DNA damage and mutagenesis induced by procarbazine in \( \lambda \)lacZ transgenic mice: Evidence that bone marrow mutations do not arise primarily through miscoding by \( O^6 \)-methylguanine

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The DNA damaging and mutagenic activities of procarbazine, a methylating drug employed in cancer chemotherapy and suspected of causing therapy-related leukaemia, were investigated in the liver and bone marrow of \( \lambda \)lacZ transgenic mice (Muta\(^\text{TM}\)Mouse). The drug was administered using two different protocols, a ‘high-dose’ one involving 5 daily doses of 200 mg/kg, expected to cause depletion of the repair enzyme \( O^6 \)-alkylguanine–DNA alkyltransferase (AGT) and thus favour the selective accumulation of the premutagenic lesion \( O^6 \)-methylguanine (\( O^6 \)-meG) relative to other adducts, and a ‘low-dose’ one involving 10 daily doses of 20 mg/kg procarbazine. Substantial accumulation of \( O^6 \)-meG was observed in both tissues examined 6 h after the end of the ‘high-dose’ treatment, with the liver accumulating somewhat higher levels than the bone marrow (28.0 ± 1.8 fmol/\( \mu \)g DNA and 18.5 ± 1.1 fmol/\( \mu \)g DNA respectively). However, significant increases in mutant frequency 10 days after the end of treatment were observed only in the bone marrow, reaching a 16-fold increase over background following the 5 \( \times \) 200 mg/kg treatment. Sequence analysis of the mutations induced after this treatment revealed a mixed spectrum, in which G:C→A:T transitions (characteristic of \( O^6 \)-meG miscoding) were only a secondary feature: Among 20 mutants analysed, only six such mutations were found, including three at Cpg sites, which might have arisen from deamination of 5-methylcytosine. The other mutations observed included 1 A:T→G:C transition, five inversions (one G:C→T:A, one double G:C→C:G, two A:T→T:A, one A:T→C:G), five deletions and three insertions. The mechanistic and clinical significance of these findings is discussed.

Introduction

Procarbazine [\( N \)-isopropyl-\( \alpha \)-(2-methylhydrazino)-\( \beta \)-toluamidine hydrochloride] is a cytostatic drug used in the treatment of a number of human cancers, including Hodgkin’s lymphoma where, in the form of combination treatments such as MOPP (a combination of procarbazine and mechloethamine, vincristine and prednisone), it has made a particularly important contribu-

tion to the remarkable progress achieved in terms of long-term survival of patients (1,2). However, this success has been complicated by the appearance, during the 10 years following successful chemotherapy, of an ~5–10% increased risk of acute non-lymphocytic leukaemia (ANLL*), as well as other types of cancer (3,4). Although procarbazine is not the only potentially carcinogenic component of MOPP, its well recognized genotoxicity (5,6), together with evidence from follow-up studies of ex-Hodgkin’s lymphoma patients (7), cause concern that it may make a significant contribution to the mechanism of MOPP carcinogenesis. Procarbazine is mutagenic, elastogenic and teratogenic in a wide range of test systems of varying complexity and a wide-spectrum carcinogen in rodents and monkeys, causing tumours of the haemopoietic system, the mammary gland, the lung and the nervous system (8).

In vivo procarbazine undergoes a complex series of metabolic changes that result in the generation of a number of chemically reactive species, including methylating agents and free radicals (9). The major circulating metabolite in the serum of animals or humans treated with procarbazine is methyloxazoprocarbazine, a relatively stable intermediate that can spontaneously give rise to a methylating species, probably the methyl-diazonium ion \([ \text{CH}_3 \text{N}_2^+ \])^\text{+}. The main type of macromolecular damage known to be produced by procarbazine is DNA methylation, with the liver accumulating the highest amounts of such damage, followed closely by the bone marrow and lymph nodes, two tissues of interest as targets for the carcinogenic and chemotherapeutic action of the drug (10,11). Procarbazine produces in DNA \( O^6 \)-methylguanine (\( O^6 \)-meG) and \( N7 \)-methylguanine at a ratio of ~0.12, typical of an \( S_N^1 \)-type methylating agent and similar to that caused by such powerful mutagens and carcinogens as methylnitrosourea and dimethyl-nitrosamine (10). It is therefore strongly suspected that DNA methylation plays a major role in the mutagenic and carci-

*Abbreviations: ANLL, acute non-lymphocytic leukaemia; AGT, \( O^6 \)-alkylguanine–DNA alkyltransferase; \( O^6 \)-meG, \( O^6 \)-methylguanine; NT, not tumorigenic; pfu, plaque forming units; \( O^6 \)-meG, \( O^6 \)-methylguanine; MOPP, mechloethamine-vincristine-prednisone-procarbazine.

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is similar to that of the rat (16), suggesting that this lesion may play a similar mutagenic role in humans treated with procarbazine as it does in the rat. Evidence compatible with an etiological role of $O^6$-meG in the induction of ANLL after MOPP chemotherapy was produced by Sagher et al. (17) who found that MOPP-treated individuals who went on to develop ANLL had lower lymphocyte levels of $O^6$-alkylguanine–DNA alkyltransferase (AGT), the enzyme responsible for repair of $O^6$-meG, than similarly treated individuals who did not develop ANLL. This observation implied that individuals with low levels of AGT may be at higher risk of procarbazine-induced carcinogenesis by accumulating higher amounts of $O^6$-meG during MOPP chemotherapy. Further support for this suggestion was obtained through the direct measurement of the formation of $O^6$-meG in blood leukocyte DNA of procarbazine-treated patients (18) and the demonstration that individuals with low pre-treatment AGT levels accumulate high amounts of $O^6$-meG, an observation also observed in patients treated with another methylating drug, dacarbazine (19,20).

Summarizing, data on the quantitative accumulation of $O^6$-meG provide some evidence compatible with this lesion being important in procarbazine-induced carcinogenesis in experimental animals and MOPP-induced ANLL in humans. However, this evidence is far from conclusive. A key event in the initiation of chemical carcinogenesis is the conversion of pre-mutagenic DNA damage into a mutation in the target tissues through the replication of damage-containing cells. The advent of the technology of transgenic rodents with transgenes, which can serve as readily observable targets for chemical mutagens, provides a new way for investigating the ability of carcinogens to induce mutations in tissues susceptible to their carcinogenic effects. Furthermore, the examination of the specific types of sequence changes caused by a mutagen provides a powerful tool for the assessment of the role of specific DNA lesions in the induction of mutagenesis, which are under the full complexity of metabolic and repair pathways that operate under in vivo conditions. Procarbazine has been reported to induce a significant increase in mutant frequency in the bone marrow of $\lambda$acZ mice (Muta$^\text{TM}$Mice) 7 days after the completion of treatment consisting of five daily doses of 200 mg/kg (21). However, no information on the relationship between mutagenesis and DNA adduct induction in any tissue of such treated animals was given in this report. In the present study we have examined the accumulation of $O^6$-meG as well as the frequency and types of mutations in the liver and bone marrow of $\lambda$acZ transgenic mice treated with procarbazine. We find that, even though after five daily treatments with 200 mg/kg procarbazine, there were slightly lower amounts of $O^6$-meG accumulated in the bone marrow than the liver: a strong mutagenic response was induced only in the former tissue, and the spectrum of induced mutations was not dominated by the characteristic fingerprint of $O^6$-meG (G:C→A:T transitions).

**Materials and methods**

**Chemicals**

Samples of procarbazine hydrochloride were obtained from Hoffman La Roche, Basle and from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, National Cancer Institute, USA. The drug was stored desiccated in an amber bottle at −20°C and dissolved (protected from light) in physiological saline just before use.

**Animal treatment**

Male $\lambda$acZ transgenic mice (Muta$^\text{TM}$Mouse) (16), 9–10 weeks old, purchased from Hazleton, were maintained on normal laboratory chow. Groups of four mice were treated i.p. with multiple doses of procarbazine (10×20 mg/kg or 5×200 mg/kg at 24-h intervals) or with physiological saline only. One animal from each group was killed 4 h after the last treatment and used for measurement of $O^6$-meG in liver and bone marrow DNA, while the remaining three animals were killed 10 days after the last treatment for mutation analysis in the same tissues. Tissues of interest were immediately placed in liquid nitrogen and stored at −70°C until analysis.

**Measurement of $O^6$-meG**

Following DNA extraction from liver and bone marrow of individual animals by a standard method involving multiple proteinase K treatments and phenol–chloroform extractions, $O^6$-meG was measured by the competitive repair assay as previously described (22) using E. coli AGT (ada protein). Each DNA sample was analysed in duplicate and duplicate measurements agreed to within 10% of each other.

**Mutagenesis in the $\lambda$acZ transgenic mouse**

High molecular-weight genomic DNA was isolated from liver and bone marrow as previously described (23), dissolved in 10 mM Tris–HCl (pH 7.5) and 4 mM EDTA to a final concentration of 1.5 mg/ml and stored at 4°C. A 5 µl aliquot of each of the DNA solutions was taken and the λ prophages were rescued in vitro using the Giga-Pack II Gold packaging extract (Stratagene, La Jolla, CA). Screening for lac$^-$ mutant phages was carried out by using the phenyl-β-D-galactopyranoside (P-gal)-based positive selection system (24) and all mutants were confirmed on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) plates. Generally >3×10$^5$ plaque forming units (pfu) were analysed in each case.

For the sequence-characterization of representative mutants, the location of the mutation in the $\alpha$, $\beta$, or $\omega$ regions of the lacZ gene was determined using a β-galactosidase complementation assay as described by Douglas et al. (25).

**Results**

$O^6$-Methylguanine accumulation in liver and bone marrow DNA

Animals were treated with two dosing schedules of procarbazine, one involving five daily doses of 200 mg/kg, anticipated to cause substantial depletion of AGT and thus favour the preferential accumulation of $O^6$-meG, and one involving 10 daily doses of 20 mg/kg, expected to generate relatively low levels of $O^6$-meG. In order to obtain an indication of the adduct levels formed and their tissue distribution, from each group of treated animals one was killed 4 h after the last treatment and $O^6$-meG was measured in liver and bone marrow DNA. The results are shown in Table I, where it can be seen that the high-dose treatment resulted in the accumulation of large amounts of $O^6$-meG in both the liver and the bone marrow, the latter tissue accumulating approximately two-thirds of the adducts of the liver. Although changes in AGT were not measured, given the pre-treatment levels of AGT in $\lambda$acZ transgenic mice (5.1 fmol/µg DNA for the liver and 0.7 fmol/µg DNA for the bone marrow (V.L. Soulidiotis et al., unpublished results)) it is likely that substantial depletion of this enzyme occurred in both tissues. The death of one of the animals treated with low doses of procarbazine prevented the measurement of the resulting adducts in this case.

**Mutant frequency analysis**

The remaining animals per group were killed 10 days after the last treatment and mutant frequency in the lacZ transgene was determined in the liver and bone marrow. As can be seen in Table I, no statistically significant increase in mutant frequency was observed in the liver after either treatment, although one animal in each case gave a particularly high mutant frequency value. In the bone marrow, a 5-fold increase was observed in both animals treated with 10×20 mg/kg procarbazine ($P < 0.005$), while a much greater increase occurred in each of the three animals treated with 5×200 mg/
kg procarbazine. The 16-fold average increase from 41 ± 22
mutants/10^6 pfu to 650 ± 76 mutants/10^6 pfu (P < 0.0005)
agrees well with the report of Myhr (21) who found a mutant
frequency of 772 mutants/10^6 pfu 7 days after completion of a
similar treatment.

**Discussion**

In an effort to investigate the mechanism of procarbazine
mutagenesis and to obtain an indication of the role of O^6^-meG
in it, we examined adduct and mutation induction in the liver
and bone marrow of λlacZ transgenic mice after treatment
with multiple doses of this agent. O^6^-Methylguanine was
measured 6 h after the last of five or 10 daily treatments.
Previous studies with a number of methylating agents, including
procarbazine, have shown that repeated daily treatments result
in a steady accumulation of O^6^-meG, which is reflected in the
adduct levels present at the end of the treatment period (11,26,27). Furthermore, O^6^-meG appears to have similar
effects on DNA damage and mutagenesis induced by procarbazine,
which is reflected in the results obtained with the use of procarbazine.
This is consistent with the findings of other studies that have shown
a similar treatment.

**Table II. Bone marrow lacZ mutants in mice treated with procarbazine
(5×200 mg/kg) and killed 10 days after last treatment**

<table>
<thead>
<tr>
<th>Mutant no.</th>
<th>lacZ region</th>
<th>Mutation</th>
<th>Position</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α</td>
<td>C:G→T:A</td>
<td>127</td>
<td>CTGCGGTAA</td>
</tr>
<tr>
<td>2</td>
<td>α</td>
<td>G:C→T:A</td>
<td>229</td>
<td>GCCGCCAAC</td>
</tr>
<tr>
<td>3</td>
<td>β</td>
<td>C:G→A:T</td>
<td>487</td>
<td>CGGCGCCTG</td>
</tr>
<tr>
<td>4</td>
<td>β</td>
<td>Insertion C</td>
<td>507–508</td>
<td>CCACCGACA</td>
</tr>
<tr>
<td>5</td>
<td>β</td>
<td>C:G→T:A</td>
<td>619</td>
<td>TCAGAATAT</td>
</tr>
<tr>
<td>6</td>
<td>β</td>
<td>A:T→C:G</td>
<td>621</td>
<td>AGGATATGT</td>
</tr>
<tr>
<td>7</td>
<td>β</td>
<td>Deletion T</td>
<td>641</td>
<td>GCATTTTC</td>
</tr>
<tr>
<td>8</td>
<td>β</td>
<td>Deletion C</td>
<td>777</td>
<td>ACTAATCTC</td>
</tr>
<tr>
<td>9</td>
<td>β</td>
<td>A:T→T:A</td>
<td>785</td>
<td>CGCGAACA</td>
</tr>
<tr>
<td>10</td>
<td>β</td>
<td>Deletion C</td>
<td>830</td>
<td>GCGACCGG</td>
</tr>
<tr>
<td>11</td>
<td>β</td>
<td>C:G→T:A</td>
<td>928</td>
<td>CGCGCAAT</td>
</tr>
<tr>
<td>12</td>
<td>β</td>
<td>G:C→A:T</td>
<td>1164</td>
<td>AGAACAAAT</td>
</tr>
<tr>
<td>13</td>
<td>β</td>
<td>Insertion TQ</td>
<td>1164–1165</td>
<td>AGAAACAAAC</td>
</tr>
<tr>
<td>14</td>
<td>β</td>
<td>A:T→G:C</td>
<td>1210</td>
<td>GCTGTGGA</td>
</tr>
<tr>
<td>15</td>
<td>β</td>
<td>C:G→G:C</td>
<td>1217, 1218</td>
<td>TACACGCTG</td>
</tr>
<tr>
<td>16</td>
<td>β</td>
<td>A:T→T:A</td>
<td>1242</td>
<td>TGATATGGG</td>
</tr>
<tr>
<td>17</td>
<td>β</td>
<td>Deletion G</td>
<td>1480</td>
<td>TGAGCGGG</td>
</tr>
<tr>
<td>18</td>
<td>β</td>
<td>Insertion C</td>
<td>1631–1632</td>
<td>ATACGCC</td>
</tr>
<tr>
<td>19</td>
<td>β</td>
<td>C:G→T:A</td>
<td>1652</td>
<td>AACACCTT</td>
</tr>
<tr>
<td>20</td>
<td>α</td>
<td>Deletion G</td>
<td>2034</td>
<td>ATGTGCTC</td>
</tr>
</tbody>
</table>
mutagenesis comparisons, they provide an adequate indication of the relative magnitude of DNA damage that occurred in the two tissues examined.

Despite the fact that the liver accumulated the highest levels of O6-meG (up to 28.0 ± 1.8 fmol/µg DNA, corresponding to ~53 000 residues of O6-meG per 10^9 lacZ transgenes, after the high-dose treatment), no statistically significant increase in liver mutagenesis could be detected with either dosing regimen. It has been suggested that the induction of toxicity-related liver cell proliferation may be a necessary prerequisite for mutagenesis (28). Although no specific information on the hepatotoxicity of procarbazine is available, the liver does not appear to be among the main targets of the toxicity of this compound, which are the gonads, the lung and the haematopoietic system (8). It thus seems possible that the low rate of liver cell proliferation after procarbazine treatment did not favour the efficient conversion of these adducts into mutations.

In contrast to what was observed with the liver, a dose-related increase in bone marrow mutagenesis was induced by both procarbazine treatments employed. Thus, despite the accumulation after the high dose treatment of 30% lower amounts of O6-meG in the bone marrow than the liver (18.5 ± 1.1 fmol/µg DNA, corresponding to 35 000 adduct residues per 10^9 transgenes), a 16-fold increase of mutagenesis over background was observed in the former tissue. The much greater susceptibility of the bone marrow to procarbazine mutagenesis is probably due in part to its greater basal rate of cell proliferation. In addition, the bone marrow is a major target for the acute toxicity of procarbazine (8,29,30), which is expected to lead to restorative cell proliferation and favour mutation fixation, thus accounting for the susceptibility of the bone marrow to procarbazine mutagenesis. A number of studies (reviewed in reference 8) have shown that the haematopoietic system is also a major target of procarbazine carcinogenesis in the mouse. Interestingly, the same appears to be true in the monkey (31), in contrast to the rat where induction of leukaemia was relatively rare (8).

In addition to the rate of cell proliferation being an important factor that determines the relative tissue susceptibility to procarbazine mutagenesis, it is also possible that the accumulation of O6-meG is not a good measure of the critical premutagenic damage induced by procarbazine, i.e. that miscoding by this lesion is not the primary mechanism of mutagenesis by this agent. Such an explanation would be compatible with the mutation spectrum observed (Table III), in which G:C→A:T transitions (the fingerprint of O6-meG miscoding) constitute only 30% of the total. For comparison, it is noted that AGT-depleting doses of the methylating agents N-nitrosodimethylamine or N-methyl-N-nitrosourea gave rise to 80% and 95% G:C→A:T transitions in the liver and the spleen, respectively, of lac transgenic mice (28,32). As three of the six G:C→A:T mutations induced by procarbazine were at CpG sites, it is possible that some or all of these might have arisen by deamination of 5-methylcytosine, thus decreasing the proportion of mutations likely to have derived from direct O6-meG mutagenesis even further. On the other hand, two of the remaining G:C→A:T mutations were at the 3′-G of pu-G dinucleotides (a site known to be favoured for O6-meG mutagenesis) and can probably be attributed to this lesion. Although O6-meG was the only adduct measured in the present study, it is expected that other DNA adducts (particularly N7-methylguanine and N3-methyladenine) were also produced in substantial amounts during the multiple treatments. Furthermore, procarbazine is known to undergo a complex pattern of metabolism, which in addition to the production of methylating intermediate(s), also leads to the formation of free radical species (9). If genotoxic species other than a methylating agent are also formed during the metabolism of procarbazine, they might well undergo substantial reaction with guanine (the most nucleophilic base in DNA) and give rise to some of the G-centred sequence changes observed. If alternative pathways of procarbazine metabolism result in the addition to DNA of the bulky side chain attached to the second hydrazine nitrogen, the resulting lesions might, for example, account for the frequent observation of frameshifts at or next to G (seven out of eight insertions or deletions), including four instances of frameshifts at GG dinucleotides, sites known to favour polymerase slippage (33).

A mixed mutation spectrum with a high proportion of frameshifts, especially those associated with polymerase slippage, is characteristically observed in mutants derived from cells deficient in mismatch repair (34,35). Methylating agents such as N-methyl-N-nitrosourea are well known to readily cause mutational inactivation of mismatch repair genes in mammalian cells, giving rise to mutants that, in addition to being hypermutable, are methylylation tolerant and thus would have a growth advantage in a background of toxic methylation insult (36). The mixed mutation spectrum and the relatively high frequency of frameshifts observed in this study (Table III) would thus be compatible with the possibility of an additional pathway of procarbazine mutagenesis involving the inactivation of mismatch repair and subsequent generation of procarbazine-resistant cells prone to replication errors. While compatible with our observations and in line with known mutagenic properties of methylating agents, this hypothesis would need to be tested by specially designed experiments. An alternative possibility to consider is that the repetitive cycles of abortive mismatch repair that take place on the strand opposite O6-meG (36), believed to be the main basis of the cytotoxicity of this lesion, may give rise to DNA rearrangements in cells that survive this process.

Mutations in the ras oncogene have been detected in blood leukocytes of patients who had undergone cytotoxic therapy for lymphoma, which probably included procarbazine (38). Of the nine mutations found, seven were transversions (five G:C→T:A, one G:C→C:G, one A:T→C:G and two A:T→G:C transitions). Although the small number of mutations characterized and the uncertain nature of the administered chemotherapy

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**Table III. Summary and comparison of spontaneous and procarbazine-induced lacZ mutation spectra in bone marrow**

<table>
<thead>
<tr>
<th>Type of mutation</th>
<th>Spontaneous Myhr et al. (21) n = 20</th>
<th>Procarbazine-induced Myhr et al. (21) n = 8</th>
<th>This work n = 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transitions</td>
<td>16</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>G:C→A:T</td>
<td>15 (14)</td>
<td>1</td>
<td>6 (3)</td>
</tr>
<tr>
<td>A:T→G:C</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Transversions</td>
<td>2</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>G:C→T:A</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>G:C→C:G</td>
<td>–</td>
<td>1</td>
<td>1 (double)</td>
</tr>
<tr>
<td>A:T→T:A</td>
<td>–</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>A:T→C:G</td>
<td>–</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Deletions</td>
<td>2</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>Insertions</td>
<td>–</td>
<td>–</td>
<td>3</td>
</tr>
</tbody>
</table>

*Within CpG dinucleotide.*
do not permit any definitive conclusions, it is evident that this mutation spectrum does not resemble the one observed in the present study. On the other hand, the absence of ras gene G:C→A:T mutations is in concordance with the low proportion of such mutations in the present study and supports the suggestion that direct miscoding by O6-meG may not be the main source of procarbazine-induced mutations. If confirmed by further studies, this suggestion might have important implications regarding the possibility of modification of chemotherapy protocols involving procarbazine with the aim of enhancing their therapeutic efficacy. Since there is evidence that O6-meG contributes substantially to the toxicity of procarbazine (39,40), in principle it would be possible to enhance this drug’s cytostatic efficacy through the depletion of AGT, along lines similar to those currently the subject of extensive experimental studies and clinical trials with nitrosourea-based drugs (41). However, in contrast to the latter drugs that bring about their mutagenic effects primarily via DNA cross-links, rather than O6-alkylguanine-type lesions, any enhancement of procarbazine-induced O6-meG accumulation would carry with it the undesirable risk of a substantial increase in mutagenicity. If, on the other hand, O6-meG plays only a minor role in procarbazine mutagenesis, as implied by the results reported here, such risks may be smaller than until now anticipated. This would open the way to protocol modifications that might be particularly valuable in the treatment of brain tumours, where procarbazine-mediated chemotherapy has not met with the success that it has in the case of Hodgkin’s lymphoma.

In conclusion, the data reported here indicate that the mouse bone marrow is highly susceptible to procarbazine mutagenesis and that mutations induced may not be primarily derived from direct miscoding by O6-meG. The molecular mechanism of the observed mutagenesis, and whether the same mechanism determines mutagenesis in genes involved in procarbazine carcinogenesis in man, are important questions that remain to be elucidated.

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References


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