cis-Dichlorodiammineplatinum(II) Chemotherapy in Experimental Murine Myeloma MOPC 104E

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SUMMARY

Data are presented indicating marked antineoplastic activity for cis-dichlorodiammineplatinum(II) in MOPC 104E myeloma. One-eighteenth of the dose that produced 100% cures can be combined with noncurative, low doses of cyclophosphamide and 1,3-bis(2-chloroethyl)-1-nitrosourea to produce antineoplastic activity of the same degree as that produced by much higher dose regimens which regularly produce cures. Since, in the past, results of therapeutic trials in plasma cell tumors in humans have paralleled results in this animal model, clinical trials of cis-dichlorodiammineplatinum in multiple myeloma appear warranted.

INTRODUCTION

Current methods for treating multiple myeloma are unsatisfactory. Although the combination of an alkylating agent and an adrenal cortical steroid can induce objective responses in 50 to 60% of patients, these responses are virtually always incomplete and transient (2, 4). Attempts to improve results of treatment with the addition of other agents such as BCNU, procarbazine (1), and vincristine (10) have not been successful. Further, current studies indicate that treatment with an alkylating agent and prednisone is of benefit for no longer than 1 year (2). Accordingly, there is a need for the development of new drugs and strategies which will be effective in myeloma. Previously, we used the MOPC 104E mouse myeloma to mimic a clinical trial comparing the relative effectiveness of Cytoxan, BCNU, and prednisolone, and Alkeran and prednisolone (8). Although both regimens were effective, the addition of BCNU did not improve the therapeutic outcome. The clinical results now confirm this prediction (3). Therefore, because of our initial successful mouse-man correlation, we decided to use this same system to obtain possible leads for the development of better regimens for the treatment of human myeloma. For this purpose we selected for study adriamycin, bleomycin, mithramycin, and cis-platinum. Of these, the only one to show significant activity was cis-platinum. The purpose of this paper is to describe the results of treatment of MOPC 104E using this compound alone and in combination with other myeloma-active agents and to discuss the implication of our findings.

MATERIALS AND METHODS

The Murine Model. Murine myeloma MOPC 104E is a transplantable plasmacytoma which produces a monoclonal IgM. This monoclonal IgM has the unique characteristic of reacting with bacterial Dextran B-1355 (a generous gift from Dr. Allene Jeanes, United States Department of Agriculture, Peoria, Ill.). Sheep RBC conjugated with Dextran B-1355 can be used in the presence of 104E IgM and complement, thus permitting the precise quantification of total body idiotypic IgM. The techniques used in this system have been previously published and are simple, accurate, and reproducible to the level of 1.3 μg IgM per mouse (7, 9). The virtue of this system is that total cell kill can be monitored in individual mice without the sacrifice of the animal, thus permitting precise comparisons of effectiveness and toxicities between regimens in both individual and groups of mice.

These experiments utilized thirty 6-week-old BALB/c female mice obtained from Laboratory Supply Co., Indianapolis, Ind., and were maintained on Labblock feed (Wayne Feed Co., Chicago, Ill.) and water ad libitum. A tumor cell suspension was prepared as described earlier (9). Each mouse was given 1.5 x 10⁶ tumor cells i.v., and the animals were randomly separated into groups of 4 or 5/treatment program. The tumor IgM was monitored as previously described, and treatment was initiated 32 days after tumor implantation. All drugs were dissolved in physiological NaCl solution.

Chemotherapy Regimens. Doses of BCNU and Cytoxan previously found to produce a maximum cell kill were progressively reduced until only a minimal effect was detected. The dose of cis-platinum originally used was obtained by converting a commonly used single human dose in man according to the method of Freireich et al. (6). The starting dose used was 9 mg/kg. As with BCNU and Cytoxan, the dose was titrated to the level which produced a minimal but noticeable response. The regimens then tested were: (a) untreated control: Regimen 1; (b) single drugs, administered i.p.: Regimen 2, Cytoxan, 2.5 mg/kg; Regimen 3, single dose BCNU, 3 mg/kg; Regimen 4, cis-platinum, 0.5 mg/kg; and Regimen 5, single dose cis-platinum, 1.0 mg/
kg; and (c) combination chemotherapy regimens: Regimen 6, BCNU, 3 mg/kg, and Cytoxan, 2.5 mg/kg; Regimen 7, BCNU, 3 mg/kg, Cytoxan, 2.5 mg/kg, and cis-platinum, 0.5 mg/kg; Regimen 8, BCNU, 3 mg/kg, Cytoxan, 2.5 mg/kg, and cis-platinum, 1.0 mg/kg; and Regimen 9, BCNU, 3 mg/kg, Cytoxan, 2.5 mg/kg, and adriamycin, 10 mg/kg. Adriamycin is ineffective in the treatment of MOPC 104E. Therefore, Regimen 9 is a 3-drug control for Regimens 7 and 8. All drugs were given i.p. simultaneously in the soluble form and only 1 time as single drugs or in combination.

RESULTS

BALB/c mice with disseminated MOPC 104E myeloma are treated with different doses of Cytoxan and BCNU as single drugs to find the dose with minimal effect on the neoplasm. The dose response for each of the 2 drugs is summarized (Table 1). There is no measurable drop in the tumor-associated IgM when the tumor-bearing hosts are treated with BCNU, 3.0 mg/kg, or Cytoxan, 2.5 mg/kg. However, we see a 100% disease-free survival up to 100 days where the animals are treated with BCNU, 25 mg/kg, or Cytoxan i.p., 100 mg/kg. The noneffective BCNU and Cytoxan doses of 3.0 and 2.5 mg/kg, respectively, are used in this report as a combination treatment and serve as a control for the cis-platinum studies.

The combination of BCNU and Cytoxan used did have a therapeutic effect, but there was only a half-log₁₀ decrease in the total idiotypic IgM (Chart 1). There was some prolongation of survival, but all animals were dead of disease by Day 67 with only a 21-day median increase in life-span.

The dose-response relationships for cis-platinum are summarized (Table 2) where the response rate, percentage of disease-free survival at 100 days, and the 100-day survival after tumor implantation are given. Doses of 2.0, 4.5, and 9.0 mg/kg were all associated with 80% survival at 100 days. However, a significant percentage of relapses occurred in each treatment group except those treated with 9.0 mg/kg. In the untreated control group (not shown) all animals died of progressive disease between the 36th and 54th day after tumor transplantation with a median survival of 36 days. The results in the minimally effective BCNU-Cytoxan combination control group (Chart 1) were then compared with those obtained using low-dose, triple-drug combinations of cis-platinum, BCNU, and Cytoxan.

The different combination treatments and the drug doses are listed under "Materials and Methods." The response rate, percentage of disease-free survival at 100 days, and the 100-day survival after tumor implantation for the combination treatments are given (Table 3). The addition of cis-platinum, 0.5 mg/kg, with BCNU and Cytoxan gave the results depicted in Chart 2. Responses were seen in all of the animals with stabilization of the tumor idiotypic IgM at a level of approximately 10 μg/mouse. The animals were treated on Day 32 after tumor implantation, and the tumor immunoglobulin dropped to the base line within 18 days after treatment. One mouse died of recurrence immediately after treatment.
Effect of drug combination treatments on MOPC 104E myeloma

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Response (%)</th>
<th>% free of disease (~100 days)</th>
<th>% survival (~100 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCNU + Cytoxan</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BCNU + Cytoxan + cis-platinum</td>
<td>100</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>BCNU + Cytoxan + cis-platinum</td>
<td>100</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>BCNU + Cytoxan + adriamycin</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- Treated animals were monitored for 100 days after tumor implantation for relapses and survival.
- Drug doses are given under "Materials and Methods." Combinations are listed in the order as they appear under "Materials and Methods."

After regression (50 days), and the remaining 3 mice survived more than 100 days with no evidence of recurrent tumor.

In Regimen 8, BCNU (3.0 mg/kg) and Cytoxan (2.5 mg/kg) were combined with cis-platinum (1.0 mg/kg) and the mice were monitored for idiotypic IgM (Chart 3). All 4 animals responded to the treatment, and the idiotypic IgM stabilized at about 10 μg/mouse, 18 days after the treatment. One mouse with the largest tumor burden died of apparent toxicity with no tumor recurrence, on Day 46. The other 3 animals survived more than 100 days with no evidence of recurrent disease.

Chart 4 depicts the combination of adriamycin (10 mg/kg) with the basic combination of BCNU and Cytoxan. Adriamycin as a single agent has no effect on this myeloma. Eighty % (4 of 5) of the animals responded to the combination with a transient drop in the idiotypic IgM. One mouse died of tumor on Day 36 (4 days after treatment), 3 mice apparently died of both toxicity and neoplasm, and only 1 mouse survived up to Day 67 (not shown), and its regression and recurrence pattern was similar to that of animals seen in Chart 1.

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survived up to Day 67 and died of recurrent tumor growth. The results of the addition of an ineffective drug, Adriamycin, indicate the requirement of cis-platinum in the combination with low-dose BCNU and Cytoxan.

DISCUSSION

The most urgent current problem related to the treatment of multiple myeloma is the discovery of drugs, regimens, and strategies capable both of increasing the response rate and its duration and improving the cell kill. To date, no regimen has achieved superiority to that of melphalan and prednisone. Since the MOPC 104E myeloma model had previously provided data confirming the truly synergistic nature of melphalan and prednisolone and had also correctly predicted that the addition of BCNU to Cytoxan and prednisone or Cytoxan alone would not be superior to Alkeran and prednisone when compared in the clinical trial, we have undertaken the use of this system as a rapid means of searching for leads for the development of new therapeutic approaches to human myeloma. We are encouraged by the activity of cis-platinum in the initial experiments as recorded in this paper. These results indicate that it is a promising agent to combine with the currently used myelosuppressive human regimens. cis-Platinum in humans at doses equivalent to those used in this study would not be expected to add to the myelotoxicity of alkylating agents and would also not be expected to produce significant nephrotoxicity in a disease in which nephropathy can play a significant role.

We have no data to indicate the mechanism of action whereby cis-platinum exerts its therapeutic effects in this system. Its chemotherapeutic effects are probably not exerted through alkylation. It has been found to specifically inhibit DNA synthesis as well as to inhibit DNA polymerase (5). Accordingly, it may be postulated that the addition of both BCNU and cis-platinum, each of which inhibits DNA polymerase, may interfere with DNA repair after alkylation with Cytoxan. On the other hand, the effect of cis-platinum alone at higher doses suggests that its activity in this system is directly cytotoxic.

We are aware of no systematic attempt to study the effect of cis-platinum alone or in combination in human myeloma. Accordingly, the results in these preliminary experiments have prompted us to begin development of a clinically feasible study of this agent in patients who have myeloma but no evidence of renal disease.

REFERENCES


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