Studies with the Murine Leukemogenic Rauscher Virus

II. Chemotherapy of Virus-induced Lymphoid Leukemia

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SUMMARY

Eight drugs were tested against the Rauscher virus-induced leukemia. In virus-inoculated mice a limited course of drug therapy was initiated after the mice had become leukemic. Netropsin, Melphalan, 6-mercaptopurine, and 5-fluorouracil produced a twofold increase in the survival time of leukemic animals. Cytoxan and Vincristine were moderately effective; p-anisaldehyde thiosemicarbazone and methylglyoxalbisguanylhydrazone were only minimally effective. Inhibition of splenomegaly was observed in animals subjected to effective therapy.

In a previous report assay systems were described for the chemotherapeutic testing of drugs in mice given inoculations of Rauscher virus (1). The parameters of drug effectiveness employed were survival time of the animals, extent of splenomegaly, and transmissibility of the Rauscher disease. In this study the drugs investigated were methotrexate (MTX), 6-mercaptopurine (6-MP), triethylenemelamine (TEM), and 2-chloro-4', 4''-di-2-imidazolin-2-yl-terephthalanilide (NSC-60,339). 6-MP, TEM, and NSC-60,339 were effective in increasing survival time when a limited course of treatment was initiated at 5 or 17 days following virus inoculation.

MTX was ineffective when treatment was initiated 3 days following virus inoculation but did increase the number of long-term survivors when treatment was initiated at the later time. Pathology studies had indicated that at 3 days following virus inoculation no leukemia was apparent. However, definite evidence of leukemia occurred by the 17th day following virus inoculation. It was considered, in the latter instance, that increases in survival time produced by drug therapy could reflect inhibition of established leukemic disease as well as any inhibition of the etiological agent, the virus.

In view of the importance of host-tumor-virus interrelationships in chemotherapy, it was considered desirable to extend the study of the Rauscher virus-induced disease with the use of a variety of potentially effective chemotherapeutic agents. This report presents the results of an investigation of Melphalan, Cytoxan, Vincristine, 5-fluorouracil, 6-mercaptopurine, and methylglyoxalbisguanylhydrazone tested in a population of mice with Rauscher virus-induced leukemia. Two antiviral agents, Netropsin and p-anisaldehyde thiosemicarbazone, were also tested in this system. Netropsin protected mice infected with vaccinia virus (9), and thiosemicarbazones have been reported to be effective against virus disease (5, 12).

MATERIALS AND METHODS

Preparation of standard virus.—The preparation of standard pools of virus has been described previously (3, 8).

Virus inoculum.—Preparation of the virus inoculum was carried out as follows:

A frozen aliquot of the virus concentrate was diluted with sterile 0.8 per cent saline to a 10⁻¹.₃ (0.05 gm equivalent of spleen/ml) concentration. Two-tenths ml. of diluted virus was inoculated intraperitoneally into 7- to 8-week-old BALB/c male or female mice weighing between 23 and 25 gm.

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1 Obtained from NIH breeding colonies.
Randomization of test animals.—In all experiments mice were randomized shortly after inoculation and were then distributed into appropriate groups.

Drugs and treatment.—Netropsin, Melphalan, Cytoxan (cyclophosphamide), Vincristine, and 5-fluorouracil (5-FU) were diluted in 0.9 per cent saline. 6-Mercaptopurine (6-MP) was dissolved in dilute alkali; p-anisaldehyde thiosemicarbazone (NSC-712) and methylglyoxalbisguanylhydrazone diacetate trihydrate (NSC-30,689) were suspended in 0.5 per cent methyl cellulose. 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

Histopathology.—For histological examination virus-inoculated mice were picked at random from the population in each experiment. In the first experiment control virus-inoculated mice were sacrificed 17 days after virus inoculation, and complete autopsies were performed. The tissues were fixed in Zenker-formol solution, and the sections were stained with hematoxylin and eosin. In agreement with previous observations (3, 8), histological examination of the tissues revealed that at 17 days post-virus inoculation the normal splenic architecture of the virus-inoculated animals was almost completely absent. Few or no Malpighian bodies were seen. The splenic tissue consisted predominantly of large immature lymphoid cells with scant cytoplasm and large vesicular nuclei, the nucleoli of which were large and prominent. Mitotic figures were numerous. A few degenerating cells were interspersed with both immature leukocytes and erythrocytes. The lymph nodes were characterized mainly by hyperplasia of lymphoid follicles and dilation of sinusoïds. With the exception of marked erythrocytopoiesis in the liver, the other tissues were not unusual.

In the second experiment control virus-inoculated mice were sacrificed 88 days after virus inoculation. Histological examination of the spleen revealed massive areas of hemorrhage and fibrosis. Numerous foci of lymphoid leukemic cells frequently interspersed with immature erythrocytes were prevalent. An examination of tail blood smears showed the presence of an appreciable number of lymphoblasts, bands, myelocytes, and metamyelocytes.

Chemotherapy of virus-inoculated mice.—The results of treatment on the survival time of BALB/c male mice given inoculations of virus in the first experiment are presented in Chart 1. Treatment was initiated on the 17th day following virus inoculation and continued daily for 5 days. The

![Chart 1](chart1.png)

* Netropsin (2-[4-(4-quinidinocetamidino-1-methyl-2-pyrrolecarboxamido)-1-methyl-2-pyrrolecarboxamido]propionamide) was obtained from Mr. John Davenport, Charles Pfizer and Co., Inc., Maywood, N.J. Vincristine sulfate (7) was obtained from Eli Lilly and Co. The remaining compounds were obtained from the Cancer Chemotherapy National Service Center through the following sources: Melphalan (p-di[2-chloroethyl]-amino-2-phenylalaine), Chester Beatty Research Institute; cyclophosphamide (N-(2H,1,2-oxazaphosphorine, 2bis[2-chloroethyl]amino)tetrahydro-2-oxide) (Cytoxan), Mead Johnson and Co.; 5-fluorouracil (5-FU), Hoffman-LaRoche, Inc.; methylglyoxalbisguanylhydrazone diacetate trihydrate (NSC-30,689), Frederic A. French, Maywood, N.J.; p-anisaldehyde thiosemicarbazone (NSC-712), Merck, Sharp and Dohme, Rahway, N.J.; 6-mercaptopurine (6-MP), Burroughs Wellcome Company.

CHART 1.—Influence of chemotherapeutic agents on the survival time of 7- to 8-week-old BALB/c male mice given inoculations of 0.8 ml of a 10⁻¹⁰ dilution of a stock virus preparation. Chemotherapy was initiated 17 days following virus inoculation and continued daily for 5 days (days 17 through 21). There were ten animals per experimental group, except in the control group, which contained 30 animals. Figures in parentheses represent the number of mice surviving to 180 days.
range of individual deaths in the untreated controls (Chart 1), from time of virus inoculation, was from 37 to 178 days. The median survival time (MST) for the group was 78 days.

Melphalan (3.2 mg/kg) and 5-FU (25 mg/kg) exerted the most extensive therapeutic effect, producing an approximately twofold increase in MST. Treatment with higher dose levels of the drug produced earlier deaths, indicative of drug toxicity. Netropsin, at dose levels of 3.12, 6.25, and of NSC-712 tested. Nondiseased drug-treated animals showed tolerance to the highest dose level of NSC-712 tested, indicating that higher dose levels might have been more effective. All the drug-treated, infected mice surviving for longer than 180 days showed signs of the virus-induced disease as evidenced grossly by enlarged spleens.

In the second experiment BALB/c female mice were treated with one dose level of Melphalan, 6-MP, and Vincristine (Chart 2). Melphalan or 6-MP treatment was initiated on the 38th day following virus inoculation and continued daily for 5 days. It had been planned to treat with Vincristine daily for 5 days. However, four of the nine mice in the group died 1 day after the first injection, and treatment was discontinued (Chart 2).

In this experiment, despite the delay in administering the drug (3 weeks prior to the median time of death of controls) as compared with the first experiment (8 weeks prior to the median time of death of controls) (Chart 1), effective therapy was obtained with Melphalan. Treatment with Melphalan (3.2 mg/kg, Chart 1, and 5.4 mg/kg, Chart 2) resulted in a twofold increase in survival time. In both experiments, treatment with 0.5 mg/kg Vincristine produced early deaths. Treatment with 6-MP (Chart 2) resulted in a 2 1/2-fold increase in survival time. All the drug-treated, infected mice surviving for 179 days (Chart 2) showed signs of the virus-induced disease as evidenced grossly by enlarged spleens and microscopically by the presence of pathologic numbers of lymphoblasts, bands, myelocytes, and metamyelocytes in smears of tail blood.

It was of interest to determine whether a correlation occurred between spleen reduction and increase in survival time as a result of drug treatment. The dosage level of each drug which produced the greater increase in survival time (Charts 1 and 2) and its effect on splenomegaly is presented in Table 1.

Treatment with Netropsin, Melphalan, Vincristine, and 5-FU reduced splenomegaly with a concomitant increase in the lifespan of the animals (Table 1, Exp. 1). Treatment with Melphalan and 6-MP (Table 1, Exp. 2) also reduced splenomegaly, with a concomitant increase in the survival time of the animals. Vincristine (0.5 mg/kg) reduced splenomegaly but produced early deaths which were attributed to drug toxicity.

Male BALB/c control mice had a MST of 78 days (Chart 1). In contrast, female mice of the same strain had a MST of 60 days (Chart 2). This difference is in agreement with the observation of Rauscher\(^{3}\) that female BALB/c mice are more

\(^{3}\) F. J. Rauscher, personal communication.
susceptible to the virus-induced disease and succumb earlier than BALB/c males.

DISCUSSION

In the current study, eight drugs were tested against primary virus-induced leukemia in mice. With each drug, treatment was initiated when mice showed definite evidence of leukemia. In the first experiment drug therapy was withheld until 8½ weeks prior to the median day of death of controls. Netropsin, Melphalan, and 5-FU were effective in producing a twofold increase in survival time of leukemic animals. Cytoxan and Vincristine were moderately effective; NSC-712 and NSC-30,689 were minimally effective.

In the second experiment, in which drug therapy was withheld until 3 weeks prior to the median day of death of controls, Melphalan and 6-MP produced at least a twofold increase in survival time of leukemic animals. A similar effect was obtained with 6-MP in leukemic mice in a previous study (3).

A previous report from this laboratory (2) showed that Cytoxan was also effective in increasing the survival time of mice inoculated with the Moloney virus and in decreasing the weight of thymus, liver, and spleen. NSC-30,689 and 5-FU caused a moderate increase in survival time (2).

In the current study, inhibition of splenomegaly appeared to accompany prolongation of the lifespan of Rauscher virus-induced leukemic mice. Whether the reduction in splenomegaly, by drug therapy, had any effect on the leukemic process has not, as yet, been elucidated. Sugiura (11) tested the effectiveness of various drugs in mice inoculated with the Friend virus and showed that

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**TABLE 1**

<table>
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<tr>
<th>Exp. No.</th>
<th>Group No.</th>
<th>Drug</th>
<th>Daily Dose (mg/kg)</th>
<th>No. Treatments*</th>
<th>Av. sp. palp. score†</th>
<th>Median Survival Time (Days)</th>
<th>Survivors/Total‡</th>
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* No. of treatments from day 17 for Experiment 1, and from day 38 for Experiment 2.
† Av. sp. palp. score = Average spleen palpation score; the figures represent the average spleen palpation of the survivors of the group. Enlargement based on an arbitrary scale ranging from 1+ to 4+. Average spleen weight, based on palpations obtained from a separate study, were: ± (0.20 gm. or less), 1+ (0.3 gm.), 2+ (0.6 gm.), 3+ (1.0 gm.), and 4+ (2.0—4.0 gm.).
‡ The number of survivors when the experiment was terminated at 180 days and 179 days for Experiments 1 and 2, respectively.

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cristine, 0.25 mg/kg; and 5-FU, 25 mg/kg) also produced a 4, 10, 10, 10, and 5 per cent loss in body weight, respectively. However, in most cases the mice regained weight rapidly. Although the loss in body weight was not extensive, it was considered possible that it may have influenced splenomegaly and survival time of the leukemic animals. A number of investigators have shown that reduced food intake and body weight loss resulting from drug administration may retard tumor growth (4, 10, 13). In any investigation of the selectivity of antineoplastic chemotherapeutic agents it is desirable to determine to what extent nonspecific host toxicity, as reflected in reduction in food intake and weight loss of the host, may account for the observable antitumor effects. Such an investigation is currently in progress and is designed to determine what effect body weight loss may have on splenomegaly and survival time of animals with virus-induced leukemia.

In a previous study (3) the four drugs MTX, 6-MP, TEM, and NSC-60,839 appeared to be more effective in animals histologically diagnosed as leukemic than in animals treated shortly after virus inoculation. It was suggested that the drug treatment may have produced a more pronounced effect in retarding the established leukemic disease than in inhibiting the etiological agent, the virus.

In preliminary experiments Vincristine, Cytoxan, and NSC-80,689 were tested for antiviral activity by beginning treatment of the mice shortly after virus inoculation (1). Vincristine, which was active against the virus-induced disease, also appeared to exert antiviral activity. Metoprine and Cytoxan, although effective against the virus-induced disease, did not exert any antiviral effect. The activity of NSC-80,689 was limited to a minimal effect against the virus-induced disease. It did not show any antiviral activity. The difference in response to therapy before and after the virus has induced leukemia indicates the importance of further studies of host-tumor-virus interrelationships in chemotherapy.

REFERENCES

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