Effect of Furosemide on the Plasma Concentration and Urinary Excretion of Purine Bases, Adenosine, and Uridine

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To examine whether furosemide affects the plasma concentration and urinary excretion of purine bases, adenosine, and uridine, we administered 20 mg furosemide intravenously to 6 healthy subjects. Furosemide decreased the plasma concentration of hypoxanthine by 39% and increased plasma renin activity (PRA) and the plasma concentration of protein by 3.4-fold and 9%, respectively, at 90 minutes after administration. Furthermore, it decreased the urinary excretion of hypoxanthine, xanthine, and uric acid by 47%, 49%, and 49%, respectively, and the fractional clearance of xanthine and uric acid by 44% and 47%, respectively, during the 1-hour period between 60 and 120 minutes after administration. However, furosemide did not affect the plasma concentration or urinary excretion of adenosine and uridine. In addition, in an in vitro incubation study of erythrocytes, furosemide (10 μg/mL) did not affect the concentration of hypoxanthine in the incubation medium or the activity of erythrocyte purine nucleoside phosphorylase and 5′-nucleotidase. These results imply that xanthine may share a renal transport pathway with uric acid. Further, it is suggested that the furosemide-induced decrease in hypoxanthine may be ascribable to a decrease in adenosine triphosphate (ATP) degradation related to the inhibition of chloride transport in the body.

MANY PREVIOUS STUDIES[1-4] have demonstrated that furosemide increases serum uric acid and suggest that furosemide-induced hyperuricemia is ascribable to a decrease in uric acid clearance due to a decrease in extracellular volume. However, it remains undetermined as to whether furosemide affects the plasma concentration and urinary excretion of oxypurines (hypoxanthine and xanthine), adenosine, or uridine. Oxypurines are purine bases, adenosine is one of the purine nucleosides, and uridine is one of the pyrimidine nucleosides. These substances may not only have a role in the regulation of intracellular nucleic acids but could also reflect purine and pyrimidine degradation, since they are used in the synthesis of nucleic acids and are produced in purine and pyrimidine degradation.

Previous studies[5,6] have demonstrated that intrarenal adenosine is elevated in situations of increased metabolic demand such as increased tubular sodium and chloride reabsorption and hypoxia, and it has also been suggested that an increase in sodium-chloride transport through the thick ascending limb of Henle's loop and macula densa cells causes an increase in adenosine triphosphate (ATP) degradation in the kidneys. Sodium-chloride transport is present not only in the kidneys but also in other organs.[7-9] Therefore, furosemide may inhibit sodium-chloride transport in these organs and decrease the energy demand of this active transport, resulting in a decrease of purine degradation (ATP degradation), including reduced production of oxypurines and adenosine. In addition, it has been suggested that furosemide may affect the plasma concentration of uridine and adenosine, since it inhibits the transport of nucleosides in erythrocytes.

Accordingly, in the present study, we investigated the effect of furosemide on the plasma concentration and urinary excretion of purine bases and adenosine together with a pyrimidine nucleoside, uridine.

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SUBJECTS AND METHODS

Six men aged 32 to 50 years (body weight, 49 to 73 kg) participated in the study after informed consent was obtained. They each had normal laboratory data. After an overnight fast except for water, their urine was voided completely and the first urine sample was collected 1 hour later. After collection of the first urine sample, furosemide (20 mg) was intravenously injected. The second urine sample was collected 1 hour after administration of furosemide, and the third urine sample was collected 1 to 2 hours after furosemide. 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 furosemide.

Blood and Urine Analyses

Hypoxanthine, xanthine, and uridine levels in plasma and urine were determined using high-performance liquid chromatography (HPLC) as described previously.[10] In brief, the column was a Wakosil 5C-18-200 (4.6 × 250 mm; Wako Pure Chemicals, Osaka, Japan). The flow rate was 1 mL/min and the mobile phase 0.02 mmol/L KH_2PO_4 (pH 2.2). Plasma adenosine was determined by the same method already described, except that the mobile phase was set to 0.04 mmol/L KH_2PO_4 (pH 1.95) and the Wakosil 5C-18-200 column was 4.6 × 150 mm. To measure the plasma concentration of hypoxanthine, xanthine, and uridine, the plasma was separated after blood sampling with a heparinized syringe as described previously.[10] However, to prevent a decrease in plasma adenosine, we added 620 μL physiological saline containing 2′-deoxycoformycin (16 μg/mL final concentration), EDTA (16 mmol/L final concentration), and dipyridamole (0.48 μmol/L final concentration) to 1 mL blood immediately after sampling as described previously.[10] After centrifugation at 4°C, the plasma was immediately separated and used to measure the concentration of adenosine.

The urinary concentration of adenosine and uridine were determined as described previously. In brief, the chromatograph consisted of 2 CCPM pumps (Tosoh, Tokyo, Japan), an SC-8020 system controller (Tosoh), 2 spectrophotometric detectors (UV-8010 and UV-8020; Tosoh), and a VC-8020 column-switching valve (Tosoh). The chromatographic columns were a Wakosil 5-C-18-200 (4.6 × 250 mm; Wako Pure Chemicals) as the first column and a Tosoh TSK Gel (ODS-120A, 4.6 × 250 mm) as the second column. In both columns, the mobile

phase was 0.02 mmol/L KH_2PO_4 (pH 2.2), the flow rate 1 mL/min, and the detection wavelength 254 nm. Twenty to 100 μL of the urine sample was injected into the first column. At the fraction time in which adenosine was eluted via the first column, the 2 columns were connected and the elute from the second column was monitored at 254 nm. The urinary concentration of uridine was also measured by HPLC with column-switching.

The HPLC method was the same as described above, before except that the pH of the mobile phase was 4.7 instead of 2.2. The concentration of uric acid and creatinine in both plasma and urine was measured by the uricase method using a Uric Acid B Test Wako Kit (Wako Pure Chemicals) and an enzymatic method using a Diacolor Liquid CRE Kit (Toyobo, Osaka, Japan), respectively. Plasma renin activity (PRA) was measured by Shionogi Biomedical Laboratories (Osaka, Japan). Other parameters were measured in our hospital laboratory.

In Vitro Studies

Since the plasma concentration of furosemide was approximately 5 μg/mL at 5 minutes after intravenous administration of 40 mg furosemide, erythrocytes were incubated at 37°C for 1 hour with or without the addition of 10 μg/mL furosemide, as previously described.[11] In brief, fresh heparinized blood was centrifuged for 10 minutes at 1,700 × *g* and the plasma, buffy coat, and top one-fifth layer of erythrocytes were removed. Infranatant cells were washed twice with 3 vol ice-cold saline and resuspended in a solution containing (final concentration) 98 mmol/L NaCl, 10 mmol/L KCl, 50 mmol/L Tris hydrochloride, 30 mmol/L glucose, 2 mmol/L $MgCl_2$, and 1 mmol/L NaH_2PO_4 (pH 7.4) with or without the addition of 10 μg/mL furosemide. The hematocrit of these suspensions was 15%. The suspensions were then incubated for 1 hour. The concentration of hypoxanthine in the incubation medium was measured by HPLC at 5 and 60 minutes after incubation, and an increase in hypoxanthine in the incubation medium was determined. The activity of 5′-nucleotidase and purine nucleoside phosphorylase in erythrocytes was determined with or without the addition of 10 μg/mL furosemide as previously described.[12,13] These experiments were performed in duplicate.

Chemicals

Adenosine was obtained from Boehringer (Mannheim, Germany). Furosemide was purchased from Hoechst Japan (Tokyo, Japan). Other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Statistical Analysis

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

RESULTS

Plasma Concentration of Purine Bases, Adenosine, and Uridine

Furosemide decreased the plasma concentration of hypoxanthine by 33% at 30 minutes after administration as compared with the control value, and further decreased by 39% at 90 minutes after administration. However, it did not affect plasma uric acid, xanthine, adenosine, or uridine during the study. In the control study, these parameters did not change significantly (Table 1).

Urinary Excretion of Purine Bases, Adenosine, and Uridine

Furosemide decreased the urinary excretion of hypoxanthine, xanthine, and uric acid by 47%, 49%, and 49%, respectively, during the 1-hour period from 60 to 120 minutes after administration as compared with the respective control values before administration. However, it did not significantly affect the 1-hour urinary excretion of hypoxanthine, xanthine, or uric acid at 60 minutes after administration, or adenosine or uridine during the study. In the control study, these parameters did not change significantly (Table 2).

Table 1. Plasma Concentration of Purine Bases (μmol/L), Adenosine (nmol/L), and Uridine (μmol/L) in Furosemide and Control Studies (n = 6)

Parameter	Period 1	Period 2	Period 3
Furosemide study			
Hypoxanthine	1.44 ± 0.42	0.96 ± 0.32*	0.88 ± 0.30*
Xanthine	0.61 ± 0.08	0.62 ± 0.08	0.62 ± 0.08
Uric acid	333 ± 24	351 ± 41	357 ± 43
Adenosine	98 ± 39	88 ± 25	87 ± 27
Uridine	4.76 ± 0.40	4.78 ± 0.36	4.90 ± 0.52
Control study			
Hypoxanthine	1.60 ± 0.38	1.52 ± 0.34	1.56 ± 0.34
Xanthine	0.62 ± 0.13	0.60 ± 0.18	0.58 ± 0.16
Uric acid	327 ± 27	327 ± 26	327 ± 21
Adenosine	90 ± 31	92 ± 33	88 ± 30
Uridine	4.86 ± 0.44	4.82 ± 0.34	4.84 ± 0.40

NOTE. Results are the mean ± SD. Periods 1, 2, and 3 denote 30, 90, and 150 minutes, respectively, after the beginning of each study.
*$P < .05$.

Urinary Excretion of Electrolytes and Urine Volume

Furosemide increased the 1-hour urinary excretion of sodium, potassium, and chloride and urine volume by 10.1-, 9.8-, 4.6-, and 5.0-fold, respectively, at 60 minutes after administration as compared with the respective control values. Further, these increases were 3.6-, 3.7-, 3.0-, and 1.7-fold, respectively, from 60 to 120 minutes after administration. In the control study, these parameters did not change significantly (data not shown).

Table 2. Urinary Excretion (μmol/h) of Purine Bases, Adenosine, and Uridine in Furosemide and Control Studies (n = 6)

Parameter	Period 1	Period 2	Period 3
Furosemide study			
Hypoxanthine	6.14 ± 1.5	4.63 ± 1.47	3.26 ± 1.06*
Xanthine	2.89 ± 0.73	2.04 ± 0.69	1.48 ± 0.49*
Uric acid	174 ± 30	142 ± 30	88 ± 33*
Adenosine	0.17 ± 0.06	0.15 ± 0.05	0.13 ± 0.03
Uridine	0.13 ± 0.04	0.11 ± 0.04	0.12 ± 0.04
Control study			
Hypoxanthine	6.07 ± 1.34	6.01 ± 1.26	6.04 ± 1.28
Xanthine	2.88 ± 0.61	2.87 ± 0.61	2.85 ± 0.48
Uric acid	176 ± 36	158 ± 48	169 ± 34
Adenosine	0.16 ± 0.05	0.17 ± 0.05	0.17 ± 0.05
Uridine	0.14 ± 0.04	0.14 ± 0.04	0.14 ± 0.03

NOTE. Results are the mean ± SD. Periods 1, 2, and 3 denote the first period (1 hour after beginning the furosemide and control studies), the second period (1-2 hours after beginning the furosemide and control studies), and the third period (2-3 hours after beginning the furosemide and control studies), respectively.
*$P < .01$.

PRA and Plasma Concentration of Electrolytes and Protein

Furosemide increased PRA and plasma protein by 3.2-fold and 8%, respectively, at 30 minutes after administration, compared with the respective control values, and it also increased these parameters by 3.4-fold and 9%, respectively, at 90 minutes after administration. However, furosemide did not affect the plasma concentration of sodium, potassium, or chloride at 30 and 90 minutes after administration. In the control study, these parameters did not change significantly (data not shown).

Creatinine Clearance and Fractional Clearance of Purine Bases, Adenosine, and Uridine

Furosemide did not decrease creatinine clearance significantly, whereas it decreased the fractional clearance of xanthine and uric acid by 44% and 47%, respectively, from 60 to 120 minutes after administration. However, furosemide did not affect the fractional clearance of xanthine or uric acid at 60 minutes after administration, nor that of hypoxanthine, adenosine, or uridine at any time during the study (Table 3). In the control study, these parameters did not change significantly (data not shown).

In Vitro Purine Metabolism in Erythrocytes

The increase in the concentration of hypoxanthine in the incubation medium with the addition of 10 μg/mL furosemide (2.01 μmol/L) was the same as the control value (1.98 μmol/L). In addition, the percent ratio of the activity of erythrocyte 5′-nucleotidase and purine nucleoside phosphorylase with the addition of 10 μg/mL furosemide was 99% and 100%, respectively, compared with the respective control values.

DISCUSSION

In the present study, two important observations were made. First, we observed a decrease in the fractional clearance of uric acid and xanthine by furosemide without a significant change in their plasma concentration. Second, we found a decrease in the plasma concentration and urinary excretion of hypoxanthine by furosemide without a significant change in the fractional clearance (Tables 1 and 3). The present study, as well as a previous study by other investigators,[14] demonstrated that furosemide decreases the fractional clearance of uric acid, indicating that either the reabsorption of uric acid was accelerated or its secretion was inhibited by furosemide. Further, in the present study, furosemide markedly increased the excretion of urine, sodium, potassium, and chloride, leading to a decrease in extracellular volume as reflected by an increase in plasma protein. The decrease in extracellular volume is thought to cause a decrease in the clearance of uric acid.[1-4] However, the details of its mechanism remain undetermined. In previous studies,[15-18] pyrazinamide and DL-lactate decreased the urinary excretion of uric acid and xanthine and glucagon had the opposite effect, suggesting that uric acid shares a renal transport pathway with xanthine but not hypoxanthine. Since furosemide decreased the fractional clearance of xanthine and uric acid but not hypoxanthine (Table 3), the present study also suggests the presence of this common renal transport pathway.

Table 3. Creatinine Clearance and Fractional Clearance of Purine Bases, Adenosine, and Uridine in the Furosemide Study (n = 6)

Parameter	Period 1	Period 2	Period 3
Creatinine clearance (mL/min)	105 ± 8	99 ± 6	96 ± 8
Fractional clearance (mL/min/mL/min · 100)			
Hypoxanthine	69.8 ± 18.5	81.5 ± 19.7	64.6 ± 16.8
Xanthine	73.1 ± 14.7	53.1 ± 16.5	41.3 ± 10.3*
Uric acid	7.7 ± 1.3	6.6 ± 1.4	4.1 ± 0.9*
Adenosine	29.7 ± 9.0	30.6 ± 12.8	27.4 ± 8.9
Uridine	0.46 ± 0.19	0.39 ± 0.17	0.42 ± 0.19

NOTE. Results are the mean ± SD. Periods 1, 2, and 3 are the same as in Table 2. Each fractional clearance denotes the clearance of each parameter/creatinine clearance × 100.

*$P < .01$.

Hypoxanthine is an end product of purine degradation in many tissues that contain a negligible amount of xanthine dehydrogenase, in contrast to the liver and small intestine, which contain a large amount. When ATP is degraded in these tissues, the plasma concentration of hypoxanthine is elevated. Therefore, plasma hypoxanthine is a sensitive marker of purine degradation (ATP degradation) in these tissues. Since hypoxanthine is a product of purine degradation, a furosemide-induced decrease in the plasma concentration and urinary excretion of hypoxanthine with no significant change in its fractional clearance suggests that the decrease may be ascribable to decreased purine degradation in the body or inhibition of hypoxanthine production, such as the furosemide-induced inhibition of purine nucleoside phosphorylase or 5′-nucleotidase activities. However, furosemide did not affect hypoxanthine production in erythrocytes or the activity of erythrocyte purine nucleoside phosphorylase and 5′-nucleotidase, suggesting that furosemide does not inhibit any steps of hypoxanthine production. In the kidney, tubular sodium-chloride reabsorption constitutes the active process with the greatest energy demand. 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 other various organs,[7-9] the inhibition of sodium-chloride transport by furosemide may decrease the production of hypoxanthine, leading to its decreased plasma concentration and urinary excretion.

Although it has been suggested that furosemide decreases endogenous adenosine in the kidneys by inhibiting tubular chloride reabsorption and then decreasing ATP consumption, the plasma concentration and urinary excretion of adenosine was not decreased by furosemide in the present study. The concentration changes of adenosine in the kidney may only be measurable by renal tissue measurements of adenosine, although the urinary excretion of adenosine reflects the intrarenal concentration of adenosine.[6] Therefore, we cannot assume that the lack of a significant decrease in the urinary excretion of

adenosine suggests that furosemide does not decrease ATP consumption sufficiently to affect the urinary excretion of adenosine in the kidney or its plasma concentration in the body. On the other hand, a decrease in the plasma concentration and urinary excretion of hypoxanthine suggests that furosemide may decrease ATP consumption sufficiently to affect its plasma concentration and urinary excretion, since the main route of ATP degradation is ATP → adenosine diphosphate (ADP) → adenosine monophosphate (AMP) → inosine monophosphate → inosine → hypoxanthine, and not ATP → ADP → AMP → adenosine.

A previous study[7] demonstrated that furosemide inhibits not only chloride transport but also nucleoside (adenosine and uridine) transport in erythrocytes, and that adenosine and uridine transporters are dependent on chloride flux. Therefore, furosemide may affect the plasma concentration of adenosine and uridine. However, the present study demonstrates that furosemide did not affect the plasma concentration and urinary excretion of uridine or adenosine (Tables 1 and 2), indicating that either the inhibition of chloride flux by furosemide is not sufficient to change the plasma level of uridine and adenosine, or the chloride transport system that is linked to nucleoside transport may not be important as a regulatory factor for the plasma concentration of uridine.

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