SUMMARY

The effect of Imuran therapy of BALB/c mice bearing four strains of transplantable plasma cell tumors has been studied. MOPC-315 is quite sensitive to Imuran and a number of 90-day cures were obtained with only two doses of the drug. MPC-11 had a variable sensitivity. MOP-1 and APC-14 did not respond to Imuran therapy. Tumor regression correlated well with a decrease in total serum protein and M-protein concentrations. Imuran was less toxic than 6-mercaptopurine at comparable dosages. Cyclophosphamide was as effective as Imuran at low dosages. These data suggest that Imuran may be very effective therapy for some strains of murine and human plasma cell tumors.

INTRODUCTION

Mouse PCT's resemble human myeloma tumors and therefore are good models for experimental chemotherapy. Murine and human PCT produce elevated serum protein with accompanying "M"-spike (14), Bence Jones proteinemia (6, 13), kidney lesions (3), and bone lesions (9). Although chemotherapy of murine tumors has failed to reveal new drugs for the treatment of human myeloma, studies (1, 7, 10, 18-20, 23) have shown that the drugs most effective in human myeloma (2), melphalan and cyclophosphamide, are also the most effective in producing remission of murine PCT.

Previous studies from our laboratory have shown that at least 1 murine plasma cell tumor undergoes significant regression following treatment with 9α-fluoroprednisolone (8). This corticoid-induced regression suggested that other immunosuppressive agents might be effective in preventing proliferation of neoplastic plasma cells. In this paper, we provide evidence that 1 line of plasma cell tumor is sensitive to treatment with Imuran.

MATERIALS AND METHODS

Tumors and Host. The murine PCT MOPC-315 was used for this study. This tumor which was kindly provided by Dr. H. Eisen, Washington University School of Medicine, St. Louis, Mo., produces an IgA-type myeloma globulin that precipitates DNP-coupled proteins (15). Tumors appeared between 9 and 22 days following s.c. transplantation into BALB/c mice (received through the Mammalian Genetics and Animal Production Section, Drug Research and Development, National Cancer Institute). Usually, the tumor-bearing mice died within 1 to 4 weeks after appearance of palpable tumor.

Test Compounds. Imuran (azathioprene) provided by Burroughs Wellcome Company, Research Triangle Park, N. C., was suspended in phosphate-buffered NaCl solution brought into solution by dropwise addition of 0.2 N NaOH, and diluted to a final concentration of 10 mg/ml. A solution of 6-MP (Sigma Chemical Co., St. Louis, Mo.) was prepared as described for Imuran. Cytoxan (cyclophosphamide) was a product of Mead-Johnson Laboratories, Evansville, Ind.

Chemotherapy. Mice bearing tumors with an average diameter of 0.8 to 1.2 cm were divided into 2 groups. Test substances were administered i.p. 5 times/week. The average diameter of each tumor was calculated from caliper measurements of 3 separate diameters at 2- to 3-day intervals. The effectiveness of each test compound was based on: (a) remission of tumor; (b) percentage of increase in MST; (c) number of 90-day survivors; and (d) disappearance of abnormal serum protein. The MST was calculated from the 1st day of treatment. Mice that survived for more than 90 days were classified as cured and excluded from the calculation of the MST.

Serum Analysis. Blood was collected weekly in heparinized capillary tubes. Following centrifugation, hematocrits were measured on a Spiracrit reading device. Total serum protein was determined on an appropriately diluted sample of serum by the method of Lowry et al. (11) using lyophilized normal mouse serum as a standard.

M-protein Analysis. The MOPC-315 PCT produces a myeloma protein that specifically precipitates with DNP-proteins and has a homogeneous binding site of high affinity (4). A simple assay was developed for measuring the level of MOPC-315 protein in serum based upon the hemagglutination of DNP-BSA-coupled sheep red blood cells (22). DNP-hapten-conjugated bovine serum albumin and DNP-BSA-coupled sheep red blood cells were prepared.
as described by Yamada and Yamada (21). Ten µl of test serum were diluted with 40 µl of phosphate-buffered saline, and 2-fold serial dilutions were made using a microtiter system. Twenty-five µl of 5% DNP-BSA-coupled erythrocytes were added to each well and hemagglutination reactions were read after a 24-hr incubation at 4°. Antibody titers (n) were expressed as the greatest dilution which gave a visible agglutination, where the dilution was equal to 1/(5 x 2^x).

RESULTS

Comparative Effect of Imuran, 6-MP, and Cytoxan. Chart 1 shows the regression of MOPC-315 during administration of different doses of Imuran. Curve A represents the normal growth curve of the MOPC-315 tumor. Curves B, C, and D show the effect of treatment with Imuran, 50, 100, and 150 mg/kg, 5 times/week for 3, 2, and 2 weeks, respectively. Each group contained 6 to 8 mice. The standard error, omitted from the figure, was usually less than 5% of the mean. Significant regression did not occur until 7 to 10 days after the start of treatment, but the progress was rapid thereafter.

Table 1 contrasts the therapeutic effect of Imuran with that of 6-MP, the principle metabolite of Imuran (5), and with cyclophosphamide which is known to cause regression of a number of PCT's (10, 16, 18, 19, 23). The mean survival time, number of mice, duration of treatment, number, and percentage of 90-day cures are given for each group. All doses of Imuran prolonged survival and resulted in a high percentage of 90-day cures. Deaths in these groups were attributed to drug toxicity since lower total dosage resulted in a high cure rate. Imuran-treated mice lost 10 to 20% of their body weight. An equivalent weight loss induced by fasting did not result in tumor regression. Significant remission was also obtained with 6-MP at some of the dose levels tested. Toxicity was a significant factor with 6-MP as evidenced by the low cure rate. Cyclophosphamide was shown to be toxic when given at a dose level of 100 mg/kg. At lower dose levels it was about as effective as Imuran.

Effect of Low Doses of Imuran and 6-MP on MOPC-315. The relative effectiveness of 1 dose or 2 doses given 3 days apart of either Imuran or 6-MP is presented in Table 2. With 1 injection of either drug a cure rate of 16% was obtained. The MST of both groups was about double that of the control group. With 2 doses the percentages of 90-day survivors were 90 and 100% for Imuran and 6-MP, respectively.

Effect of Treatment on Serum Profile. Hematocrits of mice bearing MOPC-315 fell from a normal value of 55% to 36% with increasing tumor size. Tumor-bearing mice treated with 6-MP or Imuran exhibited severely depressed

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dosage (mg/kg)</th>
<th>Wks administered&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mice</th>
<th>Cures (90 day) (%)</th>
<th>MST</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>(0.2 ml), 0.15 M</td>
<td>3</td>
<td>21</td>
<td>0 (0)</td>
<td>14.7 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Imuran</td>
<td>150</td>
<td>1</td>
<td>8</td>
<td>7 (88)</td>
<td>27.8 ± 2.7</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Imuran</td>
<td>150</td>
<td>2</td>
<td>12</td>
<td>8 (67)</td>
<td>13.8 ± 0.8</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Imuran</td>
<td>100</td>
<td>2</td>
<td>1</td>
<td>7 (88)</td>
<td>23.7 ± 8.0</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Imuran</td>
<td>100</td>
<td>2</td>
<td>19</td>
<td>16 (84)</td>
<td>28.0 ± 0.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Imuran</td>
<td>50</td>
<td>3</td>
<td>7</td>
<td>5 (72)</td>
<td>22.7 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6-MP</td>
<td>150</td>
<td>2</td>
<td>6</td>
<td>0 (0)</td>
<td>45.0 ± 2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6-MP</td>
<td>100</td>
<td>2</td>
<td>6</td>
<td>3 (50)</td>
<td>4.3 ± 0.7</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>6-MP</td>
<td>62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2</td>
<td>5</td>
<td>3 (60)</td>
<td>45.0 ± 2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6-MP</td>
<td>50</td>
<td>3</td>
<td>6</td>
<td>2 (33)</td>
<td>51.0 ± 8.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6-MP</td>
<td>31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3</td>
<td>5</td>
<td>4 (80)</td>
<td>25.5 ± 13.5</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Cytoxan</td>
<td>100</td>
<td>2</td>
<td>7</td>
<td>1 (14)</td>
<td>39.3 ± 4.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cytoxan</td>
<td>50</td>
<td>2</td>
<td>5</td>
<td>4 (80)</td>
<td>45.0 ± 2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cytoxan</td>
<td>25</td>
<td>3</td>
<td>5</td>
<td>3 (60)</td>
<td>25.5 ± 13.5</td>
<td>&gt;0.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Drugs were administered 5 times/week for the number of weeks indicated.

<sup>b</sup> Mean ± S.E.

<sup>c</sup> Equivalent on a mole basis to Imuran, 100 mg/kg.

<sup>d</sup> Equivalent on a mole basis to Imuran, 50 mg/kg.
hematocrits (15 to 30%). This effect was most evident 3 to 4 weeks after the start of treatment at a time when tumor regression was almost complete. With Cytoxan administration, hematocrits fell only slightly. Total serum protein levels were increased about 2 to 9 g/100 ml above normal values at the start of treatment. This elevation was maintained with control mice; while treatment with Imuran, Cytoxan, or 6-MP returned serum protein levels to normal (about 6.5 g/100 ml). Myeloma protein levels in untreated mice increased as tumor size increased (Chart 2A). In general, the fall in M-protein levels of treated mice paralleled the rate of tumor regression.

Effect of Rechallenge With MOPC-315. Four months after the start of treatment, cured mice were retransplanted with MOPC-315 tumor. Mice of the same age and weight were used as controls. The results of this study (Table 3) demonstrate that both the mean number of days needed for tumor to reach a 1-cm diameter and the MST's of previously treated mice were longer than the values for the control group.

Effect of Imuran on Other PCT. All 4 tumors used for this study were palpable within 1 to 2 weeks after s.c. transplant of tumor fragments. Tumor strains MOPC-315, APC-14, and MPC-11 exhibited similar growth rates with death usually occurring 1 to 4 weeks after appearance of palpable tumor. Although the growth rate of the MOP-1 tumor was the same as the other 3 strains, death was usually postponed by about 2 weeks. Imuran (150 mg/kg; 5 doses) was administered to mice bearing the PCT's MOP-1, APC-14, or MPC-11. No effect on the growth rate of either MOP-1 or APC-14 was observed. Individual MPC-11 tumors exhibited a variable response to Imuran. Of 8 MPC-11 tumors treated, 1 regressed completely while 4 others exhibited remissions greater than 50% of the starting size; however, no 90-day cures were obtained.

DISCUSSION

The aim of this study was to evaluate the tumoricidal effect of Imuran on mouse plasma cell tumors. We found that 2 tumor lines were unresponsive to treatment, a 3rd line (MPC-11) exhibited significant regression, while a 4th line (MOPC-315) was highly sensitive.

All 4 plasma cell tumors tested were responsive to treatment with Cytoxan. This confirms and extends the observations of others as to the effectiveness of alkylating agents in both mouse (1, 7, 16, 18–20, 23) and human (2) myeloma. Significantly, the PCT MOPC-315 was responsive to treatment with Imuran on 6-MP over a range of doses. Complete tumor remission was obtained in almost all cases and 90-day cure rates were usually greater than 70%. Teller (19) tested 6-MP on 3 plasma cell tumors and reports a 52 to 82% increase in MST of the host BALB/c mice. To our knowledge, this is the only report of the use of either Imuran or 6-MP on murine plasma cell tumors. Reported studies of the use of 6-MP or Imuran in human myeloma are also sketchy. Osserman and Hines (12) described a case of human myeloma with cryoglobulinemia in which remission was obtained with 6-MP. Rundles and Dugdale (17) reported that 6-MP was helpful in treating human myeloma. No detailed data were given.

In addition to estimating tumor regression by shrinkage of palpable tumor, semiquantitative measurements of serum myeloma protein levels were made. An excellent correlation...
between these levels and tumor size was observed. Following an initial lag period, serum M-protein levels rapidly declined. Control mice exhibited an increase in abnormal protein levels which paralleled the gross increase in tumor size. NaCl-(control), 6-MP-, and Imuran-treated mice also exhibited various degrees of anemia. This effect was most pronounced when Imuran was administered. Although hematocrits fell to as low as 20%, this fall was transient and after 4 to 6 weeks hematocrits had returned to near-normal levels.

When drug toxicity was not a factor (low doses), Imuran and 6-MP were equally effective in inducing tumor remission. At higher doses, treatment with Imuran proved to be superior to 6-MP treatment. These results suggest that the effect of Imuran is mediated through the slow hydrolysis of Imuran to 6-MP. This is in agreement with the finding of Elion (5), of a higher therapeutic index of Imuran than for 6-MP.

Elion (5) has demonstrated that sensitivity to 6-MP can usually be correlated with the presence of the enzyme hypoxanthine-guanine phosphoribosyltransferase which converts 6-MP to the ribonucleotide derivative 6-thioinosine monophosphate. Unpublished studies from our laboratory have shown that all 4 tumors tested for sensitivity to Imuran and 6-MP had approximately similar levels of this enzyme. This suggests that the biochemical locus of the sensitivity to Imuran in these tumors is not at the phosphoribosyltransferase site. Furthermore, the low level of Imuran (4 mg) needed to produce complete remission of MOPC-315 argues against a competition between 6-MP and hypoxanthine for the transferase enzyme. A more likely site would usually be inhibition of one of the enzymes involved in de novo purine synthesis by 6-thioinosine monophosphate.

The results of these mouse experiments indicate that Imuran is highly effective against 1 line of plasma cell tumor. Further studies are needed to determine whether other murine PCT's are also sensitive. We plan to determine the sensitivity of human myelomas to Imuran.

REFERENCES


Imuran-induced Regression of Plasma Cell Tumor MOPC-315

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