Effect of Allopurinol and Benzbromarone on the Concentration of Uridine in Plasma

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To investigate whether allopurinol and benzbromarone affect the concentration of uridine in plasma, allopurinol or benzbromarone were administered to patients with gout for 3 to 6 months. Allopurinol decreased the concentrations of uridine and uric acid in plasma and the urinary excretion of uric acid, but increased the plasma concentration and urinary excretion of oxypurines and orotidine. Benzbromarone decreased the concentration of uric acid in plasma and increased the excretion of uric acid in urine. However, it did not affect the plasma concentration of uridine or oxypurines or the urinary excretion of oxypurines or orotidine. These results suggest that orotidilytic decarboxylase was inhibited by allopurinol and oxypurinol ribonucleotides and/or that phosphoribosyl pyrophosphate (PRPP) was consumed by conversion from hypoxanthine, allopurinol, and oxypurinol to the respective ribonucleotides, resulting in a decrease in the de novo synthesis of pyrimidine leading to the decreased concentration of uridine in plasma. Furthermore, it was suggested that benzbromarone did not affect the de novo synthesis of pyrimidine or purine.

ALLOPURINOL is an analog of hypoxanthine and is oxidized to oxypurinol by xanthine oxidase, and both substances inhibit xanthine oxidase activity, leading to hypouricemia and hyperoxypurinemia. Hypoxanthine and allopurinol are converted to the respective ribonucleotides (imosine 5′-monophosphate [IMP] and allopurinol ribonucleotide) using phosphoribosyl pyrophosphate (PRPP) by hypoxanthine guanine phosphoribosyl transferase, and oxypurinol is converted to oxypurinol ribonucleotide by orotate phosphoribosyl transferase (OPRT). These ribonucleotides and depletion of PRPP inhibit the de novo synthesis of purine. In addition, administration of allopurinol also inhibits orotidilytic decarboxylase (orotidine 7-monophosphate decarboxylase) and OPRT,[1-3] leading to excessive urinary excretion of orotidine and orotic acid.[1-4] Since allopurinol is administered to patients with gout to treat hyperuricemia, orotidine, orotic acid, and oxypurines are excreted excessively in urine together with decreased urinary excretion of uric acid in these patients. Therefore, de novo synthesis of pyrimidine may be disturbed by administration of allopurinol, followed by a decrease in the degradation of uridine 5-monophosphate (UMP), leading to a decrease in the plasma uridine level. Therefore, in patients with gout, allopurinol may decrease the plasma concentration of uridine, as well as uric acid. In contrast, benzbromarone, a strong uricosuric agent, is not thought to affect the synthesis or degradation of purine or pyrimidine, although it inhibits xanthine oxidase in vitro.[5] Accordingly, we investigated whether allopurinol decreases the uridine level in plasma in patients with gout, and compared the effect of allopurinol with that of benzbromarone on the concentration of uridine in plasma.

SUBJECTS AND METHODS

Subjects and Protocol

The subjects were 38 male gout patients with normal creatinine clearance (mean age, 45 ± 8 years). All patients met criteria for primary gout as outlined by the American Rheumatism Association.[6] After informed consent was obtained, all medication was withheld 2 months before the study. The study was performed just before and 3 to 6 months after administration of allopurinol (200 or 300 mg/d) to 22 patients or benzbromarone (50 mg/d) to 16 patients. One-hour urine was collected twice and blood was drawn at the midpoint of each of these 1-hour urine collections, following an overnight fast except for water. After blood sampling, 24-hour urine was collected. The concentration of uric acid was determined in plasma and urine, and 24-hour excretion and clearance of uric acid were calculated. Alcoholic beverages were withheld 1 week before the study. Based on replies to a questionnaire, purine intake was 140 to 260 mg/d and was not different before versus after allopurinol or benzbromarone administration.

Blood and Urine Analyses

Blood samples were drawn by syringe after an overnight fast and placed in test tubes containing EDTA. The plasma was then immediately separated to prevent leakage of uridine and hypoxanthine from the blood cells. Plasma concentrations of uridine, hypoxanthine, and xanthine were determined by high-performance liquid chromatography (HPLC) as described previously.[7] Urinary orotidine was also determined at 275 nm spectrophotometrically by HPLC as follows. After centrifugation, the clear supernatant of urine was diluted 10 times, and then 20 μL was injected onto an ion-pair reversed-phase HPLC column. The column was a Beckman IP (4.6 mm × 25 cm). The mobile phase was 0.02 mol/L KH_2PO_4, pH 5.2, containing 8 mmol/L tetrabutylammonium hydrogen sulfate, and the flow rate was 1.0 mL/min. The lower detection limit was 0.1 nmol/mL. Plasma concentrations of uric acid and creatinine were determined with an autoanalyzer using specific enzymatic methods. Urinary concentrations of uric acid and creatinine were similarly determined.

Chemicals

All chemicals, including uridine, hypoxanthine, xanthine, and uric acid, were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Statistics

Values are expressed as the mean ± SD. The significance of differences for the means of the variables was analyzed by a two-tailed *t* test. The relationship between variables was expressed by a correlation coefficient. *P* less than .05 was considered statistically significant.

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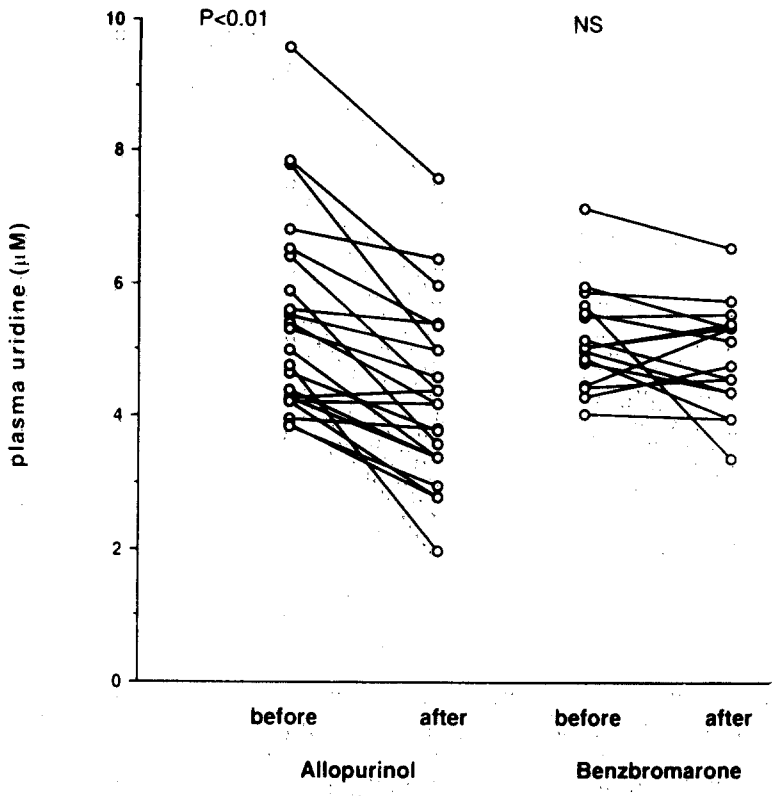

Fig 1. Effect of allopurinol and benzbromarone on plasma concentration of uridine.

RESULTS

Effect of Allopurinol and Benzbromarone on the Concentration of Uridine in Plasma

Allopurinol decreased the concentration of uridine in plasma from 5.58 ± 1.56 to 4.34 ± 1.30 μmol/L ($P < .01$; Fig 1), whereas benzbromarone did not affect the plasma concentration of uridine (5.22 ± 0.78 *v* 5.00 ± 0.80 μmol/L; Fig 1).

Effect of Allopurinol and Benzbromarone on the Concentration of Purine Bases in Plasma

The concentration of uric acid in plasma decreased 0.63-fold after administration of allopurinol ($P < .01$; data not shown). In contrast, hypoxanthine and xanthine in plasma increased 2.56-fold ($P < .01$) and 6.60-fold ($P < .01$), respectively, after administration of allopurinol (data not shown). The concentration of uric acid in plasma also decreased 0.54-fold ($P < .01$) after administration of benzbromarone, but oxypurine levels in plasma did not change (Table 1).

Table 1. Effect of Benzbromarone on the Concentration of Uric Acid, Hypoxanthine, and Xanthine in Plasma

Parameter	Before Treatment	After Treatment
Uric acid	513 ± 64	276 ± 60*
Hypoxanthine	1.31 ± 0.17	1.47 ± 0.59
Xanthine	0.87 ± 0.17	1.16 ± 0.31

NOTE. Values are expressed in μmol/L.
*$P < .01$.

Effect of Allopurinol and Benzbromarone on Urinary Excretion of Purine Bases

Urinary excretion of uric acid/body surface area decreased 0.53-fold ($P < .01$) after administration of allopurinol (data not shown), whereas it increased 1.26-fold ($P < .01$) after administration of benzbromarone (Table 2). Urinary excretion of hypoxanthine/body surface area and xanthine/body surface area increased 4.47-fold ($P < .01$) and 7.46-fold ($P < .01$), respectively, after administration of allopurinol (data not shown), whereas it did not change after administration of benzbromarone (Table 2). Urinary excretion of total purine bases (uric acid, hypoxanthine, and xanthine) decreased 0.70-fold ($P < .01$) after administration of allopurinol (data not shown), whereas it increased 1.25-fold ($P < .01$) after administration of benzbromarone (Table 2).

Table 2. Effect of Benzbromarone on Urinary Excretion of Hypoxanthine, Xanthine, and Uric Acid

Parameter	Before Treatment	After Treatment
Uric acid	2.07 ± 0.50	2.61 ± 0.46*
Hypoxanthine	42 ± 16	38 ± 17
Xanthine	36 ± 12	35 ± 24
Purine bases	2.14 ± 0.51	2.68 ± 0.47*

NOTE. Values for uric acid and purine bases are expressed in mmol/m²/d, and values for oxypurines (hypoxanthine and xanthine) are expressed in μmol/m²/d.
*$P < .01$.

Effect of Allopurinol and Benzbromarone on the Clearance of Uric Acid

Renal clearance of uric acid was not different before versus after administration of allopurinol (7.8 ± 2.4 *v* 6.3 ± 1.9 mL/min), whereas it increased from 5.9 ± 1.6 to 14.3 ± 3.9 mL/min ($P < .01$) with administration of benzbromarone.

Effect of Allopurinol and Benzbromarone on Urinary Excretion of Orotidine

Allopurinol increased urinary excretion of orotidine from 0.39 ± 0.17 μmol/m²/d to 5.87 ± 3.27 μmol/m²/d ($P < .001$). In contrast, benzbromarone did not affect urinary excretion of orotidine (0.38 ± 0.11 *v* 0.38 ± 0.11 μmol/m²/d).

DISCUSSION

In the present study, allopurinol decreased uridine and uric acid concentrations in plasma and urinary excretion of purine bases (uric acid + oxypurines), and increased the plasma concentration and urinary excretion of oxypurines and urinary excretion of orotidine. In contrast, benzbromarone did not affect plasma concentrations of uridine and oxypurines or urinary excretion of oxypurines, although it decreased the plasma concentration of uric acid and increased urinary excretion of uric acid.

Since allopurinol did not affect the clearance of uric acid in the present study, a decrease in urinary excretion of purine bases indicates that allopurinol decreased de novo synthesis of purine, suggesting that IMP and allopurinol and oxypurinol ribonucleotides were produced using PRPP as described previously.[8-14] Recently, it was demonstrated that the concentration of uridine was higher in patients with gout of the overexcretion type than in normal subjects,[15] and also that the abrupt loss of adenosine triphosphate (ATP) enhances pyrimidine degradation, leading to an increase in the concentration of uridine in plasma.[16,17] Overexcretion hyperuricemia that is caused by purine degradation is classified as either due to enhanced de novo purine synthesis or to excessive ATP consumption. Therefore, it is suggested that the concentration of uridine in plasma increases in patients with enhanced de novo purine synthesis and excessive consumption of ATP.

Enhanced de novo purine synthesis causes an acceleration of purine degradation, resulting in an increased production of uric acid. Since de novo purine synthesis requires PRPP, PRPP synthesis must be enhanced along with increased de novo synthesis. Accordingly, enhanced PRPP synthesis may increase de novo pyrimidine synthesis. The resultant enhancement of de novo pyrimidine synthesis may accelerate pyrimidine degradation, leading to increased production of uridine. In contrast to enhanced de novo purine synthesis, consumption of PRPP by administration of allopurinol may play a role in the decrease in de novo synthesis of pyrimidine. Furthermore, since administration of allopurinol inhibits OPRT and orotidilytic decarboxylase, de novo synthesis of pyrimidine from orotic acid to UMP is disturbed, leading to the increased excretion of orotidine and orotic acid. In addition, oxypurinol does not accelerate the salvage of uridine to UMP by uridine kinase.[3] These effects seem to disturb the de novo synthesis of pyrimidine, leading to a decrease in the degradation of pyrimidine, resulting in a decrease in the concentration of uridine in plasma.

Although many studies[18-21] were performed on the uricosuric action of benzbromarone, there has been no study on whether benzbromarone affects the de novo synthesis of purine or pyrimidine. Therefore, we investigated the effect of benzbromarone on purine bases, uridine, and orotidine in the present study. Although benzbromarone inhibits xanthine oxidase in vitro,[5] the results indicate that benzbromarone did not affect the plasma concentration of oxypurines or uridine or urinary excretion of oxypurines despite its uricosuric action. Since benzbromarone was administered over more than 2 months, the metabolism of purine bases seems to be in a steady state. Therefore, a benzbromarone-induced increase in the clearance of uric acid may not enhance urinary excretion of uric acid, because the plasma concentration of uric acid was decreased by benzbromarone. Nevertheless, an increase in urinary excretion of uric acid was observed after long-term administration of benzbromarone. Therefore, a benzbromarone-induced increase in urinary excretion of uric acid does seem ascribable to a shift from extrarenal excretion to renal excretion of uric acid, accompanied by a decrease in the concentration of uric acid in plasma. In addition, it does not seem to significantly affect de novo synthesis of purine or pyrimidine, xanthine dehydrogenase activity, or orotidilytic decarboxylase activity, since benzbromarone did not affect urinary excretion of oxypurines or orotidine or the plasma concentration of oxypurines or uridine.

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