6-Thioguanine-induced DNA Damage as a Determinant of Cytotoxicity in Cultured Chinese Hamster Ovary Cells

Nelwyn T. Christie,2 Stephanie Drake, Raymond E. Meyn, and J. Arly Nelson

INTRODUCTION

The toxicity of TG3 or 6-mercaptopurine in some cultured cell systems has been attributed previously to the incorporation of these compounds into DNA as thioguanate, rather than incorporation into RNA (21, 27–28). While an inhibition of de novo purine biosynthesis has been reported for both drugs, this effect appears to be reversible and not related to the delayed cytotoxic response observed (21, 27, 32). The reduction of the purine nucleotide pool sizes by TG treatment of human epidermoid carcinoma cells (H.Ep. No. 2) cells did not correlate with growth inhibition or loss of viability (21). Additionally, 6-methylmercaptopurine riboside produced a greater reduction of nucleotide pool sizes than did TG, without reducing the capacity for cell division (21). An effect of TG on nucleotide availability has been proposed as the basis for the reversible S-phase block, whereas an irreversible block in the second G2 phase after TG addition was proposed as the basis of cytotoxicity (32). These studies have provided strong support for the involvement of the incorporation of TG into DNA in its cytotoxic action and have essentially eliminated an effect on de novo purine biosynthesis as a major factor in the toxicity of this base analogue in the cells studied. In contrast, the mechanism for toxicity due to the related compound 6-mercaptopurine may vary, depending on the cell type studied, because the levels of incorporation of thioguanylate into the DNA varies markedly in different cell lines (6, 18).

EXAMINATION OF CHROMATIN STRUCTURE FOLLOWING TREATMENT WITH TG

The examined in cultured Chinese hamster ovary cells by direct measurement of the incorporation of the compound into DNA and by analysis of the resulting DNA damage. The predominant lesions as monitored by alkaline elution were DNA strand breaks. Very few, if any, interstrand or DNA-protein cross-links could be definitively observed. The cytotoxicity of TG as measured by colony-forming ability appeared closely related with its incorporation into DNA and the DNA strand scission events. As TG concentrations were increased, cytotoxicity, DNA incorporation, and strand scission reached a plateau; this result is consistent with earlier reports that TG produces a reversible block of DNA synthesis. Strand breaks appeared to be related to the incorporation of TG into DNA, since the addition of 1 μM cycloheximide during a 24-hr treatment with 3 μM TG prevented the cytotoxicity, prevented incorporation of TG into DNA, and eliminated the strand breaks. Alkali-labile sites were detected in the DNA of TG-treated cells by alkaline elution at pH 12.8, suggesting that depurination of TG residues by a glycosylase mechanism may occur. It is also postulated that TG residues are recognizable by the long-patch repair system, since UV-sensitive cells deficient for long-patch repair were more sensitive to TG than were wild-type cells. Furthermore, caffeine (1 mM) was shown to enhance the lethality of TG (3 μM), as monitored by colony formation, without altering levels of TG incorporation into DNA or the strand scission as measured immediately after treatment. This result, coupled with the known delayed cytotoxic response of TG, suggests that gaps may occur in newly synthesized DNA opposite TG residues and that the repair of these gaps by a postreplication repair mechanism is inhibited by caffeine.

MATERIALS AND METHODS

Cell Culture. The 3 cell lines of CHO (AA8, UV20, and EM9) used for these studies were the kind gift of Dr. L. H. Thompson of the Lawrence Livermore Laboratories. The AA8 wild-type line served as the parental stock for isolation of DNA repair mutants sensitive to UV irradiation (UV20) or alkylating agents (EM9) (25). The EM9 cells were also more sensitive than were AA8 cells to other DNA-damaging agents (24). All lines were grown in a minimum essential medium or McCoy’s Medium 5A containing 1% antibiotic-antimycotic mixture (Grand Island Biological Co., Grand Island, NY) and 10% fetal bovine serum (Grand Island Biological Co., heat inactivated at 56° for 30 min). The doubling time for

1Supported by NIH Research Grant CA-28034 and CA-23270 from the National Cancer Institute.
2To whom requests for reprints should be addressed.
3The abbreviations used are: TG, 6-thioguanine; CHO, Chinese hamster ovary; CHX, cycloheximide; HU, hydroxyurea; CAF, caffeine; dAdo, deoxyadenosine; PRR, postreplication repair.

Received February 1, 1984; accepted May 29, 1984.
these lines ranged from 13 to 16 hr. Survival following drug treatments was determined by a colony-forming assay in which approximately 100 cells were plated in each well of a 24-well dish (6). Drug treatments were applied after overnight incubation at 37°. Cell survival was expressed as the fraction of colonies observed after 4 to 5 days of growth in the treated samples compared to the untreated, and using a minimum colony size based on the growth of controls.

Alkaline Elution Assay. This assay followed the procedure of Kohn and Ewig (14). Approximately 10⁶ cells were grown in 10-cm dishes for 24 hr in the presence of 0.02 μCi of [3H]deoxythymidine/ml and then placed in medium without isotope for 6 to 20 hr before drug treatments were initiated. Monolayers were removed by trypsinization, and 8.5 × 10⁶ cells were applied after overnight incubation at 37°. Cell survival was expressed as colony-forming ability (Chart 1). Within a narrow concentration range (0.3 to 3 μM), the survival is reduced to 17%. In DNA, the incorporation of TG was consistently less than into DNA.

RESULTS

DNA Lesions after Exposure of CHO Monolayers to TG. We have previously shown the critical importance of the incorporation of TG into DNA to its cytotoxic action (21); however, the effect of this antimetabolite on DNA structure has not been examined in detail. The incorporation of TG into DNA as a function of dose correlated with cytotoxicity monitored by colony-forming ability (Chart 1). Within a narrow concentration range (0.3 to 3 μM), the survival is reduced to 17%. In DNA, the incorporation of TG increased steadily from 0.05 pmol TG/100 pmol dAdo for 0.75 μM TG to 1.38 for 3 μM TG. While incorporation of TG into RNA was also observed, the extent of incorporation was consistently less than into DNA.

![Chart 1. Incorporation of TG into RNA and DNA and cytotoxicity in CHO cells. CHO cells were exposed to TG for 24 hr and then harvested for quantitative recovery of RNA and DNA. After sequential degradation of each nucleic acid fraction, the nucleosides were analyzed by high-performance liquid chromatography (13). Briefly, cells were extracted with 0.8 N perchloric acid, and the acid-insoluble pellet was hydrolyzed with 0.4 N potassium hydroxide to solubilize the RNA fraction. The DNA was reprecipitated with 0.4 N hydrochloric acid and 10% trichloroacetic acid. The RNA hydrolysate was treated with alkaline phosphatase, and the DNA was treated with DNase I, 5′-nucleotidase, and phosphodiesterase to yield nucleosides. The samples were analyzed by a tandem arrangement of a Partisil-10 SAX column (Whatman, Inc., Clifton, NJ), followed by a C₁₈Bondapak column (Waters Associates, Milford, MA). The hydrolyzed samples of DNA and RNA were subjected to oxidation by potassium permanganate according to the method of Tidd and Dechar (26), to form derivatives of TG. The highly fluorescent derivatives of 6-thioguanosine or β-2′-deoxythioguanosine were eluted from the Partisil 10 SAX by 30 mM ammonium dihydrogen phosphate, pH 3.7, plus 2% methanol. Adenosine or dAdo were then eluted from the C₁₈Bondapak column using a linear gradient to 30 mM ammonium dihydrogen phosphate, pH 3.7, plus 20% methanol. Detection utilized fluorescence (6-thioguanine and β-2′-deoxythioguanosine) or UV absorbance (adenosine and dAdo). This procedure allowed simultaneous detection of TG residues and adenine residues in a given sample (13). The variability in these measurements for DNA was 1 to 3.5 pmol TG/100 pmol dAdo, with the greatest variability occurring for TG concentrations of 3 μM or more. A range of 4.1 to 10.8 pmol TG/100 pmol dAdo was observed at 12 μM, with an average of 8.0. Variability in the levels of TG incorporation into DNA was considerably lower for experiments performed within time periods of 4 to 8 weeks and with the same preparation of medium. This suggests that the higher variabilities may be due to differences in cellular uptake of TG, or to differences in the growth rate of the cells, which may be caused by fluctuation in serum components. When no standard deviations are given for the data presented, the repeat experiments were done in close proximity and the variability is negligible.

![Graph](https://example.com/graph.png)
Effect of TG Residues on DNA Structure. The effect of TG on DNA structure was analyzed by alkaline elution, and the results are shown in Chart 2A. The effect of a 0.75 μM treatment (data not shown) was detectable and intermediate between the control and the 1.5-μM samples. Increasing concentrations of TG up to 6 μM caused progressively faster elution rates, indicating that the relative number of strand breaks increased as the level of TG incorporation increased. When a concentration of 12 μM TG was used, the elution rate was slower and resembled the elution profile of the DNA of the 1.5 μM treatment. Since this pattern of increased retention of DNA at the higher concentration could indicate the presence of DNA-protein or interstrand cross-links, these possibilities were examined using 24-hr treatments for 3 concentrations of TG, 3, 6, and 12 μM. The results of the 6 μM treatment are shown in Chart 2B. The similarity in DNA retention at 12 hr is similar for the 2 irradiated samples in this experiment and others using various TG concentrations, suggesting that cross-links were not a major component of TG-induced DNA damage. Nevertheless, since the elution profile of the TG-treated and irradiated cell sample was nonlinear, while the control sample receiving only X-rays was linear, the production of a low level of cross-links was not ruled out. In addition, the slight curvature of the elution profile of the sample receiving only TG was also compatible with a very low level of cross-links, although a nonrandom distribution of DNA strand breaks could also produce this result. DNA-protein cross-links were not detected, since the addition of proteinase K to equivalent fractions of cells for each of the conditions in Chart 2B did not significantly alter elution profiles (data not shown). Similar experimental protocols were followed for cells treated with 3 or 12 μM (data not shown), and in neither case was there strong evidence for cross-linking, although again, a low level of such lesions could not be ruled out. A reduction in the number of strand breaks for TG concentrations of 12 μM or greater was observed in several experiments. We can thus conclude that increased elution rates for DNA from cells treated with TG was determined predominantly by the number of DNA strand breaks induced in vivo, or upon addition of alkali.

Relation between TG Incorporation and DNA Strand Breaks. Since the production of cross-links was insufficient to explain the increased retention of DNA from cells treated with 12 μM TG, the possibility of reduced incorporation of TG was explored. The elution rate of samples treated with 3 μM TG was essentially the same as those for samples treated with 12 μM TG, and the incorporation of TG into DNA was compared for these 2 doses. The level of incorporation for the 2 treatments was similar, with 4.9 pmol TG/100 pmol dAdo for the 3 μM treatment, and 3.9 for the 12 μM treatment. As the intracellular concentration of TG is increased, rather than continued incorporation, a blockage of DNA synthesis may occur which produces a plateau in the level of incorporation of TG. We have previously reported that TG at high concentrations can inhibit DNA synthesis (20).

The relationship between levels of incorporation and the production of single-strand breaks was further investigated by use of inhibitors of DNA synthesis. CHX is known to lower rates of DNA synthesis through its primary action as an inhibitor of protein synthesis (30), while HU is considered a specific inhibitor of DNA synthesis (5). CHO monolayers were treated for 24 hr with 6 μM TG in combination with 1 μM CHX, 0.125 mM HU or 0.25 mM HU, or each of these treatments alone. The results of this experiment are given in Table 1. While the TG treatment alone produced considerable breakage of DNA, the coadministration of CHX with TG reduced TG incorporation from 5.3 to 0.6 pmol TG/100 pmol dAdo, and correspondingly reduced the relative incorporation.
amount of the DNA damage. When CHX was applied alone to cells, the elution rate of the DNA was similar to that of untreated samples. For the cell cultures treated with HU, there was a progressive increase in the amount of incorporation of TG into DNA as the concentration of HU was decreased, and this was accompanied by an increase in the amount of DNA strand breaks. Thus, in all cases the extent of DNA breakage was proportional to the extent of incorporation of TG into DNA. The prevention of the DNA damage was also associated with a block of the cytotoxicity. For example, simultaneous treatment of TG and CHX produced a cloning efficiency of 77% of untreated samples, compared to 1.7% for the TG treatment alone. Therefore, not only was the extent of TG incorporation into DNA approximately proportional to DNA strand scission, but a reduction in incorporation produced less cytotoxicity. In other experiments using different concentrations of TG, coadministration of CHX treatments with TG reduced both DNA strand scission and the level of incorporation of TG into DNA.

Influence of DNA Repair Systems. From prior studies (21, 27–28) and from the experiments described above, we concluded that the cytotoxic action of TG was directly related to its incorporation into DNA, and that prevention of the incorporation also prevented the cytotoxicity. As an additional test of this hypothesis, we have first examined the response of DNA repair systems to the presence of thioguanylate in the DNA. In one series of experiments, CAF, an inhibitor of DNA postreplication repair was used, and in the second, TG cytotoxicity was examined in DNA repair-deficient mutants.

The combination of CAF and TG was shown to reduce survival in a concentration-dependent manner (Chart 3). Concentrations of 1 mM CAF or less reduced cell survival only slightly, yet at 0.25 mM in the presence of 0.5 µM TG, the survival was reduced to 38%, compared to 67% for TG alone. If the primary effect of CAF is to inhibit the postreplication repair of TG-induced lesions, it should not alter the incorporation levels of TG into DNA, or alter the alkaline elution pattern of the DNA immediately after treatment. Monitoring incorporation and DNA strand scission required higher concentrations of TG, and these results are seen in Table 2. The combined treatment of 3 µM TG and 1 mM CAF yielded essentially the same elution rate as for a treatment of 3 µM TG without CAF present. The levels of incorporation were 1.9 pmol TG/100 pmol dAdo for 3 µM TG, and 2.2 for cotreatment with 3 µM TG and 1 mM CAF, indicating that CAF at this concentration does not interfere with incorporation of TG. Thus, it is not expected that the enhanced cytotoxicity of the joint treatments at low concentrations of TG was due to an increased level of incorporation of TG into DNA in the presence of CAF, but may have resulted from a lowered repair capacity.

The nature of the TG lesion was also probed by using cell lines characterized as defective in excision repair. Two types of DNA repair mutants derived from the same parental line of CHO were examined for cytotoxicity to TG, UV20, a UV-sensitive mutant, and EM9, sensitive to alkylating agents (24, 25). The parental line AA8 and the 2 mutants were tested in the colony-forming assay after 24-hr treatments of TG. No difference in sensitivity was found between the AA8 cells and the EM9 cells, but the UV20 cells were more sensitive to the drug (Chart 4A). The survival of the UV20 cells was less than that of the parental cells at all doses tested; at 0.03 µM TG, the lowest dose tested, 39% of the treated UV20 cells formed colonies, compared to 88% for AA8 cells. The relative survival for both cell types did not change significantly after a dose of 3 µM. Incorporation of TG into DNA was also analyzed for each of the concentrations used in the survival study (Chart 4B). Incorporation in both cell lines initially increased with TG concentration, but levels of both incorporation and cytotoxicity form a plateau at high concentrations of TG.

The level of incorporation of TG into DNA correlated with DNA strand scission, as shown in Table 3 for AA8 and UV20 cells. The variability in these experiments is somewhat less than usual, perhaps because they were performed within a short time period with the same lot of serum, as discussed in "Materials and Methods." For both cell lines the amount of DNA retained was

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incorporation into DNA (pmol TG/100 pmol dAdo)</th>
<th>Cloning efficiency (% of control)</th>
<th>% of DNA retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>100</td>
<td>91</td>
<td>91</td>
</tr>
<tr>
<td>1 mM CAF</td>
<td>76</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>3 µM TG</td>
<td>1.9</td>
<td>8.0</td>
<td>78</td>
</tr>
<tr>
<td>3 µM TG + 1 mM CAF</td>
<td>2.2</td>
<td>2.1</td>
<td>73</td>
</tr>
</tbody>
</table>
DNA Damage by TG

Table 3
Relationship of TG incorporation into DNA and the production of DNA strand breaks

<table>
<thead>
<tr>
<th>Incorporation (pmol TG/100 pmol dAdo)</th>
<th>DNA retention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA8</td>
</tr>
<tr>
<td>Untreated</td>
<td>93.4 ± 1.3^a</td>
</tr>
<tr>
<td>&lt;0.1-0.5</td>
<td>92.7 ± 0.5</td>
</tr>
<tr>
<td>0.5-1.0</td>
<td>89.1 ± 3.1</td>
</tr>
<tr>
<td>1.0-5.0</td>
<td>73.5 ± 5.7</td>
</tr>
<tr>
<td>5.0-10.0</td>
<td>65.0 ± 2.5</td>
</tr>
</tbody>
</table>

^a Incorporation values were pooled from several experiments using different treatment concentrations for a 24-hr exposure to TG.
^b Amount retained on the filters after 12 hr of elution with alkali.
^c Average ± S.D. of 2 to 4 values.

Table 4
Time course for incorporation of TG into DNA and RNA and its effect on cloning efficiency and DNA elution rate

Each parameter was determined as for Table 2 and represents an average of 2 determinations.

<table>
<thead>
<tr>
<th>Treatment time (hr)</th>
<th>Incorporation (pmol TG/100 pmol dAdo or adenosine)</th>
<th>Cloning efficiency (% of DNA)</th>
<th>% of DNA retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.05, 0.05</td>
<td>DNA</td>
<td>RNA</td>
</tr>
<tr>
<td>1</td>
<td>0.12, 0.42</td>
<td>100</td>
<td>88</td>
</tr>
<tr>
<td>6</td>
<td>1.75, 1.03</td>
<td>18</td>
<td>75</td>
</tr>
<tr>
<td>12</td>
<td>1.56, 1.53</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>18</td>
<td>1.32, 1.28</td>
<td>2</td>
<td>77</td>
</tr>
<tr>
<td>24</td>
<td>1.44, 1.15</td>
<td>1</td>
<td>69</td>
</tr>
<tr>
<td>48</td>
<td>2.17, 1.04</td>
<td>&lt;0.1</td>
<td>61</td>
</tr>
</tbody>
</table>

^a CHO monolayers were treated with 3 μM TG for the indicated times.

Chart 4. Relationship between CHO cell survival and TG incorporation into DNA. The DNA repair-deficient mutant UV20 and parental cell line AA8 were treated with TG for 24 hr. A fraction of cells from each treatment was tested for colony-forming ability (4) and for measures of TG incorporation into DNA immediately after the 24-hr treatment (5) for both AA8 (8) and UV20 (8) cells. Values represent averages of 2 to 4 determinations. The range of values for cytotoxicity was within 1 to 4%, and for incorporation into DNA was typically 1 to 3 pmol/100 pmol dAdo. The experiments for AA8 and UV20 cells were performed using a single serum lot to avoid any possible differences from variations in serum components.

reduced as more TG became incorporated into the DNA. These results are consistent with the DNA strand breaks being involved in the cytotoxicity. In addition, for each of the ranges of incorporation given, the DNA retention was greater in UV20 than in AA8 cells, indicating fewer DNA strand breaks in UV20 cells. This difference may reflect an interaction of the enzymes of the long-patch excision repair with the DNA of the AA8 cells.

Another series of experiments was initiated to evaluate the time course for the production of breaks in relation to levels of incorporation into DNA and the colony-forming ability. CHO monolayers were treated with 3 μM TG for periods of 1 to 48 hr (Table 4). The extent of TG incorporation into DNA reached a maximum at 6 hr, but remained relatively constant through 18 hr, at which time most cells would have completed one cell cycle. The cloning efficiency at 6 hr was 18%, possibly reflecting the number of cells that were not in S phase during treatment with TG. When the treatment period was extended to 12 hr, nearly all cells would have entered S phase, and the cloning efficiency dropped to 3%. The analyses of DNA strand breaks performed after each timed treatment indicated that some of the TG lesions leading to strand breaks occurred without a cell traversing the entire cell cycle.

The production of DNA strand breaks may be due to direct enzymatic action, such as an endonuclease cleavage as a first step in the repair of the TG lesion. Alternatively, removal of thio- guanylate residues by a glycosylating mechanism might yield sites of depurination which are susceptible to alkali degradation (11). This latter possibility was investigated by performing alkaline elution analyses at pH 12.1 and 12.8, following 24-hr treatments of TG. There was an increase in the elution rate at the higher pH for both of the treatments examined. The fraction of DNA retained after 12 hr of elution decreased from 58 to 33% for the 3 μM treatment, and from 58 to 46% for the 12 μM treatment. Untreated cell samples showed no difference for the 2 pH conditions. These results indicated that some alkali-sensitive sites were present in DNA from TG-treated cells.

DISCUSSION

The goal of this study was to analyze the effect of TG incorporation into DNA in relation to its cytotoxicity, and to investigate the effect of thio- guanylate residues on DNA structure as a possible clue to its toxicity. Alkaline elution analysis revealed that the predominant lesions are DNA strand breaks observed after 24 hr of exposure of CHO cells to TG at concentrations between 1.5 and 12 μM. There was a linear increase in strand scission until a concentration of 6 μM was reached; concentrations above 6 μM produced breaks comparable to those observed at 1.5 or 3 μM (Chart 1). Analysis of the incorporation of TG into DNA over this concentration range, and the resulting cytotoxicity assayed by colony-forming ability, indicated that some feature of the TG
cytotoxicity reduced the rate of its own incorporation into DNA. Wotring and Roti Roti (32) reported that TG produced an S-phase block, although the block was reversible following removal of TG and was not associated with the delayed cytotoxicity of TG. Thus, TG may inhibit DNA synthesis at high levels, thereby self-limiting its toxicity (20). At lower concentrations this effect on DNA synthesis does not prevent the majority of the cells from traversing a complete cell cycle to the point of the G2 block (1, 32). Furthermore, Maybaum and Mandel (19) have shown that CHO cells treated with 4 μM TG were still able to divide.

More than one element in the cellular DNA repair system may recognize the lesions in DNA resulting from TG treatments. Since it is known that the hydrogen bond between the sulfhydryl group of TG and the amino group of cytosine is longer than a normal G—C hydrogen bond (23), it is plausible that thioguanylate bases are excised because of a distortion produced in the DNA helix. Two types of excision repair have been described (22). Long-patch repair is exemplified by repair of UV-induced thymine dimers, in which the stretches of newly synthesized DNA are of the order of 20 to 100 nucleotides. This type of repair can be contrasted to the short patches of 1 to 4 nucleotides of new synthesis following exposure to alkylating agents or X-rays. We have utilized mutant CHO cells sensitive to UV and deficient in long-patch repair (UV20) or sensitive to alkylating agents (EM9) to evaluate the nature of the lesion produced by TG. A similar sensitivity to TG for AA8 and EM9 cells indicated that the repair system defective in EM9 cells (24) does not interact with TG lesions.

Since the UV20 cells were more sensitive than AA8 cells to TG in the cytotoxicity assay, it appears that the long-patch repair system may be involved in the repair of TG lesions in DNA. The excision repair system involved in repair of UV damage is not expected to recognize alkylated bases, but rather to be reserved for damage producing more extreme perturbations of DNA structure. While the presence of a single thioguanylate residue is not expected to have a pronounced effect on the topography of DNA (3, 23), adjacent TG residues may exert sufficient stress in the helix to be recognized by the same repair system that excises thymine dimers. Since UV20 cells do not exhibit repair replication following UV radiation (25), these cells may lack a dimer-specific endonuclease. The presence of fewer DNA strand breaks in these cells compared to wild-type AA8 cells suggests that some breaks in AA8 cells may be due to an endonuclease mechanism. Cleavage may occur at TG clusters, since it has been proposed that adjacent TG residues may alter stacking interactions (3).

From our measurements of the incorporation level of TG into DNA there is approximately one TG residue/100 nucleotides for a highly toxic dose of 3 μM. This level of incorporation is very high, and therefore it is possible that clusters of TG occur and possibly clusters, are recognized by the long-patch repair system. In UV20 cells compared to wild-type AA8 cells suggests that some breaks in AA8 cells may be due to an endonuclease mechanism. Cleavage may occur at TG clusters, since it has been proposed that adjacent TG residues may alter stacking interactions (3).

If, as in the case of UV damage, lesions not removed before DNA synthesis result in the generation of gaps opposite the lesion, these gaps may be responsible for the chromatin disruption observed in G1 cells (11, 19), and for the cytotoxicity. The repair of gaps in newly synthesized daughter strands is referred to as PRR and can be initiated in late S or G2 (11, 22). Studies of CAF interactions with TG cytotoxicity were initiated, since CAF has been shown to inhibit PRR in rodent cells (4, 9, 29), although not in normal human cells (2, 10). CAF potentiated DNA damage in Chinese hamster V79 cells and increased cytotoxicity from UV irradiation and from certain alkylating agents (8), presumably by prevention of the closure of gaps produced during DNA synthesis (16). In our studies, CAF addition during 24-hr TG treatments produced an enhanced cytotoxic response which was not accompanied by an increased rate of alkaline elution immediately after treatment. Also, CAF did not alter the extent of TG incorporation (Table 2). The enhancement of lethality by CAF may signify that TG lesions persist after DNA synthesis resulting in gaps, and that in the absence of CAF some lesions are repaired by a PRR mechanism.

In summary, the effect of thioguanylate residues on DNA structural integrity is complex, and the resulting DNA lesions may be recognized by more than one repair system. Cytotoxicity of cultured cells to TG follows the incorporation of TG into DNA and the production of strand breaks. One possible basis for the breaks may be sites of base removal that are alkali labile. A second possibility is that certain sites of TG residues in DNA, possibly clusters, are recognized by the long-patch repair system which produces endonuclease cleavage.

REFERENCES

16. Lehmann, A. R., and Kirk-Bell, S. Effects of caffeine and theophylline on DNA


6-Thioguanine-induced DNA Damage as a Determinant of Cytotoxicity in Cultured Chinese Hamster Ovary Cells

Nelwyn T. Christie, Stephanie Drake, Raymond E. Meyn, et al.


Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/44/9/3665

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

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

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