The Effect of 6-Mercaptopurine and Allopurinol on Granulopoiesis

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SUMMARY

The effect on granulopoiesis of 6-mercaptopurine alone, and in combination with allopurinol, was studied in mice using an in vitro colony-forming assay for granulocytic progenitor cells. The combination of 6-mercaptopurine and allopurinol produced significantly greater suppression of de novo granulopoiesis than did 6-mercaptopurine alone. Allopurinol in very high doses was found to be myelosuppressive. The possible implications of these findings are discussed.

INTRODUCTION

Allopurinol [4-hydroxypyrazolo(3,4-d)pyrimidine], a xanthine oxidase inhibitor, is now generally used for the prevention and treatment of hyperuricemia in patients with gout and malignant disorders (6, 11). The enzyme xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine. Similarly, xanthine oxidase converts 6-MP to thiouric acid (5, 10). Thiouric acid accounts for a sizeable proportion of the urinary products of 6-MP and is itself inactive as an inhibitor of tumor growth (3). The inhibition of the oxidation of 6-MP to thiouric acid results in the potentiation of its action in vivo in man and mice. The effect of allopurinol on the metabolism of 6-MP was studied in patients with chronic granulocytic leukemia (9). When allopurinol was combined with 6-MP the 24-hr urinary excretion of 6-MP increased from 7% to 29%, while the excretion of thiouric acid decreased from 25% to 3% (9). Seven patients with chronic granulocytic leukemia were treated with 6-MP (50 mg/day) alone. When allopurinol (400 mg/day) was added to the regimen, the combination produced a drop in the granulocyte level equivalent to 4 to 5 times the dose of 6-MP alone (9). Levine et al. (7) treated a group of children with acute leukemia in remission with 6-MP (2.5 mg/kg/day) and allopurinol (10 mg/kg/day). Profound pancytopenia developed rapidly in the 1st 3 patients; but when the dose of 6-MP was decreased by 50%, no untoward side effects were noted. Because of these findings it is accepted clinical practice to decrease the dose of 6-MP whenever allopurinol is also administered (8, 9).

The metabolism of 6-MP and the effect of allopurinol on its excretion has been shown to be very similar in mice and man (3). It has also been shown that the antitumor activity of 6-MP is increased by the simultaneous administration of allopurinol in mice. When allopurinol was administered with 6-MP to mice bearing adenocarcinoma 755, a 4-fold increase in tumor kill was noted when compared to 6-MP alone (3). Recently, however, Walker et al. (12) administered 6-MP alone and the combination of 6-MP and allopurinol to normal New Zealand rabbits. They noted that rabbits receiving the combination had higher neutrophil and mononuclear cell counts in their peripheral blood, greater weight gain, and more prolonged survival than did rabbits receiving 6-MP alone.

In this study we have examined the effect of 6-MP, allopurinol, and their combination on granulopoiesis in mice. Measurement of the peripheral white cell count is the most frequently used method for assessing the myelosuppressive effect of a chemotherapeutic agent. Many variables may alter the peripheral white cell compartment, including the rate of proliferation and maturation of the granulocytic progenitor cells, the release of mature white cells into the peripheral blood, the size of the marginal pool, and the rate of destruction of white cells. A method is now available for the quantitation of granulocytic progenitor cells from the bone marrow of animals and man. When bone marrow cells are cultured in agar and incubated for variable periods of time, colonies of granulocytic and mononuclear cells are formed (1, 8). Each colony is derived from a CFC or a granulocytic progenitor cell. This in vitro culture system therefore gives an accurate assessment of de novo granulocyte production. We have utilized this technique to study the effect of 6-MP, allopurinol, and their combination on granulopoiesis in mice.

MATERIALS AND METHODS

Animals. Eight-week-old male BALB/c X DBA/2 F₁ (hereafter called CD2F₁) mice (supplied by the National Cancer Institute) weighing approximately 25 g were used in these experiments.

Chemotherapeutic Agents. 6-MP (Ben Venue Laboratories, Bedford, Ohio) in powder form was dissolved in sterile water to the desired concentration.
Allopurinol (Burroughs & Wellcome Co., Research Triangle Park, N. C.) in powder form was dissolved in 0.1 N sodium hydroxide and the pH was adjusted to 10.5 by the addition of 2 N hydrochloric acid. The required concentration of allopurinol was obtained by the addition of sterile water.

The Administration Schedule of Chemotherapeutic Agents. The mice were divided into 4 groups: Group 1 (controls) had their femoral CFC assayed before and 24 hr after 2 injections of 0.9% NaCl solution were administered 12 hr apart. All chemotherapeutic agents were injected into the tail vein in 0.5 ml of solution every 12 hr for a total of 4 injections. Group 2 of mice received allopurinol (40 mg/kg, 4 times), Group 3 received 6-MP (40 mg/kg, 4 times), and Group 4 received the combination of allopurinol and 6-MP (40 mg of each drug/kg, 4 times). To test the effect of different concentrations of allopurinol on the femoral CFC, groups of CD2F₁ mice were given injections of 40, 100, 200, 300, and 400 mg of allopurinol per kg. Twenty-four hr later the mice were killed, the bone marrow cells were cultured in agar, and the number of colonies formed was determined.

Preparation of Bone Marrow Cell Suspension. Twenty-four hr after the 1st injections were given, and daily thereafter, the tips of the tails of the mice were cut off; peripheral blood was aspirated with a hemocytometer pipet and diluted and the number of white cells per cu mm was counted. These mice were then killed by cervical dislocation and both femora were aseptically removed from 3 mice in each group. The tip of each femoral shaft was removed and a 23-gauge needle mounted on a 3-ml syringe was inserted into 1 end of the bone. The marrow plug was gently flushed out of the shaft with repeated aspirations into the syringe. This resulted in the pooling of bone marrow cells from 6 femurs for each group of mice. Nucleated cell counts were performed with the aid of an electronic cell counter and the number of nucleated cells per femur was determined.

Culture Medium and Plating Technique. An agar mixture was prepared containing the following ingredients: 40% agar (0.8% purified agar, Difco Laboratories, Inc., Detroit, Mich.), 40% modified Eagle’s medium, alpha medium (2-fold concentration) (Flow Laboratories, Rockville, Md.), 8% fetal calf serum (Flow Laboratories, Rockville, Md.), 8% L-cell conditioned medium, 4% horse serum (Grand Island Biological Co., Grand Island, N.Y.).

The L-cell conditioned medium provided the source of colony-stimulating activity and was prepared from the supernatant of a monolayer of mouse L-cells. The cell suspension of mouse marrow cells was diluted to 10 times the desired final concentration. The final number of nucleated cells in each plate was 5 x 10⁵. Immediately before plating, 0.5 ml of the cells was mixed with 4.5 ml of the agar mixture. Then 1 ml of this mixture was pipetted into each of 4 Petri dishes. The dishes were incubated at 37°C in a humidified incubator with a flow of air containing 7.5% CO₂. At the end of 7 days incubation the plates were examined with an inverted microscope at 40 X magnification, and colonies containing 50 or more cells were scored. The mean and the standard error of the mean were then calculated from the results of 3 experiments.

RESULTS

The number of nucleated cells per femur after repeated injections of allopurinol, 6-MP, and the combination of allopurinol and 6-MP is shown in Chart 1. Allopurinol alone had no significant effect, while the combination of 6-MP plus allopurinol decreased the number of nucleated cells/femur to a greater extent than did 6-MP alone. This was statistically significant at the nadir of myelosuppression on Days 3 and 4 (p < 0.001).

The number of CFC per femur was also significantly reduced for the combination over that for 6-MP alone on Days 3 and 4 (Chart 2). Allopurinol produced a slight decrease in the number of CFC. The number of circulating peripheral white cells was decreased with 6-MP alone and with the combination (Chart 3). Although there was a slightly greater suppression of the peripheral white cell count with the combination (starting on Day 4) than with 6-MP alone, this was not statistically significant. Allopurinol alone produced depression of the white cell count, which was most noticeable on Day 3.
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Chart 3. The effect of allopurinol, 6-MP, and the combination of allopurinol and 6-MP on the number of peripheral white cells. Shaded area, mean ± S.E. for the controls. Arrows, time of administration of chemotherapeutic agents.

Allopurinol in high doses (100 mg/kg) produced a 25% reduction of CFC. Larger doses of allopurinol did not result in greater suppression of CFC (Chart 4). With very high doses of allopurinol (300 to 400 mg/kg) the mice appeared toxic and, in 1 experiment, 2 mice died.

DISCUSSION

Our results would suggest that the combination of 6-MP and allopurinol is more myelosuppressive than 6-MP alone. The maximum suppression of the number of CFC is evident on Days 3 and 4, after which recovery begins. The maximum myelosuppressive effect on the peripheral white cells occurred around Day 6. The fact that the combination had a greater myelosuppressive effect on the CFC than on the circulating peripheral white cells may be due to the presence of other operative factors (maturation of stem cells, release of white cells into the peripheral blood, marginal leukocyte pool, rate of destruction of peripheral white cells) that may modify the number of peripheral white cells. It may be that the study of the granulopoietic CFC in culture is a more sensitive assay for the quantitation of the myelosuppressive effect of chemotherapeutic agents than the peripheral white cell count. The dose of allopurinol that produced myelosuppression in these experiments (100 mg/kg) would never be used in clinical medicine.

It is possible that the differences between our studies and those of Walker et al. (12) may be due to differences in the animal species investigated. The final answer as to whether the combination of 6-MP and allopurinol is more myelosuppressive in man must await carefully controlled clinical trials.

ACKNOWLEDGMENTS

We would like to thank Dr. George Lyon (Burroughs-Wellcome Company) for his kind cooperation and help during this study. We would also like to thank Dr. Philip R. Dodge and Dr. Teresa J. Vietti for their support and critical review of the manuscript.

ADDENDUM

Since submission of our manuscript for publication, a study has been reported by the Boston Collaborative Drug Surveillance program (J. Am. Med. Assoc., 227: 1036–1040, 1974). This study substantiates the fact that allopurinol increases the incidence of bone marrow depression in patients with neoplastic diseases who received chemotherapy.

REFERENCES

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