Sensitivity of Bone Marrow Hematopoietic Colony-forming Cells from Mice, Dogs, and Humans to Carminomycin, Marcellomycin, Aclacinomycin A, and N,N-Dibenzyl-daunorubicin and Its Relationship to Clinical Toxicity

John C. Marsh, Barbara J. Brown, and Marianne M. Nierenburg

Departments of Internal Medicine [J. C. M., B. J. B., M. M. N.] and Pharmacology [J. C. M.], Yale University School of Medicine, New Haven, Connecticut 06510

ABSTRACT

The sensitivity of bone marrow granulocyte-macrophage colony-forming cells to 4 anthracyclines, carminomycin, marcellomycin, aclacinomycin A, and N,N-dibenzyl-daunorubicin, was studied using the agar diffusion chamber technique which allows exposure of target cells to drug metabolized by the chamber-bearing host after i.v. injection. Colony-forming cells from mice, dogs, and humans were all found to have exponential dose-response curves for the agents studied, with variation of the slopes between species and agents. Species sensitivities as determined by the assay related well to the available toxicological and clinical data for specific drugs. The rank order of sensitivity of human marrow colony-forming cells to five anthracyclines tested in this and a previous study related very closely to doses producing moderate leukopenia in Phase I and II clinical studies. A dose of 200 mg/sq m of N,N-dibenzyl-daunorubicin would be expected to produce moderate leukopenia in future clinical trials. This assay may be useful in predicting human bone marrow toxicity of new agents before actual clinical trial because of the ability to study the survival of human colony-forming cells directly.

INTRODUCTION

The widespread use of the anthracyclines doxorubicin (3) and daunorubicin (5, 11) in cancer chemotherapy has led to the synthesis and/or purification of other anthracyclines in the hope that antitumor activity might be retained while toxicity, particularly cardiac toxicity, might be lessened. Although cardiac toxicity is, indeed, a serious problem in the clinical use of the anthracyclines, myelosuppression, particularly neutropenia and consequent infection, is probably responsible for more morbidity and mortality associated with their use. Toxicological studies in large and small animals have been used to assess the potential for adverse effects in humans as new agents are developed. Recently, we have used an assay (17, 21) to measure the effect of antineoplastic drugs on leukocyte-committed CFC* from mice, dogs, and humans in the hope of correlating these effects with toxicological and clinical hematological data. The assay allows in vivo metabolism of the drug under study and interaction of the agent with marrow suspended in agar in an ADC implanted in the peritoneal cavity of a mouse. We present in this study dose-response data on 4 anthracyclines, 3 of which (carminomycin, marcellomycin, and aclacinomycin A) are in early clinical trial. The fourth, B}_{2}D is in the preclinical phase of development and has not yet undergone full toxicological evaluation. Marcellomycin and aclacinomycin A are also of interest because they have recently been shown to be active inducers of differentiation of human acute promyelocytic leukemia cells (37). Doxorubicin and carminomycin, on the other hand, are not. Some of these data have been presented in preliminary form elsewhere (22, 24).

MATERIALS AND METHODS

Experimental Animals. Male and female CD-1 mice, 6 to 15 weeks old (Charles River Laboratories, Wilmington, Mass.), were used as marrow donors and as recipients for diffusion chambers. Healthy mongrel dogs were marrow donors.

Bone Marrow Preparation, Culture Technique, and Experimental Design. The procurement and processing of bone marrow and the use of the ADC assay have been described previously (21). Marrow from mice, dogs, and humans were exposed to drugs in the chamber, and mouse marrow was also exposed to each agent in situ. Colonies were counted at 7, 9, and 14 days respectively since optimal growth is seen at those times. One experiment was done for each species and each drug, except for B}_{2}D in which 2 experiments each were done for mouse marrow in situ and human marrow. Four or 5 mice were used for each drug dose, and each point of a dose-survival curve was derived from 4 or 5 chambers. The D60 value was used to compare the slopes of the dose-survival curves. This is analogous to the radiobiology term, D60, which is the dose of radiation required to reduce a cellular population to 37%.

Carminomycin, marcellomycin, and aclacinomycin A were gifts from Bristol Laboratories (Syracuse, N. Y.). Carminomycin was supplied as the hydrochloride. The 5 mg in a vial were dissolved with 5 ml of 0.9% NaCl solution, with which further dilutions were made. Marcellomycin tartrate was supplied in a 20-mg vial to which 10 ml of the same NaCl solution was added and in which further dilutions were made. Aclacinomycin A was supplied as the bulk powder. It was dissolved in 0.9% NaCl solution at a concentration of 2 mg/ml, and further dilutions were with the NaCl solution. B}_{2}D was supplied by SRI International (Menlo Park, Calif.) as the hydrochloride. It was dissolved in 66% polyethylene glycol 200 (J. T. Baker Chemical Co., Phillipsburg, N. J.) in 0.9% NaCl solution in a concentration of 8 mg/ml. Further dilutions were made in the same solution, of which control animals were also given injections. All drugs were injected i.v. into the recipient mice.

RESULTS

CFC

Carminomycin. Dose-response curves for bone marrow CFC from all 3 species were exponential (Charts 1 and 2), with mouse...
Sensitivity of Bone Marrow Cells to Anthracyclines

1.0 - 0.01

Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>D37 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse in situ</td>
<td>1.1</td>
</tr>
<tr>
<td>Mouse chamber</td>
<td>1.6</td>
</tr>
<tr>
<td>Dog chamber</td>
<td>1.0</td>
</tr>
<tr>
<td>Human chamber</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Chart 1. Dose-survival curves of dog and human bone marrow CFC in the chamber 18 hr after carminomycin. Points, mean of 4 or 5 chambers; bars, S.E.; some error bars have been omitted for clarity.

Chart 2. Dose-survival curves of mouse bone marrow CFC in the chamber and in situ 18 hr after carminomycin. Points, mean of 4 or 5 chambers; bars, S.E.; some error bars have been omitted for clarity.

JUNE 1983

Sensitivity of Bone Marrow Cells to Anthracyclines

1.0 - 0.01

Chart 1. Dose-survival curves of dog and human bone marrow CFC in the chamber 18 hr after carminomycin. Points, mean of 4 or 5 chambers; bars, S.E.; some error bars have been omitted for clarity.

marrow being least sensitive compared to dog and human marrow [D37 of 1.6, 1.0, and 1.1 mg/kg, respectively (Table 1)]. Mouse marrow in situ was somewhat more sensitive (D37 1.1) than that in the chamber.

Marcellomycin. Dose-response curves were exponential for marrow of all 3 species in the chamber (Charts 3 and 4) with D37 values of 10, 5, and 4.5 mg/kg for mouse, dog, and human marrow CFC, respectively (Table 1). Mouse marrow cells were, therefore, less sensitive than dog or human cells, as seen with carminomycin. Mouse marrow CFC in situ, however, were less sensitive than those exposed in the chamber, with a plateau at about 60% survival (Chart 4).

Aclacinomycin A. Dose-response curves over the range 5 to 50 mg/kg were exponential for marrow CFC in the chamber from all 3 species, with mouse marrow cells considerably less sensitive than those of either dog or human [D37 values of 34, 8, and 6 mg/kg, respectively (Table 1)]. Mouse marrow cells in situ were more sensitive (D37 12 mg/kg) than those in the chamber, and the dose-response curve was exponential.

B2D. Dose-response curves for B2D were all exponential over the range 5 to 30 mg/kg. The CFC from human marrow in the chamber were more sensitive than those from either mouse or dog marrow which were of equal sensitivity (Table 1). Mouse marrow cells in situ were considerably more sensitive than those in the chamber (D37 values of 9 and 10 versus 26).

Bone Marrow Cellularity

Femoral bone marrow cellularity in the in situ mouse experiments is shown in Table 2. Carminomycin was the most potent agent on a weight basis with respect to reduction of cellularity, while marcellomycin and aclacinomycin were least active and B2D was intermediate.

Relationship of Human Leukopenia-producing Doses to Marrow Dose-Response Curves

If the 4 anthracyclines studied plus 2 others reported previ-
DISCUSSION

This study has produced values for the slopes of dose-response curves for 4 anthracyclines which may be used to estimate the sensitivity of granulocyte-macrophage precursors of the 3 species studied to these agents. A similar study for doxorubicin (Adriamycin) and a derivative, AD32, was reported earlier (21). It is important to remember that the drug is presented to the marrow in the chamber as it is metabolized by the chamber-bearing mouse after i.v. administration and that differences in metabolism between species, different routes of administration or schedules, and different hematological determinations may make in vivo comparisons of species sensitivity difficult. For example, changes in mouse blood total leukocyte count may not reflect absolute neutrophil concentration as well as they do in dog or human blood, since neutrophils make up only about 30% of mouse blood (12) in contrast to dog and human blood, where they normally range from 60 to 80%.

Carminomycin is an anthracycline antibiotic which was developed (21) are ranked in ascending order of the $D_{37}$ value for human marrow obtained by the assay, a close relationship is observed to the dose reported to produce moderate leukopenia (3000/cu mm) in Phase I and II studies (Table 3). Carminomycin is the most potent, with marcellomycin next and AD32 the least potent. Aclacinomycin A and Adriamycin are intermediate, and their relationship is the only exception to the rank orders of potency for $D_{37}$ and leukopenia-producing doses being in agreement. B2D has not yet achieved clinical trial, but if it does one might predict that a dose on the order of 200 mg/sq m would produce moderate leukopenia.

Table 2

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Carminomycin</th>
<th>Marcellomycin</th>
<th>Aclacinomycin A</th>
<th>Adriamycin</th>
<th>B2D</th>
<th>AD32</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>50</td>
<td>105</td>
<td>89</td>
<td>102</td>
<td>102</td>
<td>73</td>
</tr>
<tr>
<td>5.0</td>
<td>113</td>
<td>104</td>
<td>94</td>
<td>106</td>
<td>106</td>
<td>77</td>
</tr>
<tr>
<td>10.0</td>
<td>107</td>
<td>105</td>
<td>89</td>
<td>102</td>
<td>102</td>
<td>73</td>
</tr>
<tr>
<td>15.0</td>
<td>110</td>
<td>104</td>
<td>94</td>
<td>106</td>
<td>106</td>
<td>77</td>
</tr>
</tbody>
</table>

* Pooled femoral cellularity of 5 mice/group.

Table 3

<table>
<thead>
<tr>
<th>Drug</th>
<th>$D_{37}$ (mg/sq m)</th>
<th>Leukopenia-producing (mg/sq m)</th>
<th>Median WBC</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carminomycin</td>
<td>3.3</td>
<td>20</td>
<td>2.9</td>
<td>13</td>
</tr>
<tr>
<td>Marcellomycin</td>
<td>13.5</td>
<td>40</td>
<td>3.0</td>
<td>29</td>
</tr>
<tr>
<td>Aclacinomycin A</td>
<td>18.0</td>
<td>100</td>
<td>3.0</td>
<td>10, 15</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>28.5</td>
<td>60-75</td>
<td>3.0</td>
<td>18, 30</td>
</tr>
<tr>
<td>B2D</td>
<td>45, 51</td>
<td>400</td>
<td>3.2</td>
<td>4</td>
</tr>
<tr>
<td>AD32</td>
<td>66</td>
<td>400</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**J. C. Marsh et al.**

Chart 3. Dose-survival curves of dog and human bone marrow CFC in the chamber 18 hr after marcellomycin. Points, mean of 4 or 5 chambers; bars, S.E.; some error bars have been omitted for clarity.

Chart 4. Dose-survival curves of mouse bone marrow CFC in the chamber 18 hr after marcellomycin. Points, mean of 4 or 5 chambers; bars, S.E.; some error bars have been omitted for clarity.
Marcellomycin is an anthracycline trisaccharide derived from the bohemian acid complex (28). It has significant activity against L1210 leukemia (7), B16 melanoma, Lewis lung carcinoma, and colon carcinoma 26 (33). RNA synthesis, particularly that of nucleolar RNA, is quite sensitive to the drug (33).

The effects of marcellomycin on the leukocyte count are shown in Table 4. Thus, on a surface area basis, the order of in vivo species WBC sensitivity following a single i.v. dose of marcellomycin appears to be human, dog, and mouse, which is found in this study for the sensitivity of macrophage-neutrophil precursors in the chamber.

Aclacinomycin A is an anthracycline trisaccharide developed in Japan (31, 32) which has recently entered clinical trial in this country. There is substantial antitumor activity and, like the other anthracycline analogues in this study, there is less animal cardiotoxicity, and the hope is that it may be less toxic to human hearts than are doxorubicin and daunorubicin. Phase I clinical trials have indicated that an intermittent single i.v. dose schedule will produce a median blood leukocyte nadir of 3,000/cu mm at a dose of 100 mg/sq m (10, 15). To our knowledge, canine and human data that would allow a comparison are not available, although a single i.p. dose of 47.4 mg/sq m in mice produced a severe, persistent leukopenia (20% of control at 5 and 7 days) (35).

A study of the effects of this agent on leukemic and normal CFC in mice has recently appeared (27). Bone marrow CFC in culture (granulocyte-macrophage precursor cells assayed in vitro) showed a similar sensitivity 24 hr after i.v. injection as the CFC in the ADC in our study at 18 hr, although only the dose range from 1 to 5 mg/kg was studied.

B2D is a recently developed anthracycline derivative which is more effective than the parent compound daunorubicin against P388 leukemia (1, 2) and less cardiotoxic in the rat (19). Metabolic studies in rats have suggested that it is a prodrug converted to several active metabolites. B2D is essentially inactive in vitro (2). To our knowledge, toxicological data for mice and dogs are not available, and there are no clinical data. The D37 values suggest that human macrophage-granulocyte precursors are more sensitive than those of mice or dogs which are of equal sensitivity. Future studies will be of interest to see whether this prediction is valid.

Differences in the D37 values obtained for mouse marrow CFC suspended in agar in the chamber and in situ are not readily explained. For Adriamycin, studied earlier (21), there is essentially no difference. The cells are more sensitive in situ to aclacinomycin A, B2D, and marcellomycin and less sensitive than those in the chamber to marcellomycin and AD32 (21). This variation has also been observed for other types of drugs, such as antimetabolites (17, 20, 23), and Vinca alkaloids (17). Since the chamber-in situ difference is not consistent between different drugs, multiple factors may play a role, including differences in adsorption to the agar. Gordon and Blackett (17) found changes in the dose-response curves for mouse marrow exposed to 5-fluorouracil and vinblastine with this technique when agar was omitted but found little change with cyclophosphamide.

As far as they can be compared with available in vivo data, the slopes of our dose-response curves for individual species and specific drugs are consistent with bone marrow toxicity studies in toxicological and clinical studies. Several of the drugs have not been studied appropriately in dogs or mice to allow the comparison, that is, studies which examine blood neutrophil concentration or even total leukocyte count after a single i.v. injection have not been done. The most impressive correlation is that derived from human studies comparing human marrow D37 values with doses producing the same degree of leukopenia (Table 3). With one exception in the intermediate range, the rank order for 5 anthracyclines correlates very well, suggesting that this assay would be useful for predicting the potential for leukopenia production in patients before clinical trials begin, with newly developed anthracyclines and perhaps with other agents. It is of interest to point out that a drug which produces no clinical or toxicological myelosuppression, spirogermanium, has no effect on the survival of CFC from any of the 3 species in the ADC assay (22). The variation in CFC sensitivity from the 3 species to specific drugs reported here and elsewhere (20–24) indicates that toxicological data alone may be misleading in predicting an effect on bone marrow.
appropriate human dose for some agents, at least as far as hematological toxicity is concerned. The very mild myelosuppression produced in mice by marcellomycin (6, 35) contrasts strongly with its potent activity in producing leukopenia in clinical trials (29).

Because of the ability to expose cells to drugs as they are metabolized, we have used this assay to study tumor CFC sensitivity to antineoplastic agents in melanoma, with excellent clinical correlation (25, 26). We have also compared it for a variety of solid tumors to the well-known in vitro technique.6 In addition, it has proven useful for comparing cytotoxicity to clonogenic cells with differentiation (36).

ACKNOWLEDGMENTS

We are grateful to Drs. William Bradner and John Schung of Bristol Laboratories for supplying marcellomycin, carminomycin, and aclacinomycin A and to Dr. John Peters of SRI International for supplying B2D. In addition, these workers provided much helpful information and discussion.

REFERENCES


Sensitivity of Bone Marrow Hematopoietic Colony-forming Cells from Mice, Dogs, and Humans to Carminomycin, Marcellomycin, Aclacinomycin A, and $N,N$-DibenzylDaunorubicin and Its Relationship to Clinical Toxicity

John C. Marsh, Barbara J. Brown and Marianne M. Nierenburg


Updated version  Access the most recent version of this article at: [http://cancerres.aacrjournals.org/content/43/6/2962](http://cancerres.aacrjournals.org/content/43/6/2962)