EFFECT OF 4-HYDROXYPYRAZOLO[3,4-d]PYRIMIDINE (ALLOPURINOL) ADMINISTRATION ON GROWTH OF EHRlich Tumor Cells

Akira Kizu, Akihiko Kusaba, Masahiko Nakamura, Shuji Kato, Katutoku Sawada, and Hamao Ijichi

Second Department of Internal Medicine, Kyoto Prefectural University of Medicine*

Effect of the administration of 4-hydroxypyrazolo[3,4-d]pyrimidine (allopurinol) on the growth of Ehrlich ascites tumor cells was investigated in an in vivo system. Oral administration of allopurinol (0.1% in diet) suppressed the growth of both ascites and solid types of the tumor after the implantation of Ehrlich tumor cells in mice. The inhibitory action depended on the dose but was lost rapidly when the administration was interrupted. Possible mechanisms involved in the inhibitory effect of allopurinol on tumor growth were briefly discussed.

4-Hydroxypyrazolo[3,4-d]pyrimidine (allopurinol), a potent inhibitor of xanthine oxidase, has been used clinically in the treatment of gout as a regulator of uric acid biosynthesis. In vivo, allopurinol is converted to oxipurinol (4,6-dihydroxypyrazolo[3,4-d]-pyrimidine) or allopurinol ribonucleotide, which has been ascertained to inhibit xanthine oxidase (EC 1.2.3.2) and glutamine PRPP amidotransferase (EC 2.4.2.14), respectively. This latter enzyme participates in the first step of de novo purine biosynthesis. It has been reported that xanthine oxidase activity in Bittner mammary tumor is lower than that in control tissues, and that xanthine oxidase activity was inversely correlated with the rate of tumor growth in a series of hepatomas.

In the present work, the effect of in vivo administration of allopurinol on the growth of Ehrlich ascites tumor cells was investigated, since it is thought the suppression of de novo biosynthesis of purine rather than inhibition of xanthine oxidase may occur in tumor cells.

Materials and Methods

Allopurinol was obtained from the Wellcome Foundation, England. Ehrlich ascites tumor cells were kindly donated by the Central Research Division, Takeda Chemical Ind., Osaka. DDYs mice weighing about 20 g received subcutaneous (inguinal region) or intraperitoneal implants of 6 × 10⁶ Ehrlich ascites cells under aseptic conditions. Animals were given water and Oriental chow freely and allopurinol was administered by mixing with ordinary lab chow. Preliminary experiments indicated that the addition of 0.1% allopurinol in the diet gave a daily ingestion of approximately 1 mg allopurinol/20 g body weight. Total packed cell volume (TPCV) was estimated in a hematocrit capillary tube after centrifuging the ascitic fluid at 2,500 rpm for 10 min. For solid tumors, the largest diameter of the mass was measured with calipers according to the procedure of Alexander. Significance of difference in results was determined by the Student's t-test.

Results

Table I shows the effect of administration of allopurinol with diet on the growth of mice. The increase in body weight in control and experimental groups consisting of 12 mice each was observed over a period of 40 days. All the animal survived during the entire experimental period and no statistically significant effect of the allopurinol administration was noted.
Table I. Effect of Allopurinol on Growth of Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Increment of body weight (g)</th>
<th>Days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Control (12)</td>
<td>0.6 ± 2.0</td>
<td>3.6 ± 1.2</td>
</tr>
<tr>
<td>Treated (12)</td>
<td>1.3 ± 1.0</td>
<td>3.3 ± 2.2</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD. Number in parentheses indicates that of mice.

Treated: Orally administered 0.1% allopurinol in the diet.
The values were not significant at P < 0.05.

Table II. Effect of Allopurinol on Ehrlich Tumor Growth

<table>
<thead>
<tr>
<th>Group</th>
<th>Pretreatment with allopurinol for 45 days</th>
<th>Ascites type</th>
<th>Solid type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Increment of body weight (g)</td>
<td>Diameter of tumor (cm)</td>
</tr>
<tr>
<td>Control (12)</td>
<td></td>
<td>12.7 ± 3.8</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>Treated (12)</td>
<td></td>
<td>4.8 ± 3.2</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Control (6)</td>
<td></td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.02</td>
</tr>
<tr>
<td>Treated (4)</td>
<td></td>
<td>12.0 ± 2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.7 ± 3.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean ± SD. Number in parentheses indicates that of mice.

Treated: Orally administered 0.1% allopurinol in the diet. The body weight was measured 14 days and the diameter measured 20 days after inoculation of tumor cells.

To examine the effect of allopurinol on the growth of Ehrlich tumor cells (ascites and solid types), tumor cells were implanted intraperitoneally into two groups of 12 mice each and subcutaneously into the inguinal region in another two groups of 12 mice each. One of these groups was subsequently administered 0.1% allopurinol in the diet. The increase in body weight was followed as an index of the accumulation of ascitic fluid resulting from the growth of tumor cells (ascites type). As shown in Table II, the increment in body weight at 14 days after implantation of tumor cells was suppressed significantly (P < 0.001) in the allopurinol-treated group compared with that of the control group. Table II also shows the diameter of tumors (solid type) at 20 days after implantation. Growth of the tumor was significantly suppressed in the group receiving allopurinol (P < 0.02). In one of the animals from the allopurinol-treated group complete absence of tumor growth was noted. However, exclusion of this animal from the statistical handling by the Smirnoff's test did not alter significance of the difference. Above results indicate that the administration of 0.1% allopurinol in the diet suppresses the growth of both ascites and solid types of Ehrlich tumor cells. As shown in Table II, tumor growth in the group pretreated for 45 days with allopurinol closely resembled that in the group which received allopurinol only after the implantation.

Similarly TPCV of ascitic fluid measured in these animals (21st day after the implantation) indicated that the values in the pretreated group are in essential agreement with that found in the non-pretreated group. These results suggest that the suppressive effect of allopurinol was virtually the same whether or not the animals received the drug for 45 days before the implantation of tumor.
ALLOPURINOL AND EHRlich TUMOR CELLS

Fig. 1. Dose-response curve of allopurinol on the growth of Ehrlich ascites tumor cells

The animals (9 in each group) received the indicated concentrations of allopurinol in the diet for 21 days after the tumor implantation. Each point and bar indicate mean ± SD obtained from 9 determinations.

cells and imply that allopurinol has no cumulative and/or prophylactic effect on tumor growth in these animals.

To ascertain dose-dependency of the action of allopurinol on the growth of Ehrlich tumor cells (ascites type), groups of 9 mice were fed various concentrations of allopurinol in the diet after intraperitoneal implantation of Ehrlich tumor cells. The mean TPCV of tumor cells in the ascitic fluid was measured as an index of tumor growth after 21 days of allopurinol administration. As shown in Fig. 1, the inhibitory effect of allopurinol on tumor growth increased with the increase of allopurinol dose when more than 0.01% of this compound was administered in the diet.

To investigate the effect of interruption of allopurinol administration on growth of Ehrlich tumor cells, tumor cells were implanted intraperitoneally in two groups of 12 animals, after which one group of these animals received 0.1% allopurinol in the diet for 10 days. The feeding with allopurinol was then discontinued and the experimental group received the same diet as the control for a further 10 days. As shown in Fig. 2, there was a marked suppression of the increment of body weight resulting from tumor growth during the administration of allopurinol. On removing allopurinol from the diet, however, the growth of tumor increased rapidly and the rate of increment was identical with that of the controls. These results indicate that the inhibitory effect of allopurinol on tumor growth is reversible and disappears within 1~2 days after its removal.

Discussion

To make any deductions concerning the effect of allopurinol on tumor growth from

Fig. 2. Effect of removal of allopurinol on Ehrlich tumor growth

The animals were maintained on the standard diet or the standard diet containing 0.1% allopurinol for 10 days after the tumor implantation.
the increment in body weight would not be reasonable but, as an index of antitumor effect, weight gain followed as an indication of the accumulation of ascitic fluid from the growth of tumor cells, TPCV, and the diameter of tumor mass were examined in the present preliminary studies. In the present study no effect of allopurinol on weight gain in mice was noted under the experimental conditions employed. Results obtained with this dosage clearly demonstrated that orally administered allopurinol inhibits the growth of Ehrlich ascites tumor cells in vivo.

Although the exact mechanism of action of this agent on tumor growth is not clear at present, some speculations on the mechanism may be offered. A direct correlation has been demonstrated between the inhibition of tumor growth and xanthine oxidase inhibition for a series of pyrazolopyrimidines.8) On the other hand, allopurinol has been reported to produce neither inhibition nor stimulation of adenocarcinoma-755.7) No effect of allopurinol, however, has been reported on the growth rate of transplantable rodent tumor13) and a series of hepatomas.11) In this regard, it can be said that the present result is the first report on the effect of allopurinol on the growth of Ehrlich tumor cells in an in vivo system.

When allopurinol is given in vivo, it is rapidly converted to oxipurinol or allopurinol ribonucleotide. Oxipurinol is a competitive inhibitor of xanthine oxidase like allopurinol and has various known biological activities.3,4,9,14) By the administration of allopurinol, de novo purine biosynthesis may also be inhibited by one of the following three possible mechanisms. First, allopurinol ribonucleotide may inhibit glutamine PRPP amidotransferase which is involved in the first step of purine biosynthesis.8) The second possibility is that allopurinol or oxipurinol inhibits xanthine oxidase resulting in the productions of IMP, AMP, GMP, and other ribonucleotides which inhibit glutamine PRPP amidotransferase.16) The last possibility is that PRPP consumption coupled with the conversion of allopurinol to allopurinol ribonucleotide may lead to a decrease in the substrate of purine biosynthesis due to depletion of PRPP.6)

In recent studies on purine biosynthesis using Ehrlich ascites tumor cells, Reem11) reported 37% inhibition of glutamine-dependent phosphoribosyl-1-amine synthesis by allopurinol ribonucleotide when a cell-free preparation was used. The degree of inhibition was virtually equal to that of IMP, but with oxipurinol the inhibitory effect of allopurinol was reduced. Furthermore, in vitro experiments with intact cells, addition of hypoxanthine inhibited the incorporation of 14C-glycine into N-formylglycinamide ribonucleotide while allopurinol and oxipurinol did not.

By considering these facts, it is highly unlikely that the inhibitory effect of allopurinol on the growth of Ehrlich ascites tumor cells in the in vivo system is a simple reflection of the inhibition of xanthine oxidase by allopurinol or oxipurinol. The inhibition of tumor growth may result from the conversion of allopurinol to allopurinol ribonucleotide which inhibits glutamine PRPP amidotransferase, or from depletion of intracellular PRPP at the time of conversion to allopurinol ribonucleotide, which may inhibit de novo purine biosynthesis in tumor cells.

The authors express their gratitude to Professor Kinya Kuriyama, Department of Pharmacology, Kyoto Prefectural University of Medicine, for helpful suggestions during the experiments and help in preparing the manuscript.

(Received July 3, 1976)

REFERENCES

ALLOPURINOL AND EHRLICH TUMOR CELLS