Methyl DNA Adducts, DNA Repair, and Hypoxanthine-Guanine Phosphoribosyl Transferase Mutations in Peripheral White Blood Cells from Patients with Malignant Melanoma Treated with Dacarbazine and Hydroxyurea


Imperial Cancer Research Fund, Clinical Oncology Unit, University of Oxford, Churchill Hospital, Oxford OX3 7LJ, United Kingdom [P. A. P., A. L. H., A. S., K. M., T. S. G.]; Laboratory of Chemical Carcinogenesis, Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, Athens 11635, Greece [V. L. S., S. A. K.]; MGC, Department of Radiation Genetics and Chemical Mutagenesis, State University of Leiden, Leiden, the Netherlands [A. D. T.]; and TNO Nutrition and Food Research, P. O. Box 5815, 2280 HV Rijswijk, the Netherlands [J. H. M. v. D.]

ABSTRACT

Dacarbazine (DTIC) is a DNA-methylating drug used in the treatment of malignant melanoma. Among the DNA adducts induced by DTIC are N7-methylguanine (N7-meG) and O6-methylguanine (O6-meG). The latter adduct, in particular, may be important in the mutagenic as well as the cytotoxic activity of DTIC. Repair of O6-meG is carried out by the enzyme O6-alkylguanine-DNA-alkyltransferase (AGT) by a process which results in its autoinactivation. N7-meG is lost from DNA partly spontaneously and partly by enzymatic depurination followed by excision repair of the resulting apurinic site. The purpose of this study was to determine the in vivo kinetics of formation and repair of O6-meG and N7-meG and the changes in AGT in peripheral WBCs with repeated doses of DTIC, and to determine the effects on these processes of concomitant administration of hydroxyurea. In addition, we examined the induction of mutations at the HPRT gene locus. Thirty-four patients with malignant melanoma received 1.0 g/m² DTIC i.v. every 3 weeks. Hydroxyurea was added to the second and subsequent doses of DTIC in 19 patients. The concentrations of O6-meG, N7-meG, and AGT in peripheral blood lymphocytes were determined up to 24 h after each of the first two doses of DTIC. Mutations at the HPRT gene locus were determined using the T-cell clonal assay. Peak O6-meG levels were detected 1 and 4 h after the first and second dose of DTIC, respectively. AGT concentrations declined to 56.7% (range, 40.3–76.9%) and 55.0% (range, 45.4–58.9%) of pretreatment levels 24 h after the first and second doses of DTIC, respectively, and were still approximately 25% below their initial levels just prior to administration of the second dose of DTIC. An increase in formation of O6-meG was observed at all time points after the second dose of DTIC (P = 0.0001), which was not affected by cotreatment with hydroxyurea (P > 0.5). There was a negative correlation between pretreatment AGT levels and the O6-meG concentration at 24 h after therapy (r = −0.554, P = 0.014). N7-meG levels peaked at 6 h after DTIC therapy and were not significantly influenced by the cycle number. Cotreatment with hydroxyurea tended to be associated with lower levels of N7-meG (P = 0.08). There was no correlation between either O6-meG or N7-meG levels and the grade of neutropenia. On the basis of a limited series of blood samples analyzed, there was no firm evidence that chemotherapy with DTIC resulted in induction of HPRT mutations in lymphocytes. In conclusion, repeated administrations of DTIC resulted in higher concentrations of O6-meG, probably due to reduction in cellular AGT. Hydroxyurea did not significantly influence the kinetics of O6-meG and N7-meG adduct formation. There was no significant induction of HPRT gene mutations with DTIC. This study suggests that sequencing of DTIC doses should be evaluated using the time course of cellular AGT depletion and DNA adduct formation to achieve higher cytotoxic efficiency.

INTRODUCTION

DTIC is a purine analogue which continues to be one of the most active drugs in the treatment of malignant melanoma. In vivo, DTIC is metabolically activated by microsomal N-demethylation to 5-(3-methyl-1-triazeno)imidazole-4-carboxamide. 5-(3-methyl-1-triazeno)imidazole-4-carboxamide methylates DNA, producing O6-meG and N7-meG adducts as well as probably other DNA adducts (1, 2). O6-meG is a directly miscoding DNA lesion which, during cell replication, gives rise to G:A mutations (3, 4). In addition to its mutagenic and carcinogenic potential, O6-meG appears to contribute significantly to the cytotoxicity of DTIC and other DNA-methylating agents (3, 5). Quantitatively,
Optimum scheduling of DTIC to achieve maximum antitumor activity requires adequate knowledge of the in vivo kinetics of the formation and repair of critical DNA-damaging adducts. In addition, biochemical modulation of the key steps involved in the stability of cytotoxic DNA adducts, such as AGT activity and possibly repair of N7-meG or other DNA adducts, might provide rational approaches to improve the antitumor activity of DTIC. The aims of this study were (a) to determine the kinetics of O6-meG and N7-meG formation and repair in peripheral WBCs from patients treated with repeated cycles of DTIC; (b) to determine the influence of hydroxyurea on the repair of N7-meG in vivo; (c) to investigate the relationship between the levels of these DNA adducts, myelotoxicity, and antitumor response to DTIC; and (d) to evaluate the potential genotoxic effects of therapy with DTIC.

**PATIENTS AND METHODS**

**Patients.** Thirty-four previously untreated patients (male:female, 21:13; median age, 56 years) with histologically proven malignant melanoma took part in this study. Median Eastern Cooperative Oncology Group performance status was 0 (range, 0–2). Patients had pretreatment normal hepatic function, unless due to malignant infiltration of the liver, and normal renal function. A written informed consent was obtained from each patient prior to entry into the study. The study was approved by the Central Oxford Research Ethical Committee.

Chemotherapy was given on an adjuvant basis to 16 patients who had a high risk of tumor recurrence after complete surgical clearance. The remaining 18 patients who received therapy had metastatic malignant melanoma, with at least one bi-dimensionally measurable site of disease.

**Drug Administration.** Patients received DTIC (Dtic-dome; Bayer, Berkshire, United Kingdom) at 1.0 g/m² in 0.5 liter isotonic saline (0.9%) by i.v. infusion over 1 h, every 3 weeks. Nineteen patients received hydroxyurea with the second and subsequent doses of DTIC. Hydroxyurea (Hydrea; Bristol-Myers Squibb, Syracuse, NY) was administered as a 6 g i.v. bolus over 15 min followed by an i.v. infusion of 8 g in 0.5 liter isotonic saline over 4 h and 20 g in 1 liter isotonic saline infused i.v. over 20 h, to a total dose of hydroxyurea of 34 g. When used in combination, the infusion of DTIC was started 3 h after the bolus dose of hydroxyurea to coincide with expected plasma concentrations of greater than 1 μM (19). Five patients received only one dose of DTIC because of severe toxicity (one patient) or rapid tumor progression (four patients).

**AGT Assay.** Ten ml venous blood were collected into a heparinized tube just before and at the different times after the start of DTIC infusion (1, 2, 4, and 24 h). Peripheral blood lymphocytes were isolated by centrifugation of whole blood on Lymphoprep (Nycomed Pharma S, Torshov, Norway). Lymphocytes were washed twice with PBS solution [10 mM sodium phosphate (pH 6.9) and 0.15 M sodium chloride] and once with RPMI 1640 medium. Cells were resuspended in 1 ml freezing medium containing FCS (90%) and DMSO (10%) and stored at −70°C until analysis. AGT was assayed from soluble extracts of lymphocytes according to a previously described method (20) using as a substrate [3H]-methylated calf thymus DNA which has been heat treated to remove N-alkylpurines.

N7-meG is repaired at a slower rate than O6-meG with an in vivo half-life in peripheral WBCs of approximately 53 h (15). N7-meG is lost from DNA by depurination, either spontaneously or catalyzed by N-alkylpurine glycosylase to leave an apurinic site. Such sites are repaired by an excision repair process during which the damaged DNA strand is incised by an endonuclease. The DNA strand containing the damage is removed, and the opposite intact strand acts as template for DNA synthesis followed by ligation to complete the repair process (3, 16, 17).

Hydroxyurea, an inhibitor of ribonucleotide reductase, blocks semiconservative DNA synthesis by a critical reduction in the intracellular concentrations of deoxyribonucleotides. In experimental systems hydroxyurea inhibits, at sufficiently increased concentrations, the completion of DNA excision repair by blocking DNA synthesis (18). We have previously demonstrated the feasibility of effectively inhibiting DNA synthesis in tumors and bone marrow progenitor cells in vivo by the continuous i.v. infusion of hydroxyurea (19). We have also shown that the steady-state serum hydroxyurea concentrations achieved with such a regimen exceeds 1 μM, which is within the range of DNA synthesis inhibitory concentrations in vitro (19). The in vivo effect of hydroxyurea on DNA synthesis may therefore be therapeutically exploited in blocking DNA excision-repair synthesis by using a combination of hydroxyurea and DNA-damaging drugs.
**RESULTS**

**O6-meG in Blood Leukocyte DNA.** Peak O6-meG levels were seen 1 and 4 h from the start of DTIC infusion, after the first and second doses of DTIC, respectively (Fig. 1). There was an approximately 2.0- and 1.5-fold interindividual variation in the peak O6-meG levels in cycle 1 (2.25 ± 0.13 fmol/μg DNA, mean ± SE; range, 1.44–3.91) and cycle 2 (2.84 ± 0.06 fmol/μg DNA, mean ± SE; range, 2.09–3.41), respectively. Fig. 1 shows that the concentrations of O6-meG after the second dose of DTIC were significantly higher than after the first dose at all time points including 24 h after therapy (P = 0.0001). A similar increase in O6-meG concentrations was detected in all patients who received DTIC without hydroxyurea during their second cycle of therapy (data not shown) when analyzed separately.

None of the 24 patients whose WBC DNA was analyzed prior to the second dose of DTIC had measurable levels of O6-meG. In two patients for whom third cycle data were available, there was no further increase in O6-meG adduct levels after the third dose of DTIC.

**AGT Activity.** Lymphocyte AGT concentrations were determined in 21 patients prior to the first cycle of therapy. The mean concentration of AGT was 8.04 ± 0.26 fmol/μg DNA, with approximately a 2-fold variation among patients. There was a progressive reduction in lymphocyte AGT concentrations which was detectable at 1 h after starting the DTIC infusion (Fig. 2). The lowest mean AGT concentrations were observed 24 h after DTIC administration and were 56.7% (40.3–76.9%) and 55.0% (45.4–58.9%) of pretreatment levels after the first and second doses of DTIC, respectively. In 11 patients the lowest AGT values were observed at 6 h and were followed by some recovery by 24 h (data not shown). The AGT levels immediately before the second dose of DTIC (3–4 weeks after the first dose) in all 13 patients for whom second cycle measurements were available were approximately 25% lower than those prior to the first cycle (P = 0.0001; Fig. 2). AGT levels determined in two patients prior to the third course of treatment revealed little change when compared to the levels prior to the courses.
second dose of DTIC. The changes in lymphocyte AGT concentrations were not affected by the coadministration of hydroxyurea (P > 0.5).

Relationship between Lymphocyte AGT Activity and Peripheral WBC O6-meG Accumulation. The relationship between pretreatment AGT concentrations and the decrease in O6-meG levels were determined. There was a significant negative correlation between the lymphocyte AGT concentrations before the first dose of DTIC and the 24-h concentrations of O6-meG adducts (r = −0.554, P = 0.014; Fig. 3). However, the inverse relationship between the corresponding parameters for the second treatment did not reach statistical significance (r = −0.124, P = 0.7).

N7-meG Levels and Influence of Cotreatment with Hydroxyurea. Unlike O6-meG, measurable concentrations of N7-meG were present in total WBC cell DNA before starting DTIC at a mean concentration of 1.53 ± 0.28 fmol/μg DNA (range, 0.0–5.63 fmol/μg DNA). Following administration of the first dose of DTIC, the highest N7-meG levels (56.9 ± 6.56 fmol/μg DNA; range, 24.7–165.9 fmol/μg DNA) were seen 6 h after the start of DTIC infusion followed by a slow decay (Fig. 4). N7-meG levels were approximately 20-fold higher than the corresponding O6-meG levels. There was no significant difference in the N7-meG levels between the first and second cycles of DTIC (P = 0.09, pooled data). However, there was a trend toward lower N7-meG concentrations in 14 patients at all time points during the second cycle of therapy when DTIC was combined with hydroxyurea (P = 0.08; Fig. 4A).

Relationship between O6-meG and N7-meG DNA Adduct Concentrations in Peripheral WBCs. There was no significant correlation between the peak O6-meG and N7-meG levels after the first dose of DTIC in 27 patients (r = 0.241, P = 0.24; Fig. 5). The mean ratio of O6-meG:N7-meG observed 2 h after the first dose of DTIC was 0.062 (range, 0.019–0.111) and decreased to 0.04 at 24 h in accordance with the higher rate of repair of O6-meG relative to N7-meG during this time interval (Fig. 6). Mean O6-meG:N7-meG ratios were higher during the second cycle of DTIC as a result of the higher concentration of O6-meG.

Relationship between Adduct Levels and Myelotoxicity due to DTIC. Because of the confounding effects of hydroxyurea on bone marrow toxicity, the relationship between the WHO grade of myelotoxicity and O6-meG and N7-meG adduct levels was investigated after the first dose of DTIC. Severe myelotoxicity (grade 3 or 4) was infrequent in this study, and only one patient developed fever which complicated neutropenia and required parenteral antibiotic therapy. The pretreatment AGT levels and peak O6-meG and N7-meG concentrations in patients who developed myelotoxicity were close to the population mean. However, a 37-year-old female patient undergoing adjuvant therapy with DTIC experienced prolonged grade 2 neutropenia after the first cycle of DTIC with the highest peak O6-meG level (1.44 fmol/μg DNA), which was 2-fold higher than that of the population mean.

Fig. 3 The relationship between the pretreatment peripheral WBC AGT concentrations and O6-meG DNA adduct levels 24 h after the first dose of DTIC. Error bars represent standard error of the mean. Regression analysis was used to determine the relationship between the two parameters.

Fig. 4 N7-meG DNA adducts in peripheral WBCs after two i.v. doses of DTIC (1 g/m²) 3 weeks apart. A, fourteen patients who received the second dose of DTIC also received hydroxyurea (34 g i.v. over 24 h). B, four patients received single agent DTIC during their second cycle of treatment. The t test was used to determine the difference between the adducts after the first and second cycles of DTIC, respectively. Error bars represent standard error of the mean.
Tumor response to therapy was determined in 18 patients who received 1-6 cycles of chemotherapy. Objective response was assessed in two patients with hepatic metastases, giving an overall remission rate of 17%. Thirteen patients had progressive disease, and two patients had stable disease. The peak and 24-h concentrations of O6-methylguanine (O6-mG) and N7-methylguanine (N7-mG) DNA adducts were close to the population mean. Objective tumor responses were assessed after the third course of treatment.

**Relationship between Adduct Levels and Objective Response to Chemotherapy.** Patients received a median of three cycles of chemotherapy (range, 1–6). Objective response assessment was undertaken after the third course of treatment. Tumor response to therapy was determined in 18 patients who were treated for metastatic malignant melanoma. Patients had at least one measurable disease site, and standard criteria for objective response assessment were used. Only one patient achieved a complete response in metastatic lymph nodes, and two patients had a partial response in hepatic metastases, giving an overall remission rate of 17%. Thirteen patients had progressive disease, and two patients had stable disease. The peak and 24-h concentrations of O6-mG and N7-mG in patients with objective tumor responses were close to the population mean.

**HPRT Gene Mutations.** Overall, 15 patients were investigated for the occurrence of HPRT mutations after treatment with DTIC (Table 1). CEs were measured in pretreatment blood samples from 12 patients: mean CE ± SD was 14.3 ± 11.0% (range, 2.1–42.1%). When the three pretreatment samples for which mutation frequencies could not be measured are excluded, the mean CE increased to 17.2 ± 11.2% (range, 6.9–42.1%). Thirteen blood samples were obtained 24 h after the completion of the first dose of DTIC. The mean CE for these samples was 11.3 ± 10.9% (range, 0.3–32.5%). After exclusion of five samples for which mutation frequency could not be measured, a mean CE of 9.0 ± 8.2% (range, 0.3–27.0) was obtained. Overall, CEs for samples obtained 24 h after DTIC were lower than those for samples collected before therapy. CEs for samples taken before the second or later cycles of DTIC varied considerably even within the same individual, and no distinct pattern could be discerned (Table 1).

Data for mutant frequencies are less complete than those for CEs because the quality of the blood samples did not permit the selection of enough cells for the reliable detection of mutations. In six patients, it was possible to measure mutation frequencies before chemotherapy and at one or more times during chemotherapy. Nonoverlapping 95% confidence intervals of successive mutation frequencies in individual patients are usually interpreted as indicating a significant difference (27). Unfortunately, the data show considerable variations in mutation frequencies between samples collected from patients in our study with significant differences noted only in one patient (patient 8, Table 1). However, no measurement of the pretreatment mutation frequency could be obtained, and therefore it was impossible to conclude that DTIC was mutagenic in this patient. Given the results above, it was impossible to detect a definite enhancement of the mutation frequencies that could be ascribed to treatment with DTIC.

**DISCUSSION**

Patients with metastatic malignant melanoma are frequently treated with DTIC-based drug regimens. Nevertheless, objective responses to this drug when used alone have been disappointingly low (15–20%) and short-lived (28). DTIC has been used on a 3–4-week schedule in the majority of clinical studies on an empirical basis rather than well-defined biochemical end points. With the recognition of O6-methylguanine (O6-mG) and its suicidal repair in the mechanism of methylating agent, cytoxicity investigators have been particularly interested in regimens based on sequential administrations of agents that result in the depletion of cellular AGT which could theoretically enhance the sensitivity of tumor cells to subsequent therapy with methylating drugs (11).

This study investigates the formation and repair of O6-mG and N7-mG adducts and changes in the repair enzyme AGT in patients with malignant melanoma treated with moderately high doses of DTIC with or without hydroxyurea. Peripheral WBCs were used as a surrogate to monitor the biochemical effects of DTIC and the modulation of repair of DNA adducts in vivo. There is evidence to suggest correlation in DNA adduct formation between different tissues (29, 30). The relevance of studies using peripheral WBCs to study methylating drug antitumor cytoxicity depends critically on the extent to which the DNA adduct and repair activity levels in WBCs correlate with those of tumor cells.
This study shows that DTIC resulted in significant reductions of lymphocyte AGT levels which were broadly in agreement with previous reports (13, 14). Those studies have demonstrated that the decrease in lymphocyte AGT was patient, dosage, and cycle dependent. The reduction in lymphocyte AGT concentration after DTIC in our study was generally lower than that reported by Lee et al. (13, 14) for a smaller group of patients treated with lower doses of DTIC (400–800 mg/m²). The mean time to nadir AGT levels in their studies was also shorter (2–6 h) compared to our results (at least 24 h). Furthermore, in the present study, it is possible that the concentrations of AGT at 24 h did not represent true nadir levels because measurements beyond 24 h were not undertaken. The likeliest reason for the differences in the results between our study and those reported by Lee et al. (13, 14) lies in variations in the treatment protocol used, with higher doses of DTIC in our study.

The complete recovery in lymphocyte AGT concentrations after 3 weeks of DTIC in our study is in contrast to animal data4 which indicate that treatment of rats with doses of DTIC that significantly deplete lymphocyte AGT was followed by complete recovery of AGT within 72 h. Gerson (31) has shown that the rate of AGT recovery in human lymphocytes in vitro was strongly dependent on the proliferative status of these cells, which proceeded rapidly over a period of 3–4 days in the presence of phytohemagglutinin stimulation. In the absence of mitogenic stimulation, the enzyme recovery rate was significantly slower. Therefore, it is possible that prolonged inhibition of lymphocyte proliferation by DTIC resulted in a reduced rate of regeneration of cellular AGT. Alternatively, it may be that bone marrow recovery after DTIC results in a differential change in the circulating population of WBCs with the appearance of new and immature cells which are known to be relatively deficient in AGT (32). Based on observations of the two patients who were studied after the third dose of DTIC, it is possible that the reduction in steady-state AGT levels after one dose of DTIC would not be enhanced further with 3-weekly additional doses of DTIC.

Peak O₆-meG levels were seen 1 h after the first dose of DTIC. In all of our patients, peak concentrations of O₆-meG were higher, and time to peak levels were longer after the second dose of DTIC (Fig. 1). This increase in O₆-meG adduct formation is explained, at least in part, by the 25% decrease in AGT levels at the start of the second DTIC dose. Our findings are in variance to the study of Lee et al. (13) where an increase in O₆-meG was seen only in some of the patients. A modulating effect of AGT on O₆-meG accumulation was also suggested when an inverse correlation was demonstrated between the 24-h O₆-meG levels and the pretreatment AGT levels (Fig. 3). It is noticeable that over the range of AGT values observed in our study (5–11 fmol/μg DNA), the effect of AGT on O₆-meG accumulation, in different individuals, although real, is relatively small and appears to be confined to individuals lying at the extremes of the AGT range. Inspection of the data reported by Lee et al. (14) reveals a similar picture, with the effect on AGT being most pronounced in individuals with the highest and lowest enzyme levels. It is likely that the effects of interindividual variations of AGT are to some extent masked by variability in drug metabolism, since the relatively small cycle-to-

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Table 1  Frequencies of HPRT mutations in peripheral blood WBCs from patients with malignant melanoma treated with DTIC at a dose of 1 g/m² administered i.v. every 3 weeks

<table>
<thead>
<tr>
<th>Patient</th>
<th>Days after the start of DTIC</th>
<th>CE (%)</th>
<th>Mutations frequency/10⁶ cells (with 95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>22.6</td>
<td>NMa</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>21.7</td>
<td>8.8 (4.4–17.9)</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>14.4</td>
<td>13.6 (8.8–25.9)</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>20.2</td>
<td>11.9 (8.4–17.0)</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>9.8</td>
<td>22.6 (11.4–46.0)</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>6.8</td>
<td>21.6 (13.6–37.0)</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>6.9</td>
<td>11.4 (5.0–25.9)</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>2.1</td>
<td>NM</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>10.1</td>
<td>41.2 (19.3–87.9)</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>7.0</td>
<td>6.9 (2.6–18.7)</td>
</tr>
</tbody>
</table>

aNM, no measurements performed on a given sample.

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Unpublished data.
cycle variation in AGT within the same individuals had a very clear effect on O6-meG accumulation (Fig. 1).

In contrast to O6-meG, which was never detected prior to DTIC treatment, N7-meG was detected at low concentrations prior to starting therapy in most patients, probably as a result of environmental exposures to methylating chemicals (33). The N7-meG concentrations over the various time points after DTIC were comparable to those reported by van Delft et al. (15) for a smaller group of patients treated with DTIC doses ranging from 250 to 800 mg/m². There was also a longer time to peak N7-meG levels (6 h) and slower decay compared to the O6-meG, indicating a slower rate of repair of the N7-meG DNA. This also is in agreement with the report of van Delft et al. (15) that N7-meG is lost slowly from peripheral blood lymphocytes, with a half-life of approximately 53 h. Walles and Ringborg (34) showed that maximum DNA damage measured as single-strand DNA breaks occurred 5 h after 250 mg/m² DTIC, and that such damage (probably an intermediate of N7-meG repair) accumulated with repeated daily administrations of DTIC. The concentrations of N7-meG did not increase after the second dose of DTIC, which suggests that the corresponding increase observed in O6-meG was unlikely to be a result of a change in the metabolic activation of DTIC. As an S9-methylating agent, DTIC would be expected to give rise to an O6-meG:N7-meG ratio of 0.11 in the absence of any repair of O6-meG (3). The mean O6-meG:N7-meG ratio observed 2 h after the first dose of DTIC therapy was 0.062 (range, 0.019–0.111) and decreased to 0.04 at 24 h in accordance with the higher rate of repair of O6-meG in relation to N7-meG (Fig. 6). The mean O6-meG:N7-meG ratio was higher during the second cycle of DTIC as would be expected from the lower AGT and higher O6-meG levels observed during this cycle. However, it is striking that this difference decreased gradually during the posttreatment period, implying that the loss of O6-meG repair during the second treatment cycle was particularly rapid during the early time points.

In patients treated with a combination of DTIC and hydroxyurea, no effect on the formation or loss of O6-meG was seen. On the other hand, in these patients there appeared to be a tendency for lower accumulation of N7-meG, whose significance is difficult to assess in view of the small numbers of patients involved. If there is a true effect of hydroxyurea of reducing the concentrations of N7-meG, one possible mechanism would be an increase in the amount of N-alkylpurine glycosylase by blocking the cycling cells at the G1-S interphase and resulting in higher activities of glycosylases (35, 36).

There are no published studies on the mutagenic effects of DTIC in humans but there are reports indicating mutagenicity in mouse lymphoma cells in vitro and in Drosophila melanogaster (37). The results of the present study of the genotoxic potential of DTIC are inconclusive because the low CEs of most of the blood samples obtained from patients did not allow us to measure the mutant frequencies with a significant degree of confidence. The absence of significant changes in mutant frequencies in this study may be due to the generally poor quality of the blood samples. On the other hand, DTIC may be truly nonmutagenic at the dose and schedule used in this study. It is also possible that mutated lymphocytes were killed by the treatment itself. One other possibility is that DTIC-induced mutations require a longer time to be expressed in the peripheral lymphocytes. The latter possibility is supported by recent work which indicated that induction of HPRT mutations after cyclophosphamide or ifosfamide treatment could only be detected approximately 4 months after the onset of chemotherapy (26). Most of our patients were studied over a period of 1–3 months, and additional studies are therefore required over longer periods of time. In three patients, where samples were obtained at 6, 8, and 10 months, we did not detect an increase in the mutant frequencies. These results support the use of DTIC in other tumor types, such as Hodgkin’s disease, with the aim of reducing risk of secondary leukemia.

Analysis of results from this study revealed no significant correlation between either O6-meG or N7-meG adduct levels and neutropenia due to DTIC. Lack of correlation may have resulted from other factors contributing to myelosuppression induced by DTIC. It is noteworthy that one patient with prolonged grade 2 neutropenia and thrombocytopenia after one dose of DTIC had peak O6-meG levels twice the population mean. This observation would suggest a possible contribution of high levels of O6-meG adducts to DTIC-induced myelotoxicity.

Based on the results of this study, we can conclude that it may be possible to schedule the dosing of DTIC so as to achieve maximum reduction in AGT and formation of O6-meG in an effort to optimize its efficacy. One approach might be to administer DTIC at lower doses using shorter intervals.

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310 Dacarbazine-induced DNA Damage in Peripheral WBCs


Methyl DNA adducts, DNA repair, and hypoxanthine-guanine phosphoribosyl transferase mutations in peripheral white blood cells from patients with malignant melanoma treated with dacarbazine and hydroxyurea.

P A Philip, V L Souliotis, A L Harris, et al.


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