ALLOPURINOL INDUCES RENAL TOXICITY BY IMPAIRING PYRIMIDINE METABOLISM IN MICE

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Summary

We investigated the relationship between the toxic effect of allopurinol and pyrimidine metabolism in mice. Allopurinol-induced increases in plasma transaminase levels in dinitrofluorobenzene (DNFB)-sensitized mice were not affected by uridine. In contrast, plasma creatinine and BUN tended to decrease 18 hr after the last injection of uridine. Both plasma and urinary orotidine (OD) were detected in DNFB-sensitized mice after administration of a single dose of allopurinol. In contrast, TEI-6720, a newly synthesized xanthine oxidase/xanthine dehydrogenase inhibitor, caused neither pyrimidine metabolism abnormality nor renal impairment in DNFB-sensitized mice. Also, normal mice administered high doses of allopurinol showed abnormal pyrimidine metabolism together with renal toxicity which could be ameliorated by uridine, indicating that allopurinol essentially causes pyrimidine metabolism abnormality leading to renal impairment. In DNFB-sensitized mice, allopurinol increased urinary OD excretion to an extent similar to that in normal mice administered the same dose of allopurinol. However, renal impairment by allopurinol was more striking in DNFB-sensitized mice than in normal mice. Histopathological observations showed that allopurinol induced calculus formation in the collecting tubules and papillary duct. Calculus formation was increased by DNFB and decreased by uridine. These observations indicate that the enhancement of the renal toxicity of allopurinol by DNFB-sensitization may be due to some biological interactions between DNFB and allopurinol. In humans, it is possible that there are some biological interactions which serve to enhance the toxicity of allopurinol, resulting in the development of allopurinol hypersensitivity syndrome (AHS). In contrast, TEI-6720, had no effect on pyrimidine metabolism and showed no toxic effect.

Key Words: pyrimidine metabolism, xanthine dehydrogenase/xanthine oxidase, allopurinol, renal toxicity
Hyperuricemia caused by a decrease in renal excretion of uric acid, an excessive rate of uric acid production, or a combination of both, has been considered to be the most important risk factor for the onset of gout (1, 2). Agents such as uricosurics and xanthine oxidase/xanthine dehydrogenase (XOD/XDH) inhibitors, have been used to reduce serum uric acid levels. Currently, the only commercially available XOD/XDH inhibitor is allopurinol, developed in the early 1960's (3).

Allopurinol is widely used and generally well-tolerated. However, when used in patients with renal insufficiency it may have life-threatening toxic effects, such as vasculitis, toxic epidermal necrolysis (TEN), eosinophilia, hepatitis, reduced renal function and bone marrow suppression, known as allopurinol hypersensitivity syndrome (AHS) (4-7). Allopurinol is metabolized to oxypurinol, an active metabolite (8), which is excreted via the kidney into urine in the same manner as uric acid (9, 10). Oxypurinol tends to accumulate in patients with renal insufficiency, and elevated oxypurinol levels seem to be a prerequisite for the occurrence of AHS (4). Although the exact mechanism responsible for the development of AHS is unknown, both allopurinol and oxypurinol are suggested to undergo conversion to their corresponding nucleotides in vivo and affect pyrimidine metabolism. It is well known that allopurinol causes an increase in urinary excretion of orotic acid (OA) and/or orotidine (OD) in humans (11-15). The possibility that effects on pyrimidine metabolism may contribute to the pathogenesis of AHS cannot be ruled out.

In an effort to clarify the mechanism of AHS, we have investigated the toxicity of allopurinol using a DNFB-induced contact hypersensitivity mouse model and found that allopurinol increased ear swelling and mortality in this model (16). We also found that allopurinol induced liver injury and renal impairment in DNFB-sensitized mice (submitted for publication). In the present study, we assumed that the toxic effects of allopurinol observed in previous studies in mice may result from the impairment of pyrimidine metabolism, and we found that allopurinol showed renal toxicity due to, at least in part, impaired pyrimidine metabolism. In addition, the effects of TEI-6720 (2-(3-cyano-4-isobutoxyphenyl)-4-methyl-5-thiazole carboxylic acid), a newly synthesized XOD/XDH inhibitor with a non-nucleic acid structure (Figure 1; 17), in this model were also investigated and compared with those of allopurinol.

![Chemical structures of allopurinol (A) and TEI-6720 (B).](image-url)
Experimental Animals

Male BALB/c mice, 8 weeks of age (Charles River Japan, Kanagawa, Japan), were used for all experiments. The animals were kept in an air-conditioned room and given standard chow (CE-2, Clea Japan, Shizuoka, Japan) and water *ad libitum* for the duration of the study.

Materials

The following materials were used: TEI-6720 (synthesized by Teijin Ltd.), allopurinol (Sigma Chemicals Co., St. Louis, MO, U.S.A.), uridine, dinitrofluorobenzene (DNFB, Wako Pure Chemical Industries, Osaka, Japan). TEI-6720 or allopurinol was suspended in 0.5% methylcellulose (MC) solution, and uridine was dissolved in saline, prior to use.

Experiment 1: Effect of Allopurinol on Pyrimidine Metabolism in DNFB-Sensitized Mice

The abdomen of each mouse was shaved with electric clippers and an electric razor on day -2. Mice were sensitized on days -1 and 0 by painting 100 μl of 0.5% DNFB, dissolved in ethanol, onto the shaved abdomen. Allopurinol (30 mg/kg/day) was orally administered, simultaneously with DNFB sensitization, for 2 days. Control animals received only the dosing vehicle at a constant volume of 10 mL/kg. Uric acid (600 or 1200 mg/kg/day) dissolved in saline was intraperitoneally injected simultaneously with sensitization and allopurinol-dosing, for 2 days. Six and 18 h after the last sensitization, animals were anesthetized with ether and blood samples were collected by cardiac puncture into a 1 mL plastic syringe with a 26G needle, filled with 1/10 volume of 3.8% sodium citrate solution. After centrifugation, the plasma samples were obtained and plasma GPT, GOT, creatinine and BUN were measured using an automatic biochemical analyzer (Hitachi Automatic Analyzer 7070).

In the next experiment, mice were sensitized by painting 100 μl of 0.5% DNFB, dissolved in ethanol, onto the shaved abdomen. TEI-6720 (5 or 10 mg/kg), allopurinol (15 or 30 mg/kg) or vehicle was orally administered, simultaneously with DNFB sensitization. Urine was collected in metabolic cages from the time of drug administration to 24 hr post-administration. Urine volume and urinary creatinine were measured. Blood samples were collected by cardiac puncture into a heparinized 1 mL plastic syringe with a 26G needle, under ether anesthesia, 0, 1, 2, 6 and 24 hr after sensitization and drug administration, and plasma creatinine and BUN were measured. OA and OD in urine and plasma were measured by HPLC.

Experiment 2: Effect of Allopurinol on Pyrimidine Metabolism in Normal Mice

Allopurinol (3, 30, 300 mg/kg) or vehicle was orally administered to mice. Control animals received only the dosing vehicle at a constant volume of 10 mL/kg. Urine was collected in metabolic cages from the time of drug administration to 24 hr post-administration. Urine volume and urinary creatinine, OA and OD were measured. Heparinized blood samples were collected 0, 1, 2, 6 and 24 hr after drug administration and creatinine, BUN, OA and OD were measured.
In the next experiment, uridine (600 or 1200 mg/kg) was intraperitoneally injected into mice, simultaneously with oral administration of allopurinol (300 mg/kg). Twenty-four hours after administration, blood samples were collected and plasma creatinine and BUN were measured.

In the last experiment, TEI-6720 (1, 10, 100 mg/kg), allopurinol (3, 30, 300 mg/kg) or vehicle was orally administered to mice. Urine was collected in metabolic cages from the time of drug administration to 24 hr post-administration. Urine volume and urinary creatinine, OA and OD were measured. Heparinized blood samples were collected 24 hr after drug administration and creatinine, BUN, OA and OD were measured.

Histopathological examination of the kidney was performed in the case of some animals.

Statistical Analyses

For evaluation of the data, Dunnett's multiple comparison test was performed for differences in the effects of several doses of uridine on allopurinol-induced increase in the plasma GOT, GPT, creatinine or BUN. Student's or Welch's t-test, or Wilcoxon's test was performed to evaluate the differences between control-group and allopurinol-group. The results are expressed as means ± standard deviations. Histopathological findings were analysed using Wilcoxon's test for evaluating the differences between intact-group and allopurinol (300 mg/kg)-group. Kruskal-Wallis test and non-parametric type (joint ranking) Dunnett's test were performed for differences in the effects of several doses of uridine on allopurinol-induced histopathological alterations.

Results

Effect of Uridine on the Toxicity of Allopurinol in DNFB-Sensitized Mice

In DNFB-sensitized mice, allopurinol caused liver damage, as indicated by an increase in plasma GPT and GOT (TABLE I). Uridine had almost no effect on the allopurinol-induced increase in the plasma levels of these enzymes. Plasma creatinine and BUN were markedly increased by allopurinol (Figure 2). Uridine tended to decrease plasma creatinine and BUN 18 hr after the last administration, however, its effect was minimal 6 hr after the last administration.

Features of Abnormal Pyrimidine Metabolism in DNFB-Sensitized Mice

We next investigated the features of abnormal pyrimidine metabolism in DNFB-sensitized mice (Figure 3). Plasma creatinine was increased 1 or 2 hr after administration of a single dose of allopurinol and reached a relatively high level at 6 hr post-administration. TEI-6720 did not increase plasma creatinine. Plasma OD was detected following allopurinol administration, and the amount was increased at a high dose of allopurinol. The increase in plasma OD induced by allopurinol was observed 2 hr after administration, it reached a high level at 6 hr, and continued to be elevated until 24 hr post-administration. This kinetics profile was parallel to that of plasma creatinine. Plasma OD was not detected in the TEI-6720 group. In addition, urinary OD was detected only in the allopurinol group. In the allopurinol group, the
increase in OA in plasma and urine was not marked compared with that of OD.

**Abnormal Pyrimidine Metabolism Induced by Administration of a Single Dose of Allopurinol in Normal Mice**

We next examined the effect of administration of a single dose of allopurinol on pyrimidine metabolism in normal mice (Figure 4). High doses (30 and 300 mg/kg) of allopurinol increased plasma creatinine within 1 or 2 hr after administration, and at 6 hr these parameter values had reached a relatively high level. The time course of changes in BUN was similar to that of plasma creatinine (data not shown). However, the extent of renal impairment induced by allopurinol in normal mice was less than that in DNFB-sensitized mice given the same dose of allopurinol. In brief, although allopurinol at 30 mg/kg caused renal impairment which continued through to 24 hr post-administration in the DNFB-sensitized mice, in normal mice, renal function tended to recover within 24 hr. Allopurinol at 300 mg/kg markedly decreased urinary creatinine excretion (data not shown). Plasma OD increased within 6 hr after administration of a high dose (30 or 300 mg/kg) of allopurinol. The properties of the kinetics of plasma OD were the same as those of the parameters of renal impairment. Urinary OD excretion levels were also strikingly increased following administration of allopurinol at 300 mg/kg, indicating that abnormal pyrimidine metabolism occurred. Plasma and urinary OA were also increased in 300 mg/kg allopurinol group.

**TABLE I.**

Effect of Uridine on the Allopurinol-Induced Increase in Plasma GOT and GPT in DNFB-Sensitized Mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg/day, p.o.)</th>
<th>Uridine (mg/kg/day, i.p.)</th>
<th>N</th>
<th>GOT (IU/L)</th>
<th>GPT (IU/L)</th>
</tr>
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<tbody>
<tr>
<td>&lt;6 hr&gt;</td>
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<td>-</td>
<td>-</td>
<td>7</td>
<td>49 ± 5</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>113 ± 8</td>
<td>10 ± 5</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>30</td>
<td>(saline)</td>
<td>7</td>
<td>180 ± 32</td>
<td>101 ± 15</td>
</tr>
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<td>600</td>
<td></td>
<td>7</td>
<td>208 ± 71</td>
<td>108 ± 19</td>
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<tr>
<td></td>
<td>1200</td>
<td></td>
<td>7</td>
<td>182 ± 57</td>
<td>103 ± 32</td>
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<td></td>
<td></td>
<td>7</td>
<td>57 ± 36</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>EtOH</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>71 ± 25</td>
<td>49 ± 10</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>116 ± 29</td>
<td>72 ± 18</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>30</td>
<td>(saline)</td>
<td>6</td>
<td>81 ± 17</td>
<td>61 ± 19</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td></td>
<td>7</td>
<td>82 ± 18</td>
<td>63 ± 7</td>
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<tr>
<td></td>
<td>1200</td>
<td></td>
<td>7</td>
<td>92 ± 18</td>
<td>63 ± 7</td>
</tr>
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</table>

Mice were sensitized on days -1 and 0 by painting 100 μl of 0.5% DNFB onto the shaved abdomen. Allopurinol (30 mg/kg/day) was orally administered, simultaneously with DNFB sensitization, for 2 days. Uridine (600 or 1200 mg/kg/day) dissolved in saline was intraperitoneally injected simultaneously with sensitization and allopurinol-dosing, for 2 days. Six and 18 h after the last sensitization, blood samples were collected and plasma GOT and GPT were measured. #P<0.05, ##P<0.01 and ####P<0.001: Statistically different from Allopurinol 30 mg/kg/day group (Student's or Welch's t-test); *P<0.05: Statistically different from Allopurinol 30 mg/kg group (Dunnett's multiple comparison test). Data are shown as mean ± standard deviation.
Effect of uridine on the allopurinol-induced increase in plasma creatinine (top) or BUN (bottom) in DNFB-sensitized mice. Mice were sensitized on days -1 and 0 by painting 100 μl of 0.5% DNFB onto the shaved abdomen. Allopurinol (30 mg/kg/day) was orally administered, simultaneously with DNFB sensitization, for 2 days. Uridine (600 or 1200 mg/kg/day) dissolved in saline was intraperitoneally injected simultaneously with sensitization and allopurinol-dosing, for 2 days. Six and 18 h after the last sensitization, blood samples were collected and plasma creatinine and BUN were measured. #P<0.05, ##P<0.01 and ###P<0.001: Statistically different from Allopurinol 30 mg/kg/day group (Welch's t-test or Wilcoxon's test). Data are shown as mean ± standard deviation.
Time course of changes in plasma creatinine (top left) and plasma OD (bottom left) after administration of TEI-6720 or allopurinol, and urinary OA (top right) and OD (bottom right) for the 24-hr period after administration of TEI-6720 or allopurinol in DNFB-sensitized mice. Mice were sensitized by painting 100 µl of 0.5% DNFB onto the shaved abdomen. TEI-6720 (5 or 10 mg/kg), allopurinol (15 or 30 mg/kg) or vehicle was orally administered, simultaneously with DNFB sensitization. Urine was collected in metabolic cages from the time of drug administration to 24 hr post-administration. Blood samples were collected by cardiac puncture into a heparinized 1 mL plastic syringe with a 26G needle, under ether anesthesia, 0, 1, 2, 6 and 24 hr after sensitization and drug administration, and plasma creatinine was measured. OA and OD in urine and plasma were measured by HPLC. Urinary OA and OD were expressed as µg/mg of creatinine/24 hr. Data are shown as mean ± standard deviation (N=3).
Allopurinol on Pyrimidine Metabolism

Fig. 4

Time course of changes in plasma creatinine (top left) and plasma OD (bottom left) after administration of allopurinol, and urinary OA (top right) and OD (bottom right) for the 24-hr period after administration of allopurinol in normal mice. Allopurinol (3, 30, 300 mg/kg) was orally administered to mice. Urine was collected in metabolic cages from the time of drug administration through to 24 hr post-administration. Urine volume and urinary creatinine, OA and OD were measured. Heparinized blood samples were collected 0, 1, 2, 6 and 24 hr after drug administration and creatinine, OA and OD were measured. Urinary OA and OD were expressed as µg/mg of creatinine/24 hr. Data are shown as mean ± standard deviation (N=3).

Intraperitoneally injected uridine was significantly effective in reducing the allopurinol-induced increase in plasma creatinine and BUN (Figure 5). These results indicate that renal impairment by allopurinol in mice may be due to abnormal pyrimidine metabolism.

We next compared the effects of allopurinol on renal function and pyrimidine metabolism with those of TEI-6720. TEI-6720 did not cause renal impairment or abnormal pyrimidine metabolism, at doses up to 100 mg/kg (Figure 6).

Plasma creatinine and BUN levels in DNF-sensitized or normal mice administered...
allopurinol were plotted against the urinary OD excretion levels in these mice, as shown in Figure 7. In the DNFB-sensitized mice, allopurinol increased urinary OD excretion to an extent similar to that in normal mice administered the same dose of allopurinol. However, plasma creatinine and BUN levels were increased by allopurinol more markedly in DNFB-sensitized mice than in normal mice.

Normal mice administered allopurinol at 300 mg/kg showed tubular necrosis, vacuolar degeneration, tubular dilation, calculi and hyaline deposits in the papillary duct. Calculi were observed diffusely in renal medulla and cortex. Especially in the collecting tubules and papillary duct, calculus formation was marked (Figure 8). These histopathological changes, especially calculus formation, were reduced by uridine injection (Figure 9). DNFB-sensitized mice administered allopurinol at 30 mg/kg showed histopathological features similar to those of normal mice administered allopurinol at 300 mg/kg (Figure 10). The results are summarized in TABLE II.

Discussion

Allopurinol, when used in patients with renal insufficiency, may have life-threatening toxic effects, known as allopurinol hypersensitivity syndrome (AHS) (4-7). However, the exact mechanism responsible for the development of AHS is unknown. In an effort to clarify the mechanism of AHS, we have investigated the toxicity of allopurinol using a DNFB-induced contact hypersensitivity mouse model and found that allopurinol increased ear swelling and mortality in this model (16). In addition, allopurinol was found to induce liver injury and renal impairment in DNFB-sensitized mice (submitted for publication). Allopurinol and oxypurinol have been suggested to affect pyrimidine biosynthesis in addition to XOD/XDH, and the possibility that an effect on nucleic acid metabolism may contribute to the pathogenesis of AHS cannot be ruled out.

It is well known that allopurinol causes an increase in urinary excretion of OA and/or OD in humans (11-15). The concentrations of OA and/or OD in erythrocytes and in plasma are also reported to be increased by allopurinol (14, 18). In addition, it is reported that allopurinol causes a decrease in plasma uridine concentrations (15). These abnormalities in pyrimidine metabolism caused by allopurinol are thought to be due to some metabolites of allopurinol or oxypurinol, possibly produced by HGPRT or orotate phosphoribosyltransferase (OPRT). In in vitro studies, allopurinol-1-ribonucleotide, oxypurinol-1-ribonucleotide and oxypurinol-7-ribonucleotide were found to act as potent inhibitors of yeast-, rat- or human-derived orotidine-5'-monophosphate decarboxylase (OMPDC), the second enzyme of the uridine-5'-monophosphate (UMP) synthase complex in mammals (11, 19, 20). The ribonucleosides corresponding to these nucleotides have been detected in the urine or plasma of humans administered allopurinol (13, 14, 21, 22). Although pyrimidine metabolism abnormality is encountered in healthy subjects as well as in patients, and its contribution to AHS has been unclear, it should be noted that in patients with renal insufficiency, striking increases in the levels of orotidine and oxypurinol-ribonucleosides in blood or urine upon administration of allopurinol have been observed (14, 18). We assume that the pyrimidine metabolism abnormality induced by allopurinol is one of the causes of AHS and the toxic effects of allopurinol observed in previous studies in mice, and thus we conducted the present study. Although several reports have demonstrated allopurinol-induced toxicity in animal studies (23-27), the relationship between toxicity and pyrimidine metabolism abnormality has not been investigated so far.
Effect of uridine on the allopurinol-induced increase in plasma creatinine (top) or BUN (bottom) in normal mice. Uridine (600 or 1200 mg/kg) was intraperitoneally injected into mice, simultaneously with oral administration of allopurinol (300 mg/kg). Twenty-four hours after administration, blood samples were collected and plasma creatinine and BUN were measured. ##P<0.01 and ###P<0.001: Statistically different from Allopurinol 300 mg/kg group (Welch's t-test); ###P<0.001: Statistically different from Allopurinol 300 mg/kg group (Dunnett's multiple comparison test). Data are shown as mean ± standard deviation (N=6).
In the present study, allopurinol increased plasma GPT, GOT, creatinine and BUN in DNFB-sensitized mice. Simultaneously injected uridine had almost no effect on the allopurinol-induced increase in transaminase levels, however, plasma creatinine and BUN tended to decrease 18 hr after the last injection of uridine. The beneficial effect of uridine on the kidney was minimal 6 hr after the last administration. The lack of effect of uridine on the increase in transaminase levels suggests that abnormal pyrimidine metabolism may not have any relation to liver damage in DNFB-sensitized mice. Plasma levels of these parameters peaked at 6 hr, but declined time-dependently by 6 to 18 hr post-administration in DNFB-sensitized mice, indicating that liver damage may be a phenomenon that occurs temporarily. In contrast, the increase in
Fig. 7
Individual plasma creatinine (top) and BUN (bottom) values 24 hr after administration of allopurinol plotted against urinary OD excretion for the 24-hr period after administration of allopurinol in DNFB-sensitized or normal mice.
Fig. 8
Histopathological observations of the kidney in mice (Allopurinol 300 mg/kg, p.o.)
top: papillary duct, bottom: renal cortex;  x165, HE staining.)
Histopathological observations of the kidney in mice (Allopurinol 300 mg/kg, p.o.; Uridine 1200 mg/kg, i.p.) (top: papillary duct, bottom: renal cortex;  x 165, HE staining).
Fig. 10
Histopathological observations of the kidney in mice (0.5% DNFB application; Allopurinol 30 mg/kg, p.o.) (top: papillary duct, bottom: renal cortex;  ×165, HE staining).
### TABLE II.
Histopathological Features of the Kidney in Mice Administered Allopurinol.

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<th>Treatment</th>
<th>Item</th>
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<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Necrosis, tubular epithelium</td>
<td>6 0 0 0 0 0</td>
<td>2 1 2 1 0</td>
<td>5 1 0 0 0</td>
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<tr>
<td>Degeneration, tubular epithelium</td>
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<td>1 1 4 0 0</td>
<td>4 2 0 0 0</td>
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Sections of kidney were stained with HE, and scored according to the grades - , ±, +, ++ and +++, for each of the nine histological features as follows: -, normal; ±, minimal; +, slight; ++, moderate; and +++, severe. Results are shown as the number of animals showing the corresponding histopathological feature among six mice.

a) **P<0.01: Statistically different from Intact (Wilcoxon's test)

b) NS: Not Significant; *P<0.05, **P<0.01 and ***P<0.001: Statistically different from Allopurinol (300 mg/kg) (non-parametric type (joint ranking) Dunnett's test)

plasma creatinine and BUN continued through to 18 hr post-administration. Therefore, renal impairment by allopurinol may be due to a combination of abnormal pyrimidine metabolism and other mechanism(s). The latter may be temporal as in the case of liver damage, and the former may be long-lasting. Accordingly, it is thought that uridine did not affect renal impairment within 6 hr, but served to ameliorate the impairment within 18 hr after the last administration in this study.

If pyrimidine metabolism abnormality occurred as above, one may assume that repeated administration of allopurinol and successive sensitization with DNFB may not be necessary. Therefore, we next investigated the features of abnormal pyrimidine metabolism in mice.
administered a single dose of allopurinol and given a single dose of DNFB for sensitization. As a result, renal impairment was caused also in this protocol. Both plasma and urinary OD were detected following allopurinol administration, and were increased at a high dose of allopurinol. The time course of changes in plasma OD was similar to that of plasma creatinine. These results suggest that allopurinol caused pyrimidine metabolism abnormality and this resulted in renal impairment in DNFB-sensitized mice. In contrast, TEI-6720 caused neither abnormal pyrimidine metabolism abnormality nor renal impairment in DNFB-sensitized mice.

In a previous study, a high dose of allopurinol was shown to cause renal impairment in normal mice (submitted for publication). Therefore, we next examined the effect of administration of a single dose of allopurinol on pyrimidine metabolism in normal mice compared with that in DNFB-sensitized mice. High doses (30 and 300 mg/kg) of allopurinol caused abnormal pyrimidine metabolism together with renal toxicity which could be ameliorated by uridine also in normal mice. These observations indicate that allopurinol essentially causes pyrimidine metabolism abnormality leading to renal impairment. In DNFB-sensitized mice, allopurinol increased urinary OD excretion to an extent similar to that in normal mice administered the same dose of allopurinol. However, BUN was increased by allopurinol more markedly in DNFB-sensitized mice than in normal mice. These observations indicate that the enhancement of the renal toxicity of allopurinol by DNFB-sensitization may not be due to the increase in blood concentrations of allopurinol or its metabolite(s) by DNFB, but rather due to some biological interactions between DNFB and allopurinol.

In the present study, the doses at which allopurinol caused renal toxicity seemed to be higher than those at which allopurinol exerts its hypouricemic efficacy in mice. Because allopurinol, which is well recognized to be safe, infrequently induces life-threatening toxicity in clinical use, high doses of allopurinol were selected in order to detect the toxicity which could be hardly observed at efficacy doses of allopurinol in the present study. As a result, the toxicity of allopurinol in DNFB-sensitized mice was apparently observed at lower doses than those of allopurinol which caused toxicity in the normal mice. These results suggest that safety range of allopurinol could become lower under certain conditions.

Histopathological observations showed that allopurinol induced calculus formation in the collecting tubules and papillary duct. Calculus formation was increased by DNFB and decreased by uridine. Because plasma levels of creatinine and BUN were in good agreement with the extent of calculus formation, renal impairment may have relation to calculus formation. It is reported that administration of a high dose of allopurinol to rodents results in death and this is considered to be attributable to xanthine calculi-induced renal impairment due to inhibition of XOD/XDH (28). However, another XOD/XDH inhibitor, TEI-6720, whose XOD/XDH inhibitory efficacy is more potent than allopurinol (29), showed no effect on plasma creatinine or BUN at doses up to 100 mg/kg in this study. In addition, TEI-6720 and allopurinol showed similar dose-response curves for the decrease in uric acid or allantoin levels, and the associated increase in xanthine (submitted for publication), indicating that TEI-6720 and allopurinol induce changes with similar characteristics although the dosage required differs. Therefore, calculus formation by allopurinol observed in the present study may not be related to XOD/XDH inhibitory activity. However, it is possible that oxyipurinol has physicochemical effects, such as promotion of xanthine crystal formation. It is reported that a large dose of allopurinol induces oxyipurinol calculus formation clinically (30, 31), suggesting the involvement of
oxypurinol itself in calculus formation in the allopurinol-treated mice in this study. However, its possibility may be low because the extent of calculus formation at the same dose of allopurinol was increased by DNFB and decreased by uridine. Accordingly, the possibility that the calculus consists of precipitated OA is also low. Thus, we were not able to identify the component(s) of the calculi observed in this study. However, the possibility that an increase in xanthine, oxypurinol and/or OA may serve as a trigger for calculus formation cannot be ruled out. In the present study, we showed that allopurinol induced nephrotoxicity in mice. However, impaired renal function is not always characterized as a sign of AHS, in comparison with skin rash or liver injury (7). Besides, in humans, impaired renal function by allopurinol is caused immunologically, and this is not thought to coincide with the renal failure that appears following administration of a single dose of allopurinol in mice. Especially, in humans, renal calculus is hardly induced by allopurinol. Although the cause of these discrepancies is unknown, it is thought that allopurinol toxicity might appear in the most susceptible organ in each species. In the case of mice, this may be the kidney, and increased levels of xanthine caused by allopurinol may serve as a trigger for renal disorder, as mentioned above. Although xanthine levels are likely to be increased also by TEI-6720, we consider that allopurinol impairs the restoration of some kidney function(s) and this results in severe renal calculus formation, through abnormal pyrimidine metabolism in addition to the increase in xanthine levels. Therefore, we consider that allopurinol-induced calculus may not be formed directly by the abnormal pyrimidine metabolism, but be caused as a result of the deterioration of some kidney function(s) by the pyrimidine metabolism perturbation.

DNFB is known to possess irritant properties, causing inflammation and an increase in ornithine decarboxylase activity, the key enzyme in polyamine synthesis (32). These phenomena are also known to occur in response to lipopolysaccharide (LPS) or pro-inflammatory cytokines, interleukin (IL)-1 or tumor necrosis factor (TNF)-α, and are considered to be a protective or healing response. It is reported that LPS, IL-1 or TNF-α enhanced the hepatotoxicity of galactosamine (GαN), which consumes uridine nucleotides (33). Induction of ornithine decarboxylase by LPS or these cytokines suppressed by GαN, leading to impairment of the protective function, resulted in enhancement of the hepatotoxicity of GαN through the effect on pyrimidine biosynthesis. Although the exact mechanism(s) by which DNFB augments the toxicity of allopurinol is unknown, this report suggests that DNFB may also cause metabolic changes via inflammation, leading to enhanced toxicity of allopurinol.

Some adverse effects of allopurinol are thought to be immunological. Since allopurinol and its metabolites structurally resemble natural purine bases, ribonucleosides or ribonucleotides, there is the possibility of increased susceptibility to allergic reactions (34). On the other hand, the reason why AHS tends to occur in patients showing accumulation of oxypurinol is not considered to be only because of an allergic reaction, but also because of another factor that amplifies the allergic reaction, which is most likely abnormal pyrimidine biosynthesis. It has been suggested that the safety range of allopurinol is lower in patients with impaired renal function (35). In such cases, in addition to accumulation of oxypurinol, systemic metabolic changes due to impaired renal function are also involved, probably resulting in an even greater possibility of AHS, as in the present study.

In conclusion, allopurinol was found to cause abnormal pyrimidine metabolism leading to renal impairment, which was enhanced by DNFB. Although it should be careful in applying
the results observed in mice to the interpretation of AHS in humans, one may assume that there may also be some biological interactions which serve to enhance the toxicity of allopurinol, resulting in the development of AHS. In contrast, the newly synthesized XOD/XDH inhibitor, TEI-6720, had no effect on pyrimidine metabolism and showed no toxic effect.

References