Combination Chemotherapy for Clinically Established Graft-versus-Host Disease in Mice

H. Glucksberg and A. Fefer

Division of Oncology, Department of Medicine, University of Washington School of Medicine, Seattle, Washington 98195

SUMMARY

The efficacy of combined chemotherapy against established graft-versus-host disease was studied with a view to its eventual application to tumor-bearing hosts subjected to adoptive chemoimmunotherapy. Adult BALB/c mice inoculated on Day 0 with nonlethal cyclophosphamide (CY) and C57BL/6 spleen cells exhibited clinical graft-versus-host disease by Days 5 to 7, and 95% of them died, with a median survival time of 15 days. Therapy with optimal doses of either CY or procarbazine alone on Days 6 to 9 saved about 30% of the mice. The combination of procarbazine and CY, both administered on Days 6 to 9, was significantly more effective than optimal doses of either CY or procarbazine alone, as reflected by a doubling of the percentage of 60-day survivors (to 62%). Cortisone acetate was therapeutically ineffective when used alone and did not influence the results obtained with the effective CY-procarbazine combination.

INTRODUCTION

One experimental approach to tumor immunotherapy in animals has involved the adoptive transfer of lymphoid cells from variably immunized donors to tumor-bearing hosts subjected to chemotherapy or X-irradiation (7, 9). The purpose of the drugs or X-ray is to decrease the tumor load and, in the allogeneic situation, to depress the immunological responsiveness of the host so as to delay the rejection of the infused donor lymphoid cells by the host. Allogeneic lymphocytes infused after drugs or X-irradiation can have a significant antitumor effect (2-4, 6, 9, 17, 18). However, in the application of such an approach to grossly histoincompatible donor-host combinations, a major stumbling block is the almost certain development of severe or fatal GVHD, which is extremely difficult to control. In non-tumor-bearing animals, cytotoxic agents will often prevent GVHD if they are administered early, but they are generally far less effective if administered after the GVHD has become clinically detectable (1, 14, 16, 19, 20). Unfortunately, early interference with the antitumor activity of the infused lymphoid cells.

The results presented below show that the combination of the CY (4). Presumably, early administration of CY interfered with the antitumor effect of the foreign lymphoid cells. Therefore, it may be essential to delay any anti-graft-versus-host therapy that might interfere with the survival or antitumor activity of the infused lymphoid cells.

Accordingly, the potential efficacy of cytotoxic agents for treatment of GVHD after it is already clinically detectable was studied in a reproducible model for rapidly fatal GVHD in non-tumor-bearing mice. The model involves adult BALB/c mice given a nonlethal dose of CY and C57BL/6 spleen cells. A previous report showed that treatment of the resultant GVHD with small doses of either CY or procarbazine saved about 30% of the mice (11). By analogy with the demonstrated superiority of combination chemotherapy against some neoplastic disorders (5, 13) and also by analogy with suggestive data for the superiority of combination versus single-agent chemotherapy for immunosuppression (10), we tested the possibility that combination chemotherapy for GVHD might be more effective than optimal treatment with single agents. The results presented below show that the combination of procarbazine and CY was significantly more effective than optimal doses of CY or procarbazine used alone.

MATERIALS AND METHODS

Animals. BALB/c (H-2d) and C57BL/6 (H-2b) mice, 10 to 14 weeks old, were obtained from Texas Inbred Mice, Houston, Texas. They were housed in covered plastic cages, 9/cage (or fewer), and were given tap water and Purina mouse chow.

Drugs. CY (Mead Johnson and Co., Evansville, Ind.), procarbazine (Hoffman-La Roche, Inc., Nutley, N.J.), and cortisone acetate were dissolved in distilled water in the appropriate concentrations and injected i.p. (0.01 ml/g body weight) within 30 min of preparation.

Preparation of Spleen Cells. Spleen cells were prepared as previously described (11). The concentration of viable cells was determined by trypan blue exclusion and was adjusted with Hanks' balanced salt solution. For induction of GVHD, the cells were injected i.p. in a volume of 0.3 ml/mouse.

Preparation of Antibody against Donor γ-Globulin. Sera from BALB/c mice immunized against C57BL/6 γ-globulin by

1 These studies were supported by Grants CA 10777 and CA 05231 from the NIH, USPHS, and by Institutional Cancer Grant IN-26M. Some of these data were presented at a meeting of the American Association for Cancer Research in Boston, Mass., on May 4, 1972.

2 Advanced Clinical Fellow of the American Cancer Society.

3 Scholar of the Leukemia Society of America.

4 The abbreviation used is: GVHD, graft-versus-host disease.

Received November 21, 1972; accepted January 16, 1973.
RESULTS AND DISCUSSION

The efficacy of different doses of CY or procarbazine on established GVHD is shown in Table 1. BALB/c mice that received CY plus C57BL/6 spleen cells and no treatment developed GVHD by Days 5 to 7 and 93% of them died, with a median survival time of 16 days. To determine the dose of CY or procarbazine which, as a single agent, would exert the maximal therapeutic effect, we gave mice with GVHD various doses of CY or procarbazine on Days 6 through 9. The results (Table 1) show that doses of 25 or 30 mg of CY or 50 to 125 mg of procarbazine per kg per day, administered on Days 6 to 9, were optimal and resulted in a significant increase in 60-day survivors. Higher or lower doses of CY, or higher doses of procarbazine were either less effective or no more effective than the above-mentioned doses.

The efficacy of optimal single-drug therapy was compared with that of the 2 drugs in combination. The results of 5 concurrent experiments are shown in Table 2. All animals with untreated GVHD died with a median survival time of 16 days. Therapy with optimal doses of either CY or procarbazine alone on Days 6 to 9 permitted 26 to 29% of the mice to survive 60 days. The combination of CY (administered on Days 6 and 8) and procarbazine (administered on Days 7 and 9) was no more effective than optimal doses of either procarbazine or CY alone. However, the combination of procarbazine and CY, both administered on Days 6 to 9, was significantly (p < 0.01) more effective than optimal doses of either procarbazine or CY alone. As reflected by a doubling of 60-day survivors to 62%. Cortisone acetate, an established immunosuppressive agent, was not therapeutically effective against GVHD when used alone, nor did it significantly influence the results obtained with the effective CY:procarbazine combination.

We evaluated chimerism by testing for the presence of donor-type γ-globulin in host sera. On Day 8, sera from mice with untreated GVHD had donor-type γ-globulin in titers of 1:16 to 1:64. Similar tests were performed on sera obtained on Days 34 to 42 from mice with GVHD that had been successfully treated with drugs, as well as from the few mice that had recovered without therapy. None of the sera exhibited detectable donor-type γ-globulin, which suggested that there had been only transient persistence of donor-type spleen cells.

Although the observed beneficial effects of drug therapy for GVHD may be mediated by or associated with a more rapid loss of chimerism, the disappearance of chimerism in the few mice surviving GVHD without therapy suggests that transient chimerism may be a characteristic of the model per se, as a function of the relatively low dose of immunosuppressive conditioning and of major donor-host histoincompatibility. Although persistent chimerism is essential when the immunosuppressive conditioning is lethal, as in human bone marrow transplantation after supralethal total-body irradiation (21), or when persistence of donor cells is essential, as in marrow transplantation for aplastic anemia (22), it probably is not necessary in situations in which allogeneic lymphocytes are used as tumor immunotherapy as an adjunct to nonlethal

---

Table 1
Chemotherapy of established GVHD
All mouse groups (except that indicated by footnote) received drug on Days 6, 7, 8, and 9.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg/day)</th>
<th>No. of mice surviving 60 days/no. tested</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>6/91</td>
<td>7</td>
</tr>
<tr>
<td>CY</td>
<td>15</td>
<td>4/16</td>
<td>25</td>
</tr>
<tr>
<td>CY</td>
<td>25</td>
<td>29/85</td>
<td>34</td>
</tr>
<tr>
<td>CY</td>
<td>30</td>
<td>6/18</td>
<td>33</td>
</tr>
<tr>
<td>CY</td>
<td>40</td>
<td>4/19</td>
<td>21</td>
</tr>
<tr>
<td>CY</td>
<td>50</td>
<td>3/21</td>
<td>14</td>
</tr>
<tr>
<td>CY</td>
<td>50</td>
<td>3/19</td>
<td>15</td>
</tr>
<tr>
<td>Procarbazine</td>
<td>50</td>
<td>5/14</td>
<td>35</td>
</tr>
<tr>
<td>Procarbazine</td>
<td>75</td>
<td>3/8</td>
<td>37</td>
</tr>
<tr>
<td>Procarbazine</td>
<td>100</td>
<td>14/38</td>
<td>36</td>
</tr>
<tr>
<td>Procarbazine</td>
<td>125</td>
<td>6/18</td>
<td>33</td>
</tr>
<tr>
<td>Procarbazine</td>
<td>150</td>
<td>1/15</td>
<td>6</td>
</tr>
</tbody>
</table>

* Drug given on Days 6, 7, and 8.
immunosuppressive therapy (3, 4, 6, 8). Indeed, in the latter situation, transient chimerism and a short-term but potent graft-versus-host reaction which can be terminated by chemotherapy may yield optimal antitumor effects and host survival (3, 4).

The results presented warrant further attempts in other models to use combination chemotherapy for the treatment of GVHD in tumor-bearing and non-tumor-bearing animals conditioned with nonlethal or supralethal doses of immunosuppressive agents.

ACKNOWLEDGMENTS

We would like to thank Mrs. Susan Geisler for her excellent technical assistance.

REFERENCES


Combination Chemotherapy for Clinically Established Graft-versus-Host Disease in Mice

H. Glucksberg and A. Fefer


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/33/4/859

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.