The Effect of Treatment with a Combination of 6-Mercaptopurine and Porfiromycin on an Established Friend Leukemia Virus Infection

Robert W. Sidwell, Glen J. Dixon, Patricia Compton, and Frank M. Schabel, Jr.

SUMMARY

The effect of parenteral therapy with combinations of 6-mercaptopurine and porfiromycin on established infections of Friend leukemia virus in Swiss mice was studied. In one experiment both drugs were markedly effective in reducing virus-induced splenomegaly and virus titers in the plasma and spleens, but treatment with the combination of drugs was more effective than treatment with either drug used alone, when comparative nonlethal drug levels were used. In a second experiment these results were confirmed, and reduction in blood hematocrit values and in hepatomegaly were used successfully as additional criteria for evaluation. In all experiments treatment was not initiated until 14 days after virus inoculation when the virus infection was established in the animals as shown by demonstrable splenomegaly and spleen and plasma virus titers.

INTRODUCTION

A problem acknowledged by a number of investigators in the field of cancer chemotherapy has been the possible role viruses may play in the etiology of cancer. This possible viral etiology has serious implications when the control of human cancer is sought through chemotherapy, since, if the viral etiology of the disease is recognized, both the elimination of the virus-induced neoplastic disease (cell cure) and the elimination of the oncogenic virus, which may be capable of reinduction of the disease, must be accomplished to bring about a complete cure of the disease (4, 9, 24). One approach to these problems would be to develop effective compounds and methods of treatment against a neoplastic disease of lower animals which is known to be induced by a virus, assuming that there would be some relation between these methods and those used for a similar human disease. In the past, the results of chemotherapy studies with cell-induced mouse leukemias have usually correlated well with the results of chemotherapy against acute human lymphatic leukemia (31).

The murine reticulum cell leukemia induced by the Friend virus has been employed in a series of chemotherapy studies to attempt to attain the above goals of determining effective therapeutic agents and to develop acceptable methods of treatment against this type of neoplastic disease (5, 11, 19, 21—23, 26). These studies have indicated that two classes of compounds, purine analogs or antagonists and biologic alkylating agents, are highly effective against the Friend virus-induced disease (22). The available data suggest that the purine compounds inhibit the formation of some cellular component necessary for virus synthesis (23). Less is known concerning the mode of action of the alkylating agents against the Friend disease, although our studies have indicated they probably act against the disease in a different manner than the purine analogs. These observations prompted a series of experiments to determine if treatment with a combination of one of each of these compounds would be more effective against an established infection with the Friend virus than treatment with either compound used alone. In order to evaluate whether chemotherapy with a drug combination is synergistic, a fixed criterion must be employed as a baseline for measurement. A fixed limit of toxicity for each drug, as well as the drug combination, was used in the present studies as this baseline. Hence, the most effective drug or drug combination would be that which was significantly effective against the disease at drug levels which are the least toxic. Drug toxicity can be measured in many ways; for the present studies, described in this report, lethality was the primary criterion, although host weight loss was also considered.

MATERIALS AND METHODS

Animals. Random-bred 18- to 21-gram ICR Swiss mice obtained from Southern Animal Farms, Prattville, Alabama, were used in these studies.
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**Virus.** The Friend leukemia virus (FLV) employed was the spleen filtrate preparation described in detail previously (22). This virus produces a malignant proliferation in reticulum cells involving the spleen and liver; a marked polycythemia is also induced.

**Compounds.** The purine analog employed was 6-mercaptopurine (purine-6-thiol hydrate, 6-MP, NSC 755) and the alkylating agent used was porfiromycin (methylmitomycin C, NSC 56410). The activity of each compound against the Friend virus has been previously reported (5, 11, 19, 20, 22, 23, 26). Both compounds were supplied by the Cancer Chemotherapy National Service Center, National Cancer Institute. In the first experiment carried out in this study, the suspending vehicle for 6-MP was sterile 0.4% carboxymethylcellulose (CMC) in phosphate-buffered saline; porfiromycin was dissolved in sterile distilled water. In the second experiment, CMC was used for both compounds.

**Methods.** Experiment 1. Mice were inoculated i. p. with 0.2 ml of a cell-free spleen suspension containing about $10^6 \text{ID}_{50}$ of FLV. Fourteen days after virus inoculation, 20 mice were killed, their spleens weighed, and the FLV titers of these spleens and the plasma from the same animals were determined by splenomegaly induction in recipient indicator Swiss mice inoculated i. p. (5, 22). The remaining animals were then randomized and divided into groups of 10 for each drug dosage. Twenty animals were treated with drug vehicle only and served as virus controls, and 20 uninfected mice of the same weight were treated with each dosage of drug to serve as toxicity controls. The latter controls were killed 40 days after initiation of treatment, which began 14 days after virus inoculation. A series of doses of each drug was administered i. p. once daily for 9 days, each dosage given in mg/kg based on a daily individual animal weight. The dosage levels of each drug and combination of drugs used were designed to range from toxic to nontoxic levels, the LD$_{10}$ being approximately the median dose. Since both 6-MP and porfiromycin have been used in many experiments in our laboratory, the LD$_{10}$ level of each drug was well established (Chart 1). Less work has been done

![Chart 1](https://image-url.com/chart1.png)

**Chart 1.** Summarization of cumulative mortality plots and computer-calculated line of best fit employed to determine the LD$_{10}$ dose levels of 6-mercaptopurine and porfiromycin, derived from a series of separate toxicity experiments. Each point represents 10 or 20 mice.
with the drug combination, but we felt this LD_{10} level was also moderately well fixed, based on lethality in Swiss mice in both these and other experiments. When two drugs were given to the same animals, each was injected separately. Virus control mice were injected with drug vehicle only in the same number of injections. Animals treated with a single drug were also injected with drug vehicle on the same schedule as those mice receiving multiple drugs so that all received the equal stress of multiple injections. Twenty-three days post-virus inoculation all the virus-infected mice were killed and the spleens and plasma were removed. The spleens were weighed and the virus titers determined in the spleens and plasma. The evaluation of the comparative effectiveness of each drug and combination of drugs was carried out by comparing the effects of equally toxic dosages of each.

**Experiment 2.** A second experiment was carried out in which the drugs were suspended in CMC and mixed immediately prior to injection; the mice therefore received only one drug injection per day. This alteration was devised to reduce the trauma to the animal resulting from multiple drug injections. Since the spleen was markedly enlarged when treatment was initiated, the drugs were injected s.c. in this second experiment to avoid puncture of the spleen. The criteria for the evaluation of drug effectiveness in this second experiment included reduction in splenomegaly and in spleen and plasma virus titers, and, in addition, reductions in blood hematocrit values and hepatomegaly were also determined.

### RESULTS

Treatment with each compound singly or in combination was effective in reducing the mean spleen weights and the virus titers in the spleens and plasma of the infected mice. The results of the first experiment are summarized in Table 1. The comparative effectiveness of treatment with the drug combination compared with the treatment with the individual drugs was evaluated on the basis of relative mortalities of the toxicity control animals. When the splenomegaly reduction per dose of each drug and drug combination was plotted using the LD_{10} dose of each as the "base" dose, the therapy with the drug combination apparently had a potentiating effect (Chart 2). This synergism was also indicated by reductions in spleen and plasma FLV titers brought about by therapy with nonlethally toxic dose levels of the drug combination. Using significant virus titer reduction as the criterion for evaluation, the therapeutic index (highest nontoxic drug dose divided by the lowest active drug dose) was 1 for 6-MP, 1 or 2 for porfiromycin, and 4 or greater for the drug combination. Since different vehicles were used for each drug, a separate set of virus control animals (treated with vehicle only) was utilized for each group, thus accounting for the difference in splenomegaly and spleen and plasma virus titers in the three simultaneously run control groups shown in Table 1.

In the second experiment, treatments with each drug and with the combinations of drugs again reduced the spleen

<table>
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<tr>
<th>Drug dosagea (mg/kg/day)</th>
<th>Toxicity control mortalityb (D/T)</th>
<th>Av. spleen wt. (mg)</th>
<th>Splenomegaly reduction (P)c</th>
<th>Virus titera</th>
<th>Virus titersd</th>
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<td></td>
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Table 1

The effect of 6-mercaptopurine and porfiromycin used singly and in combination on established Friend leukemia virus infections in Swiss mice. Mice were inoculated i.p. with approximately 10^6 ID_{50} of virus and held 14 days prior to treatment. Average spleen weight of 20 mice sacrificed 14 days postvirus inoculation = 741 mg.

aDrugs were administered i.p. once daily for 9 days beginning 14 days postvirus inoculation. Each drug was administered in a separate injection to all mice.

bToxicity control mice held 40 days after initiation of treatment.

cProbability (t test) that reductions in splenomegaly or in spleen and plasma virus titers were due to chance.

dExpressed as the maximum dilution of plasma or spleen homogenates that induced significant splenomegaly in recipient Swiss mice (22).
DISCUSSION

Chemotherapy with drug combinations has been used effectively by a number of investigators in recent years (16, 28, 30), and combinations of purine analogs, including 6-MP, and alkylating agents for use in such studies is not unique. Using 6-MP in combinations with dopan (5-bis[β-chloroethyl] amino-6-methyluracil) or sarcolysin (DL-p-[β-chloroethyl] amino-phenylalanine), an additive effect was demonstrated against Ehrlich carcinoma, Crocker sarcoma, and Sarcoma 180 (25, 30). Uracil mustard and 6-MP combinations were more effective against Sarcoma 180 than either compound used alone (3). Schabel (18) has reported the effectiveness of the combination of 6-MP and Cytoxan against carcinoma 755 in mice. Other alkylating agents used in combination with 6-MP that produced synergistic effects in tumor systems have includ-

weight and the virus titers, and in addition reduced the mean hematocrit and the hepatomegaly of the virus-infected mice (Table 2). When the splenomegaly data were again plotted against the equivalent toxicities of each drug and the drug combination, the therapy with the drug combination was again more effective than treatment with either drug used alone (Chart 3). Treatment with the drug combination was also markedly effective in reducing the spleen and plasma virus titers at all doses employed, indicating by other perimeters the potentiating effect of the drug combination. Comparing virus titer reduction, in this experiment the therapeutic index was about 2 for 6-MP, 0 for porfiromycin, and 8 or greater for the drug combination. The use of a common vehicle (CMC) in this second experiment had the advantage of requiring only a single group of virus control animals.

Chart 2. The effect of intraperitoneal treatment with 6-mercaptopurine and porfiromycin used singly and in combination on established Friend leukemia virus infections in mice. Data presented as dose response plots of mean splenomegaly inhibition (10 mice per point) vs drug dosages. △, porfiromycin-treated mice; ○, 6-mercaptopurine-treated mice; □, mice treated with combination of the above two drugs.

Chart 3. The effect of subcutaneous treatment with 6-mercaptopurine and porfiromycin used singly and in combination on established Friend leukemia virus infections in mice. Data presented as dose response plots of mean splenomegaly inhibition (10 mice per point) vs drug dosages. △, porfiromycin-treated mice; ○, 6-mercaptopurine-treated mice; □, mice treated with combination of the above two drugs.
ed N-oxapentamethylen-N',N''-diethylenetriphosphoramid, cyclophosphamide, myleran, and nitrogen mustard (2, 7, 8, 10, 13). Porfiromycin has been used with success in combination with 6-thioguanine, a purine analog, in experiments with Sarcoma 180 and L1210 lymphoma (17). The present studies have demonstrated that the combination of 6-MP and porfiromycin is more effective against the virus-induced Friend disease than is either compound used alone. This is presumably the first such demonstration of synergistic effect against a virus-induced neoplasm. Particularly significant in these studies was the effect against an established infection of the virus, that is, the spleen had increased in size at least five-fold, the spleen and plasma virus titers were in excess in 1.5 log10, hepatomegaly was apparent, and the blood hematocrit had increased prior to initiation of treatment. Treatment with the higher nonlethally toxic drug doses was sufficient to essentially eliminate all signs of the disease from the animal. As reported previously (21), excessive host weight loss resulting from drug toxicity can alter the development of splenomegaly; in the present study the animals treated with the nonlethally toxic doses of the compounds did not lose a significant amount of body weight, thus strengthening the premise that the antiviral and antitumor activity seen was a result of specific drug action.

6-Mercaptopurine is thought to inhibit purine synthesis de novo (1) and to inhibit some of the interconversions of purine nucleotides (15). We have previously demonstrated this compound to be effective when administered in a single injection at an early stage in the virus production in the mouse (23), suggesting the drug affects either the replication of viral RNA, the synthesis of an RNA component required for enzyme synthesis in the cell, or the synthesis of new protein needed for virus production. In unreported studies we have noted that porfiromycin is most effective against the Friend disease if administered during the period of time when the virus titers in the spleen and plasma are increasing at their most rapid rate. In our experience, the drug has not been virucidal, i.e., the compound was ineffective when incubated up to one hour with FLV prior to inoculation of the virus into test mice. Similar nonvirucidal results using other animal viruses have been reported with a related compound, Mitomycin C (27). In our experience, the drug has not been virucidal, i.e., the compound was ineffective when incubated up to one hour with FLV prior to inoculation of the virus into test mice. Similar nonvirucidal results using other animal viruses have been reported with a related compound, Mitomycin C (27). In animal cells, the mitomycins become converted to highly reactive bifunctional alkylating agents which form covalent bonds with nucleic acids and proteins, with a resultant inhibition of DNA replication as well as a secondary inhibition of RNA synthesis (27). Production of viruses containing single-stranded RNA usually is little, if at all, affected by moderate concentrations of mitomycins (6, 12), although the synthesis of the viruses of fowl plague (14) and Rous sarcoma (29) reportedly is sensitive to these drugs. No work has been reported on the specific mechanism of action of the mitomycins on the RNA-containing Friend virus.
REFERENCES


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