Comparative study of the formation and repair of $O^6$-methylguanine in humans and rodents treated with dacarbazine

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The mutagenic, carcinogenic and cytotoxic activity of dacarbazine, a drug employed in cancer chemotherapy, may be related to the induction in DNA of $O^6$-methylguanine ($O^6$-meG), a quantitatively minor but biologically important lesion. In the present study the kinetics of $O^6$-meG formation and repair in blood leukocyte DNA were examined in 20 Hodgkin’s lymphoma patients treated i.v. with 180 ± 13 (mean ± SD) mg/m² dacarbazine and compared with those observed in various tissues of rodents treated with different doses of the drug. In Hodgkin’s lymphoma patients adduct levels reached a value of 0.27 ± 0.14 fmol/µg DNA 2 h after dacarbazine administration, while the rate of subsequent loss suggested an adduct half-life of ~30 h. Measurement of adduct levels in the same individuals after successive courses of treatment spaced 3 weeks apart (up to 10 treatment courses) demonstrated a consistent individual response and statistical analysis of variance confirmed that intra-individual variation in adduct accumulation after a given dose of dacarbazine accounted for only 5% of the total variance observed. In contrast, inter-individual variation accounted for 70% of the observed variance, with adduct levels 2 h after drug treatment varying ~7.5-fold among adduct-positive individuals. No significant depletion of lymphocyte $O^6$-alkylguanine-DNA alkyltransferase (AGT) occurred after patient treatment with dacarbazine. No significant relationship between adduct levels and clinical response to treatment was observed. In rats treated with single or multiple doses of dacarbazine causing varying degrees of AGT depletion the highest levels of $O^6$-meG were seen in the liver, followed by the lymph nodes, bone marrow and blood leukocytes, which showed up to ~2-fold lower levels. A similar tissue distribution was also observed in mice and in a single rabbit. These observations suggest that $O^6$-meG levels assayed in blood leukocytes of therapeutically treated humans reflect those present in the lymph nodes (target tissue for chemotherapy) and the bone marrow (target tissue for leukemogenesis) and may be utilized as a measure of the drug dose reaching these tissues. The quantitative data reported in this study show that under conditions of no depletion of AGT $O^6$-meG accumulates in blood leukocyte DNA of humans at a rate similar to that observed in rats, suggesting that human susceptibility to any $O^6$-meG-mediated genotoxic effects of dacarbazine may be comparable with that of the rat.

Introduction

Dacarbazine [5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide] is an alkylating cytostatic drug used in the treatment of various types of human cancer, including malignant melanoma, Hodgkin’s lymphoma, soft tissue sarcoma, osteogenic sarcoma and neuroblastoma. High dose dacarbazine (up to 1 g/m², usually administered as a single agent) is the chemotherapy of choice in malignant melanoma, with a response rate of ~15–20% (1,2). In the case of Hodgkin’s lymphoma chemotherapy with dacarbazine (usually at single doses of 150–200 mg/m² per treatment cycle in combination with other drugs, e.g. Adriamycin, bleomycin and vincristine as in the widely used ABVD protocol) is sometimes preferred instead of the more commonly used procarbazine-based MOPP protocol, because of its lower reproductive toxicity (3). Although dacarbazine-based chemotherapy also appears to be less leukemogenic than MOPP (4), it is not completely devoid of risk of induction of leukemogenic activity (mainly acute myeloid leukemia) in long-term survivors and a weak correlation of such activity with the dose of dacarbazine received has been reported (5). However, the currently available evidence does not permit the confident assessment of the contribution (if any) of dacarbazine to the carcinogenic activity of such mixed chemotherapy protocols.

Dacarbazine is cytotoxic and mutagenic in various in vitro assays (6–8), while chronic bioassay studies have shown that it has considerable carcinogenic potency in laboratory animals (9–11). For example, following oral or i.p. administration to rats dacarbazine produced tumors of the breast, thymus, spleen, brain and mammary gland (9), while in mice it produced tumors of the lung, hematopoietic tissues and uterus (10,11). Dacarbazine is converted through a series of changes initiated by liver enzymatic $\alpha$-hydroxylation of one of its methyl groups into a methylating agent (probably the methylidiazonium ion), which methylates DNA to produce, among other lesions, the biologically important adduct $O^6$-methylguanine ($O^6$-meG*) (12). The formation of this adduct in the DNA of experimental animals treated with dacarbazine has been known for a long time (13,14), while its formation in blood leukocyte DNA of patients with Hodgkin’s disease or malignant melanoma treated with dacarbazine has been demonstrated recently (15–17). $O^6$-Methylguanine plays an important role in mutagenesis, carcinogenesis and cytotoxicity induced by methylating agents. It is a directly miscoding lesion whose presence in DNA during cell replication directs incorporation of thymine, thus resulting in the generation of G:C→A:T transition mutations (18–20). Evidence for an in vivo role of $O^6$-meG in the genotoxicity of methylating agents has recently come from studies in transgenic mice containing the lacZ or lacl genes as targets for mutagenesis, which have shown that methylating...
agents such as N-nitrosodimethylamine or N-methyl-N-nitrosourea induce predominantly G:C→A:T transitions after acute treatment (21). The presence of O^6^-meG in particular types of tissues or cells during experimental carcinogenesis frequently correlates with the appearance of cancer (22,23). Direct support for a role of this adduct in the initiation of cancer was recently obtained using transgenic mice in which expression of the human repair enzyme for O^6^-meG, O^6^-alkylguanine-DNA alkyltransferase (AGT), prevented induction of cancer or precancerous lesions by methylating carcinogens in the thymus, liver or colon (24–26). Important evidence for a role of O^6^-meG in cell killing comes from experiments showing that expression of AGT in mammalian cells protects them against the cytotoxic effects of alkylating agents, including dacarbazine (27–30). The cytotoxic effect of O^6^-meG is thought to be indirect and mediated by its inappropriate processing by DNA mismatch repair (31).

In view of the evidence that O^6^-meG may contribute to the mutagenic and carcinogenic activity of dacarbazine, as well as to the mechanism by which this drug brings about its therapeutic effects, it seems useful to evaluate the accumulation of this adduct in humans during therapeutic exposure and in animals treated with dacarbazine at doses comparable with those employed in human chemotherapy. Such data may permit a better understanding of the contribution of O^6^-meG in determining the overall therapeutic efficacy, as well as the chronic toxicity of dacarbazine chemotherapy, and, by permitting better dose-to-dose and surrogate tissue-to-target tissue extrapolations, facilitate the design of improved therapeutic protocols. Furthermore, animal-to-human comparisons of the accumulation of this precarcinogenic DNA adduct may assist in attempts at the assessment of the leukemogenic risk associated with dacarbazine treatment. In this paper, adduct data obtained from Hodgkin’s lymphoma patients and experimental animals treated with dacarbazine are presented and quantitative and qualitative comparisons between them are discussed.

Materials and methods

Dacarbazine employed in animal experiments was obtained from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program (National Cancer Institute, Bethesda, MD). All other reagents were research grade materials obtained from Sigma (St Louis, MO).

Animal experiments

Female Sprague–Dawley rats (150 g), male BALB/c mice (20 g) and a single female New Zealand rabbit (2 kg) were maintained on normal laboratory chow. For each dose or time point, pairs of rats or mice were treated i.p. with a solution of dacarbazine, freshly dissolved in MAC buffer (5 mg/ml mannitol, 10 mg/ml citric acid) and protected from light. Controls received MAC buffer. Animals were killed by cervical dislocation at appropriate times after treatment. In multiple treatment experiments, rats were dosed once a day for 10 days and killed 24 h after the last treatment. A single rabbit was treated with dacarbazine and blood was collected from an ear vein at various time points. The same animal was similarly treated again 2 months later, on which occasion the animal was killed 24 h after treatment and internal tissues collected. In all cases, collected tissues (blood, liver, neck lymph nodes and femur bone marrow) were immediately placed in liquid nitrogen. For AGT measurements, lymphocytes were isolated from fresh blood by centrifugation on Lymphoprep gradients (Nycomed AS, Oslo, Norway) prior to freezing. All tissues were stored at −70°C until analysis, which was carried out within 2–3 weeks of collection.

Human data

Blood samples were collected from 20 patients on ABVD treatment for Hodgkin’s disease stage I or II as described previously (15). Each treatment cycle involved a single i.v. injection of 300 mg dacarbazine (corresponding to ~180 mg/m^2), followed 2 h later by i.v. injection of bleomycin, vincristine and Adriamycin (ABVD protocol). This treatment was repeated every 15 days.

Fig. 1. Formation and loss of O^6^-meG in peripheral blood leukocyte DNA of Hodgkin’s lymphoma patients treated with dacarbazine (180 mg/m^2).
(A) Adduct kinetics in five patients during consecutive courses of treatment. (B) Mean adduct levels in 20 patients (error bars, SD). The dotted line indicates the limit of adduct detection.

for up to 10 cycles. Samples of venous blood were collected just before and 2, 24 and 48 h after dacarbazine treatment. In most cases, two 4 ml samples of blood were drawn into a heparinized syringe. One was immediately stored at −20°C and transferred to a −70°C freezer on the same day. This sample was used for extraction of DNA. The second blood sample collected was stored at 4°C for not more than 2 h and subsequently used for isolation of lymphocytes and determination of AGT.

Measurement of O^6^-meG and AGT

DNA was extracted from tissues by a previously described method involving treatment with proteinase K and RNase A and multiple phenol/chloroform extractions (32). Measurement of O^6^-meG in DNA of individual animals was carried out by the competitive repair assay according to Souliotis et al. (33), using partially purified E. coli AGT (ada protein). The limit of detection by this assay, using 10 μg DNA per assay, was 0.05 fmol/μg DNA. Provided sufficient DNA was available, each sample was analyzed at least twice. AGT was determined in tissue extracts using as substrate 3H-methylated calf thymus DNA (32).

Results

O^6^-meG and AGT in blood leukocytes of Hodgkin’s lymphoma patients treated with dacarbazine

Twenty Hodgkin’s lymphoma patients received dacarbazine (180 ± 13 mg/m^2, mean ± SD) in the context of one or more courses of ABVD chemotherapy. Detectable levels of O^6^-meG were found in blood leucocyte DNA at some time point after all but four instances of dacarbazine treatment, two of which concerned successive treatment courses of the same patient. The kinetics of O^6^-meG formation and repair in five patients treated for up to four successive courses are shown in Figure 1A. In agreement with our earlier observation (15), highest adduct levels were observed in samples collected 2 h post-treatment, while adduct levels 24 and 48 h post-treatment were...
always lower (sometimes below the limit of detection). As shown in Figure 1B, mean adduct levels 2 h post-dacarbazine during the first course of treatment were $0.27 \pm 0.14$ fmoi/µg DNA (0.43 ± 0.21 µmol/molG) and decreased by 40% during the following 22 h. The adduct-forming response of different patients was relatively consistent from course to course (Figure 1A), e.g. patients 1 and 2 consistently showing relatively high or low adduct formation, respectively, during three successive treatment cycles. On the other hand, there was significant inter-individual variability, with adduct levels 2 h post-treatment covering an ~7.5-fold range (excluding the few samples with non-detectable adducts). Statistical analysis indicated that inter-individual variation accounted for 70% of the total variance, while only 5% was accounted for by intra-individual variation. No significant AGT depletion was caused by the treatment (results not shown). Furthermore, no statistically significant correlation between pre-treatment lymphocyte AGT and $O^\beta$-meG formation could be demonstrated.

**Relationship between $O^\beta$-meG accumulation and clinical response**

Figure 2 shows the relationship between the clinical response of different patients and $O^\beta$-meG accumulation after the first course (A) or after completion of treatment (B). As can be seen in Figure 2A, two patients showed complete or partial remission after the first course of treatment, even though no adducts could be detected in their blood leukocyte DNA. One (but not the other) of these patients had significant adduct levels after the second course of treatment (the only additional course for which adduct data are available) and both showed complete remission at the end of the full treatment. Figure 2A also shows that four patients who did not respond to the first treatment course had substantial adduct levels. All four of these patients showed complete remission by the end of the full treatment and, with the exception of one of them after a single treatment course, they all showed significant adduct formation after all subsequent treatment courses for which data are available. Thus no correlation between adduct formation and clinical response after the first course of treatment was observed. A similar lack of correlation was observed when clinical response to the full treatment was compared with mean adduct levels after individual treatment courses (Figure 2B) (data from 2–10 courses were available for all but four patients): two of three patients with overall partial remission or recurrence showed substantial adduct levels after 2–4 treatment courses, while a third patient had no detectable adducts following the first course of treatment (the only course for which data were available). On the other hand, one patient with no detectable adducts after the first two treatment courses showed full remission upon completion of treatment.

Figure 3 shows $O^\beta$-meG levels in various tissues and changes in liver AGT of rats (A) and mice (B) after single treatment with dacarbazine (118 mg/m²). (C) $O^\beta$-MeG levels in blood leukocytes and changes in blood lymphocyte AGT in a rabbit treated with dacarbazine (118 mg/m²) [error bars, SD of measurements from two animals (rats) or multiple measurements from one animal (rabbit)].
Table I. AGT levels (mean ± SD) in tissues of the rat, mouse and rabbit prior to treatment with dacarbazine

<table>
<thead>
<tr>
<th>Species/tissue</th>
<th>Rat fmol/μg DNA</th>
<th>Rat fmol/mg protein</th>
<th>Mouse fmol/μg DNA</th>
<th>Mouse fmol/mg protein</th>
<th>Rabbit fmol/μg DNA</th>
<th>Rabbit fmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>10.1±0.3</td>
<td>59.7±1.3</td>
<td>4.1±0.4</td>
<td>54.8±1.4</td>
<td>31.3±2.1</td>
<td>271.0±9.4</td>
</tr>
<tr>
<td>Blood lymphocytes</td>
<td>3.2±0.5</td>
<td>39.6±4.0</td>
<td>2.6±0.2</td>
<td>44.6±2.1</td>
<td>5.3±0.6</td>
<td>196.8±6.6</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>2.2±0.4</td>
<td>33.7±3.8</td>
<td>1.9±0.3</td>
<td>29.8±0.7</td>
<td>5.3±0.4</td>
<td>58.4±2.0</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>1.3±0.4</td>
<td>55.6±2.1</td>
<td>0.9±0.1</td>
<td>61.9±2.7</td>
<td>2.6±0.2</td>
<td>90.9±3.6</td>
</tr>
</tbody>
</table>

Table II. Tissue distribution of $\O^6$-meG and changes in lymphocyte AGT in a single rabbit after a dose of 7.9 mg/kg (118 mg/m$^2$) of dacarbazine (mean ± SD of multiple determinations)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Liver fmol/μg DNA</th>
<th>Blood leukocytes fmol/μg DNA</th>
<th>Lymph nodes fmol/μg DNA</th>
<th>Bone marrow fmol/μg DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.09±0.10</td>
<td>0.60±0.10</td>
<td>0.89±0.17</td>
<td>0.53±0.05</td>
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<tr>
<td>0.5</td>
<td>0.54±0.04</td>
<td>0.93±0.16</td>
<td>1.35±0.18</td>
<td>1.23±0.10</td>
</tr>
<tr>
<td>1</td>
<td>0.54±0.04</td>
<td>0.93±0.16</td>
<td>1.35±0.18</td>
<td>1.23±0.10</td>
</tr>
<tr>
<td>2</td>
<td>1.09±0.10</td>
<td>0.60±0.10</td>
<td>0.89±0.17</td>
<td>0.53±0.05</td>
</tr>
<tr>
<td>4</td>
<td>1.23±0.10</td>
<td>1.23±0.10</td>
<td>1.23±0.10</td>
<td>1.23±0.10</td>
</tr>
<tr>
<td>24</td>
<td>196.8±9.2</td>
<td>167.7±12.5</td>
<td>152.6±9.4</td>
<td>140.4±10.2</td>
</tr>
<tr>
<td>48</td>
<td>142.1±13.9</td>
<td>142.1±13.9</td>
<td>142.1±13.9</td>
<td>142.1±13.9</td>
</tr>
</tbody>
</table>

$\O^6$-meG and AGT in experimental animals after single, low doses of dacarbazine

As already indicated, no significant depletion of blood lymphocyte AGT was observed in Hodgkin’s lymphoma patients examined in this study. In order to obtain a better understanding of the kinetics of formation and repair and the tissue distribution of $\O^6$-meG under corresponding conditions of full AGT activity adduct formation was examined in three species (rats, mice and rabbits) after low dose treatment with dacarbazine.

DNA adduct levels induced in different tissues of rats by treatment with a single i.p. dose of dacarbazine (20 mg/kg, 118 mg/m$^2$) covered a 2-fold range, with the liver showing highest adduct levels, followed by the lymph nodes, while the bone marrow and blood leukocytes had lower, similar levels (Figure 3A). Maximum adduct formation was observed 2 h after drug treatment in all tissues and thereafter $\O^6$-meG decreased significantly, with ~40–60% (depending on the tissue) being lost between 2 and 24 h. During the 48 h following dacarbazine administration liver AGT was almost unchanged, showing a <10% decrease (Figure 3B). Although AGT changes were not measured in the other three tissues, comparison of the pre-treatment AGT levels (Table I) with the adduct levels observed suggests that any changes in these tissues are likely to have been small.

Pairs of mice and a single rabbit were also treated with single doses of dacarbazine (36.9 mg/kg and 7.9 mg/kg respectively), selected so as to be equivalent on a body surface area basis to that given to rats (118 mg/m$^2$) (34). Peak $\O^6$-meG levels were seen 1 h after dacarbazine treatment in mice (all tissues) (Figure 3B) and 2 h in rabbit leukocytes (Figure 3C). The tissue distribution of $\O^6$-meG in mice (Figure 3B) and the rabbit (only at 24 h) (Table II) was similar to that observed in the rat, with adduct levels in blood leukocytes again being similar to those in the bone marrow. On the other hand, the range of adduct levels observed in the mouse was slightly wider than that seen in the rat, probably reflecting the greater depletion of liver AGT (nearly 90% during the 48 h following dacarbazine administration) (Figure 3B). A more limited AGT decrease (~35% during the 48 h following treatment) was seen in rabbit blood lymphocytes (Figure 3C). Comparison of the observed adduct levels with the pre-treatment levels of AGT in the other tissues examined (Table I) suggests that varying degrees of AGT depletion (limited in the rabbit but more extensive in the mouse) probably occurred after this treatment with dacarbazine.

In order to examine the relationship between dacarbazine dose and $\O^6$-meG formation various doses of the drug (10–40 mg/kg, corresponding to 59–236 mg/m$^2$), selected so as to span those received by Hodgkin’s disease patients, were administered to rats. The data presented in Figure 4 show that...
in all tissues examined $O^6$-meG levels were linearly related to the dose, suggesting that any changes in AGT did not grossly affect $O^6$-meG accumulation.

$O^6$-meG and AGT in rats after single, high doses of dacarbazine

Single agent, high dose treatment with dacarbazine (up to 1000 mg/m$^2$), repeated over a number of courses spaced ~3 weeks apart, is the major mode of chemotherapy of malignant melanoma (1,2). Such treatment has been shown to result in extensive depletion of AGT in blood lymphocytes (and probably in other tissues) (17,35,37). In order to study $O^6$-meG and AGT changes in rat tissues during comparable treatment animals were administered dacarbazine at a high dose (350 mg/kg, 2065 mg/m$^2$), selected so as to cause significant depletion of AGT. The tissue distribution of adducts (Figure 5A) was qualitatively similar to that seen after low dose treatment and covered an ~3-fold range. Peak adduct levels were seen 6 h after drug treatment, while the nadir of AGT occurred 24 h after dacarbazine administration (Figure 5B). Thereafter AGT recovered rapidly and reached pre-treatment levels within 72 h. When the same treatment was administered to animals on a further two occasions spaced 3 weeks apart the kinetics of $O^6$-meG and AGT changes observed on each occasion were similar to those observed following the first treatment (data not shown).

$O^6$-meG and AGT in rats after multiple doses of dacarbazine

Cancer patients are sometimes treated with chemotherapy protocols involving the use of multiple doses of dacarbazine (100–250 mg/m$^2$ each) daily for up to 10 days. Data on the accumulation of methylation adducts (including $N7$- and $O^6$-meG) in blood leukocyte DNA during such treatment have recently become available (van Delft, Souliotis and Kyrtopoulos, unpublished results). In order to obtain an indication of the relative methylating activity of dacarbazine in humans and rats during such treatment, the drug was administered to rats for 10 days at a dose rate of 20 mg/kg/ day (118 mg/m$^2$/day). As can be seen in Figure 6A, $O^6$-meG increased smoothly in all tissues examined, in the lymph nodes and bone marrow appearing to approach a plateau between days 8 and 10. The tissue distribution of DNA methylation was similar to that observed after single dose treatments (compare Figure 6A with Figures 3 and 5). As shown in Figure 6B, a steady depletion of AGT occurred in all tissues analyzed, minimum AGT levels (as low as 30–35% of pre-treatment levels) being observed after administration of the last dose of dacarbazine. Thereafter AGT recovered completely in all tissues within 4–6 days.

Discussion

The importance of dacarbazine in the chemotherapy of a number of common forms of human cancer makes it highly desirable to seek ways to achieve chemotherapeutic schemes which optimize its cytotoxic effectiveness while minimizing its chronic toxicity. One approach towards this end is based on the measurement in treated patients of the accumulation of drug-induced DNA adducts. Of the DNA adducts known to
be induced by dacarbazine, 8-meG is of particular interest because it is believed to contribute significantly to the genotoxic as well as the cytotoxic (hence chemotherapeutic) properties of the drug. Therefore, despite the fact that 8-meG is shorter lived than other methylated DNA adducts, measurement of the concentration in treated patients of this biologically relevant adduct may be important, because it provides a measure of the biologically effective drug dose received at a critical cellular site (DNA).

In this study we have presented extensive data on the kinetics of formation and repair of 8-meG in blood leucocyte DNA of Hodgkin’s lymphoma patients treated with dacarbazine. Ideally it would be desirable to have measurements of dacarbazine-induced lesions in tumor tissue as well as in normal tissues which may form targets for the drug’s toxic effects. However, the possibility of such measurements is limited by difficulties in obtaining sufficient amounts of tumor tissue and by ethical considerations. In order to overcome these limitations we have supplemented our measurements in a human surrogate tissue (blood leukocytes) with a detailed study of adduct accumulation and repair in different tissues of experimental animals treated with dacarbazine at doses approaching those to which humans are therapeutically exposed.

In a previous limited study with 11 Hodgkin’s lymphoma patients (15) we reported the detection of 8-meG in almost every individual treated with ~180 mg/m^2 dacarbazine in the context of ABVD chemotherapy. That pilot study was significantly extended in the present report, through the examination of 20 patients, sampled usually 0, 2, 24 and 48 h after treatment for up to 10 cycles (a total of 261 samples). No 8-meG could be detected prior to dacarbazine treatment, even in patients starting a second or subsequent course of treatment. On the other hand, this adduct was detected in almost all samples collected 2–24 h after treatment. Maximum levels of 8-meG were observed in samples collected 2 h post-dacarbazine. Although the frequency of sampling was not sufficient to determine the exact time of maximal adduct formation and, hence, its rate of subsequent loss, the observed changes in adduct levels suggest that the half-life of 8-meG in AGT-proficient human lymphocytes is <30 h. This agrees well with the rate of loss of the same adduct from human blood leucocyte DNA observed after exposure to procarcabazine under conditions which also did not cause AGT depletion (37). Intra-individual variability between different treatment courses was limited, accounting for only 5% of the total adduct variance, in contrast to inter-individual variability, which accounted for 70% of the observed variance. Analogous inter- and intra-individual variabilities have been observed for 8-meG induced in Hodgkin’s lymphoma patients by procarcabazine (37) as well as by high doses of dacarbazine in melanoma patients (17).

No significant AGT depletion in human blood lymphocytes was induced by dacarbazine treatment, probably due to the large excess of AGT present in most individuals examined (usually 5–15 fmol/μg DNA) relative to the observed adduct levels (usually <0.5 fmol/μg DNA). Significant depletion of lymphocyte AGT was recently reported by Lee et al. (35,36) and Philip et al. (17) in melanoma patients treated with doses of dacarbazine ~6-fold higher than the mean dose used in the current study (up to 1000 mg/m^2). Although a protective effect of AGT against 8-meG formation has been reported in patients treated with procarcabazine (38) or high doses of dacarbazine (16,17), in the current study no correlation of 8-meG accumulation with pre-treatment lymphocyte AGT was found. It is possible that any such relationship was concealed by individual differences in dacarbazine metabolism. It is also possible, in principle, that the comparison between adduct accumulation and AGT levels may have been complicated by the fact that, for practical reasons, adducts were measured in total blood leukocytes and AGT in blood lymphocytes (which constitute only ~30% of the total blood leucocyte count). We consider that such a complication is unlikely because: (i) 5-(3-hydroxymethyl-3-methyl)imidazole-4-carboxamide, the metabolite of dacarbazine believed to spontaneously break down to the ultimate methylating agent (39), is of moderate chemical stability and thus likely to reach and alkylate different types of blood cells to the same extent; (ii) the various sub-categories of human blood leukocytes appear to have broadly similar AGT contents (40; P. Georgiadis and S.A. Kyrtopoulos, unpublished observations), suggesting similar ability to repair 8-meG under AGT-proficient conditions. Therefore, it seems more likely that the absence of a significant relationship between adduct and AGT levels may reflect other complicating factors, such as individual pharmacokinetic or metabolic variability.

The accumulation of 8-meG and changes in AGT were systematically examined in three species of experimental animals (rats, mice and a single rabbit) after administration of single or multiple doses of dacarbazine spanning the range of exposures suffered by patients undergoing dacarbazine chemotherapy. In the rat no significant depletion of liver AGT was observed after a low dose of dacarbazine (118 mg/m^2), while depletion in the other tissues examined is believed to have been limited at this dose. On the other hand, treatment with a single high dose (2065 mg/m^2) and multiple treatments (118 mg/m^2 daily for 10 days) induced significant AGT depletion in all tissues analyzed. A similar tissue distribution of 8-meG was found in all species examined regardless of the extent of AGT depletion induced, with the liver (main site of metabolism) showing highest adduct accumulation, followed by the lymph nodes and then by the bone marrow and blood leukocytes, which showed lower broadly similar levels (Figures 3–6). Maximum 8-meG levels occurred later (6 h) following administration to rats of a high dose of dacarbazine (2065 mg/m^2) while after low dose exposure (118 mg/m^2, 2 h), probably due to more prolonged drug metabolism after high dose treatment. 8-Methylguanine was lost from the tissues examined with half-lives in the range 20–40 h (depending on the tissue and the dose employed), such loss being fastest in the liver (tissue with the highest AGT content) and slowest in the bone marrow (tissue with the lowest AGT content).

Following its depletion in various rat tissues after administration of single or multiple doses of dacarbazine AGT recovered fully within 4–6 days of cessation of exposure. Valavanis et al. (32) studied AGT depletion and recovery after treatment of rats with another methylating cytostatic drug, procarcabazine, and found that this agent induced a prolonged decline of AGT followed by very slow recovery (~20-fold slower than that induced by N-methyl-N-nitrosourea), possibly due to a slow rate of de novo biosynthesis of the enzyme. Examination of the data in Figures 3, 5 and 6 (including analysis by mathematical modeling; our unpublished results) shows that AGT recovery after dacarbazine treatment is slightly (~1.5-fold) slower than after N-methyl-N-nitrosourea, suggesting that dacarbazine influences de novo biosynthesis of this repair enzyme in the
rat much less than procarbazine. On the other hand, analogous examination of the changes in O^*^-meG and AGT in mouse liver indicates that the rate of decrease of AGT during the period 4–48 h post-dacarbazine treatment significantly exceeds the rate of loss of O^*^-meG (Figure 3B), suggesting that dacarbazine may cause AGT depletion in the mouse (as well as in the rat) by a mechanism additional to suicide repair of O^*^-meG.

The availability of detailed data on O^*^-meG and AGT levels after dacarbazine treatment in tissues of patients receiving chemotherapy and of laboratory animals treated by comparable protocols permits the examination of some of the factors affecting adduct accumulation and allows tissue-to-tissue and species-to-species extrapolations. Figure 7 shows the levels of O^*^-meG in blood leukocytes of humans and rats 24 h after single treatments with dacarbazine at different doses, plotted against the dose expressed in mg/m^2_. The human data at 225 and 800 mg/m^2 come from the study of van Delft, Soulisiotis and Kyrtopoulos (unpublished results) and at 1000 mg/m^2 from that of Philip et al. (17). It can be seen that O^*^-meG levels in blood leukocyte DNA of rats 24 h after single doses of dacarbazine were linearly related to the dose in the range 59–236 mg/m^2, a range in which AGT depletion was likely to have been limited. A dose-related increase in O^*^-meG is also observed in blood leukocyte DNA of humans treated with 180–1000 mg/m^2 dacarbazine. Comparison of the dose–response curves for rats and humans indicates that under conditions of full AGT activity (i.e., after low dacarbazine doses) O^*^-meG accumulates at a similar rate in blood leukocyte DNA of the two species. The similarity of adduct accumulation observed in the remaining animal tissues examined (including the lymph nodes and the bone marrow) to those in blood leukocytes implies that a similar relative species susceptibility to O^*^-meG accumulation may also apply to those tissues. It therefore seems likely that human susceptibility to any O^*^-meG-mediated genotoxic effects of dacarbazine is comparable with that of the rat. We note that a closely similar relative species susceptibility was also observed for O^*^-meG formation induced by procarbazine (37).

One of the purposes of this study was to assess the relevance of measurements of O^*^-meG in blood leukocyte DNA of patients therapeutically treated with dacarbazine to toxic events occurring at less readily accessible target tissues and their relationship to clinical response. The data presented here, obtained from three different rodent species and under a wide range of AGT activities, suggest that blood leukocytes are a good surrogate tissue for the assessment of O^*^-meG accumulation in important target tissues of dacarbazine-treated patients, such as the bone marrow and the lymph nodes. On the other hand, no correlation between the formation of this adduct in blood leukocytes and therapeutic response could be established in this limited cohort. One important complicating factor of the present study that should be noted relates to the fact that dacarbazine was administered to Hodgkin’s lymphoma patients in the context of a multi-drug protocol which also included adriamycin, bleomycin and vincristine. It is therefore possible that any variation in the clinical effectiveness of dacarbazine related to the extent of DNA damage could have been masked by the effects of the other drugs. On the other hand, similar observations of no (or only marginal) correlation between methylated DNA adduct formation and clinical response were also reported in recent studies utilizing limited numbers of melanoma patients receiving single drug dacarbazine treatment (17,41). In contrast, larger scale studies of ovarian cancer patients receiving single drug cis-platin treatment (42,43) have demonstrated a correlation between drug-induced DNA damage and therapeutic response. It remains to be seen whether further investigation of the relationship between O^*^-meG accumulation and clinical response to dacarbazine, especially in single agent treatment protocols, if based on sufficiently large numbers of patients, may provide information useful for the design of clinical protocols of improved efficacy and safety.

Acknowledgements

The expert technical support of Stella Kaila and Margarita Bekyrou is acknowledged. The work was financially supported by grants from the European Union to SAK under contracts nos EV5V-CT91-0012 and EV5V-CT91-0013.

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