Inactivation of Ultraviolet Repair in Normal and Xeroderma Pigmentosum Cells by Methyl Methanesulfonate

J. E. Cleaver

Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, San Francisco, California 94143

ABSTRACT

Excision repair of ultraviolet damage in the DNA of normal and xeroderma pigmentosum (Groups C, D, and variant) cells was inactivated by exposure of cells to methyl methanesulfonate immediately before irradiation independent of the presence of 0 to 10% fetal calf serum. The inactivation could be represented by a semilog relationship between the amount of repair and methyl methanesulfonate concentration up to approximately 5 mM. The inactivation can be considered to occur as the result of alklylation of a large (about 10^6 daltons) repair enzyme complex, and the dose required to reduce repair to 37% for most cell types was between 4 and 7 mM. No consistent, large difference in sensitivity to methyl methanesulfonate was found in any xeroderma pigmentosum complementation group compared to normal cells, implying that reduced repair in these groups may be caused by small inherited changes in the amino acid composition (i.e., point mutations or small deletions) rather than by losses of major components of the repair enzyme complex.

INTRODUCTION

Excision repair of UV damage to DNA of Escherichia coli (6), human cells (8, 13), and hamster cells (14) can be inactivated by exposure to an alkylating agent before or after irradiation. Inactivation has been demonstrated by measurement of pyrimidine dimer excision, repair replication, and unachieved synthesis, and occurs with MMS, dimethyl sulfate, and N-methyl-N'-nitro-N-nitrosoguanidine. The mechanism of inactivation in both E. coli and mammalian cells is most readily explained in terms of alklylation of excision repair enzymes. A tentative application of target theory leads to an estimate of about 10^6 daltons for the whole complex of enzymes involved (8, 13).

XP is a human disease in which hypersensitivity to sunlight and UV light is caused by defects in a component of the repair complex. It is possible, therefore, that some of the complementation groups could have enzyme complexes lacking one of the component polypeptides. A recent preliminary investigation suggested that this might be the case for one Group C cell line, XP7CA, but not for a Group D, XP1BR, or variant XP line (5). This was consistent with an earlier hypothesis about the structure of the enzyme complexes in these groups (9). In the present study, therefore, I investigated the inactivation of excision repair by MMS in a variety of XP and normal cell lines.

MATERIALS AND METHODS

Human fibroblast cell lines were established from skin biopsies in our own laboratory or obtained from the Human Genetic Mutant Cell Repository, Camden, N. J. These normal cell lines GMS2881, JD, and 2498 were all from young adults, and HFI was from human foreskin. The XP cell lines obtained from the cell bank (XP7CA, XP8BE, XP1AA, XP1BR, and XP115L0) were from patients 10 to 30 years old. Cell line 1404 was from Dr. J. German, New York Blood Center, and originated from Dr. Min, Beijing, China, and both this line and XP1AA were confirmed as Group C by complementation assays against known Groups A, C, and D. XP24MA was a gift from Dr. Jung, Mannheim, Germany, from his "pigmented xerodermoid" series that we have demonstrated to be identical to XP variants (4). Cells were grown in Eagle's minimal essential medium with 10% fetal calf serum and assayed routinely to ensure that they were M. M. pharmaceutical. No consistent, large difference in sensitivity to methyl methanesulfonate was found in any xeroderma pigmentosum complementation group compared to normal cells, implying that reduced repair in these groups may be caused by small inherited changes in the amino acid composition (i.e., point mutations or small deletions) rather than by losses of major components of the repair enzyme complex.
ethanol (1:3 v/v) and processed for autoradiography (2). Unscheduled synthesis in lightly labeled G₁ and G₂ cells was estimated by counting grains over 40 cells.

RESULTS

Exposure of human fibroblasts to UV, MMS, or both in combination produced increases in the ^3H:14C ratios that represented unscheduled synthesis (2) (Chart 1; Table 1). UV and MMS repair were clearly measurable in normal and XP cells. Combined exposure to MMS and UV resulted in a decrease in unscheduled synthesis from that seen in cells exposed to UV alone (Table 1).

In a previous study (8, 13), UV and MMS repair were investigated in normal cells and XP Groups A and G cells, which perform negligible levels of unscheduled synthesis. Those results showed that the decrease in unscheduled synthesis observed after combined MMS and UV exposure was due to the decreased capacity for excision of pyrimidine dimers; however, irradiation with UV did not appear to inhibit MMS repair. Therefore, the relative amount of UV repair performed by cells exposed to UV and MMS can be calculated from the following formula, in which it is implicit that MMS repair is unaffected by concomitant UV exposure.

\[
R = \frac{[UV + MMS] - [MMS]}{[UV] - [control]}
\]

Each set of brackets represents the amount of repair in cells exposed to the treatment indicated ([MMS] = MMS alone, etc.).

\( R \), the relative amount of UV repair, was calculated from scintillation spectrometry or autoradiography for normal human cells exposed to MMS in various serum concentrations (Chart 1). Under all conditions between 0 and 10% serum during MMS exposure, the value of \( R \) decreased linearly on a semilog display. The amount of unscheduled synthesis observed after MMS exposure alone was similar for all serum concentrations. This observation is important because, in a previous brief report in which it was claimed that MMS did not inhibit UV repair, 10% serum was used during exposure (1), whereas in my own previous series of studies I used 0 to 1% serum (5, 8, 13, 14).

In these experiments, fibroblasts are exposed to high concentrations of MMS that resulted in a rapid inhibition of UV repair, such that the precise amount of residual UV repair was a rapidly varying function of both MMS concentration and exposure time. There was therefore some variation between experiments in the absolute value of the MMS dose (\( D_{0[MMS]} \)) required to reduce UV repair to 37% of the amount in cells irradiated with UV alone. This was probably due to variations in the precise duration of exposure to MMS. \( D_{0[MMS]} \) values were determined from UV repair inactivation curves (Chart 1), and the error in these values was much less than the interexperiment variation. Therefore, the values of \( D_{0[MMS]} \) were calculated both as absolute mM values in repeated experiments and as values relative to those of normal cells exposed at the same time as the various XP cells (Tables 2 and 3).

Groups C and D XP cell lines perform measurable levels of unscheduled synthesis, usually in the range of 10 to 20% of normal, although Group D cells generally excise negligible amounts of pyrimidine dimers (19). UV and MMS repair were readily detected in Groups C, D, and variant cells by the methods used in this study. The inactivation of UV repair by concomitant MMS exposure gave inactivation kinetics similar to those seen in normal cells by both scintillation spectrometry and autoradiography.

The absolute and relative values of \( D_{0[MMS]} \) were similar for the majority of normal and XP cells (Tables 2 and 3). One Group C cell line, XP71C, gave a value that seemed larger than the average for all cell types. In a smaller group of cell types investigated previously, XP71C was thought to be representative of Group C cells (5), but MMS resistance does not seem to be generally true of this group. The absolute value of

---

**Table 1**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Treatment</th>
<th>^14C (cpm)</th>
<th>^3H (cpm)</th>
<th>( \frac{\text{^3H:14C}}{^{14}C} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (249b)</td>
<td>Control</td>
<td>23.8</td>
<td>2.3</td>
<td>0.097</td>
</tr>
<tr>
<td></td>
<td>UV (13 J/sq m)</td>
<td>27.6</td>
<td>65.7</td>
<td>0.238</td>
</tr>
<tr>
<td></td>
<td>MMS (4 mM)</td>
<td>28.9</td>
<td>26.2</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>UV + MMS</td>
<td>27.3</td>
<td>51.8</td>
<td>1.90</td>
</tr>
<tr>
<td></td>
<td>( R^6 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XPBDE (Group C)</td>
<td>Control</td>
<td>53.2</td>
<td>1.9</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>UV (13 J/sq m)</td>
<td>57.4</td>
<td>17.5</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>MMS (4 mM)</td>
<td>59.9</td>
<td>27.5</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>UV + MMS</td>
<td>53.4</td>
<td>27.6</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>( R^6 )</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

*Because every cell type exhibited characteristic growth conditions, the actual \( \frac{\text{^3H cpm per cell}}{\text{~mm}} \) (determined by hemocytometer counting) was different for different cell types and served to normalize the \( \frac{\text{^3H cpm}}{\text{~mm}} \) to constant cell number per dish for a given cell type. Values are given as cpm/dish of approximately \( 5 \times 10^9 \) cells, \( x 10^{-2} \). Relative repair levels (\( R \)) can be compared because they are calculated in a way that cancels the absolute value of \( \frac{\text{^14C cpm}}{\text{~mm}} \). *

---

**Chart 1.** Top, relative amount of unscheduled synthesis (\( R \)) in cells exposed for 30 min to various concentrations of MMS in 0 to 1% serum and irradiated with 13 J UV per sq m before labeling for 80 min with 10 \( \mu \)Ci \( \text[^3H]dTThd \) per ml (60 Ci/mmol). □, 249b cells in 0% serum; △, 249b cells in 1% serum; O, JD cells in 1% serum; □, normal cells in 10% serum; ●, XP1404 Group C cells in 1% serum. **Bottom**, MMS-induced unscheduled synthesis in normal cells exposed to MMS for 30 min in 0 to 10% serum. Symbols as described above. Repair was calculated as a fraction of the UV repair determined in the same cells labeled for the same interval after irradiation with 13 J UV per sq m.
than the majority, but this value was obtained in an experiment in which the normal cell line also gave a low value. Thus, the relative value of $D_2[MMS]$ was not calculated in the mean.

Table 3

Relative $D_2[MMS]$ values for the inactivation of UV repair by MMS in various cell types

<table>
<thead>
<tr>
<th>XP cell type</th>
<th>Normal cell type</th>
<th>$D_2[MMS]$ relative to parallel normal cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>XP Group C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XP7CA</td>
<td>HF1</td>
<td>5.52</td>
</tr>
<tr>
<td>XPBBE</td>
<td>JD</td>
<td>0.89</td>
</tr>
<tr>
<td>XP1AA</td>
<td>2498</td>
<td>1.19</td>
</tr>
<tr>
<td>1404</td>
<td>JD</td>
<td>0.74</td>
</tr>
<tr>
<td>1404</td>
<td>2498</td>
<td>0.77</td>
</tr>
<tr>
<td>XP Group D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XP1BR</td>
<td>HF1</td>
<td>0.73</td>
</tr>
<tr>
<td>XP variant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XP115LO</td>
<td>HF1</td>
<td>1.21</td>
</tr>
<tr>
<td>XP24MA</td>
<td>2498</td>
<td>1.05</td>
</tr>
</tbody>
</table>

$^a$ The value for XP7CA was not calculated in the mean. Pairing of normal and XP cells was on a random basis.

$^b$ Mean ± S.D.

$^c$ Mean.

$D_2[MMS]$ obtained for the Group D cell line seemed smaller than the majority, but this value was obtained in an experiment in which the normal cell line also gave a low value. Thus, the relative value of $D_2[MMS]$ was not significantly different from normal (Table 3).

**DISCUSSION**

These studies indicate that the inactivation of UV repair by alkylating agents observed previously by dimer excision and repair replication in human cells (8, 13) and Chinese hamster cells (14) also occurs in a wide variety of repair-deficient XP cells. These inactivation kinetic values were analyzed previously by a simple target theory, assuming that complexes of UV repair enzymes constituted targets that could be inactivated by random alkylations (8, 13). The size of the target from such analysis was of the order of several million, which is not unreasonable considering that a repair complex would consist of at least the polypeptides specified by 7 XP complementation groups together with exonucleases, polymerases, ligases, and other associated factors. If the mass of each polypeptide is of the order of 50,000 to 100,000 daltons, as reported for the products of the UVR A, B, C loci in *E. coli* (15, 16), then the total size expected is close to that estimated from the target theory calculation.

Alternate explanations for the inhibition of UV repair by MMS have been discussed in detail previously (8, 13, 14) and will be summarized briefly. Changes in patch size, precursor pools, and phosphorylating enzymes are an unlikely cause of these observations because MMS directly inhibited an early step of repair, the excision of pyrimidine dimers, in both human and hamster cells (13, 14). Specific alteration of precursor supply by metabolic inhibitors, for example, has only a minor influence on dimer excision and repair replication (2, 3, 7). Saturation of a repair pathway that is common for UV and alkylation damage is also unlikely because the major products from these agents are repaired by distinct pathways, nucleotide, and base excision repair, respectively (2). In UV repair-deficient XP cells, high doses of UV light were without influence on MMS repair that proceeded at normal levels (8). Inactivation of UV repair enzymes by protein alkylation therefore remains the most reasonable interpretation of these data. This interpretation is supported by the observation that $N$-methyl-$N'$-nitro-$N$-nitrosoguanidine, a more potent protein alkylator than MMS, is also more potent at inactivating UV repair in human and hamster cells (8, 13, 14).

The observation that similar target sizes are obtained from the XP complementation Groups C, D, and variant implies that the repair observed in each group is carried out by an enzyme complex similar in size to that in normal cells. This complex would be impaired or altered in function for Groups C and D and perhaps unaffected in the variant. Groups C and D therefore probably represent mutations (i.e., point mutations or small deletions) in the corresponding polypeptides that impair function by an alteration in amino acid composition rather than by losses of major components of the repair enzyme complex. Loss of a component greater than a standard deviation of the mean $D_2[MMS]$, i.e., about 20 to 30% of control, would probably be the smallest loss detectable in this procedure. It is possible, however, that the apparent resistance of UV repair to MMS in XP7CA might be the consequence of a significant reduction in the target size of the repair complex, which could be produced by a deletion mutation or the failure to synthesize a polypeptide subunit. There is no a priori reason that deletions and point mutations could not both be found within a complementation group.

A dilemma is presented by the present data in which serum concentrations between 0 and 10% had no influence on the action of MMS (Chart 1). On the assumption that UV repair is inactivated by MMS because it reacts with proteins, high concentrations of serum proteins were expected to absorb many of the reactive species. Either MMS reacts in a distinctive manner once inside the cells, or 10% serum is still a relatively small concentration compared to the concentration of reactive species from MMS. Whatever the explanation, it is clear that these results fail to provide support for preliminary results in which 10 mM MMS apparently had no effect on UV repair in V79 Chinese hamster cells (1). In fact, in all cell types that I have investigated, normal, XP, SV40-transformed human, and Chinese hamster (8, 13, 14), exposure to more than about 8
MMS caused visible necrosis within 2 to 4 hr and cells lifted from their substrate by 24 hr.

This investigation was an attempt to analyze the structure of the gene products involved in defective repair of UV damage in XP cells. Direct isolation and characterization of these products have thus far been unsuccessful because of their in vitro instability (12, 17, 18), making indirect approaches such as those described here among the few tools available. Undoubtedly, indirect approaches will be superseded when eukaryotic excision repair systems yield to recombinant DNA and gene cloning methods, as have E. coli and T4 phage repair systems (15, 16).

Note Added in Proof

We have subsequently measured the single-strand breaks associated with excision repair of UV and MMS damage by alkaline elution and alkaline sucrose techniques. We found that cytosine arabinoside added after exposure inhibited polymerization of repair patches and increased the frequency of strand breaks after both UV and MMS damage. The number of strand breaks observed by both analytical techniques, with and without cytosine arabinoside, in cells exposed to UV and MMS together was found to be the sum of the number of breaks in cells exposed to each agent separately. Therefore, MMS must inactivate UV repair at steps beyond the initial incision by UV endonucleases (Park, S. D., and Cleaver, J. E., unpublished observations).

REFERENCES

Inactivation of Ultraviolet Repair in Normal and Xeroderma Pigmentosum Cells by Methyl Methanesulfonate

J. E. Cleaver


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/42/3/860

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.