Repair of $O^6$-Alkylguanine during DNA Synthesis in Murine Bone Marrow Hematopoietic Precursors

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ABSTRACT

$O^6$-Alkylguanine, a DNA adduct formed by nitrosoureas, becomes the site of a point mutation during DNA synthesis by preferentially base mispairing with thymine rather than correctly base pairing with cytosine. To repair this adduct, cells contain a limited amount of $O^6$-alkylguanine-DNA alkyltransferase (alkyltransferase), a protein which removes the alkyl group in a stoichiometric reaction. To prevent mutations, repair must occur before DNA replication takes place. Consequently, formation of point mutations is related inversely to the number of alkyltransferase molecules and directly to the rate of DNA synthesis. Bone marrow hematopoietic precursors, the target for nitrosourea-induced leukemia, are deficient in alkyltransferase activity. We questioned whether regenerating bone marrow is more susceptible to nitrosoureas than other organs due to persistently low levels of alkyltransferase activity during periods of increased cell proliferation and DNA synthesis. Following syngeneic bone marrow transplantation, murine hematopoietic cells underwent rapid cell proliferation but alkyltransferase activity remained well below the activity in liver. After $N$-nitrosomethylurea exposure, $[3H]$thymidine incorporation in rat bone marrow increased 3-fold and stem cell proliferation over 10-fold within 2 days of exposure, but alkyltransferase activity remained low. The relative susceptibility of bone marrow to mutagenic damage from $O^6$-alkylguanine adducts was determined by comparing the ratio of alkyltransferase activity to $[3H]$thymidine incorporation in marrow, kidney, and liver. In untreated animals, the ratio was lowest in bone marrow and decreased further 48 h after $N$-nitrosomethylurea exposure to only 21% that of kidney and 1% that of liver. Thus, proliferating hematopoietic precursors appear more likely to form point mutations following nitrosourea exposure than other rodent tissues because they undergo rapid proliferation soon after DNA damage and before $O^6$-alkylguanine adducts can be repaired. The combination of rapid cell proliferation and low DNA repair capacity may be the mechanism of nitrosourea induced leukemic transformation of the bone marrow.

INTRODUCTION

Nitrosoureas are a well known cause of leukemic transformation in rodent and human bone marrow (1, 2). Although nitrosoureas induce a number of different tumors in rodents, leukemia is the only malignancy clearly associated with nitrosourea exposure in humans (2). While the specific mechanism through which nitrosoureas transform bone marrow is unknown, transformation most probably develops as a result of mutagenic DNA damage and may be caused by mutagenic lesions.

One of the adducts produced by nitrosoureas, $O^6$-alkylguanine, is an important premutagenic adduct because it will preferentially base mispair with thymine rather than undergoing the appropriate base pairing with cytosine (3). Not only can this adduct be the site of a random point mutation but it has now also been documented to be the cause of the point mutation in the 12th codon of H-ras in MNU3 induced rat mammary carcinoma which leads to activation of this oncogene and malignant transformation of the cell (4). In the absence of DNA synthesis, monofunctional $O^6$-alkylguanine adducts do not disrupt the DNA or induce other forms of mutagenic DNA damage (3, 5). For this reason, DNA synthesis is required for expression of the mutagenic potential of $O^6$-alkylguanine at the genomic level. Consequently, as with many mutagens, the probability of developing point mutations following nitrosourea exposure depends on the number of persistent adducts at the time of DNA replication (6, 7).

A number of tissues lack the capacity to repair $O^6$-alkylguanine adducts due to low levels of the DNA repair protein $O^6$-alkylguanine-DNA alkyltransferase (alkyltransferase) (8-10). This protein catalyzes covalent transfer of the alkyl group from the substituted base to one of its own cysteine residues which causes irreversible inactivation of the protein (10, 11). The unique stoichiometric mode of action of the alkyltransferase (5, 10, 11) means that once the level of nitrosourea exposure is sufficient to cause inactivation of the alkyltransferase, persistent adducts accumulate in the cell. A threshold is thus observed in the level of mutations produced (5, 12). The number of alkyltransferase molecules in different cell types ranges from less than 5,000 to greater than 140,000, indicating that only a limited number of adducts can be repaired (13). Tissues with low levels of alkyltransferase activity are target organs for the carcinogenic effects of nitrosoureas (14-16).

Other tissues which are susceptible to the mutagenic effects of $N$-nitrosocompounds have high rates of DNA synthesis as well as low levels of alkyltransferase activity (7). Pubescent rat mammary tissue has low alkyltransferase activity (17) and undergoes hormonally dependent glandular cell proliferation, causing a high rate of MNU induced tumors (18). Sinusoidal endothelial cells of liver, the target cells for nitrosamine induced carcinogenesis, have lower levels of alkyltransferase but higher rates of DNA synthesis than the nontarget hepatocyte (6, 7, 19). Furthermore, hepatocytes increase their alkyltransferase activity when the rate of DNA synthesis increases (7, 19).

We have previously suggested that the susceptibility of bone marrow to nitrosoureas is due to their low level of alkyltransferase activity (7). In addition, cell proliferation increases dramatically in bone marrow following cytotoxic damage (22), suggesting that marrow is a target organ because it replicates in the presence of persistent mutagenic DNA damage. However, the relationship between alkyltransferase activity and DNA synthesis in these cells has not been established. To provide further information concerning the susceptibility of hematopoietic precursor cells to the mutagenic effects of nitrosoureas, we studied this relationship during periods in which these cells were undergoing different rates of cellular proliferation. First, we studied actively proliferating normal hematopoietic cells following bone marrow transplantation. Second, we compared

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The abbreviations used are: MNU, $N$-nitrosomethylurea; PBS, phosphate buffered saline; HPLC, high performance liquid chromatography; HEPES, 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid; CPU-GM, colony forming unit-granulocyte/macrophage (tibia and femur); PCA, perchloric acid.
the changes in alkyltransferase activity in liver, kidney, and
bone marrow in animals exposed to MNU at doses which
induce leukemia in rodents (2). The results indicate that the
low level of alkyltransferase activity during periods of increased
dNA replication in hematopoietic cells further contributes to
the susceptibility of these cells to leukemic transformation
following nitrosourea exposure.

MATERIALS AND METHODS

Chemicals. All chemicals were purchased from Sigma Chemical Co.,
St. Louis, MO, unless otherwise stated. MNU was stored descicated at
−20°C and dissolved in 1% acetic acid to give a concentration of 0.1 M
and diluted into PBS just prior to use. [3H]Thymidine (New England
Nuclear (78-mCi/mmol)) was stored at 4°C and used within 1 month.
Bisbenzamidochrome (Hoescht dye No. 33258; Calbiochem-
Behring, La Jolla, CA) was stored in the dark at 4°C. Methanol, H2O,
and KH2PO4 were HPLC grade (Fisher Scientific, Pittsburgh, PA).

Animals. Male CD-1 mice, 50 days old and weighing 18 g were
purchased from Charles River Breeding Laboratories (Wilmington,
MA). Sprague-Dawley rats, 100 days old and weighing 150 g, were
purchased from Zivic-Miller (Allison Park, PA). Animals were housed
in metal cages and fed water and rodent chow ad libitum, in an
environment of 12 h light and 12 h dark (21).

Animal Nitrosourea Exposure. Littermate mice or rats received a
single i.v. dose of MNU (35 mg/kg) by tail vein injection on day 0 of
the experiment.

Bone Marrow Transplantation. CD-1 mice undergoing bone marrow
transplantation were irradiated with 825 rads using a 60Co source 24 h
prior to transplantation. This dose caused bone marrow aplasia and
death of the animals within 8 days in the absence of transplantation.
Bone marrow from five unirradiated littersmates was flushed from
bilateral tibias and femurs and resuspended at 5 × 106 cells/ml in RPMI
1640 culture medium [RPMI 1640 supplemented with 15% fetal calf
serum (HyClone, Logan, UT), 50 units/ml penicillin, 50 μg/ml strep-
tomycin solution, 2 mM glutamine, and 25 mM HEPES] at 22°C. Irradiated recipients received 5 × 106 bone marrow cells in 0.1 ml by
tail vein injection. This produced 70–110 pLimpotent I hematopoietic
stem cells (colony forming units-spleen) per spleen and allowed bone
marrow reconstitution in 21 days. In some experiments, animals were
given PBS injections to test the effect of radiation alone on tissue DNA
synthesis and DNA repair.

Tissue Preparation. Mice were sacrificed by cervical dislocation. Rats
were anesthetized with ether, given injections of 200 units of heparin
by tail vein, and sacrificed by cervical dislocation. After sacrifice, mouse
or rat liver and kidney were processed as described previously (23).
Bisbenzamidochrome (Heat Systems Ultrasonics, Farmingdale, NY).
The sonicate was centrifuged at 10,000 × g for 2 min to remove cellular
debris (3, 15) and stored at −80°C. Enzyme activity was identical in
samples with and without cellular debris. Samples of extracts were
taken for determination of protein and DNA content using previously
described procedures (28, 29).

Results

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Bone marrow, spleen, or liver from 30-day-old CD-I mice or rats received
a single i.v. injection of [3H]thymidine labeled cellular DNA with each batch of
samples, it was determined that recovery of DNA was 69 ± 4.5% (SD)
of total DNA.

[3H]Thymidine incorporation in bone marrow and spleen cells was
also measured in tissue culture. Preliminary experiments indicated that
these results were similar to those obtained with the in vivo method.
Bone marrow and spleen cells were resuspended in RPMI culture
medium and incubated at 37°C. The incubation was stopped by
addition of 5 ml 0.9% NaCl solution at 4°C, the cells were recovered following centrifugation at
1000 × g for 7 min, and were then resuspended in 5 ml 5% trichloroacetic acid at 4°C. The suspension was sonicated and collected
on GF/C glass fiber filters and processed for scintillation counting as
described previously (27).

Cell Extracts. Tissue homogenates and cell suspensions were soni-
cated 3 times for 5 s at 4°C to complete cell dispiration using a
Marmasonic processor (Heat Systems Ultrasonics, Farmingdale, NY).
The sonicate was centrifuged at 10,000 × g for 2 min to remove cellular
debris (3, 15) and stored at −80°C. Enzyme activity was identical in
samples with and without cellular debris. Samples of extracts were
taken for determination of protein and DNA content using previously
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O'-Alkylguanine-DNA Alkytransferase Assay. O'-Alkylguanine-
DNA alkytransferase activity in cell extracts was measured as removal of the
[3H]methyl adduct from O'-[3H]methylguanine in DNA alkylated with
[3H]MNU as described previously (20, 30). The specific activity of the
substrate [3H]methyl-DNA was 2.7 × 106 dpm/mg DNA (10.4 dpm
of O'-[3H]methylguanine/mol). The assay mixture contained either
250, 500, or 750 μg protein of cell extract, depending on the expected
level of alkytransferase activity, 4.3 μg [3H]methyl-DNA (containing
100 fmol O'-methylguanine), and assay buffer consisting of 70 mM
HEPES (pH 7.8), 0.1 mM EDTA, 5% glycerol, 1 mM dithiothreitol,
and 25 μM spermidine in a total volume of 200 μl (10, 18, 20) as
described previously. Following a 60-min incubation at 37°C which
allows the reaction to go to completion (20), the DNA was precipitated with
15% trichloroacetic acid and the purines were liberated by acid
hydrolysis and separated by HPLC on an MCH-5 15-cm 5 μm silica-
bonded C8 column (Varian Associates, Sunnyvale, CA) using a gradient
of 100% (0.1 M KH2PO4 (pH 5.8) to 75% 0.01 M KH2PO4/25% methanol run over 15 min) (20). To compare alkytransferase
activity in different tissues, activity was expressed relative to DNA content of
the cell extract (21). One unit of alkytransferase activity was defined as
removal of 1 fmol of O'-methylguanine/μg cellular DNA. This
expression adjusts for changes in DNA content of the cell that occur
during the cell cycle and during DNA synthesis.

Hematopoietic Stem Cell Assay. CFU-GM were detected as described
previously for mouse (31) and rat (32) bone marrow cells. Briefly, 1 × 108
density mononuclear bone marrow or spleen cells were added to a
200 μl assay mixture of 0.5% agar, 0.2 ml fetal calf serum, and 0.3 M
double concentration Dulbecco’s modified Eagle’s medium supple-
mented with 15 μg/ml DEAE-chloride, and 20 μg/ml asparagine in 30-
mm Lux gridded tissue culture plates (Miles Laboratories, Naperville,
IL). The plates were incubated at 37°C in 5% CO₂ in a fully humidified
atmosphere for 7 days. Colony stimulating activity was provided as
either 10% pokeweed mitogen-rat spleen cell conditioned medium for
rat cultures (32) or 10% endotoxin treated mouse lung conditioned
medium for mouse cultures (31). Colonies were counted and confirmed
morphologically as described previously (23).

RESULTS

Alkytransferase Activity during Bone Marrow Transplantation
in Mice. We have shown previously that the level of
We studied bone marrow cell regeneration following bone marrow alkyltransferase activity in normal murine bone marrow is only 15% of that present in liver (21). To determine the relationship between this low level of alkyltransferase and cell proliferation, we studied bone marrow cell regeneration following bone marrow transplantation. The control and posttransplant proliferation of hematopoietic stem cells (CFU-GM), early hematopoietic progenitor cells, and total cell number in both bone marrow and spleen, a site of early regenerative hematopoiesis, are shown in Table 1. The increase in CFU-GM occurred between days 3 and 6 in the bone marrow and days 4 and 8 in the spleen. Total cellularity began to increase between days 5 and 8 in both organs. During regeneration, the spleen is largely replaced by hematopoietic precursors.

The relationship between [3H]thymidine incorporation into DNA and alkyltransferase activity in regenerating bone marrow and spleen cells is shown in Figs. 1 and 2. [3H]Thymidine incorporation fell sharply from control values on day 1 posttransplant (Fig. 1). During cell proliferation (days 3 to 8) [3H]thymidine incorporation increased in both bone marrow and spleen. However, throughout this time period, hematopoietic cells maintained persistently low levels of alkyltransferase activity (Fig. 2). This activity ranged from 0.7 ± 0.3 to 2.2 ± 0.6 units in the bone marrow and from 0.6 ± 0.3 to 1.3 ± 0.4 units in the spleen. Although there is induction of alkyltransferase activity in both organs, the maximal level of activity remains low relative to liver.

While regenerating hematopoietic cells maintained low levels of alkyltransferase activity, the liver in the transplanted animals underwent a significant increase in alkyltransferase activity (Fig. 2) from 5.1 to a maximum of 15.2 units on day 4. This increase was due to the radiation exposure these animals received (825 rads but not transplanted. In contrast to the results in liver, there was no increase in bone marrow alkyltransferase activity. However, throughout this time period, hematopoietic cells maintained persistently low levels of alkyltransferase activity (Fig. 2). This activity ranged from 0.7 ± 0.3 to 2.2 ± 0.6 units in the bone marrow and from 0.6 ± 0.3 to 1.3 ± 0.4 units in the spleen. Although there is induction of alkyltransferase activity in both organs, the maximal level of activity remains low relative to liver.

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Effect of MNU on Alkyltransferase Activity in Rats and Mice. Having shown that normal bone marrow maintains relatively low levels of alkyltransferase activity even during periods of rapid cell proliferation, we determined the effect of nitrosoureas on this relationship. We hypothesized that the dose of MNU shown to be leukemogenic in rodents (35 mg/kg, given 5 times at 14-day intervals (2-4)) would be sufficient to generate enough 0'-alkylguanine adducts to inactivate the alkyltransferase in hematopoietic cells. At the same time, we reasoned that nitrosoureas might induce a high rate of DNA synthesis in the bone marrow due to a proliferative burst such as that which occurs following other cytotoxic damage to the bone marrow (22). This combination would make bone marrow cells much more susceptible to the mutagenic effects of persistent 0'-alkylguanine adducts than other tissues which are not proliferating. To test this hypothesis, we studied rats and mice given a single 35-mg/kg i.v. dose of MNU. We compared the level of alkyltransferase activity relative to the level of cell proliferation in organs.
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with different susceptibilities to nitrosourea induced carcinogenesis: bone marrow (high susceptibility); kidney (intermediate), and liver (low).

MNU Treated Rats. Fig. 3 shows that total bone marrow cellularity fell within 1 day of MNU exposure, reaching a nadir at 2–4 days whereas CFU-GM were reduced to 10% of control at 2 h. CFU-GM recovered to base line at 2 days, and proliferation of these stem cells caused an increase in total cellularity between days 7 and 11. [3H]Thymidine incorporation in the bone marrow was depressed 2 h after MNU exposure (Fig. 4) but rebounded sharply to 3 times base line at 2 days. [3H]-Thymidine incorporation in liver DNA fell immediately after MNU exposure and had two peaks of activity that were twice base line at 1 and 7 days. In contrast, [3H]thymidine incorporation in the kidney fell at 2 to 5 hours post-MNU exposure, rose to basal activity levels at 2 days, but never rose above baseline. During recovery, maximal [3H]thymidine incorporation in the rat bone marrow was about 8.5-fold greater than that of the liver and kidney.

The levels of O6-methylguanine repair in bone marrow, liver, and kidney cells following MNU exposure are shown in Fig. 5. Basal alkyltransferase activity was 8.1 ± 1.9 units in rat liver compared to 2.2 ± 0.4 units in the kidney and 0.9 ± 0.1 units in the bone marrow. Alkyltransferase was inactivated 2 to 5 h after MNU exposure in each tissue, an indication that O6-methylguanine adducts have been formed and that some have been repaired leading to consumption of alkyltransferase activity. In liver, alkyltransferase activity recovered to 50% of control on day 1 and to basal levels by day 3. In kidney, alkyltransferase was depressed for 3 days and recovered on day 4, while bone marrow alkyltransferase remained at a low level for 11 days. The very low levels of activity noted in the kidney and bone marrow may indicate that alkyltransferase is synthesized at a low rate and/or consumed during repair of persisting O6-methylguanine adducts. We also measured the effect of repeated exposure to 35 mg/kg MNU i.v. on alkyltransferase activity. Animals were given injections of MNU at 14-day intervals and alkyltransferase activity was assayed following each dose of MNU. There was no induction of alkyltransferase activity in the blood cells during the repeated exposure to MNU (data not shown).

MNU Treated Mice. To determine whether these observations were limited to the rat or applied to other species, similar experiments were performed on CD-1 mice exposed to MNU (35 mg/kg). Bone marrow cell counts and hematopoietic colony forming cells (CFU-GM) were depleted after MNU exposure (Table 2). CFU-GM rebounded to 5 times base line by 11 days and [3H]thymidine incorporation recovered 3 days after exposure. Nonetheless, alkyltransferase activity, as indicated in Fig. 6, remained depressed at less than 1 unit from the time of MNU exposure throughout the recovery period. Mouse liver alkyltransferase activity was initially inactivated and then rebounded by day 2 to basal levels. Thus, mouse bone marrow, like rat bone marrow, remains at risk for mutagenic damage from O6-methylguanine adducts due to persistently low levels of alkyltransferase activity throughout the period of hematopoietic cell regeneration.

O6-Alkylguanine Repair to DNA Replication Index. We developed a quantitative index which compared tissues for the

Fig. 3. Bone marrow cell recovery following MNU exposure in the rat. Male Sprague-Dawley rats received 35 mg/kg MNU i.v. and the bone marrow was harvested at various time points. Top, total mononuclear cell count per bone; bottom, CFU-GM/10^5 mononuclear bone marrow cells; bars, SE.

Fig. 4. Organ specific [3H]thymidine incorporation following MNU exposure in the rat. Male Sprague-Dawley rats received 35 mg/kg MNU i.v. and then 50 μCi [3H]thymidine i.v. 1 h prior to sacrifice. DNA was extracted from each tissue and [3H]thymidine incorporation determined as described in “Materials and Methods.” Points, means of 4 replicate samples taken from a representative experiment; bars, SE.

Fig. 5. Alkyltransferase activity in rat tissues after MNU exposure. Tissues from rats treated as in Fig. 3 were assayed for alkyltransferase activity as described in “Materials and Methods.” Points, means of 4 experiments; bars, SE. O6mG, O6-methylguanine.

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CD-I mice were given injections of MNU, 35 mg/kg, at time 0. At the times indicated, the hematopoietic cells in the bone marrow were harvested, counted, and assayed for CFU-GM and [3H]thymidine incorporation.

The index is the C'°-alkylguanine repair to point mutations in replicating DNA rather than being removed by processes such as excision repair. The index was calculated as the ratio of alkyltransferase activity/µg cellular DNA to [3H]thymidine incorporated/µg DNA/h and has units of fmol O°-methylguanine/dpm/h. Animals were treated as described in Fig. 4.

The likelihood that O°-alkylguanine adducts would become sites of point mutations in replicating DNA rather than being removed prior to replication is the index. The index is the O°-alkylguanine repair to DNA replication index and was calculated as the alkyltransferase activity (units/µg cellular DNA) divided by the rate of [3H]thymidine incorporation (dpm/µg cellular DNA/h). A high index indicates that O°-alkylguanine adducts are more likely to be repaired than to be converted into point mutations, whereas a low index indicates that the tissue is at high risk for creation of mutations at sites of O°-alkylguanine adducts.

Changes in the index following MNU exposure in rat tissues are shown in Fig. 7. The index in normal bone marrow was only 14% that of kidney and only 1.6% that of liver. Although the index rose briefly in kidney and bone marrow between 2 and 5 h following MNU exposure, the divergence between tissues remained evident over the next 11 days. At 2 days, the point of maximal DNA synthesis in kidney and bone marrow, the index in bone marrow was 21% that of kidney and 1.1% that of the liver. The O°-methylguanine repair:DNA replication index in mouse bone marrow was similar to that in rat bone marrow during the 11 days following MNU exposure (data not shown).

**DISCUSSION**

A critical variable in cellular susceptibility to nitrosoureas appears to be the relationship between the capacity for repair of O°-alkylguanine and the rate of DNA synthesis. This is important for two reasons. (a) The repair of O°-alkylguanine occurs by stoichiometric transfer of the alkyl group to the repair protein O°-alkylguanine-DNA alkyltransferase (5, 10, 11). This inactivates the alkyltransferase such that a cell can remove as many O°-alkylguanine adducts as there are alkyltransferase molecules (5, 35). Excess adducts persist until new alkyltransferase molecules are synthesized (12, 36). Repair of these adducts will deplete newly synthesized alkyltransferase activity until the number of persistent adducts is low or absent (6, 9). (b) O°-Alkylnucleotide adducts are not mutagenic until base mispairing of O°-alkylguanine with thymine occurs during DNA replication, causing a point mutation in the daughter strand (3, 5). Consequently, the longer a cell has to repair the adduct prior to DNA replication, the less likely it is that a point mutation will occur.

To analyze the mechanism of mutagenesis by nitrosoureas in the bone marrow, we examined the relationship between alkyltransferase activity and DNA synthesis (measured as [3H]thymidine incorporation) during bone marrow regeneration. We have shown previously that rat, mouse, and human bone marrow cells have very low levels of alkyltransferase compared to most other organs (21). In this study, hematopoietic cells induced to proliferate following either bone marrow transplantation or exposure to a single dose of MNU had a sharp rise in [3H]thymidine incorporation but maintained very low levels of alkyltransferase activity relative to the level found in kidney or liver. The bone marrow in these animals contained levels of alkyltransferase activity that were as low as we have reported in any human, rat, and mouse tissues except rat brain (21).

We studied DNA repair capacity during regeneration of both normal and drug treated bone marrow. In the bone marrow transplantation experiments, rapidly proliferating hematopoietic cells induced their alkyltransferase activity only slightly, but the absolute level of activity remained low. Following MNU exposure, when O°-methylguanine adducts should be present in the DNA (37), the alkyltransferase activity in bone marrow was also very low. In both instances, proliferating hematopoietic cells failed to induce their alkyltransferase activity into the
range seen in cells which are resistant to nitrosoureas. To quantify the relative likelihood of mutagenic damage from nitrosoureas in different tissues, we developed the O\(^\alpha\)-methylguanine repair to DNA replication index. This index reflects the likelihood that an O\(^\alpha\)-methylguanine adduct will be repaired rather than becoming the site of a point mutation during DNA synthesis. Following MNU exposure in the rat, the index was high in liver, intermediate in kidney, and very low in bone marrow. Thus, although the alkyltransferase activities in bone marrow and kidney were similar for the first 72 h after MNU exposure, the greater rate of DNA synthesis in bone marrow indicates that these cells are at a greater risk for mutagenic damage. On the basis of these data, we propose that the bone marrow is a target organ for malignant transformation following nitrosourea exposure because of the disparity between repair of O\(^\alpha\)-alkylguanine adducts and DNA synthesis, particularly in the period following nitrosourea exposure. The rapid proliferation of hematopoietic cells within 48 h of exposure indicates that point mutations are likely to occur because persisting adducts have not had time to be repaired.

The high rate of cell proliferation in the bone marrow after nitrosourea exposure means that measurement of persisting adducts may greatly underestimate the presence of point mutations in the bone marrow. Other investigators have directly measured persisting O\(^\alpha\)-alkylguanine adducts to predict mutagenic damage from nitrosourea exposure (16, 37) and have found a good correlation between persisting adducts and carcinogenic risk. Studies of persistent adducts in bone marrow have not been performed beyond 18 h (37). Our data indicate that there will be dilution of persistent adducts in bone marrow cells due to the high rate of DNA synthesis and cell proliferation even in the absence of repair. This dilutional effect causes an apparent decrease in persistent adducts even though point mutations are being formed in the newly synthesized daughter strands of DNA. For this reason, the concentration of persistent adducts may not accurately reflect either the repair capacity of the cell or the risk for mutagenic damage.

Our studies do not establish why the incidence of leukemia increases with multiple doses of nitrosoureas. This is most likely due to cumulative mutagenic damage in regenerating hematopoietic precursors and the need for more than one mutagenic event (for instance, activation of multiple oncogenes) for leukemogenic transformation. Myeloid leukemias do occur at a low frequency in thymectomized mice exposed to a single dose of MNU (36). In pregnant mice, a single dose of N\(^\beta\)-ethyl-N\(^\gamma\)-nitrosourea will induce leukemia in offspring (38). Huggins and Veda (39) observed a higher incidence of leukemia in older (100-day) male rats (the age of the animals used in our experiments) than in younger rats. In younger animals a variety of tumors, in particular mammary tumors, develop whereas in older (100-day) male rats (the age of the animals used in our experiments) tumors, in particular mammary tumors, develop whereas in older (100-day) male rats, leukemia predominates (2). Our data indicate that the schedule used by Huggins (5 doses at 14-day intervals) to MNU might cause greater mutagenic damage to the bone marrow than the Huggins protocol because subsequent MNU exposure would occur when DNA synthesis and stem cell proliferation were above normal.

These results suggest that the combination of rapid cell proliferation and the absence of DNA repair may be critical events in the leukemogenic process initiated by a number of compounds. Bone marrow may be unique in the degree to which DNA damage induces cell proliferation. In addition, it remains a target organ for carcinogenesis throughout life because it retains the capacity for rapid regeneration following DNA damage (39) and the hematopoietic stem cells may accumulate damage during repeated exposures because of their longevity (40). This may also be the leukemogenic mechanism of benzene (41), irradiation (42), and other alkylating agents (22) in addition to the nitrosoureas.

There are as yet no known methods to induce the alkyltransferase activity in hematopoietic cells. Rat liver alkyltransferase activity is induced after exposure to a variety of toxins (39), other N-nitroso compounds (7), and irradiation (33) and following partial hepatectomy (19). However, other species and organs are less responsive to alkyltransferase induction by nitrosamines (7, 33) or irradiation (33). Nontoxic methods of inducing endogenous alkyltransferase activity have not been reported. Because alkylating agent-induced leukemia remains an important clinical problem, further studies on induction of the alkyltransferase in the bone marrow are needed.

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REFERENCES

15. Goth, R., and Rajewsky, M. F. Persistence of O\(^\alpha\)-ethylguanine in rat-brain...


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