The Failure of Allopurinol to Enhance 6-Mercaptopurine Toxicity in Rabbits

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SUMMARY

We have evaluated the effect of allopurinol on the hematopoietic toxicity of 6-mercaptopurine in rabbits by studying the development of neutropenia in animals that received only 6-mercaptopurine and in those that received both drugs. The studies showed that, rather than demonstrating any potentiation of 6-mercaptopurine toxicity, allopurinol protected the animals, as evidenced by less weight loss, less depression of neutrophils, and lower mortality.

INTRODUCTION

Allopurinol [4-hydroxypyrazolo(3,4-d)pyrimidine], an inhibitor of xanthine oxidase, is used in humans to prevent a marked increase in uric acid production secondary to destruction of malignant cells. It is believed to potentiate the toxic effects of 6-MP and, when the 2 are used together, the dose of 6-MP is usually reduced. This practice derives from the following observations and deductions. (a) A given dose of 6-MP produces greater inhibition of tumor growth in mice also given allopurinol (2). (b) About one-half of an injected dose of 6-MP is excreted in the urine in 24 hr. In humans, about 5% of p.o. dose appears in urine as free 6-MP and 25% appears as 6-thiouric acid. The remainder of the excretion consists of 6-methylpurine and other degradation products (2, 3, 10). Subjects also given allopurinol excrete 25% of the 6-MP unchanged and only 5% as 6-thiouric acid. It is not clear whether allopurinol alters the rate of excretion. (c) The objective of our studies was to obtain data from controlled experiments in animals concerning the effects of allopurinol on the marrow toxicity of 6-MP. Allopurinol appeared to protect the animals, rather than to enhance 6-MP toxicity.

MATERIALS AND METHODS

Animals used in these studies were male white New Zealand rabbits weighing between 2 and 3 kg. Housed in separate cages in air-conditioned quarters, they had free access to food and water. Allopurinol and 6-MP (generously provided through the courtesy of Dr. Gertrude Elion of Burroughs Wellcome and Company, Tuckahoe, N. Y.) were dissolved each day in 0.1 N NaOH so that the required dose, in 3 to 5 ml, could be given by stomach tube. Control animals received an equal volume of 0.1 N NaOH. The gavage tube was flushed with 3 ml of water to ensure that the total dose was expelled from the tube into the stomach. Ear vein blood was obtained daily, total white cell counts were done in duplicate with a Model B Coulter cell counter, and differential counts of 200 cells were made from Wright's stained cover slip smears.

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stopped by cytotoxic drugs have a postmitotic pool of cells big enough to maintain a normal blood neutrophil count for 2.5 days. Progressing neutropenia then develops, approaching zero concentration 4 days after exposure to the drug. With doses of drug not large enough to stop marrow production completely, neutropenia of a lesser degree ensues reaching the nadir later. The aim of this study was to determine what quantity of 6-MP would produce early neutropenia and what quantity would produce neutropenia substantially later, so that it would be possible to determine whether the addition of allopurinol would potentiate the effect of the 6-MP, causing neutropenia to appear earlier.

RESULTS

Various doses of 6-MP (10 to 100 mg/kg/day) were tested, and it was found that 100 mg/kg/day produced agranulocytosis by the 6th day. In animals given 20 mg of 6-MP per kg per day, there was an exponential regression in blood neutrophils beginning on Day 4 (Chart 1). When a similar group of animals was given 50 mg of allopurinol per kg per day, along with 20 mg of 6-MP per kg per day, no potentiation of the toxic effects of 6-MP, as evidenced by an earlier neutropenia, was observed. Rather, the opposite occurred; allopurinol apparently protected the animals from the toxic effects of 6-MP. Chart 2 shows changes in mononuclear cell counts (predominantly lymphocytes). Animals given 6-MP alone developed a modest but significant reduction of lymphocytes, in comparison with those given both drugs. The volume of packed red cells in both groups fell from a mean of 37% on Day 0 to a mean of 33% on Day 11. There were no significant changes in the leukocyte counts of 6 control animals given 0.1 N NaOH or in those of 6 control animals given 50 mg of allopurinol per kg per day.

Charts 3 and 4 show additional evidence of the toxicity of 6-MP and the protection afforded by allopurinol. Animals given 6-MP alone showed progressive weight loss and progressively mounting death rate, while those given both drugs maintained weight and life.

To determine whether allopurinol was interfering with xanthine oxidase degradation of 6-MP at these doses, we determined the 24-hr excretion level of free 6-MP in the urine of 10 rabbits, as described. Each animal served as his own control with a 1-week rest between studies. One-half of the animals received 1st 6-MP alone, followed in 1 week by treatment with both drugs, while the other one-half of the
animals received both drugs initially. The data in Table 1 show that allopurinol blocks 6-MP degradation in the rabbit to about the same extent as reported in humans, with the excretion of free 6-MP in urine rising to about 20 to 25%.

**DISCUSSION**

The failure of allopurinol to potentiate 6-MP toxicity suggests that the biological activity of 6-MP cannot be related to the form in which it is excreted. The total amount of 6-MP excreted appears to be unchanged by allopurinol, and this may be a more important determinant of its biological effects than the particular form in which it is eliminated from the body. Recent studies by Coffey et al. (1) showed that allopurinol did not alter the plasma clearance of single large doses of 6-MP in humans, and they also cast doubt on earlier interpretations to the effect that the excretion of more free 6-MP in urine by patients given allopurinol reflects greater availability of 6-MP to the tissues. Elion et al. (2, 3) recently found, however, that the tumor content of labeled 6-thioinosinic acid is increased by the concomitant administration of allopurinol with labeled 6-MP.

The potentiation of the antitumor activity of purine analogs by allopurinol is not necessarily tied to an increase in toxicity. Elion et al. (2) showed this when they demonstrated that allopurinol produced a severalfold increase in inhibition of tumor growth and suppression of immune response by different 6-substituted purines without an equivalent increase in apparent toxicity. Mechanisms other than xanthine oxidase inhibition could explain the allopurinol-induced potentiation of the antitumor activity of purine analogs. The drug also inhibits the *de novo* synthesis of purines and pyrimidines (effects not necessarily dependent on xanthine oxidase inhibition, as they are demonstrable in cells lacking that enzyme) (6).

The protection from the toxic effects of 6-MP that allopurinol afforded the rabbits in our study was unexpected. Such a protective action could be explained by interference with the conversion of 6-MP to its active ribotide form. This might be effected by depletion of phosphoribosylpyrophosphate due either to consumption or inhibition of synthesis (5) or to the fact that allopurinol and hypoxanthine compete with 6-MP as substrate for the enzyme hypoxanthine guanine phosphoribosyltransferase which, in the presence of phosphoribosylpyrophosphate, converts the purines to the ribotides (4). Whatever the mechanism involved, it is clear that 50 mg of allopurinol per kg, given with 20 mg of 6-MP per kg, protected these rabbits from 6-MP toxicity. It is not known to what extent the metabolism and interaction of 6-MP and allopurinol in rabbits parallel those in humans, and these studies do not purport to define that relationship.

Anecdotes abound about the manner in which allopurinol potentiates 6-MP toxicity in man, and they may portray accurately the interaction of the drugs. However, controlled studies are not available and, until such data are obtained, this question remains unanswered. Regardless of the final answer, in order for one to avoid dangerous marrow suppression, careful, frequent monitoring of blood counts will continue to be mandatory in the management of patients taking 6-MP, with or without concomitant allopurinol.

**REFERENCES**

2. Elion, G. B., Callahan, S., Nathan, H., Bieber, S., Rundles, R. W.,
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