

Accumulation of O⁶-Methylguanine in Human DNA after Therapeutic Exposure to Methylating Agents and Its Relationship with Biological Effects

by Soterios A. Kyrtopoulos,^{1,3} Vassilis L. Souliotis,¹
Christos Valavanis,¹ Viki A. Boussiotis,² and
Gerasimos A. Pangalis²

O⁶-Methylguanine has been measured in peripheral blood leukocytes of 14 patients during one or more cycles of treatment with procarbazine (daily treatment for 10 days) and in 12 patients during one or more cycles of treatment with dacarbazine (single dose per cycle). Adduct formation at levels up to about 0.4 fmole/ μ g DNA was detected in all procarbazine- and all but one dacarbazine-treated patients at some point after treatment. O⁶-Methylguanine accumulated during procarbazine treatment in a dose-related manner (mean rate of accumulation 2.8×10^{-4} fmole/ μ g DNA per mg/m² dose) and appeared to approach a plateau by the end of the cycle (above 600 mg/m² cumulative dose). The average rate of O⁶-methylguanine formation 2 hr after dacarbazine treatment was $11 \pm 8 \times 10^{-4}$ fmole/ μ g DNA per mg/m² dose. Individuals examined on more than one treatment cycle with either drug showed broadly similar methylation responses. The rate of adduct accumulation showed a nonsignificant, negative correlation with the pretreatment lymphocyte levels of the repair enzyme O⁶-alkylguanine-DNA alkyltransferase (AGT) in the case of procarbazine and no correlation in the case of dacarbazine. No consistent lymphocyte AGT depletion was noted as a result of treatment with either drug.

No correlation between O⁶-methylguanine formation and hematological toxicity was observed. In eight patients showing full remission after treatment with dacarbazine, the value of O⁶-methylguanine (averaged over all the cycles) was 0.252 ± 0.120 fmole/ μ g DNA while in four patients showing partial or no response it was 0.087 ± 0.110 fmole/ μ g DNA ($p < 0.05$). One of the two nonresponding procarbazine-treated patients showed very low levels of O⁶-methylguanine during a cycle of observation of DNA adduct formation.

Human blood leukocytes are about 5-fold less susceptible than those of the rat to accumulation of O⁶-methylguanine during exposure to procarbazine. Furthermore, similar adduct levels were found in rat peripheral blood leukocytes and lymphocytes, bone marrow, and lymph nodes, suggesting that levels observed in human blood leukocytes may reflect those in the presumed target tissues for the leukemogenic (bone marrow) and the therapeutic (lymph nodes) effects of procarbazine. The possible use of these observations in the assessment of carcinogenic risks to humans exposed to procarbazine or environmental methylating agents such as dimethylnitrosamine is discussed.

Introduction

The measurement of DNA adducts formed in patients treated with alkylating drugs is of interest because such adducts may indicate the biological dose received at the level of the individual patient, which may be correlated with therapeutic response or long-term complications (mainly therapy-induced carcino-

genesis). During the past 2 years we have focused our attention on two methylating agents used in the chemotherapy of Hodgkin's lymphoma, procarbazine (PCZ) [N-isopropyl- α -(2-methylhydrazino)-*p*-toluamide] and dacarbazine (DCZ) [5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide] (1,2). Both drugs are converted by metabolism to S_N1-type methylating intermediates and give rise to a range of DNA adducts, including O⁶-methylguanine (O⁶-MeG) (3,4), an adduct believed to play an important role in carcinogenesis, mutagenesis, and cytotoxicity (5-7). One of the most widely used therapeutic protocols for Hodgkin's lymphoma, the MOPP protocol (using PCZ in combination with nitrogen mustard, vincristine, and prednisone), has been shown to be associated with an increased risk of acute nonlymphocytic leukemia in treated individuals, giving rise to a cumulative risk of more than 10%

¹Programme of Chemical Carcinogenesis, National Hellenic Research Foundation, Institute of Biological Research and Biotechnology, 48 Vassileos Constantinou Avenue, Athens 11635, Greece.

²Lymphoma Unit, Haematology Clinic, First Department of Internal Medicine, University of Athens School of Medicine, Athens, Greece.

Address reprint requests to S. A. Kyrtopoulos, Programme of Chemical Carcinogenesis, National Hellenic Research Foundation, Institute of Biological Research and Biotechnology, 48 Vassileos Constantinou Avenue, Athens 11635, Greece.

within 10 years of treatment (8–10). An alternative protocol, the ABVD protocol (using dacarbazine in combination with adriamycin, bleomycin, and vincristine), although not so far shown to be leukemogenic, may not be completely free of carcinogenic risk (11).

Apart from considerations of therapy-induced carcinogenesis, an additional reason that makes the study of the methylation of human DNA by PCZ and DCZ of interest is the need to develop methodologies for assessing of the risks posed to the general population by exposure to environmental methylating agents. Such exposure is known to occur widely via contact with exogenous or endogenously formed chemicals (12–15).

Here we review the current status of our ongoing studies and discuss the observed relationships between exposure dose, adduct formation, DNA repair and biological effects and discuss their usefulness in the assessment of cancer risk associated with exposure to PCZ and DCZ and to environmental methylating agents.

Materials and Methods

Procarbazine treatment of patients involved 50-mg doses, three times per day (about 80 mg/m²·day) for 10 consecutive days, followed by a 3-week rest period, a treatment normally

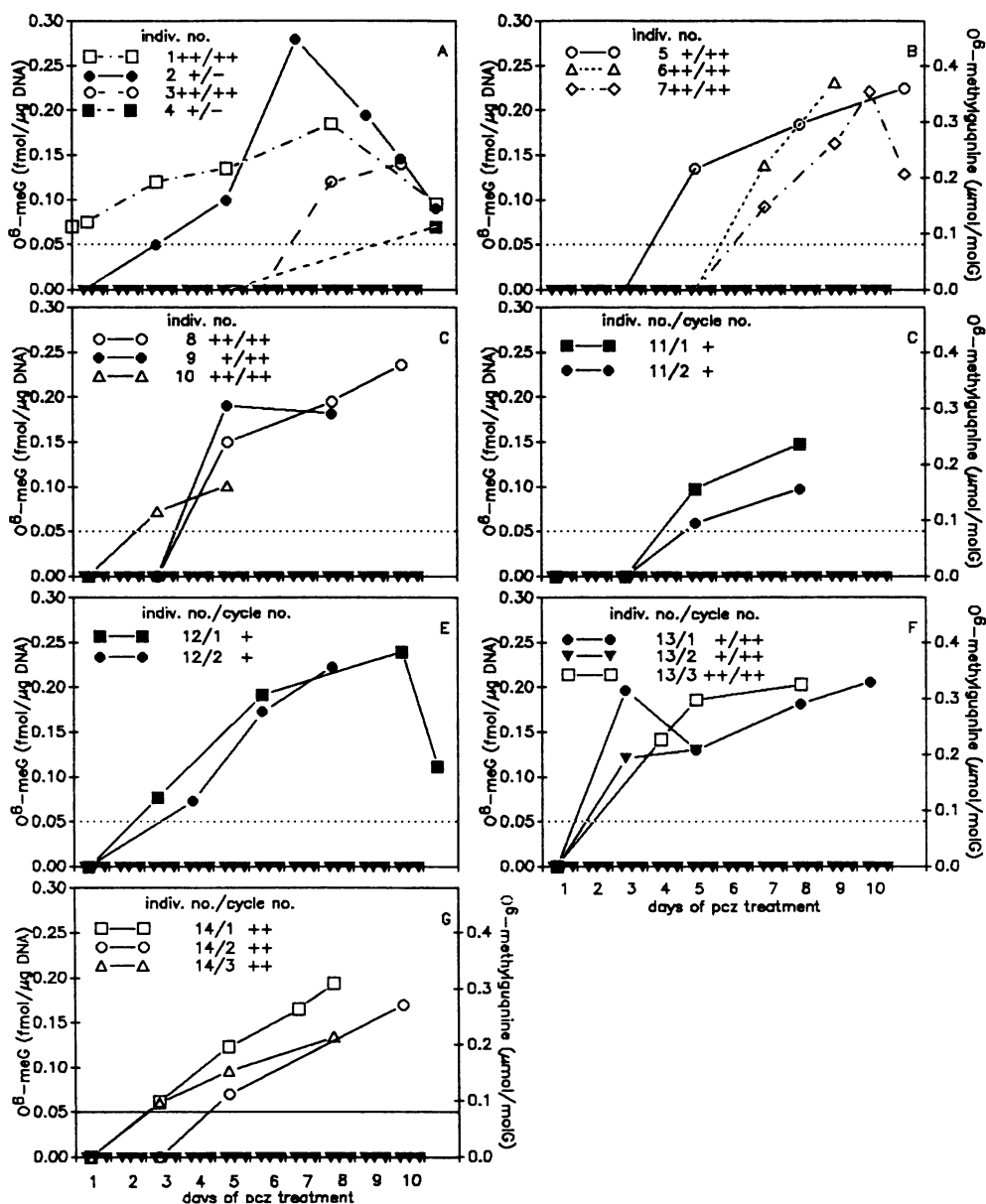


FIGURE 1. Accumulation of *O*⁶-methylguanine in blood leukocytes of 14 individuals during one or more cycles of treatment with procarbazine. (Open symbols) Individuals with full remission at the end of the cycle; (filled symbols) individuals with partial or no remission. The response of each individual after the particular cycle described and after the end of the full course of treatment is indicated as follows: (++) full remission; (+) partial remission; (-) recurrence. No overall clinical assessment is available for some patients who had not yet completed therapy at the time of writing.

repeated for six cycles. Other drugs employed in the same protocol included vincristine and cyclophosphamide or mechlorethamine hydrochloride. Each cycle of DCZ treatment involved a single injection of the drug (300 mg, about 160 mg/m²) followed 2 hr later by IV injections of bleomycin, vinblastin, and epirubicin. This treatment was repeated every 15 days for a total of four to six cycles. Blood samples were collected from each PCZ-treated patient just before the start of a cycle and on indicated days during the cycle, 4 hr after the morning intake of a 50-mg PCZ tablet. For DCZ patients, blood samples were collected just before and 1 hr after DCZ administration as well as on the next 1–2 days (where possible). In some cases a duplicate portion of blood was collected and immediately used for the isolation of lymphocytes and measurement of O⁶-alkylguanine-DNA alkyltransferase (AGT). Details of the assays used for determining O⁶-MeG and AGT have been published (1,2,16).

Procarbazine-treatment of female Sprague-Dawley rats (150 g) was carried out by daily gavage (10 mg/kg, 59 mg/m²) for 10 days. Groups of four animals were killed 24 hr after they received the indicated cumulative doses. In a separate series of experiments, rats were given single IP doses of PCZ or dimethylnitrosamine (DMN) at the doses indicated and killed after 2 or 6 hr, respectively.

Results and Discussion

Fourteen patients (twelve with Hodgkin's lymphoma and two with non-Hodgkin's lymphoma) on PCZ treatment and twelve

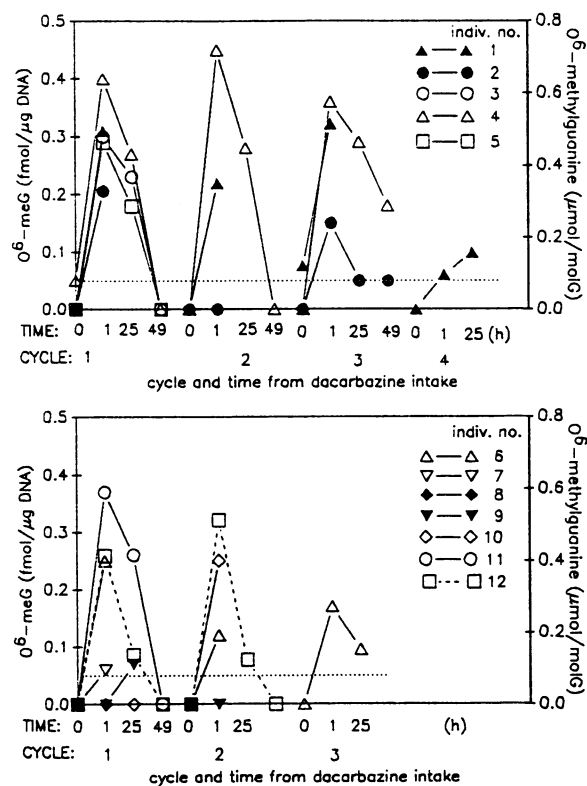


FIGURE 2. O⁶-methylguanine in blood leukocytes of 14 individuals 1–49 hr after treatment with dacarbazine for one or more cycles. (Open symbols) Individuals with full remission at the end of a cycle; (filled symbols) individuals with partial or no remission.

Hodgkin's lymphoma patients on DCZ treatment were followed for up to four cycles of chemotherapy. In almost all cases, no O⁶-MeG could be detected prior to the start of a treatment cycle, even in patients starting a second or subsequent cycle, but O⁶-MeG was detected at some point after treatment (Figs. 1 and 2). Adducts accumulated during PCZ treatment, usually reaching about 0.2–0.3 fmole/μg DNA (0.32–0.48 μmole O⁶-MeG/mole guanine) at the end of each cycle. Slightly higher adduct levels were seen 2 hr after treatment with DCZ. Where the same individuals were followed for more than one cycle of treatment, broadly similar methylation responses were observed. In all cases where data were available for the 24 hr immediately after cessation of treatment, a decrease in adduct levels was observed amounting to 44 ± 8% (n = 3) for PCZ-treated individuals and 43 ± 19% (n = 7) for DCZ-treated individuals.

As indicated in Figure 3, the accumulation of O⁶-MeG in PCZ-treated individuals as a group is linearly related to cumulative dose (with a slope of 2.8 × 10⁻⁴ fmole/μg DNA per mg/m² dose) and approaches a plateau above a cumulative dose of 600 mg/m². In DCZ-treated individuals, the average level of O⁶-MeG found 2 hr after exposure was 0.20 ± 0.14 fmole/μg DNA, corresponding to a rate of formation of 11 ± 8 × 10⁻⁴ fmole/μg DNA per mg/m² dose.

As already reported, no consistent changes in lymphocyte AGT levels were observed during PCZ treatment (1) and the average value of AGT remained constant throughout the cycle. A similar picture was obtained with patients on DCZ treatment, where a) no significant differences were seen between AGT values before and after treatment and b) AGT levels in the same patients prior to the start of repeated cycles were sometimes constant and sometimes changed in either direction (2). The overall picture that emerges from our studies is that no significant AGT depletion occurred in most cases as a result of suicide repair of O⁶-MeG adducts formed in the context of the chemotherapy protocols we examined. This can be explained by the large excess of lymphocyte AGT present in most individuals examined (usually 5–15 fmole/μg DNA) relative to the observed adduct levels and by the de novo synthesis of AGT (17). Significant depletion in lymphocyte AGT was recently reported by Lee et al. (17) in melanoma patients treated with high doses of DCZ (up to 800 mg/m²), about 5-fold higher than the doses used in our protocols.

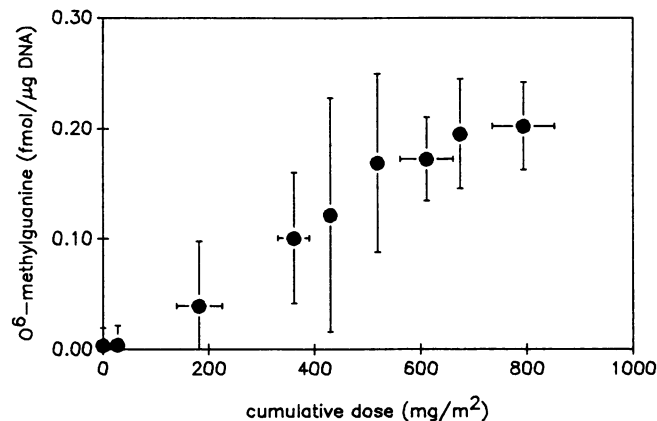


FIGURE 3. O⁶-methylguanine accumulation in blood leukocyte DNA as a function of the cumulative dose of procarbazine. Each point represents the mean of 3–14 individuals sampled for up to 3 consecutive cycles. Bar indicate SDs.

Examination of O^6 -MeG accumulation in different individuals has provided evidence that interindividual variability of AGT levels may be an important parameter influencing adduct accumulation during exposure to PCZ (1). Based on the currently available data, while an inverse trend between lymphocyte AGT and O^6 -MeG accumulation is consistently observed, this correlation does not reach statistical significance (Fig. 4). In the case of DCZ exposure, formation of O^6 -MeG does not show any correlation with lymphocyte AGT before exposure. It is possible that any underlying relationship between adduct formation and AGT repair activity is in this case confounded by differences in individual rates of DCZ metabolism.

Relationships between O^6 -MeG Accumulation and the Clinical Effects of PCZ and DCZ

Dacarbazine. No correlation between O^6 -MeG levels and the change in white blood cell (WBC) count was observed. Figure 5 shows the levels of O^6 -MeG in DCZ-treated patients classified according to clinical response. The mean value of O^6 -MeG after each cycle in individuals showing full remission of clinical symptoms at the end of the same cycle (0.236 ± 0.146 fmole/ μ g DNA) is higher than that in partial or nonresponders (0.156 ± 0.132 fmole/ μ g DNA), although the difference is not statistically significant ($p = 0.18$). If classification is based on overall clinical response and the O^6 -MeG levels are averaged over all the treatment cycles for each individual, the corresponding values become 0.252 ± 0.104 fmole/ μ g DNA and 0.087 ± 0.110 fmole/ μ g DNA, respectively ($p < 0.05$). While possibly indicative of a correlation, these comparisons are clearly limited by the small number of individuals for which full clinical data are currently available. No meaningful comparisons of AGT levels in the different groups can be made based on the currently limited available data.

Procarbazine. As already mentioned, 12 of the PCZ-treated patients had Hodgkin's lymphoma and 2 (nos. 1 and 2) had non-Hodgkin's lymphoma. Of the patients who have so far completed the full course of PCZ treatment, all have shown good therapeutic response (full disease remission) with the exception of two patients (no. 2, a non-Hodgkin's lymphoma patient and no. 4, a Hodgkin's lymphoma patient). This similarity in therapeutic responses, in combination with the similarity of methylation responses (Figs. 1A-G), makes the assessment of any relationship between the two parameters difficult. However, it is notable that the two individuals in whom therapy failed were the only ones exhibiting an abnormal methylation pattern: patient no. 2 accumulated high levels of adducts up to day 7 and subsequently showed an unexplained rapid loss of adducts, and patient no. 4 accumulated very low adduct levels throughout the treatment cycle.

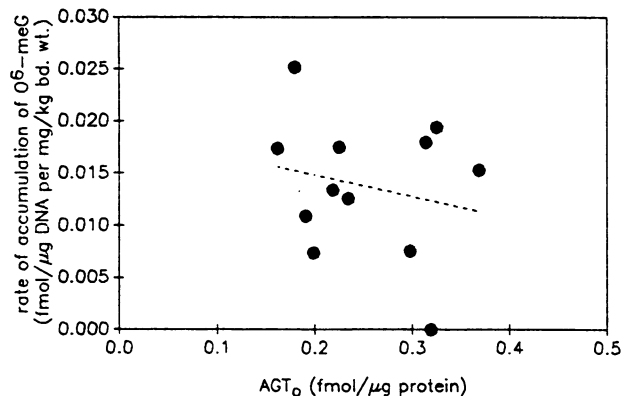


FIGURE 4. The rate of accumulation of O^6 -methylguanine (O^6 -MeG) during treatment with procarbazine in different individuals as a function of pretreatment lymphocyte O^6 -alkylguanine-DNA alkyltransferase (AGT) values. The rate of O^6 -MeG accumulation was calculated as the least-squares slope of the corresponding O^6 -MeG versus cumulative procarbazine dose curve in Figure 1 A-G. Samples collected after day 10 were excluded, as were samples collected after day 7 from individual no. 2 (for whom extensive loss of methylation was observed after day 7).

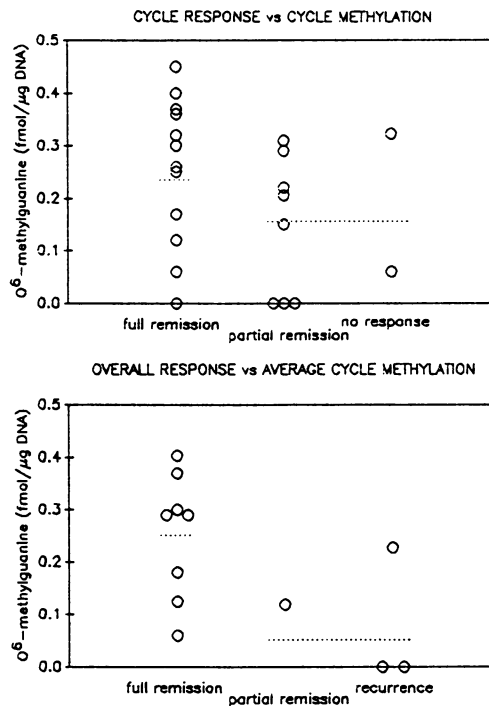


FIGURE 5. Scattergram of O^6 -methylguanine levels 2 hr after dacarbazine treatment relative to clinical response at the end of a particular cycle (upper panel) or of the full course of treatment (lower panel). In the lower panel, the O^6 -methylguanine values indicated are the averages of the values after each cycle for which they have been measured. The dotted lines indicate the means of the full responder and partial responder/nonresponder groups.

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Animal-to-Human Extrapolations for PCZ, DCZ, and Environmental Methylating Agents

A question of fundamental importance in any attempt to use blood leukocyte adduct levels as indicators of tissue-specific therapeutic effectiveness or carcinogenic risk is their relationship to adduct levels in the target cells. Fong et al. (18) have shown that doses of PCZ and methylnitrosourea resulting in similar extents of mammary carcinogenesis in female rats yield different amounts of O^6 -MeG in total mammary DNA, implying that the cell-specific distribution of adducts may be important in PCZ carcinogenesis. In order to obtain an indication of the tissue distribution of O^6 -MeG formed by PCZ, rats were treated daily *per os* with PCZ for 10 days with a dose comparable to that used in the MOPP protocol. Accumulation of similar amounts of

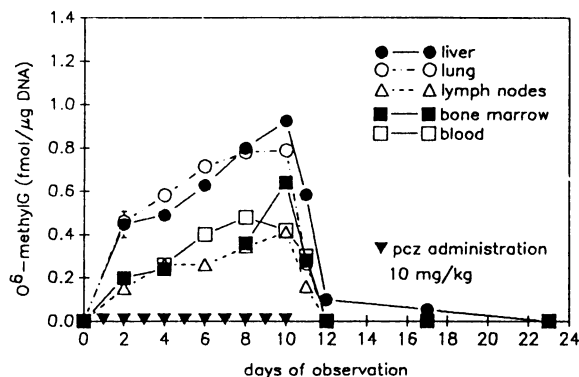


FIGURE 6. Accumulation of O⁶-methylguanine in rat DNA during treatment with dacarbazine (10 mg/kg) for 10 days. (▼) Times of procarbazine administration. For details of the treatment schedule see Materials and Methods.

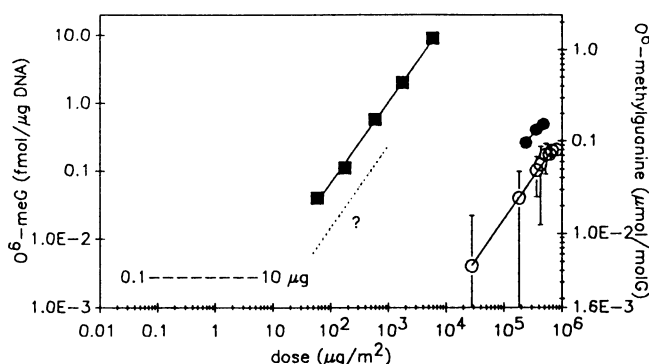


FIGURE 7. Comparative formation of O⁶-methylguanine in rat (filled symbols) and human (open symbols) blood leukocytes after repeated treatment with procarbazine (circles) and dimethylnitrosamine (squares). The dotted line refers to the methylation of human leukocyte DNA by dimethylnitrosamine and is extrapolated from that of the rat by assuming similar relative species susceptibilities as observed for procarbazine methylation. The horizontal dashed line indicates the possible range of human daily exposure to dimethylnitrosamine.

O⁶-MeG in the liver and lung and about 2.5-fold lower levels in blood leukocytes, bone marrow and leukocytes occurred (Fig. 6), suggesting that adduct levels in human blood leukocytes may be a good indicator of levels in the bone marrow (target tissue for leukemogenesis) and the lymph nodes (target tissue for chemotherapy). Furthermore, comparison of the rates of accumulation of O⁶-MeG in rat and human blood leukocytes suggests that humans have 5-fold lower susceptibility than rats to leukocyte O⁶-MeG induction by PCZ.

Figure 7 describes a first approach toward using the above data to extrapolate the expected extent of O⁶-MeG formation in human blood leukocytes after exposure to DMN. Based on the comparison of O⁶-MeG formation in rat and human blood leukocytes by PCZ and assuming similar relative species susceptibilities to DMN-induced methylation, current daily human exposure to DMN (0.1–10 μg) (12,19) would be expected to give rise to O⁶-MeG levels well below 1×10^{-9} mole/mole guanine.

This manuscript was presented at the Conference on Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment that was held in Kailua-Kona, Hawaii, 26 October–1 November 1991.

The technical assistance of Stella Kaila and Margarita Bekyrou is gratefully acknowledged. This work was partly supported by grants (to S.A.K.) by the Commission of the European Communities (contract no. EV4V-0062) and the international Agency for Research on Cancer (Collaborative Research Agreement BRI/89/09).

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