Effect of Furosemide on Renal Excretion of Oxypurinol and Purine Bases

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To examine whether furosemide affects the plasma concentration and urinary excretion of purine bases and oxypurinol, we administered allopurinol (300 mg) orally to 6 healthy subjects and then administered furosemide (20 mg) intravenously 10 hours later. Furosemide (20 mg) decreased the urinary excretion of uric acid by 40% ($P < .01$), oxypurinol by 39% ($P < .05$), and xanthine by 43% ($P < .05$) and the fractional clearance of uric acid by 45% ($P < .01$) and oxypurinol by 34% ($P < .05$) when measured 1 to 2 hours after administration. Moreover, furosemide increased the plasma concentration of uric acid by 6% at 1.5 hours after administration. These results indicate that furosemide may decrease the urinary excretion of uric acid and oxypurinol by acting on their common renal transport pathway(s). In addition, it is suggested that the effect of furosemide on oxypurinol is clinically important, since the hypouricemic effect of allopurinol may become more potent as a result.

FUROSEMIDE is a diuretic used for the treatment of heart failure, hypertension, and edema. It inhibits the absorption of chloride and sodium at Henle's loop, resulting in an increase in the rate of urine formation together with natriuresis. In addition to diuresis, furosemide dilates arterioles, followed by a decrease in blood pressure. Further, it increases the serum uric acid concentration, which is attributable to a furosemide-induced decrease in extracellular fluid and the urinary excretion of uric acid.[1-4] Many previous studies[5-7] have demonstrated that glucagon, amino acids, benzbromarone, and probenecid increase the urinary excretion of oxypurinol, xanthine, and uric acid, suggesting that uric acid and xanthine (one of the oxypurines) share a renal transport pathway with oxypurinol. Therefore, we speculated that furosemide may decrease the urinary excretion of oxypurinol, resulting in an increase in its plasma concentration.

Oxypurinol is a metabolite of allopurinol that is used for the treatment of hyperuricemia. Although both allopurinol and oxypurinol are potent inhibitors of xanthine oxidase, the biologic half-life of oxypurinol is longer. Accordingly, the overall effect of allopurinol may depend on the action of oxypurinol.[8-10] If furosemide decreases the urinary excretion of oxypurinol, the concentration of oxypurinol in plasma may increase. This finding would be clinically important, because the hypouricemic effect of allopurinol mostly depends on plasma oxypurinol levels. In previous studies,[11,12] it has been suggested that an increase in sodium-chloride transport through the thick ascending limb of Henle's loop and macula densa cells leads to an increase in adenosine triphosphate (ATP) degradation in the kidneys. In addition, we recently demonstrated that furosemide decreased the plasma concentration and urinary excretion of hypoxanthine, suggesting that it may also decrease purine degradation.[13] Therefore, we performed the present study to investigate whether furosemide decreases the urinary excretion of oxypurinol together with uric acid and whether it decreases purine degradation, using allopurinol as a xanthine oxidase inhibitor.

SUBJECTS AND METHODS

Chemicals

Furosemide was obtained from Hoechst-Marion-Roussel (Tokyo, Japan). Allopurinol was purchased from GlaxoWellcome Japan (Tokyo, Japan). Other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan).

Subjects and Protocol

Six men aged 36 to 45 years (body weight, 62 to 78 kg), each with normal laboratory data, participated in the study after informed consent was obtained. The study protocol is shown in Fig 1. In brief, allopurinol (300 mg) was administered orally to the subjects at 11:30 PM after a 4-hour fast. At 8:30 AM the next day, the urine was completely voided and the first 1-hour urine sample was collected (first period). After the first urine sample was collected, furosemide (20 mg) was administered intravenously. The second urine sample was collected 1 hour after administration of furosemide (second period), and the third urine sample was collected between 1 and 2 hours after furosemide administration (third period). The first, second, and third blood samples were drawn with heparinized syringes at the midpoint of the respective 1-hour urinary collections. Two weeks later, a control study was performed using the same protocol, except without the administration of furosemide. This control study was performed with the subjects fasting, except for water.

Blood and Urine Analyses

The concentrations of hypoxanthine, xanthine, and oxypurinol in plasma and urine were determined using high-performance liquid chromatography as described previously.[5] In brief, the column was a Wakosil 5C-18-200 (4.6 × 250 mm; Wako Pure Chemical Industries, Osaka, Japan) with a flow rate of 1 mL/min and a mobile phase of 0.02 mol/L KH_2PO_4 (pH 2.2). To measure the plasma concentration of hypoxanthine, xanthine, and oxypurinol, the plasma was immediately separated after blood sampling with a heparinized syringe. The concentrations of uric acid and creatinine in plasma and urine were measured by the uricase method using a Uric Acid B Test Wako Kit (Wako Pure Chemicals) and the enzymatic method using a Diacolor Liquid CRE Kit (Toyobo, Osaka, Japan), respectively. Plasma renin activity (PRA), angiotensin II, and aldosterone levels were measured by Shionogi Biomedical Laboratories (Osaka, Japan), while other measurements were performed in our hospital laboratory.

The percentage ratios for uric acid clearance to creatinine clearance (fractional uric acid clearance), hypoxanthine clearance to creatinine clearance (fractional hypoxanthine clearance), xanthine clearance to

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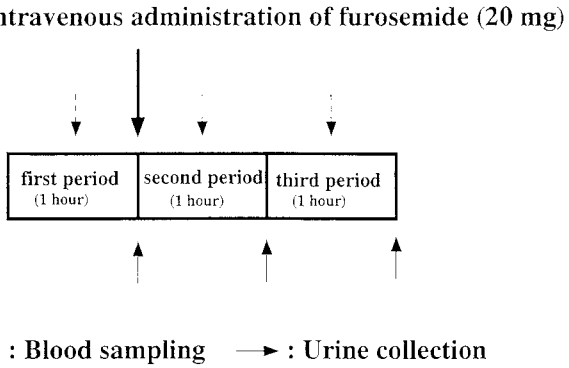

Fig 1. Furosemide-loading study protocol. Allopurinol was administered in the evening 9 hours before the study.

creatinine clearance (fractional xanthine clearance), and oxypurinol clearance to creatinine clearance (fractional oxypurinol clearance) were calculated.

Statistical Analysis

The data are presented as the mean ± SD. The significance of differences between variables was analyzed by a 2-tailed paired *t* test.

RESULTS

Effect of Furosemide on the Urinary Excretion of Purine Bases and Oxypurinol and Total Urine Output

In the furosemide-loading study, total urinary output increased by 5.3-fold ($P < .01$) and 2.6-fold ($P < .05$) in the second and third periods, respectively. The urinary excretion of uric acid, oxypurinol, and xanthine decreased by 40% ($P < .01$), 39% ($P < .05$), and 43% ($P < .05$), respectively, in the third period as compared with the first period. However, the urinary excretion of hypoxanthine did not change. In contrast, none of the respective values changed significantly in the control study (Table 1).

Table 1. Urinary Excretion of Purine Bases and Oxypurinol (N = 6)

Parameter	1st Period	2nd Period	3rd Period
Furosemide-loading study			
Urine volume (mL/h)	174 ± 45	922 ± 168†	451 ± 251*
Hypoxanthine (μmol/h)	9.16 ± 3.39	7.69 ± 3.29	6.95 ± 3.11
Xanthine (μmol/h)	17.66 ± 5.31	12.93 ± 4.79	10.10 ± 3.51*
Uric acid (μmol/h)	146 ± 31	115 ± 22	83 ± 20†
Oxypurinol (μmol/h)	35.12 ± 9.59	26.89 ± 8.39	21.50 ± 6.25*
Control study			
Urine volume (mL/h)	180 ± 58	205 ± 65	177 ± 59
Hypoxanthine (μmol/h)	9.22 ± 3.68	8.91 ± 3.45	7.22 ± 2.40
Xanthine (μmol/h)	15.86 ± 3.24	14.83 ± 2.83	12.78 ± 1.34
Uric acid (μmol/h)	151 ± 39	154 ± 42	147 ± 37
Oxypurinol (μmol/h)	34.14 ± 9.39	33.32 ± 8.15	32.34 ± 8.85

NOTE. Values are the mean ± SD.
*$P < .05$.
†$P < .01$.

Effect of Furosemide on the Plasma Concentration of Purine Bases and Oxypurinol

In the furosemide-loading study, the plasma concentration of uric acid increased by 6% ($P < .05$) in the third period as compared with the first period. However, the plasma concentration of xanthine, hypoxanthine, and oxypurinol did not change significantly throughout the study. In the control study, the plasma concentration of uric acid, hypoxanthine, xanthine, and oxypurinol did not change significantly (Table 2).

Effect of Furosemide on the Fractional Clearance of Purine Bases and Oxypurinol and the Clearance of Creatinine

In the furosemide-loading study, the fractional clearance of uric acid and oxypurinol decreased by 41% ($P < .01$) and 31% ($P < .05$), respectively, in the third period. However, the fractional clearance of hypoxanthine and the clearance of creatinine did not change significantly. In the control study, the fractional clearance of uric acid, oxypurinol, hypoxanthine, and xanthine and the clearance of creatinine did not change significantly (Table 3).

Effect of Furosemide on PRA and Plasma Angiotensin II and Aldosterone

PRA increased by 3-fold ($P < .01$) and 2.9-fold ($P < .01$) in the second and third periods, respectively, and plasma angiotensin II and aldosterone increased by 2.2-fold ($P < .05$) and 1.3-fold ($P < .01$), respectively, in the third period of the furosemide-loading study (Table 4). These parameters did not change significantly in the control study (data not shown).

Effect of Furosemide on the Urinary Excretion of Sodium, Chloride, and Potassium

In the furosemide-loading study, the urinary excretion of sodium, chloride, and potassium increased by 15.2-, 13.7-, and 2.8-fold, respectively, in the second period, and the urinary excretion of sodium and chloride increased by 4.4- and 4.4-fold, respectively, in the third period (Table 5). These parameters did not change significantly in the control study (data not shown).

Table 2. Plasma Concentration (μmol/L) of Purine Bases and Oxypurinol (N = 6)

Parameter	1st Period	2nd Period	3rd Period
Furosemide-loading study			
Hypoxanthine	2.16 ± 0.72	1.88 ± 0.64	1.52 ± 0.60
Xanthine	4.76 ± 0.82	4.24 ± 0.84	3.78 ± 0.76
Uric acid	292 ± 42	298 ± 46	310 ± 48*
Oxypurinol	24.90 ± 2.90	24.38 ± 3.42	23.94 ± 3.52
Control study			
Hypoxanthine	2.04 ± 0.44	1.92 ± 0.48	1.64 ± 0.48
Xanthine	5.30 ± 0.92	5.02 ± 1.02	4.62 ± 1.16
Uric acid	298 ± 41	298 ± 42	298 ± 42
Oxypurinol	26.22 ± 4.76	25.62 ± 4.76	25.40 ± 4.92

NOTE. Values are the mean ± SD.
*$P < .05$.

Table 3. Fractional Clearance of Purine Bases and Oxypurinol (N = 6)

Parameter	1st Period	2nd Period	3rd Period
Furosemide-loading study			
Fractional hypoxanthine clearance	63.3 ± 20.1	64.1 ± 24.8	70.8 ± 18.1
Fractional xanthine clearance	58.7 ± 21.3	50.3 ± 23.3	45.0 ± 17.1
Fractional uric acid clearance	7.3 ± 1.8	6.0 ± 1.5	4.3 ± 1.3†
Fractional oxypurinol clearance	20.5 ± 4.9	17.1 ± 5.7	14.2 ± 3.6*
Creatinine clearance	115 ± 9	109 ± 7	106 ± 8
Control study			
Fractional hypoxanthine clearance	69.3 ± 26.4	72.3 ± 24.4	70.5 ± 23.2
Fractional xanthine clearance	46.7 ± 12.4	47.1 ± 11.0	45.0 ± 9.9
Fractional uric acid clearance	7.9 ± 2.7	8.2 ± 2.6	7.9 ± 2.4
Fractional oxypurinol clearance	20.3 ± 6.1	20.4 ± 4.4	20.2 ± 5.4
Creatinine clearance	109 ± 8	107 ± 7	107 ± 7

NOTE. Values are the mean ± SD. Fractional uric acid clearance is the percentage ratio of uric acid clearance to creatinine clearance, fractional hypoxanthine clearance is the percentage ratio of hypoxanthine clearance to creatinine clearance, fractional xanthine clearance is the percentage ratio of xanthine clearance to creatinine clearance, and fractional oxypurinol clearance is the percentage ratio of oxypurinol clearance to creatinine clearance.

*$P < .05$.

†$P < .01$.

Effect of Furosemide on the Plasma Concentration of Total Protein, Sodium, Chloride, and Potassium

In the furosemide-loading study, the plasma concentration of total protein increased by 8% in the second period and 9% in the third period as compared with the first period. In contrast, plasma chloride decreased by 2% and 3% in the second and third periods, respectively, while potassium decreased by 5% in the second period, as compared with the first period (Table 6). None of these parameters changed significantly in the control study (data not shown).

DISCUSSION

In the present study, it is demonstrated that the urinary excretion and fractional clearance of uric acid and oxypurinol were decreased by furosemide (Table 3), indicating that it either accelerated the reabsorption of uric acid and oxypurinol or inhibited their secretion. Further, we observed that furosemide markedly increased the excretion of urine, sodium, potassium, and chloride (Tables 1 and 6) and decreased the plasma concentration of chloride and potassium, which led to a decrease in extracellular fluid volume that was reflected by an increase in plasma protein (Table 6). A decrease in extracellular fluid volume is thought to cause a decrease in the clearance of uric acid[1-4] since furosemide, by replacing the lost sodium and water, increases the fractional clearance of uric acid,[14] presumably by furosemide-induced vasodilation.[15] Further, since the main renal transport system of uric acid is in the proximal tubules and furosemide decreased the fractional clearance of uric acid, it is suggested that the extracellular fluid volume contraction due to furosemide affects the renal transport of uric acid in the proximal tubules, but not in Henle's loop. However, it cannot be excluded that an increase in the renal concentration of angiotensin II due to furosemide may partly play a role in decreased fractional uric acid clearance.

In the present study, the most intriguing finding, for its potential clinical importance, is the furosemide-induced decrease in the urinary excretion and fractional clearance of oxypurinol. Although the plasma concentration of oxypurinol did not change with a decrease in the urinary excretion of oxypurinol, our finding seems to demonstrate that the level of oxypurinol in the body decreases along with its urinary excretion, since oxypurinol is no longer produced 9 hours after administration of allopurinol. The present oxypurinol data indicate that a decrease in the urinary excretion of oxypurinol may retard the decrease in the plasma concentration of oxypurinol. Many studies[5-7,16] have demonstrated that glucose, glucagon, and probenecid increase the fractional clearance of uric acid, xanthine, and oxypurinol, suggesting that these latter three partly share a common pathway in the kidney. Therefore,

Table 4. PRA and Plasma Concentration of Angiotensin II and Aldosterone in the Furosemide-Loading Study (N = 6)

Parameter	1st Period	2nd Period	3rd Period
PRA (ng/mL/h)	0.8 ± 0.2	2.4 ± 0.7†	2.3 ± 0.8†
Angiotensin II (pg/mL)	2.4 ± 1.0	5.5 ± 4.0	5.3 ± 2.7*
Aldosterone (pg/mL)	91 ± 15	118 ± 30	118 ± 21†

NOTE. Values are the mean ± SD.

*$P < .05$.

†$P < .01$.

Table 5. Urinary Excretion (μmol/h) of Sodium, Chloride, and Potassium in the Furosemide-Loading Study (N = 6)

Parameter	1st Period	2nd Period	3rd Period
Na	6.5 ± 2.7	98.6 ± 10.5†	28.7 ± 8.6†
Cl	7.5 ± 3.6	102.4 ± 13.0†	32.8 ± 8.1†
K	3.6 ± 1.8	10.1 ± 3.0†	8.5 ± 6.1

NOTE. Values are the mean ± SD.

*$P < .05$.

†$P < .01$.

Table 6. Plasma Concentration of Total Protein, Sodium, Chloride, and Potassium in the Furosemide-Loading Study (N = 6)

Parameter	1st Period	2nd Period	3rd Period
Protein (g/L)	75 ± 2	81 ± 2*	82 ± 2†
Na (mEq/L)	141 ± 2	140 ± 2	140 ± 1
Cl (mEq/L)	104 ± 2	102.4 ± 1*	101 ± 2*
K (mEq/L)	4.2 ± 0.3	4.0 ± 0.3†	3.9 ± 0.2†

NOTE. Values are the mean ± SD.
*$P < .05$.
†$P < .01$.

if oxypurinol is transported via the same pathway as uric acid, the urinary excretion of oxypurinol may be affected by an extracellular fluid volume contraction due to furosemide, although it is also possible that furosemide directly acts on the renal handling of oxypurinol. As a result, a decrease in the fractional clearance of oxypurinol by furosemide may increase the plasma concentration of oxypurinol in patients receiving furosemide and allopurinol, such as gout patients with heart failure. Additional clinical studies are needed, including an assessment of the hypouricemic or adverse effects of allopurinol, in these patients.

A recent report[13] demonstrated that furosemide decreased the fractional clearance of uric acid and xanthine in healthy subjects without a preload of allopurinol, suggesting that furosemide may partly affect the common renal pathway. In the present study, furosemide did not affect the fractional clearance of xanthine (Table 3). However, xanthine excretion decreased significantly, while its fractional clearance showed a downward trend, though not statistically significant. Therefore, it is likely that our present observations are similar to those of a recent report.[13] Moreover, the same report[13] also demonstrated that furosemide decreased the plasma concentration and urinary excretion of hypoxanthine, suggesting that it may decrease the production of hypoxanthine in many tissues that contain a negligible amount of xanthine dehydrogenase, since hypoxanthine is an end product of purine degradation in these tissues. In the kidney, tubular sodium-chloride reabsorption is the active process with the greatest energy demand.[11,12] Since furosemide inhibits sodium-chloride reabsorption in the thick ascending limb of Henle's loop and the macula densa and also increases the urinary excretion of sodium and chloride, this inhibition may decrease ATP consumption, resulting in a decreased concentration of hypoxanthine in the kidney. Further, since sodium-chloride transport is present not only in the kidneys but also in various other organs,[17-19] furosemide may decrease the production of hypoxanthine, leading to its decreased plasma concentration and urinary excretion. The present study suggests that furosemide may decrease the production of xanthine, as it did not affect the fractional clearance of xanthine or the plasma concentration of oxypurinol, on which the plasma concentration and urinary excretion of oxypurines are dependent. Therefore, it is suggested that furosemide may also decrease purine degradation (ATP consumption) in tissues that contain a large amount of xanthine dehydrogenase, such as the liver and small intestine. However, the effect of furosemide seems too slight, since the urinary excretion of hypoxanthine did not decrease significantly in the present study.

In conclusion, the effect of furosemide on the urinary excretion of oxypurinol and uric acid may be clinically important, since the overall hypouricemic effect of allopurinol is mainly dependent on the plasma concentration of oxypurinol. In addition, an important implication of our results is the possible potentiation of allopurinol toxicity. In patients with impaired renal function, oxypurinol levels may become elevated and cause toxicity, and furosemide may potentiate this effect. Therefore, an examination of the exact mechanism of the decrease in the fractional clearance of uric acid and oxypurinol due to furosemide is important, as well as further study regarding the effect of furosemide on oxypurinol together with uric acid in patients receiving allopurinol and furosemide, such as gout patients with renal or heart failure.

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