Drug Interaction between Phenytoin and Allopurinol

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The combination of phenytoin (DPH) and allopurinol is used for the treatment of a neurological disease. However, interactions between DHP and allopurinol and the mechanism are little known. The repeated dosing of allopurinol at higher doses (20 and 50 mg/kg) significantly retarded the elimination of DPH from the circulation and dramatically decreased the urinary excretion of p-hydroxyphenytoin (HPPH), a major metabolite of DPH. However, a single administration of allopurinol (10 or 50 mg/kg) did not give rise to these effects. Allopurinol did not affect the hepatic extraction of DPH and renal plasma flow rate. Allopurinol (50 mg/kg/d) dosed repeatedly could not inhibit the hepatic drug metabolizing enzyme activities. The in vitro hydroxylation of DPH was inhibited only slightly and the kinetic plots gave apparently non-competitive inhibition. The less inhibitory effect of allopurinol on the in vitro hydroxylation did not agree with the in vivo data. These results indicate that the inhibitory effect of allopurinol is not mediated by cytochrome P-450 dependent monoxygenase reactions.

Keywords — phenytoin-allopurinol interaction; rat; hepatic extraction; p-hydroxyphenytoin urinary excretion; drug metabolizing enzyme activity; in vitro hydroxylation; hydroxylation inhibition; delayed phenytoin elimination; phenytoin

Introduction

Phenytoin (DPH) is the drug most effective and most widely used in the treatment of seizure disorders, and is often used in combination with other drugs. Allopurinol, a selective inhibitor of xanthine oxidase, is known to inhibit drug metabolism, thereby prolonging the duration of action of various drugs administered simultaneously.

A sex-linked familial neurological disease, first described by Lesch and Nyhan, consisting of cerebral palsy, spasticity, choreoathetosis and compulsive aggressive behavior is known to be associated with a loss of an enzyme that participates in purine metabolism, namely hypoxanthine-guanine phosphoribosyltransferase. The combination of allopurinol and DPH is used for the treatment of the neurological disease.

The present study was designed to investigate whether or not allopurinol inhibits the hepatic metabolism of DPH and if so, the inhibitory mechanism in rats. The influence of allopurinol on the disposition of DPH was also examined by determination of the plasma and urinary levels after coadministration with the inhibitor.

Materials and Methods

Materials — DPH and allopurinol were a generous gift of Dainippon Pharmaceutical Co. p-Hydroxyphenytoin (HPPH) and 5-(p-methylphenyl)-5-phenylhydantoim (methylphenytoin), an internal standard for high-performance liquid chromatography (HPLC), was purchased from Aldrich Chemical Co. Nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate (G-6-P) and G-6-P dehydrogenase were obtained from Oriental Yeast Co. Adenosine 5'-triphosphate (ATP) and 20% sodium p-aminophenurate (PAH) were purchased from Sigma Chemical Co. and Dai-ichi Pharmaceutical Co., respectively. Aniline and phenol were used following redistillation. Aminopyrine was utilized after recrystallization.

Animals and Treatment — Male Wistar rats weighing 180—230 g were used throughout this experiment. Animals had free access to MF

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diet (Oriental Yeast Co.) and water before experiments, because drugs are generally administered with or after meals. The animals were divided at random into 2 or 3 groups, each consisting of 4–6 rats. On the day before the sampling experiment, the rat jugular vein was cannulated with a silicon tubing. In a single oral administration, animals were treated with either DPH (20 or 50 mg/kg) alone or DPH and allopurinol (10 or 50 mg/kg), as an aqueous suspension in 2% acacia in a volume of 0.5 ml/100 g. In a repeated oral administration, animals were treated for 7 d with administration of DPH (20 or 50 mg/kg) alone or in combination with allopurinol (20 or 50 mg/kg). In comparable experiments, allopurinol (50 mg/kg) alone was orally administered for 7 d to rats. Blood samples (0.2 ml) were collected periodically from the silicon tubing into a heparinized syringe after the final dosing of drugs and followed by centrifugation. Urine was collected every 2 or 12 h up to 54 h after the final administration of drugs in metabolic cages. The plasma and urine samples were frozen until assay.

Preparation of Hepatic 9000 × g Supernatant and Microsomal Fractions — The animals were starved for 24 h and sacrificed by decapitation 12 h after the final administration of saline or drugs. The liver was thoroughly perfused in situ with ice-cold 0.9% NaCl solution. The liver was excised and homogenized with 4 volumes of 1.15% KC1 solution in a Potter homogenizer with a Teflon pestle. The homogenate was centrifuged at 9000 × g for 25 min. The supernatant fractions were used in the experiment. The microsomes were further fractionated according to the method of Omura and Sato.

Determination of DPH in Plasma and Urine — DPH in plasma and urine was determined by the method of Sato, with slight modifications (methylphenytoin as the internal standard was used and the samples, after dissolving in methanol, were injected into the HPLC apparatus).

Determination of HPPH in Urine — HPPH was assayed by the method of Sato, with slight modifications (cyclobarbital as the internal standard and methanol–0.02% aqueous ammonium acetate (4:6, v/v) as the mobile phase were used).

Determination of DPH in Liver — Animals were fasted for 12 h prior to the experiments in order to remove the effect of food and gastric emptying. Allopurinol and oxypurinol (50 mg/kg, each) suspended in 2% acacia and 2% acacia alone were separately administered to 3 rats orally. At 3 h after the administrations, DPH (25 mg/kg) solution was injected into the jugular vein of each rat. The liver homogenate was prepared in 0.25 M sucrose–50 mM phosphate buffer, pH 7.4, 2 h after the intravenous (i.v.) dosing. DPH concentration in the homogenate was determined according to the HPLC method.

Enzyme Assays — The concentration of cytochrome P-450 (P-450) in the 9000 × g supernatant and microsomal fractions was determined by the method of Omura and Sato. The activities of aniline hydroxylase and aminopyrine-N-demethylase were assayed at 37 °C by the methods of Ariyoshi and Takabayatake and Ikeda, respectively.

In Vitro Hydroxylation of DPH and Effect of Allopurinol and Oxypurinol — Livers were obtained from rats pretreated for 4 d with phenobarbital (20 mg/kg/d, intraperitoneally) and sacrificed on day 5. Microsomal fractions were prepared as described above. Incubation with microsomes were as follows: Potassium phosphate buffer (0.5 ml, 0.1 M, pH 7.4) containing 10 μmol NADP, 5 μmol ATP, 25 μmol G-6-P, 3 units of G-6-P dehydrogenase and 5 μmol MgCl₂, with and without allopurinol (25 or 100 μM) or oxypurinol (20 μM) dissolved in 25 μl of acetonitrile, was mixed with 0.5 ml of resuspended microsomes (8 mg protein/ml). DPH (15–200 μM) dissolved in 25 μl of 0.05 N NaOH was added to the mixture and incubated for 30 min at 37 °C. Incubation was terminated by addition of 1.0 ml of acetonitrile and followed by centrifugation. An aliquot (1.0 ml) of the supernatant obtained was then extracted with CHCl₃ containing the internal standard and HPPH was determined by the method mentioned above.

Measurement of Renal Plasma Flow Rate (RPF) — Animals were treated for 7 d with
oral administration of DPH (50 mg/kg/d) alone or in combination with allopurinol (50 mg/kg/d). At 2 h after the final dosing, 30 mg of PAH was administered intravenously. Blood samples were collected periodically. PAH in plasma was determined according to the method of Burn.\textsuperscript{10}) RPF was calculated by the following equation: \( \text{RPF} = \frac{D}{AUC_{\text{PAH}}} \), where \( D \) is the dose of PAH and \( AUC_{\text{PAH}} \) is the area under the concentration-time curve.

**Protein Determination** — Protein concentration was determined by the method described by Lowry et al.\textsuperscript{14}) with bovine serum albumin as a standard.

**Pharmacokinetic and Statistical Analyses** — Plasma concentration-time curve obtained after administration and rate constants were analyzed by an iterative least-squares regression procedure, MULTIT\textsuperscript{15}) with a desk-top digital computer PC-9801VM (NEC Corp.). \( AUC_{0-24} \) was calculated by the trapezoidal method to the last determined point and in some experiments at a low dose (20 mg/kg) the area to 24 h was added by integration \( (C_n/k_e) \), where \( C_n \) is the last observed plasma concentration and \( k_e \) is the apparent elimination rate constant, calculated from the slope of elimination phase. The means of all data are presented with their standard deviation (mean ± S.D.). Student’s \( t \) test was utilized to determine a significant difference between the groups, \( p < 0.05 \) being taken as the minimum level of significance.

**Results**

**Plasma Concentration of DPH after Single and Repeated Oral Administration of DPH Alone or in Combination with Allopurinol**

![Fig. 1. Plasma Concentration of DPH after a Single Oral Administration of DPH or in Combination with Allopurinol (ALP)](image)

Each point represents the mean ± S.D. \( (n = 3-4) \). ● and ■, DPH alone, 20 mg/kg and 50 mg/kg, respectively; ○ and □, combination, DPH 20 mg/kg and ALP 10 mg/kg and DPH 50 mg/kg and ALP 50 mg/kg, respectively.

The plasma concentrations of DPH after a single oral administration of the anticonvulsant (20 or 50 mg/kg) alone or DPH and allopurinol (10 or 50 mg/kg) are shown in Fig. 1. In all groups the plasma decay curves of DPH were found to be monoexponential. The mean maximum plasma concentration \( (C_{\text{max}}) \) of DPH was attained at 1.5 h after the 20 mg/kg dosing and at 3—5 h after the 50 mg/kg dosing. No statistically significant difference was obtained in the pharmacokinetic parameters between both groups, as shown in Table I.

The plasma concentrations of DPH after repeated oral dosing of DPH (20 mg/kg) with or without allopurinol (20 mg/kg) are shown in Fig. 2A). The coadministration with allopurinol

<table>
<thead>
<tr>
<th>Dose</th>
<th>( k_e ) (h(^{-1}))</th>
<th>( AUC_{0-24} ) (( \mu \text{g} \cdot \text{h/ml} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mg/kg</td>
<td>DPH alone</td>
<td>0.343 ± 0.075</td>
</tr>
<tr>
<td></td>
<td>Combination</td>
<td>0.317 ± 0.060</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>DPH alone</td>
<td>0.211 ± 0.052</td>
</tr>
<tr>
<td></td>
<td>Combination</td>
<td>0.188 ± 0.064</td>
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</table>

There was no significant difference between both groups. Each value represents the mean ± S.D. \( (n = 4) \).
Phenytoin-Allopurinol Interaction

Fig. 2. Plasma Concentrations of DPH after Repeated Oral Administration of DPH Alone or in Combination with Allopurinol (ALP)

Each point represents the mean ± S.D. (n = 4). ●, DPH alone, A) 20 mg/kg and B) 50 mg/kg; ○, combination, A) DPH 20 mg/kg and ALP 20 mg/kg, B) DPH 50 mg/kg and ALP 50 mg/kg.

significantly delayed the elimination of DPH compared with that after administration DPH alone. Some pharmacokinetic parameters calculated from the data are shown in Table II. The AUC of DPH after coadministration with allopurinol was increased by 1.5–2.2 times of that after DPH alone.

The plasma concentrations of DPH after repeated oral administration of DPH alone at a higher dose (50 mg/kg) or in combination with allopurinol (50 mg/kg) are shown in Fig. 2B, and some pharmacokinetic parameters are summarized in Table I. The elimination of DPH was dramatically delayed by the coadministration with allopurinol at the high dose. The AUC0–24 of the coadministration group was 3 times that of the DPH alone, respectively.

Urinary Excretion of HPPH after Repeated Oral Administration of DPH Alone or in Combination with Allopurinol

The urinary excretion of HPPH after administration of DPH (20 or 50 mg/kg) alone or DPH and allopurinol (20 or 50 mg/kg) is shown in Fig. 3, as the cumulative urinary excretion. The excretion of HPPH, the major metabolite of DPH, was extremely decreased by coadministration with allopurinol at both doses, without significant differences of urinary volumes between both groups. Especially, the amount of HPPH excreted for 54 h after administration at the higher dose (50 mg/kg) in the coadministration group was only 35% of that in the DPH group (Fig. 3B). The urinary excretion of HPPH was observed to be dose-dependent in spite of

<table>
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<tr>
<th>TABLE II. Pharmacokinetic Parameters of DPH after Repeated Oral Administration of DPH Alone or in Combination with Allopurinol (ALP)</th>
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<tr>
<td>Dose</td>
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<tr>
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<tr>
<td>20 mg/kg</td>
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<tr>
<td>50 mg/kg</td>
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</table>

a) AUC0–12 for the 20 mg/kg dose and AUC0–24 for the 50 mg/kg dose. b) p < 0.05 compared with DPH alone. Each value represents the mean ± S.D. (n = 4).
coadministration of allopurinol.

**Hepatic Uptake of DPH**

The amounts of DPH extracted by liver 2 h after i.v. dosing of DPH (25 mg/kg) in the presence or absence of allopurinol (50 mg/kg) and oxypurinol (50 mg/kg) were 0.781 ± 0.210, 0.915 ± 0.323 and 0.889 ± 0.235 mg/g liver for the DPH alone, DPH-allopurinol and DPH-oxypurinol, respectively. These results suggest that the combination of allopurinol or oxypurinol did not affect the hepatic extraction of DPH and that the delayed elimination of DPH after coadministration with allopurinol may be not related to the hepatic extraction.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Control</th>
<th>DPH alone</th>
<th>DPH + ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline hydroxylase (a^j)</td>
<td>2.537±0.613</td>
<td>2.796±0.736</td>
<td>2.575±0.446</td>
</tr>
<tr>
<td>Aminopyrine demethylase (a^j)</td>
<td>3.096±0.983</td>
<td>3.196±0.816</td>
<td>2.911±0.714</td>
</tr>
<tr>
<td>Cytochrome P-450 (b^j)</td>
<td>1.339±0.352</td>
<td>1.304±0.188</td>
<td>1.391±0.126</td>
</tr>
</tbody>
</table>

Control, 2% acacia; DPH alone, 20 mg/kg; DPH + ALP, DPH 20 mg/kg and ALP 20 mg/kg. Each value represents the mean ± S.D. \((n = 4)\). \(a^j\) nmol/min/mg protein. \(b^j\) nmol/mg protein.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Control</th>
<th>DPH alone</th>
<th>DPH + ALP</th>
<th>ALP alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline hydroxylase (a^j)</td>
<td>0.628±0.062</td>
<td>0.987±0.283</td>
<td>1.115±0.318</td>
<td>0.581±0.061</td>
</tr>
<tr>
<td>Aminopyrine demethylase (a^j)</td>
<td>0.681±0.113</td>
<td>1.346±0.327</td>
<td>1.330±0.299</td>
<td>0.732±0.106</td>
</tr>
<tr>
<td>Cytochrome P-450 (b^j)</td>
<td>0.196±0.019</td>
<td>0.309±0.081</td>
<td>0.327±0.065</td>
<td>0.191±0.018</td>
</tr>
</tbody>
</table>

Control, 2% acacia; DPH alone, 50 mg/kg; DPH + ALP, DPH 50 mg/kg and ALP 50 mg/kg; ALP alone, 50 mg/kg. Each value represents the mean ± S.D. \((n = 9)\). \(a^j\) nmol/min/mg protein. \(b^j\) nmol/mg protein. \(c^j\) \(p < 0.05\) compared with the control.
Effect of DPH and DPH Plus Allopurinol on Hepatic Drug Metabolizing Enzyme Activities

In order to estimate the inhibitory effect of allopurinol on drug metabolism, hepatic drug metabolizing enzyme activities were determined after repeated oral administration of DPH alone or in combination with allopurinol. The results are shown in Table III and IV. The significant changes in these enzyme activities were not observed after combined treatment with DPH and allopurinol at lower doses (20 mg/kg, each), however the activities were significantly enhanced with DPH at higher doses (DPH alone, 50 mg/kg or DPH and allopurinol, 50 mg/kg each), by 57—98%, probably due to the inductive effect of DPH. No significant differences in the enhanced activities were seen between the DPH alone and coadministered groups. Additionally, the repeated administration of allopurinol alone gave no inhibitory effect on the enzyme activities in the 9000 × g supernatant, as shown in Table IV. These results clearly demonstrated that allopurinol, even in repeated dosing, did not inhibit directly hepatic drug metabolizing enzyme activities in rats.

**In Vitro Hydroxylation of DPH and Effect of Allopurinol**

Since the hydroxylation activity of the hepatic microsomes was low as a result of a preliminary experiment, the induction of the enzyme activity was done with phenobarbital. In order to clarify the mechanism by which allopurinol inhibits the metabolic conversion of DPH, the *in vitro* hydroxylation of DPH was measured in the presence or absence of allopurinol. The results are shown as the Lineweaver-Burk plots in Fig. 4. The kinetic plots showed only a slight decrease in the hydroxylation rate of DPH in the presence of allopurinol (25 and 100 μM). The $V_{max}$ was 52.6 and 48.1 nmol/mg protein·min⁻¹ for the DPH alone and the combination (100 μM allopurinol), respectively, with $K_m$ value of 4.71 × 10⁻⁵ M. Although the figure gave the data on apparently non-competitive inhibition of DPH hydroxylation by allopurinol, the drug, even in a high concentration, did not exert the strongly inhibitory effect on the *in vitro* hydroxylation of DPH. The results did not agree with the data (the severe inhibition of hydroxylation) of the *in vivo* experiments (Figs. 2 and 3). Oxypurinol (20 μM) also had a inhibitory effect on DPH hydroxylation to the same extent as that by allopurinol (data not shown).

**Effect of Allopurinol on Plasma Flow Rate**

Wexler and Greenberg¹⁷ report the renal damage of rats treated with allopurinol at a high dose (400 mg/kg, for 10 d). Therefore, the effect of allopurinol on the renal plasma flow rate was estimated after repeated dosing of DPH and allopurinol (50 mg/kg, each for 7 d). The plasma clearance of PAH was similar between the DPH alone and DPH-allopurinol groups (RPF: 2.928 ± 0.129, 1.885 ± 0.340 and 2.044 ± 0.213 l/h, for the control, DPH alone and DPH-allopurinol, respectively, $n = 3$). Consequently, the 50 mg/kg dosing caused no effect on renal plasma flow rate of these rats.

**Discussion**

Allopurinol is known to inhibit drug metabolism,²,³ but several unresolved problems concerning the role of allopurinol in prolonging the metabolism of other drugs have remained. Therefore, interactions between DPH and allopurinol and the possible mechanism were investigated in rats.

Our data show that the inhibition of DPH hydroxylation was seen after the repeated dosing
of allopurinol at higher doses (Fig. 2), but not after a single administration (Fig. 1), and suggest the alteration of a biotransformation process by the accumulated drugs or metabolites. The results demonstrate that the hydroxylation of DPH was inhibited by allopurinol or its major metabolite, oxypurinol.

It is shown \textit{in vitro} that the hydroxylated metabolite of one drug can inhibit the biotransformation of another drug. Such cross-inhibition is reported for the following metabolite-drug systems: HPPH-phenylbutazone,\textsuperscript{18} oxyphenbutazone-phenytoin,\textsuperscript{18} HPPH-hexobarbital,\textsuperscript{19} HPPH-ethylmorphine,\textsuperscript{19} HPPH-zoxazolamine,\textsuperscript{20} HPPH-aniline\textsuperscript{20} and HPPH-antipyrine.\textsuperscript{21} Thus, a possibility that oxypurinol, a hydroxylated metabolite, might be involved in the inhibition of DPH hydroxylation cannot be ruled out. The much longer half-life (16.9 h) of oxypurinol,\textsuperscript{22} compared with that (1.6 h) of allopurinol, may strengthen the possibility, based on the accumulation of hydroxylated metabolite. Allopurinol and oxypurinol inhibited the hydroxylation of DPH slightly \textit{in vitro} (Fig. 4). This suggests that both agents may not directly inhibit P-450-dependent monooxygenation. In addition, both agents are not the substrate for P-450, and did not inhibit the hepatic drug metabolizing enzyme activities (Tables III and IV). These results show that the inhibitory effect of allopurinol and oxypurinol is not mediated by P-450-dependent monooxygenase reactions.

It was assumed that allopurinol might inhibit the hepatic uptake of DPH. The assumption, however, was completely denied by the data showing that the uptake of DPH was not altered on the simultaneous administration of allopurinol.

Allopurinol has been used widely and through long duration for relieving hyperuricemia.\textsuperscript{23,24} In spite of the usefulness of this drug, it is reported that allopurinol in large doses can induce a marked nephrotoxicity in experimental animals.\textsuperscript{17,25,26} Therefore, the allopurinol-induced nephrotoxicity was examined in rats treated for 7 d, in relation to the decreased excretion of HPPH. However, no significant change in the renal plasma flow was observed. This indicates that the interaction between DPH and allopurinol observed \textit{in vivo} was not due to the allopurinol-induced nephrotoxicity.

The mechanism for which allopurinol and partly oxypurinol inhibited the hydroxylation of DPH is not clear on the basis of the results obtained. There may be some mechanisms which are not fully elucidated, such as the electron transfer between P-450 and xanthine oxidase, decreased capacity for hydroxylation due to depletion of specific P-450 species and forming an inactive complex with a drug oxidizing enzyme.\textsuperscript{27}

In conclusion, the repeated dosing of allopurinol at higher doses inhibited the hydroxylation pathway of DPH and dramatically decreased the urinary excretion of HPPH. However, allopurinol gave no inhibitory effect on hepatic drug metabolizing enzyme activities and no damage to renal plasma flow in the doses used. Although these findings did not permit one to draw certain conclusions concerning the mechanism for the inhibition of hydroxylation and the delayed elimination of DPH, we present unambiguous evidence on a drug interaction between DPH and allopurinol based on the data obtained.

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