ABSTRACT

The present studies were designed to evaluate the role of cell cycle time and time of drug administration on the persistence of sister chromatid exchange (SCE)-inducing lesions in normal and lymphoma second- and third-division AKR bone marrow cells.

Normal second-division cells harvested from mice given injections of BCNU at the start of an 18-, 24-, or 28-hr 5-bromo-2-deoxyuridine (BrdUrd) infusion exhibited similar linear dose-dependent increases in SCE frequencies (p > 0.05). The faster-cycling lymphoma cells, harvested after 18-hr BrdUrd infusion, had significantly higher baseline (p < 0.05) and BCNU-induced increases (p < 0.001) in SCE frequencies than did normal cells.

Dose-dependent increases in SCE frequencies were demonstrated in third-division normal and lymphoma cells from mice infused with BrdUrd for 24 or 28 hr. Whereas lymphoma cells from mice treated with 3.3 mg BCNU per kg exhibited 31.2 ± 3.9 (S.E.) SCEs in second-division cells and 4.7 ± 0.4 reciprocal and 22.9 ± 2.0 nonreciprocal SCEs in third-division cells, a 5 times higher dose of BCNU was required to induce similar levels of 30.0 ± 0.8 SCEs in second-division cells and 4.4 ± 0.6 reciprocal and 22.6 ± 1.2 nonreciprocal SCEs in third-division normal cells.

BCNU dose-dependent increases in SCE frequencies were also observed following injection of BCNU 8 hr after the start of BrdUrd infusion. The unexpectedly higher levels of SCEs for both normal and lymphoma cells by this treatment protocol may be due to SCEs occurring at the same site in successive divisions in BrdUrd. Regardless of the protocol used, lower nonreciprocal SCE frequencies were observed in third-division cells relative to SCE frequencies in second-division cells; a possible consequence of the cytotoxicity of BCNU. Injection of BCNU produced significant changes in the proportions of normal and lymphoma cells completing one, two, and three or more divisions in BrdUrd.

Lymphoma cells were consistently more sensitive to the specific type(s) of BCNU-induced damage leading to SCEs and cell death than were normal cells. These studies indicated that differences in SCE response were not due to cell cycle time, time of drug administration, or potential for repair. It is therefore suggested that increased sensitivity of lymphoma versus normal cells may be due to increased cellular uptake of BCNU.

INTRODUCTION

We have described an AKR(Rb6.15)1Ald lymphoma model which provides a unique means of studying drug-induced DNA damage and repair, as reflected by SCE induction and cytotoxicity in normal and lymphoma cells in the same host. In preliminary studies using this model, it was found that the faster-cycling lymphoma cell population (identified by the presence of 2 metacentric chromosomes) demonstrated a 3-fold higher SCE response relative to simultaneously harvested normal bone marrow cells following 1-hr i.v. infusion of 4.4 mg BCNU per kg (1). Since cell cycle time may alter SCE response, the present studies were designed to evaluate the role of cell cycle time and time of drug administration on SCE frequencies in lymphoma and normal second- and third-division cells.

In third-division cells, SCEs appear as either nonreciprocal or reciprocal SCEs. Nonreciprocal SCEs, recognized as asymmetrical disruptions of the staining pattern, reflect SCEs that have occurred in the first or second cycle (9). The frequency of nonreciprocal SCEs in third-division cells should therefore be the same as observed in second-division cells if they are progeny of the same initially exposed population. Reciprocal SCEs, recognized as symmetrical SCEs between the 2 chromatids, are assumed to reflect SCEs that have occurred in the third division as a result of new lesions or persistent unrepaired DNA lesions inducing an SCE for the first time in the third cycle (10). Thus, in the present study, analysis of reciprocal SCEs in third-division cells is used to evaluate the relative extent of repair in normal versus lymphoma cells.

MATERIALS AND METHODS

Chart 1 illustrates the experimental design used in these studies. AKR/J tumor passage recipients were given injections of 1 x 10⁶ spleen cells from a leukemic donor 3 days prior to BrdUrd infusion. Mice were continuously infused with BrdUrd (8.1 mg/ml/hr; Sigma Chemical Co., St. Louis, Mo.) for 18, 24, 28, and 33 hr (Chart 1, 1, 2, 3, and 4) to analyze cells with corresponding cell cycle times of 9, 12, or 14 hr. Bone marrow cells were harvested 2 hr after i.p. injection of colchicine at the corresponding infusion times. Treated mice were administered 1.1 to 16.5 mg BCNU (Bristol-Myers, Syracuse, N. Y.) diluted in 0.9% NaCl solution per kg as an acute i.v. injection through the reagent line according to 3 treatment protocols. In Protocol I, mice were given injections of BCNU at the start of BrdUrd infusion. Protocol II injection times were optimized to expose (a) lymphoma cells between the first and second cycles in BrdUrd or (b) normal cells between the first and second cycles in BrdUrd. For Protocol III, BCNU was given by

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The abbreviations used are: SCE, sister chromatid exchange; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; BrdUrd, 5-bromo-2-deoxyuridine.
BCNU-induced SCEs in Normal and Lymphoma Cells

RESULTS

Protocol I. The following experiments were designed to compare SCEs induced in 2 successive cycles in normal and lymphoma cells. Reciprocal SCEs in third-division cells were evaluated to determine whether SCE-inducing lesions could persist through 3 cell cycles following exposure to BCNU.

Second-division normal metaphase cells, harvested from mice infused with BCNU for 18, 24, and 28 hr, exhibited linear BCNU dose-dependent increases in SCE frequencies (R² = 0.83, 0.78, and 0.98). While the 3 regression lines were not coincident (0.025 < p < 0.05), neither the slopes nor intercepts were significantly different in normal cell populations analyzed at the 3 infusion times (1 to 4) corresponded to 18 (1), 24 (2), 28 (3), or 33 (4) hr of BCNU infusion.

At the earliest cell harvest time (Protocol I, 18-hr BCNU), adequate numbers of second-division metaphase cells were found only at the earliest cell harvest time (Protocol I, 18-hr BCNU). Multiple regression analysis indicated that lymphoma cells had significantly higher baseline [5.7 ± 0.2 (S.E.) versus 4.2 ± 0.2 SCEs/cell, p < 0.05] and BCNU-induced (Chart 3, R² = 0.89) increases in SCE frequencies than did normal cells (p < 0.001). Whereas lymphoma cells from mice treated with 3.3 mg BCNU per kg exhibited a mean of 31.2 ± 3.9 SCEs/cell, p < 0.05] and BCNU-induced (Chart 3, R² = 0.89) increases in SCE frequencies than did normal cells (p < 0.001). At all concentrations less than 11.0 mg BCNU per kg, the reciprocal SCE frequency in normal cells was not increased over base-line levels. A dose of 3.3 mg BCNU per kg induced 4.7 ± 0.4 reciprocal and 22.9 ± 0.2 nonreciprocal SCEs in lymphoma cells. A 5-fold increase in dose to 16.5 mg BCNU per kg induced similar levels of 4.4 ± 0.6 reciprocal and 22.6 ± 1.2 nonreciprocal SCEs in normal cells. Elevated reciprocal SCE frequencies in normal and lymphoma third-division cells indicated the persistence of BCNU-produced SCE-inducing lesions through 3 cycles.

Comparisons of SCE frequencies in second-division metaphase cells with nonreciprocal SCE frequencies in third-division metaphase cells were made by multiple regression analy-
sis. For both normal and lymphoma cells, baseline SCE frequencies in second-division cells were similar to baseline non-reciprocal SCE frequencies in third-division cells ($p > 0.05$). However, BCNU-induced SCE frequencies in both lymphoma and normal second-division cells were significantly higher than the frequency of nonreciprocal SCEs observed in third-division cells (lymphoma cells, $p < 0.025$; normal cells, $p < 0.001$).

**Protocol II.** In order to evaluate initial extents of damage in normal and lymphoma cells, BCNU was injected at the beginning of the second cycle. SCE frequencies in second-division cells should therefore represent SCEs occurring in the first posttreatment cycle.

Baseline SCE frequencies in normal and lymphoma cells were similar to those observed in Protocol I. BCNU dose-dependent increases in SCE frequencies were observed in second-division normal and lymphoma cells following treatment according to Protocol II (Charts 2 and 3). Induced SCE frequencies in second-division normal cells were independent of times of BCNU injection (8 or 11 hr) and of BrdUrd infusion (18 or 23 hr). Slopes of Protocol II dose-response curves for second-division lymphoma and normal cells were both significantly greater ($p < 0.01$) than the corresponding slopes from Protocol I. These results are not consistent with the assumption that SCEs are simply a cumulative sum of SCEs occurring in the first and second cycles in BrdUrd. For example, if complete repair occurs prior to the second posttreatment cycle, Protocol II would be similar to Protocol I. Alternatively, if new SCEs were induced in the second posttreatment cycle, Protocol I would be greater than Protocol II.

Incomplete repair, as indicated by persistence of SCE-inducing lesions in the second posttreatment cycle, was detected as increased reciprocal SCE frequencies in third-division normal and lymphoma cells. In Protocol II, increased reciprocal SCE frequencies were observed in third-division lymphoma cells following exposure to 1.1 mg BCNU per kg ($t$ test, $p < 0.01$) and in third-division normal cells exposed to 11.0 mg BCNU per kg (one-way analysis of variance, $p < 0.01$), as shown in Table 1.

**Protocol III.** To determine whether BCNU-induced SCE lesions could persist over longer periods of time in dividing cells, a 26 hr delay was used prior to BrdUrd infusion. In mice infused with BrdUrd for 18 hr 26 hr after i.v. injection of 4.4 mg BCNU per kg, SCE levels were indistinguishable from baseline, there were subpopulations of cells that had high SCE frequencies.

### Table 1

| Dose BCNU (mg/kg) | Time BCNU administered (hr) | Total BrdUrd infusion (hr) | SCEs/cell | Normal | | Lymphoma | | Reciprocal | Nonreciprocal | Reciprocal | Nonreciprocal |
|-------------------|-----------------------------|-----------------------------|-----------|--------|--------|-----------|-----------|-----------|-----------|-----------|
| 0                 | 24                          | 24                          | 1.6 ± 0.3 | 3.9 ± 0.02 (2) | 1.3 ± 0.9b | 5.8 ± 0.3 (5)c |
| 1.1               | 8                           | 23                          | 1.9 ± 0.3 | 7.6 ± 0.3 (4)  | 3.4 ± 0.5  | 13.8 ± 0.8 (3) |
| 4.4               | 11                          | 34                          | 2.3 ± 0.2 | 11.0 ± 1.2 (3) | 7.1 ± 0.5  | 8.0 ± 0.4 (3) |
| 11.0              | 8                           | 34                          | 3.8 ± 0.3 | 14.4 ± 1.2 (4) | 15.4 ± 2.0 | 8.7 ± 1.2 (1) |

a Twenty-five metaphases scored for each cell type.
b Mean ± S.E.
c Numbers in parentheses, number of mice.
and BrdUrd plus BCNU treatment groups. In contrast to the decreased percentages of lymphoma versus normal metaphase cells, the cytotoxic effects of BCNU and BrdUrd did not appear to be synergistic in increasing survival of the host.

DISCUSSION

Many dividing normal bone marrow cells and almost all dividing AKR(Rb6.15)1Aid lymphoma cells complete one cell division cycle in approximately 8 hr of BrdUrd infusion (1). After 11 hr of infusion, almost all normal cells complete one cycle, and some lymphoma cells complete 2 cycles of BrdUrd incorporation. Since neither lymphoma nor normal cells are synchronously dividing cell populations, subpopulations of both cell types are expected to be in various cell cycle phases at the time of BCNU administration. By continuing BrdUrd infusion for increasing periods of time, SCEs in second- and third-division normal and lymphoma cells could be evaluated and time of BCNU administration optimized to correspond to approximate cell cycle intervals for normal and lymphoma cells.

Cell cycle time does not appear to be primarily responsible for the consistently lower responses of normal cells relative to lymphoma cells observed over all BCNU doses since, within the normal cell population, BCNU-induced SCE frequencies did not vary significantly over cell cycle times of 9, 12, and 14 hr. Likewise, comparable repair capacities of normal and lymphoma cells are suggested by third-division cell data. The fact that significantly increased reciprocal SCE frequencies relative to baseline were induced by BCNU in both Protocols I and II implies that complete repair of SCE-inducing lesions did not occur prior to the third (Protocol I) or second (Protocol II) posttreatment cycle. A 5-fold higher BCNU dose was required for normal cells to exhibit second-division and third-division reciprocal and nonreciprocal SCE frequencies comparable to those in corresponding lymphoma cells. Reciprocal SCEs in third-division cells are assumed to result from persistent lesions and from new lesions induced in the third cycle (10). However, induction of new lesions is unlikely in view of the short half-life of BCNU of less than 15 min (5). Rather, it appears that lymphoma cells are simply more sensitive to specific type(s) of BCNU-induced lesions leading to SCEs and given the same amount of initial damage, similar extents of repair are apparent by the third cell cycle. Whereas chemical decomposition of BCNU to carbonium ions and isocyanate molecules is thought to occur in situ, the increased sensitivity of lymphoma versus normal cells may be due to selective uptake of BCNU in lymphoma cells (11).

Increased SCE frequencies in lymphoma, as compared to normal cells, were observed for all BCNU administration and BrdUrd infusion protocols used. Unexpectedly, in both cell populations, Protocol II yielded higher levels of SCEs than did Protocol I. Regardless of protocol, nonreciprocal SCE frequencies in third-division cells were generally lower than corresponding SCE frequencies in second-division cells with similar cell cycle times. Populations of cells analyzed after exposure to BCNU and BrdUrd infusion for increasing periods of time are a select subpopulation of cells that are able to continue dividing. Because our studies indicate BCNU to be cytotoxic, frequencies of SCEs observed in third-division cells may not correspond to SCE frequencies in second-division cells. In addition, lower SCE frequencies in Protocol I relative to Protocol II may result simply because cells had to undergo a second division leading to selection of less damaged cells. Likewise, an enhanced sensitivity of BrdUrd-substituted DNA (i.e., Protocol II) could be responsible for observed relative responses.

Table 2

<table>
<thead>
<tr>
<th>Dose BCNU (mg/kg)</th>
<th>Length of BrdUrd infusion (hr)</th>
<th>Normal cells</th>
<th>% of lymphoma cells in 100 consecutive metaphase cells examined</th>
<th>Lymphoma cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st division</td>
<td>2nd division</td>
<td>3rd division</td>
<td>1st division</td>
</tr>
<tr>
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<td>18</td>
<td>39</td>
<td>61</td>
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<td>59</td>
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</table>

*Italicized distributions significantly different from control (χ²; p < 0.05).
An alternative explanation is suggested by the hypothesis that persistent DNA lesions may induce an SCE at the same locus in successive cell cycles. SCEs that occur at the same locus in the first and second cycles would be cancelled (8). Persistent cross-links must, of necessity, induce same locus SCEs (8). BCNU is known to induce DNA cross-links (4). If BCNU-induced cross-links are able to persist for more than one cycle, estimates of SCEs induced in the first cycle after BCNU exposure (Protocol II) may, therefore, be higher than the cumulative total of SCEs expected to occur over the first plus second cycles (Protocol I) (10). Although the exact role of cross-links in SCE induction remains to be clarified, the same-locus hypothesis is supported by studies of Conner and Cheng in which ethyl carbamate produced considerably lower SCE levels when administered by Protocol I than by Protocol II, with no evidence of cytotoxicity or enhanced sensitivity of BrdUrd.

In this study, normal AKR/J dividing cells, critical targets of antineoplastic agents, were susceptible to BCNU-induced SCEs. Two days after exposure to 4.4 mg BCNU per kg, baseline SCE frequency distributions were observed in lymphoma cells, whereas a small proportion of normal cells had increased SCE frequencies. The bone marrow has a complex renewal system which prevented us from determining whether or not the observed normal cells had divided continuously since the time of exposure to BCNU. Nevertheless, since persistence of lesions has been associated with neoplastic transformation (6) and increased SCE frequency may be correlated with tumorigenicity of certain chemicals (2), the observation of a subpopulation of normal cells with persistent SCE-inducing lesions indicates a potential risk for development of second cancers at certain dose levels of BCNU. The demonstrated differential sensitivity of lymphoma and normal cells might permit determination of a BCNU dose, which in this model is effectively toxic to lymphoma cells (and increases survival of the host) and still is nongenotoxic to normal cells. Whereas increased SCE frequencies generally paralleled increased susceptibility to the cytotoxic effects of BCNU in lymphoma cells, increased sensitivity to BrdUrd was also apparent. Further investigations into combined effects of chemotherapeutic agents, such as BCNU and BrdUrd, are required in order to more rigorously evaluate the significance of increased SCE frequencies as they relate to cytotoxicity and genotoxicity in lymphoma and normal cells.

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REFERENCES

Cellular Replication Kinetics and Persistence of Sister Chromatid Exchange-inducing Lesions in Normal and Lymphoma AKR Cells following Exposure to 1,3-Bis(2-chloroethyl)-1-nitrosourea

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