15 ± 1.91</td>
</tr>
<tr>
<td>B</td>
<td>68</td>
<td>80/80</td>
<td>0</td>
<td>12.55 ± 1.91</td>
</tr>
<tr>
<td>C</td>
<td>170</td>
<td>80/20</td>
<td>0.25 ± 0.50</td>
<td>14</td>
</tr>
</tbody>
</table>

^a Insufficient cellular growth did not allow SCE analysis.
^b 80 cells were analyzed for chromosomal breaks. None of these cultures contained BrdU. An additional 80 cells were analyzed for SCE.
numerical increase of SCE in all cultures containing MAM AC. These increases were statistically significant in four of the five experiments when either B or C cultures were compared to A (control) cultures. Although there was a numerical increase in the C culture series of Experiment 1 (E1), the increase was not significant. The same cultures in E3 exhibited insufficient mitogenesis in all four replicates so that SCE could not be assessed. However, there were over twice the number of SCE in B versus control cultures ($p < .01$). A similar result occurred in E5 ($p < .01$) while both concentrations of MAM AC significantly increased SCE in E4 ($p < .01$). Finally, only those cultures that were exposed to the higher MAM AC concentration showed elevated SCE levels ($p < .05$) in E2.

The study of 1168 metaphase spreads that were not exposed to BrdU showed that MAM AC did not cause a significant increase in the frequency of CB. Only single chromatid breaks were observed. As a matter of fact, the total number of chromatid breaks in cultures A, B and C from all individuals was 15, 12, and 9, respectively. Table 1 shows the mean frequencies of CB per 20 cell culture. There was only one culture condition (E3C) where less than 80 cells were available for examination.

Discussion

The present work has involved the study of 2188 cells and the assessment of chromosome damage in 100,648 chromosomes. We have shown that SCE is a more sensitive indicator of chromosome damage in this system since MAM AC clearly caused an increase of SCE in the cultures of all individuals while there was no significant increase in CB in other cultures (not containing BrdU, but exposed to the same concentrations of MAM AC) from the same individuals.

We agree with Wolff (1977), and Abe and Sasaki (1977), who stated that SCE and chromosomal breakage may result from different mechanisms. That aberration production is associated with cell death (Wolff 1977) would probably have been shown in our study, if higher concentrations of MAM AC had been tested. However, we chose those concentrations (Evans and Jenkins 1976) that either had no effect on blast cell transformation (B cultures, Table 1) or those that caused a significant reduction in blastogenesis (C cultures, Table 1). Higher concentrations may have caused an increase in chromosomal aberrations, but probably would have resulted in a lack of analyzable cells. That MAM AC could have induced chromosomal aberrations at higher concentrations is supported by the findings of Teas et al. (1965) in mitosing onion root tips, although it is realized that this is a plant system and the present work is mammalian.

Finally, it is interesting to note that in combination MAM AC and BrdU seem to antagonize mitosis. Four cultures from series C in E3 contained only 48 metaphases. The other four cultures that were exposed to both MAM AC and BrdU had no metaphases.

Summary

In comparison to chromosomal breakage (CB) studies, sister chromatid ex-
change (SCE) is considered a more sensitive indicator of chromosomal damage. The present study answers the question as to whether or not methylazoxymethanol acetate (MAM AC) exerts an effect on chromosomal breakage and sister chromatid exchange in short-term human leucocyte cultures derived from the same individuals. Only chromatid breaks were observed in a total 1168 metaphases. Statistical analyses of the data have indicated that there were no significant differences in the incidence of chromatid breaks when cultures treated with MAM AC were compared to control cultures ($p > .2$). However, an examination of 1020 metaphases derived from BrdU treated cultures from the same individuals revealed a significant increase in SCE with MAM AC exposure. Therefore, MAM AC has no effect on the incidence of CB, and SCE is a more sensitive indicator of chromosomal damage than CB is in this system.

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References


1982 The Type of Chromosomal Damage Caused by Methylazoxymethanol Acetate