Response of a Virus-induced Murine Lymphoid Leukemia to Drug Therapy

MICHAEL A. CHIRIGOS, JOHN B. MOLONEY, STEWART R. HUMPHREYS, NATHAN MANTEL, AND ABRAHAM GOLDIN

(Laboratories of Chemical Pharmacology and of Viral Oncology, and Biometry Branch, National Cancer Institute, Bethesda, Md.)

SUMMARY

Assay systems were described for the chemotherapeutic testing of drugs against the Moloney virus-induced leukemia and the transplantable whole-cell leukemia in mice. In the virus-induced leukemia system treatment was initiated after the induction of the leukemia.

Triethylene melamine, Cytoxan, and amethopterin produced limited increases in survival time of mice with the virus-induced leukemia. Drug therapy also inhibited the increase in weight of thymus, liver, and spleen, in mice with the virus-induced leukemia.

A number of drugs were tested in the transplantable leukemia assay system. Two alkylating agents, Cytoxan and triethylene melamine, produced the most extensive increases in survival time. Minimal effects were obtained with the folic acid antagonists, amethopterin and S',5'-dichloroamethopterin, as well as with the purine antagonist, 6-mercaptopurine. Factors which influenced the effectiveness of candidate drugs included the regimen of drug therapy and the inoculum concentration. More extensive survival time was observed in first generation hybrids (CDBA) than in the strain of origin (BALB/c). This may be attributable to differences in drug tolerance.

Problems involved in assay systems employing virus-induced tumors as tools for chemotherapeutic study are discussed.

Only in recent years has the problem of treating viral tumors with chemotherapeutic agents been studied. Groupé et al. (7, 8) reported on in vivo studies with the Rous sarcoma virus which were concerned primarily with the antiviral and antitumor activity of Xerosin. Johnson and his coworkers, in 1958 (9, 10), described the prophylactic effect of selected compounds in the induction of Rous sarcoma, employing standardized doses of the virus. Sugiura (12, 13) reported the effects of known antitumor drugs on the Friend mouse leukemia.

Although Bryan (1, 2) has described the dose-response relationships with the Rous sarcoma virus and stressed the quantitative methodology required for chemotherapeutic studies with virus-induced tumors, there has been only a limited amount of study in this area. As pointed out by Endicott (3), the inability to employ appropriate test systems has been due to the lack of information available on the biology of the tumor viruses and the induced neoplasms.

Moloney (11) recently described the recovery of a murine lymphoid leukemia virus from the neoplasm Sarcoma 37. This agent is leukemogenic in mice of various strains and of various age levels, as well as in rats. The virus-induced leukemia is freely transplantable within the mouse strain where induced and in compatible F1 hybrid mice. The generalized leukemia which results from virus inoculation or from the implantation of neoplastic cells is similar to that which occurs spontaneously in certain inbred strains of mice (11).

The successful use of the mouse leukemia L1210 under controlled conditions in assay systems for testing candidate chemotherapeutic agents (4-6) prompted investigation of similar methodology for the Moloney leukemia.
MATERIALS AND METHODS

Virus material.—A pool of virus-induced leukemic spleens, lymph nodes, and thymus of BALB/cAn mice served as source material for the preparation of cell-free concentrates of the leukemia agent. The details of the method of differential centrifugation which was employed have been previously published (11). The final concentration of the particulate fraction was 0.5-gm. equivalents per ml.; a 1-gm. equivalent per ml. concentrate is equal to 1 ml. of final suspension for every gram of tissue processed.

Leukemic cell suspension.—As previously noted, the virus-induced leukemia is freely transplantable within a mouse strain and in compatible F1 hybrids. Transplantation of neoplastic cells produces a generalized lymphoid leukemia in all recipients within a relatively short period. Repeated whole cell passage of such material results in a decrease of the latent period and time to death with leukemia. The whole cell leukemia was carried routinely in series in a limited number of adult BALB/c mice. For the purposes of the experiments described herein, such tumor material was transplanted into larger groups, i.e., greater than 100, of 4- to 8-week-old BALB/cAn1 and 6-week-old CDBA1,2 hybrid mice. Such leukemic animals served as experimental mice for the chemotherapeutic studies.

Preparation of inocula and routes of inoculation.
—Preparation of the virus inoculum was carried out as follows: frozen aliquots of the virus concentrate were diluted 10-1 with 0.05 M pH 6.8 sodium citrate buffer containing 1 mg. of hyaluronidase per 100 ml. of diluent. BALB/c mice less than 36 hours of age were given inoculations subcutaneously with a table of random numbers, at the time the disease was diagnosed. The diagnosis was based on spleen and lymph node enlargement on an arbitrary scale ranging from + to +++++. In the first two experiments (Chart 1; Chart 2, section 1 and 2) the animals were assigned and appropriate treatment initiated at stage ++ leukemia. In the third experiment (Chart 2, section 3) the animals were entered into the experiment at random from populations of leukemic mice given inoculations of virus at varying times and concentrations. In this experiment, no attempt was made at maintaining uniformity of entry stage. The disease ranged from + to +++++ at the time of assignment into the experiment.

In the experiments employing leukemic cell suspensions the mice were randomized into appropriate groups shortly after inoculation.

Drugs and treatment.—Triethylenemelamine (TEM), 5-fluorouracil (5-FU), mitomycin C, and Cytoxan were prepared in 0.9 per cent saline. Meticortelone and methyglyoxalbisguanylylhydrazine diacetate trihydrate (MGBGH) were prepared in aqueous solution. Amethopterin (MTX) and 3', 5'-dichloroamethopterin (DCM) were prepared in 2 per cent NaHCO3. Myleran was suspended in 0.1 per cent methyl cellulose. 6-Mercaptopurine (6-MP) was dissolved in dilute alkali.3

The drugs were administered subcutaneously in the axillary region in a constant volume of 0.01 ml/gm of body weight. The regimens of treatment are indicated with the individual experiments.

RESULTS

Chemotherapy of virus-induced leukemia.—Chart 1 (Parts A and B) shows the results of an experiment on the virus-induced leukemia. Treatment was initiated at leukemic stage ++ and continued daily until the end of the experiment. A considerable range was observed between the time of viral inoculation to the time of diagnosis of the disease. In the three control groups (Chart 1B) the ranges were: 65-104 days, 84-116 days, and 65-116 days, respectively. The range of death in the untreated controls, from the time they were determined to be leukemic to the time of death also varied markedly—e.g., in the three control groups (Chart 1B) the range was 20-70

1 Obtained from NIH breeding colony.
2 CDBA = (BALB/cAn X DBA/2J)F1.
3 Amethopterin and 3',5'-dichloroamethopterin were obtained from the Lederle Laboratories Division of the American Cyanamid Company. Meticortelone was obtained from the Schering Corporation. The remaining compounds were obtained through the Cancer Chemotherapy National Service Center from the following sources: triethylenemelamine, Lederle Laboratories Division of the American Cyanamid Co.; 5-fluorouracil, Hoffmann-LaRoche, Inc.; Cytoxan, Mead Johnson & Co.; methyglyoxalbisguanylylhydrazine diacetate trihydrate, Frederic A. French, Mt. Zion Hospital, San Francisco, California; Myleran and 6-mercaptopurine, Burroughs Wellcome & Company; mitomycin C, Dr. H. L. Dickison, Bristol Laboratories, Inc., Syracuse, New York.
days, 1–52 days, and 2 to greater than 84 days. The over-all median survival time (MST) for the control groups from the day of diagnosis was 22 days (Chart 2, panel 1). The results (Chart 1A and 1B, and Chart 2, panel 1) indicate that in most cases treatment with MTX, 6-MP, and Cytoxan failed to increase the survival time of the mice. Increases in MST were noted with 36 mg/kg Cytoxan and 0.6 mg/kg MTX.

The effects of the compounds were also evaluated by comparing the weights of thymus, liver, and spleen at time of death. All three drugs were effective in decreasing the weights of thymus, liver, and spleen as compared with untreated controls. The average weight of thymus, liver, and spleen of control animals ranged from 0.20 to 0.36, 2.50 to 2.89, and 0.79 to 1.13 gm., respectively. In every case where drug was administered, the wet weights of the tissues were 50 per cent or less than those of the controls. The exception was the effect shown with 0.36 mg/kg MTX, where tissue weights were similar to the controls.

In a second experiment (Chart 2, section 2) TEM showed an increase in MST of 6 days over controls at a daily dose of 0.5 mg/kg. Myleran and mricortelone were ineffective in increasing survival time.

In the third experiment (Chart 2, section 3) there were more survivors in the groups treated with MGBGH and 5-FU than in the control group. However, these data can be considered

<table>
<thead>
<tr>
<th>THYMUS WGT</th>
<th>LIVER WGT</th>
<th>SPLEEN WGT</th>
<th>SURVIVAL TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTX, 0.36 mg/kg DAILY</td>
<td>0.0184</td>
<td>224</td>
<td>0.083</td>
</tr>
<tr>
<td>MTX, 0.60 mg/kg DAILY</td>
<td>0.0056</td>
<td>1.13</td>
<td>0.44</td>
</tr>
<tr>
<td>MTX, 1.0 mg/kg DAILY</td>
<td>0.0067</td>
<td>101</td>
<td>0.31</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>THYMUS WGT</th>
<th>LIVER WGT</th>
<th>SPLEEN WGT</th>
<th>SURVIVAL TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-MP, 36 mg/kg DAILY</td>
<td>0.071</td>
<td>6.0</td>
<td>0.83</td>
</tr>
<tr>
<td>6-MP, 60 mg/kg DAILY</td>
<td>0.022</td>
<td>1.19</td>
<td>0.49</td>
</tr>
<tr>
<td>6-MP, 100 mg/kg DAILY</td>
<td>0.005</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Chart 1.—Parts A and B. Treatment of virus-induced leukemia. The points are for individual mice. The figures in the panels for thymus, liver, and spleen represent the average tissue weight for the group. The figures in the panels for survival time represent the median survival time in days after initiation of treatment.
as no more than suggestive because of the lack of uniformity in the selection of experimental material in this experiment (see "Methods").

The diminished survival time observed at the higher doses of the various drugs employed would appear to reflect cumulative toxicity resulting from continuous drug administration.

**Chart 2.** Influence of chemotherapeutic agents on the survival times of mice with virus-induced leukemia. The top section and Chart 1, A and B, show data from the same experiment. The middle and bottom sections are from individual experiments.

**Chemotherapy of transplanted virus-induced leukemia.** Studies were also undertaken on the chemotherapy of the transplantable whole cell virus-induced leukemia. The transplantable whole cell preparation is capable of producing death with leukemia consistently and within a short period of time.

The results of three experiments employing mice with the transplantable whole-cell induced leukemia are described in Chart 3. The response of the untreated control, as measured by time to death with leukemia, was more rapid and less variable than was observed with mice given inoculations of virus preparations in the previous experiments. The MST of the control groups was 7.0–8.5 days. In these experiments drug therapy was initiated 3 days postinoculation and administered daily for 5 days. Of the eight drugs tested, TEM exerted the most extensive therapeutic effect; 0.25, 0.5, and 1.0 mg/kg, respectively, produced a 2-, 4-, and 11-day increase in MST over the untreated controls. However, in the third experiment, where both male and female 5-week-old mice were used, 1.0 mg/kg TEM was capable of effecting only a 1-day increase in MST. The three concentrations of MTX tested in two separate experiments produced small but consistent increases in MST. Of the three levels of DCM tested, only the 50 mg/kg dose level produced a 2-day increase in MST. Cytoxan and meticortelone increases were minimal.

Since the whole-cell preparation was very potent it was considered possible that withholding therapy until 3 days postinoculation may have limited the therapeutic effect. Experiments were therefore conducted in which the effect of varying the time of drug initiation on survival time was tested. The results of three such experiments are summarized in Chart 4. Drug was administered daily for 5 days starting at three different times following leukemic cell inoculation. In the first experiment when therapy was initiated 1 day...
postinoculation, a 4.5-day increase in MST occurred in the group of mice which received the lowest dose of TEM. Increasing the drug concentration decreased survival time so that at 1.50 mg/kg the MST was the same as the untreated controls. The decrease in survival time with increasing dose would appear to be attributable to drug toxicity. When therapy was initiated 3 days postinoculation, a 2-day increase in MST occurred with 0.7 mg/kg, but the higher concentrations were not so effective. A reverse effect occurred when the drug was withheld until the 6th day, when the most extensive response occurred with the highest concentration of TEM (1.5 mg/kg). The toxicity of the higher doses of TEM, becoming manifest later, when treatment was withheld until the 6th day following leukemic cell inoculation, could account for the increase in survival time.

When 6-MP was employed, the three drug concentrations tested were capable of producing a 2- to 4.5-day increase in MST over controls when therapy was initiated on the 1st or the 3rd day postinoculation. The inability to produce any increase in the lifespan of mice treated with even the highest concentration of 6-MP, with treatment beginning on the 5th day postinoculation, indicates that the disease had progressed too far to respond to therapy with this drug.

The administration of MTX 1 day postinoculation was capable of prolonging lifespan 1–2 days over that observed in the untreated controls. When treatment was withheld until the 3rd or 5th day postinoculation, the higher concentration of MTX produced the most extensive effect.

An additional experiment involving alteration of regimen of drug therapy is summarized in Chart 5. 6-MP and Cytoxan were administered on limited and continuous schedules, starting at two different times following leukemic inoculation. Results shown in section 1 demonstrate that, with treatment initiated 1 day following leukemic inoculation, limited and continuous therapy with 100 mg/kg of 6-MP produced only a 2-day increase in MST. Similar results were obtained when therapy was withhold until the 3rd day postinoculation. Limited and continuous therapy with Cytoxan, initiated 1 day after leukemic inoculation, resulted in greater increases in MST than did 6-MP. Continuous treatment with 12.5 mg/kg Cytoxan was the most effective, resulting in an 8-day increase in MST. The highest concentration of 25 mg/kg was apparently too toxic even when administered for five daily treatments. The toxicity of this level of Cytoxan had apparently also limited therapeutic activity in a previous experi-
ment (Chart 3). With therapy delayed for 3 days only a 4-day increase in MST was observed with continuous treatment, and a 2-day increase in MST with limited treatment. The higher concentration of Cytoxan was again apparently too toxic.

The results shown in Chart 6 demonstrate the influence of concentration of leukemic cell inoculum on the effectiveness of therapy with Cytoxan (12.5 mg/kg daily). No significant differences in MST of treated and untreated controls were apparent in either the male or female mice at the 5, 10, 20, or 50 per cent inoculum levels. However, at the 2.0 and 2.5 per cent inoculum levels Cytoxan therapy resulted in a 19-day and 14-day increase in MST over untreated animals.

During the course of this study, it became apparent that drug toxicity limited the treatment of BALB/c mice. The experience of this laboratory has been that CDBA hybrid mice are, in general, less susceptible than either inbred parent (BALB/cAn or DBA/2J) to the toxic effects of many compounds. It was considered possible that the employment of CDBA hybrid mice as hosts for the BALB/c whole-cell induced leukemia might permit more extensive therapy with effective drugs.

The results shown in Chart 7 illustrate the effect of daily continuous therapy initiated 1 day following leukemic inoculation in BALB/c and in CDBA hybrid mice. In the untreated control CDBA and BALB/c mice, a difference of only 1 day in MST was observed. DCM, 6-MP, TEM, and MTX therapy produced a 5-, 5-, and 12-day increase, respectively, in MST in CDBA mice, whereas these drugs failed to increase survival time in the BALB/c mice. A minimal effect occurred with MTX, where a 1-day increase in MST was obtained in the CDBA mice. With the above drugs, in general, weight loss occurred earlier and was more extensive with the BALB/c mice than with the CDBA mice.

Five drugs previously tested in BALB/c mice were assayed again in CDBA mice; the results are shown in Chart 8. Daily continuous drug therapy was initiated 1 day following leukemic inoculation. TEM and Cytoxan were the most effective in increasing survival time. TEM at 0.42 mg/kg and Cytoxan at 9 mg/kg produced an 11.5- and 10.5-day increase in MST over un-

**Chart 6.—Influence of cell concentration on the effectiveness of therapy.** Mice given intraperitoneal inoculations of 0.05 ml. of different dilutions of a 1:1 leukemic whole-cell suspension. Treatment initiated 1 day postinoculation and administered continuously until the end of the experiment.
untreated CDBA mice given inoculations of the BALB/c whole-cell preparation succumbed to the disease.

**DISCUSSION**

The current studies illustrate the use of the Moloney virus-induced leukemia and the transplanted virus-induced leukemic cells in the assay of antitumor agents.

The results of the studies on the effect of drug therapy against the virus-induced leukemia emphasize the importance of several factors of primary concern in assay systems of this type:

1. The variation observed in control mice was more extensive than is desirable for sensitive assay. Standard virus concentrates of high biological activity are currently being developed which may

---

**CHART 7**—Comparison of drug effectiveness in CDBA hybrid and BALB/cAn mice. Mice given intraperitoneal inoculations of 0.05 ml. of a 1:1 dilution of leukemic whole-cell suspension. Treatment initiated 1 day postinoculation and administered continuously until the end of the experiment.

**CHART 8**—Therapeutic effectiveness of drugs in CDBA hybrid mice. Mice given intraperitoneal inoculations of 0.05 ml. of a 1:1 dilution of leukemic whole-cell suspension. Treatment initiated 1 day postinoculation and administered continuously until the end of the experiment.
decrease the variation in the controls by reducing the latent period and time to death following virus inoculation.

2. In the current experiments treatment was initiated, for each mouse, at the time of diagnosis of the disease. The employment of potent standardized virus inocula would permit initiation of therapy at specified times following the induction of leukemia. Treatment could also be initiated during the induction period in studies of the prophylactic influence of therapy.

Of the drugs tested, TEM, Cytoxan, and MTX produced moderate increases in median survival time. The results also suggested activity for MGBGH and 5-FU. The increase in weight of thymus, liver, and spleen of the virus-inoculated mice was observed to be inhibited by administration of MTX, 6-MP, or Cytoxan. It is of interest that MTX and 6-MP were also effective in limiting spleen weight with the Friend mouse leukemia (12, 18).

It would be of interest to determine to what extent drugs may be inhibitory to the virus and/or to the induced neoplastic cell. Is death attributable to viral multiplication or to leukemic cell infiltration? What role may these interrelationships play in chemotherapeutic testing of Sarcoma 37 from which the virus was recovered?

Involved in the treatment of the virus-induced leukemia are the interrelations of the host, virus, leukemic cells, and drug. The observation that the drugs employed restricted the weights of the tissues studied would indicate an inhibitory effect on the leukemic process; on the other hand, no attempt was made to determine to what extent the reductions in organ size may have been attributable to nonspecific host toxicity. Determinations of the effect of the drug on the virus may be made in virus recovery studies and in studies involving drug administration in the induction phase following viral inoculation. Such studies are currently in progress.

Also, in the study of the virus-induced disease, collateral investigations of the chemotherapy of the transplantable virus-induced leukemia would appear to be desirable. In the current studies with the transplantable leukemia the control animals succumbed earlier and within a narrower range.

Animals given inoculations of the transplantable whole-cell preparation responded to drug therapy as evidenced by increased survival time. Of the drugs tested in BALB/c mice, MGBGH, 5-FU, and metiocetronol were ineffective; MTX, DCM, and 6-MP produced minimal increases in survival time. Cytoxan and TEM were the most effective. Sugiura (12, 18) reported the inhibitory effect of TEM on the Friend mouse virus leukemia.

The current studies indicate that the concentration of whole-cell leukemic inoculum may influence therapy. Cytoxan (12.5 mg/kg) produced a greater therapeutic effect in mice given inoculations of dilute inocula.

The results also indicate that the schedule of drug administration, including the time of treatment initiation and the number of treatments, may influence the drugs in this system.

The tolerance of the mice to drug may also be an important factor. CDBA hybrid mice are apparently capable of tolerating more drug therapy than are BALB/c mice. The CDBA mice exhibited substantial increases in median survival time when treated with DCM, 6-MP, and TEM in an experiment where these drugs were ineffective in BALB/c mice. As in the BALB/c mice, TEM and Cytoxan were the most effective drugs in the CDBA mice.

The Moloney virus-induced leukemia, the transplanted leukemia, and the virus itself would appear to afford useful tools in chemotherapeutic investigation.

REFERENCES
Response of a Virus-induced Murine Lymphoid Leukemia to Drug Therapy

Michael A. Chirigos, John B. Moloney, Stewart R. Humphreys, et al.


Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/21/6/803

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.