Sensitivity of Human and Murine Hemopoietic Precursor Cells to Chemotherapeutic Agents Assessed in Cell Culture

By Makio Ogawa, Daniel E. Bergsagel, and E. A. McCulloch

The sensitivity of human and murine hemopoietic precursor cells to 1,3 bis (2 chloroethyl)-1-nitrosourea (BCNU), nitrogen mustard, 5-fluorouracil, and melphalan was assessed in culture using colony assay techniques. The results indicate that human and murine hemopoietic cells may differ in their intrinsic sensitivity to drugs. The hematologic toxicity of some chemotherapeutic agents cannot be extrapolated directly from mouse to man.

M U S E MODEL SYSTEMS are frequently used to assess the effects of chemotherapeutic agents prior to clinical trial. This usage is based on the assumption that findings in the mouse are applicable to man. This assumption can be tested since very similar assay procedures are available for human and murine granulopoietic progenitors in culture (CFU-C). Accordingly, we used the cell culture method to assess the sensitivity of human and murine CFU-C to melphalan, nitrogen mustard, 5-fluorouracil (5 FU), and 1,3 bis (2 chloroethyl)-1-nitrosourea (BCNU). Significant species differences were encountered.

MATERIALS AND METHODS

Drugs

Injectable melphalan was purchased from Burroughs Wellcome & Co., London, England. Melphalan, 100 mg, was dissolved in 10 ml sterile propylene glycol at temperatures between 60°C and 70°C and for a 1-wk period was used as a stock solution. BCNU was obtained through the courtesy of The National Cancer Institute, Bethesda, Md., and kept at -40°C. When used it was dissolved first in ethyl alcohol, then in distilled water. Nitrogen mustard was purchased from the Boots Co., Nottingham, England. 5 FU was purchased from Hoffmann-La Roche Ltd., Montreal, Canada. Subsequent dilutions of all agents were made in 0.9%, NaCl solution.

Human Marrow Cells

The study is based on marrow specimens taken from a healthy volunteer and 11 patients during the course of hematological assessment. Only patients whose marrow examinations were normal, other than absence of iron, and who had not received chemotherapy, were selected for this study. Table 1 contains the clinical information and results of CFU-C assays for these patients. We did not observe any specific effect of disease on marrow colony-forming ability or sensitivity to chemotherapeutic drugs; consequently, these marrows were considered to be suitable for com-
Table 1. Sources of Human Marrow: Clinical and Cell Culture Data

<table>
<thead>
<tr>
<th>Drug</th>
<th>Diagnosis</th>
<th>WBC/CU pm</th>
<th>PMN (%)</th>
<th>Marrow Morphology</th>
<th>CFU-C/10^5 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>Lung cancer</td>
<td>8,100</td>
<td>66</td>
<td>Normal</td>
<td>14</td>
</tr>
<tr>
<td>mustard</td>
<td>Iron deficiency anemia</td>
<td>9,300</td>
<td>67</td>
<td>No stainable iron</td>
<td>20</td>
</tr>
<tr>
<td>Lymphoma</td>
<td></td>
<td>7,900</td>
<td>58</td>
<td>Normal</td>
<td>42</td>
</tr>
<tr>
<td>Melphalan</td>
<td>Breast cancer</td>
<td>8,600</td>
<td>86</td>
<td>No stainable iron</td>
<td>41</td>
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<tr>
<td>Melanoma</td>
<td></td>
<td>8,100</td>
<td>56</td>
<td>Normal</td>
<td>34</td>
</tr>
<tr>
<td>Lymphoma*</td>
<td></td>
<td>10,600</td>
<td>78</td>
<td>Normal</td>
<td>12</td>
</tr>
<tr>
<td>Normal†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCNU</td>
<td>Blood loss anemia</td>
<td>7,400</td>
<td>76</td>
<td>Normal</td>
<td>51</td>
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<td>Renal failure</td>
<td></td>
<td>8,300</td>
<td>89</td>
<td>Normal</td>
<td>20</td>
</tr>
<tr>
<td>Normal†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 FU</td>
<td>Lymphoma</td>
<td>7,800</td>
<td>60</td>
<td>Normal</td>
<td>19</td>
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<tr>
<td>Iron deficiency anemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma*</td>
<td></td>
<td>10,600</td>
<td>78</td>
<td>Normal</td>
<td>12</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td></td>
<td>4,600</td>
<td></td>
<td>Normal</td>
<td>84</td>
</tr>
</tbody>
</table>

*Marrow used for survival curves for both melphalan and 5 FU.
†Marrow from a volunteer used for survival curves for both melphalan and BCNU.

parison with normal mouse marrow in the survival curve studies reported in this paper. From 2 to 5 ml of marrow was aspirated, mixed with 0.2 ml of heparin (1000 u/ml without preservative), (Connaught Medical Research Laboratories, Toronto, Canada) and centrifuged for 3.5 min at 150 g. The buffy coat was removed, washed, and suspended in alpha medium1 (Flow Laboratories, Rockville, Md.) before incubation with various drug concentrations. For experiments with 5 FU, alpha medium without nucleosides was used.

Assays for Human CFU-C

The technique of Iscove et al.2 was used with minor modifications, consisting of the substitution of alpha medium for McCoy's 5A and omission of bovine serum albumin. Colony-stimulating activity (CSA) was obtained from fluid overlying normal peripheral leukocytes immobilized in agar at a concentration of 10^6/ml and incubated for 7 days. Cells were cultured in the presence of 0.8%, methylcellulose, 20%, CSA, and 20%, fetal calf serum at 37°C in a moist atmosphere flushed with 7.5%, CO2. After 10 14 days of incubation, three morphologically distinct colony types were seen.3 Only granulocyte and eosinophil colonies containing more than 20 cells were scored. Colonies containing large vacuolated peroxidase-negative cells were excluded from the counts because their loose arrangement often made them difficult to distinguish from the background. Further, the significance and origin of these colonies are unknown; unlike morphologically similar colonies from mouse marrow4 their origin from granulocyte colonies has not been observed. This counting convention has also been used by Brown et al.5

Assays for Mouse CFU-C

Marrow was obtained from femoral shafts of F1 hybrids between C57BL/6JOCI and C3H/He-JOC1 (C3B6F1) and BALB/c mice from Jackson Laboratories. No strain differences were observed, and the source of cells will not be designated separately in the experiments. CFU-C was measured by the method of Worton et al.6 modified by the substitution of alpha medium for CMRL-1066 and of 20%, horse serum for fetal calf serum and omission of bovine serum albumin. CSA was derived from medium conditioned by mouse L cells.

Exposure to Drugs In Vitro

Bone marrow cells at a concentration of 10^6 nucleated cells/ml were incubated for 1 hr at 37°C with varying concentrations of the drugs under test. Incubation was carried out in the absence of nucleosides for 5 FU. The cell suspensions were washed twice in alpha medium after exposure and then plated for CFU-C as described above.
Fig. 1. (left) Sensitivity of human and mouse CFU-C to BCNU. Bone marrow cells were exposed to the drug in culture for 1 hr and then tested for the surviving fraction of CFU-C. The dotted survival curve for mouse CFU-C was taken from previously reported experiments. Circles represent the means of the surviving fractions of each human bone marrow listed in Table 1. Triangles represent the surviving fractions of mouse CFU-C. Closed symbols indicate simultaneous experiments for human and mouse bone marrow. D$_{37}$ (the dose reducing survival to 37% of initial population) is 23.5 μM/liter and 31.3 μM/liter for human and mouse CFU-C, respectively. A statistical assessment based on linear regression analysis revealed no significant difference between the two classes of cells.

Fig. 2. (right) Sensitivity of human and mouse CFU-C to nitrogen mustard. Bone marrow cells were exposed to the drug in culture for 1 hr and then tested for the surviving fraction of CFU-C. The dotted survival curve for mouse CFU-C was taken from previously reported experiments. Circles represent human, and triangles mouse CFU-C. Closed symbols indicate simultaneous experiments for human and mouse bone marrow. D$_{37}$ is 0.5 μM/liter for human and mouse CFU-C, respectively.

Irradiation Procedure
Marrow cells were irradiated with 2000 rads using a $^{60}$Co gamma ray source.

Statistical Analysis
For each group, four plates were prepared at each of three different cell concentrations. Values obtained from the plates did not deviate significantly from linearity ($p < 0.05$), and the intercept of the line obtained by the method of least squares did not differ significantly from zero (within 95% confidence limits).

RESULTS
The results are presented in Figs 1-4 in the form of survival curves with logarithm of percentage survival plotted against drug dose. The dotted survival curves for mouse marrow are taken from previously reported experiments; points shown for mouse marrow were obtained in experiments carried out simultaneously with measurements of human marrow survival. It may be seen that the new values for mouse marrow are in agreement with the previous data. The survival curves for melphalan are based on new data, and they include one simultaneous measurement of human and mouse drug sensitivity.

For BCNU the sensitivity of human and mouse cells is similar (Fig. 1); a statistical assessment based on linear regression analysis revealed no significant differences. For nitrogen mustard and 5 FU, human cells are more resistant.
Fig. 3. (left) Sensitivity of human and mouse CFU-C to 5 FU. Bone marrow cells were exposed to the drug in culture containing alpha medium without nucleosides for 1 hr and then tested for the surviving fraction of CFU-C. The dotted survival curve for mouse CFU-C was taken from previously reported experiments. Closed symbols indicate simultaneous experiments for human and mouse CFU-C. D37 is 2.38 mM/liter and 0.104 mM/liter for human and mouse CFU-C, respectively.

Fig. 4. (right) Sensitivity of human and mouse CFU-C to melphalan. Bone marrow cells were exposed to melphalan in culture for 1 hr and then tested for the surviving fraction of CFU-C. Circles represent human, and triangles mouse CFU-C. Closed symbols indicate simultaneous experiments for human and mouse CFU-C. D37 is 2.2 μM/liter and 4.2 μM/liter for human and mouse CFU-C, respectively.

than mouse cells (Figs. 2 and 3). In contrast, for melphalan, mouse CFU-C are more resistant than human CFU-C (Fig. 4).

In order to exclude the possibility that the apparent differences reflect non-specific carryover of agents, the following experiment was done. 2 × 10⁶ mouse marrow cells were exposed to the maximum dose of each of the drugs used to obtain survival curves. The cells were washed using the same procedure as for measurement of survival and then irradiated with 2000 rads to eliminate surviving colony-forming ability. Then these cells were mixed with 10⁵ normal mouse marrow cells, and the mixture plated. Controls consisted of mixtures of normal marrow and irradiated marrow not exposed to drug. The results are given in Table 2. It is evident that the presence of drug-exposed cells did not decrease colony formation. This finding makes it unlikely that significant carry over of free drug occurred.

It also seemed possible that the observed differences in survival were related to small but important variations in the conditions of drug exposure. Such differences seemed particularly likely in the case of 5 FU where small amounts of nucleosides remaining in the incubation step after the washing procedure

<table>
<thead>
<tr>
<th>Table 2. Carry-Over Study of Mouse Bone Marrow Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiated cells exposed to:</td>
</tr>
<tr>
<td>CFU-C/10⁵ cells (mean ± 1 SD)</td>
</tr>
</tbody>
</table>
might affect survival. It was feasible to test this possibility in a mixing experiment because of a specific difference in the culture systems for human and mouse CFU-C: CSA derived from human cells enhances colony formation by both human and mouse cells, while in contrast, CSA from murine sources will not sustain the growth of human cells. Accordingly, small numbers of human and mouse marrow cells were mixed and an aliquot exposed to 100 μg/ml of 5 FU; after the usual washing procedure (see Materials and Methods), the control and mixture exposed to drug were plated either with CSA derived from human or mouse sources. As additional controls, each component of the mixture was also plated with each source of CSA. The results are presented in Table 3. It is evident from the table that 100 μg/ml of 5 FU had little effect on colony formation by human cells but reduced colony formation by mouse cells to approximately 50% of control values. This value is in good agreement with the data for mouse cells depicted in Fig. 3. On the basis of the data on Table 3 we conclude that a different response of human and mouse CFU-C to 5 FU is a function of the cells rather than of the exposure procedure.

**DISCUSSION**

The results presented in this paper provide direct evidence that human and murine hemopoietic cells differ in their sensitivity to drugs. Further, no consistent pattern of difference was observed. Variations in cell cycle appear to be an unlikely explanation for the observations since the proportion of CFU-C in the DNA synthesis phase is similar in human and mouse marrow. Furthermore, previous studies of mouse CFU-C indicated that changes in cycle state were not reflected in changes in sensitivity to melphalan or nitrogen mustard. Regardless of the mechanism, these studies must be considered when extrapolating results obtained in the mouse to man. In contrast to ionizing radiation, where similar sensitivities were found for human and murine CFU-C, direct extrapolation appears to be unwarranted for chemotherapeutic agents. Further, species differences in metabolic rate may not be sufficient to permit extrapolation, although this has been suggested by the National Cancer Institute Chemotherapy Drug Development Program. Rather, specific measurements, such as those provided in this paper, are required.

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REFERENCES


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