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Plasma concentrations and urinary excretion of purine bases (uric acid, hypoxanthine, and xanthine) and oxypurinol after rigorous exercise

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Abstract

To investigate the effects of exercise on the plasma concentrations and urinary excretion of purine bases and oxypurinol, we performed 3 experiments with 6 healthy male subjects. The first was a combination of allopurinol intake (300 mg) and exercise ($\dot{V}O_2$max, 70%) (combination experiment), the second was exercise alone (exercise-alone experiment), and the third was allopurinol intake alone (allopurinol-alone experiment). In the combination experiment, exercise increased the concentrations of purine bases and noradrenaline in plasma, as well as lactic acid in blood and the urinary excretion of oxypurines, whereas it decreased the urinary excretion of uric acid and oxypurinol as well as the fractional excretion of hypoxanthine, xanthine, uric acid, and oxypurinol. In the exercise-alone experiment, exercise increased the concentrations of purine bases and noradrenaline in plasma, lactic acid in blood, and the urinary excretion of oxypurines, whereas it decreased the urinary excretion of uric acid and fractional excretion of purine bases. In contrast, in the allopurinol-alone experiment, the plasma concentration, urinary excretion, and fractional excretion of purine bases and oxypurinol remained unchanged. These results suggest that increases in adenine nucleotide degradation and lactic acid production, as well as a release of noradrenaline caused by exercise, contribute to increases in plasma concentration and urinary excretion of oxypurines and plasma concentration of urate, as well as decreases in urinary excretion of uric acid and oxypurinol, along with fractional excretion of uric acid, oxypurinol, and xanthine. In addition, they suggest that oxypurinol does not significantly inhibit the exercise-induced increase in plasma concentration of urate.

1. Introduction

A number of studies have demonstrated that rigorous exercise leads to an energy crisis and subsequent acceleration of adenine nucleotide degradation, which produces hypoxanthine as an end product in exercised muscles that possess rare xanthine dehydrogenase activity [1-3]. Xanthine dehydrogenase activity is present in the liver and intestine, where hypoxanthine is converted to xanthine and uric acid by xanthine dehydrogenase, resulting in an increase in plasma concentration of urate and xanthine together with that of hypoxanthine, which leaks into the bloodstream. An increase in the plasma concentration of oxypurines (hypoxanthine and xanthine) usually enhances the urinary excretion of oxypurines by the kidneys, whereas exercise raises the blood concentration of lactic acid because of anaerobic glycolysis, leading to an inhibition of renal uric acid excretion [4,5], which also increases the plasma concentration of urate. However, it remains undetermined whether exercise has an effect on the plasma and urinary excretion of oxypurines in the presence of high plasma concentrations of oxypurines caused by oxypurinol, a metabolite of allopurinol and a xanthine oxidase inhibitor.

Allopurinol inhibits the conversion of hypoxanthine to xanthine and then xanthine to uric acid, leading to a decreased plasma concentration of urate, and is metabolized to oxypurinol by both xanthine dehydrogenase and aldehyde oxidase. Oxypurinol is also a potent inhibitor of xanthine dehydrogenase and has a half-life of 18 hours, which is

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longer than that of allopurinol (approximately 4 hours) [6-9]. Because the overall effect of allopurinol mainly depends on the action of oxypurinol on plasma urate levels, it is clinically important to understand the renal transport of oxypurinol associated with exercise. Therefore, in the present study, we investigated the effects of exercise on the plasma concentration and urinary excretion of oxypurines in the presence of oxypurinol together with those of oxypurinol. In addition, we attempted to determine to what degree oxypurinol could inhibit the increase in plasma concentration of urate caused by rigorous exercise because the hypouricemia effect of oxypurinol toward exercise-induced hyperuricemia has not been examined in detail despite it being a clinically important action.

2. Subjects and methods

Six healthy males were enlisted as subjects, after informed consent was obtained, and subjected to 3 experiments in the study. Each had normal laboratory data including serum aspartate aminotransferase, alanine aminotransferase, creatinine, and plasma glucose. In the first experiment (combination), allopurinol (300 mg) was administered at 10:00 PM, and after an overnight fast except for water, urine was completely voided at 8:00 AM on the next day. Thereafter, urine was collected every hour for a total of 4 times (periods 1, 2, 3, and 4), and blood was drawn at the midpoint of each of those 1-hour urine collections. After the period 1 urine collection, exercise was performed at $\dot{V}O_2$max 70%, determined as described previously [3], using a bicycle ergometer for 30 minutes. Two weeks later, the second experiment (exercise alone) was performed with the same protocol, except for no administration of allopurinol. Two weeks after the second experiment, the third experiment (allopurinol alone) was done with the same protocol, except that it included the allopurinol administration and no exercise. Because allopurinol was below the limits of detection at 13 hours after administration (300 mg), we were able to examine the effects of exercise on purine bases and oxypurinol under conditions of high plasma oxypurine levels caused by oxypurinol.

2.1. Determination of uric acid, hypoxanthine, xanthine, and oxypurinol concentrations

The plasma and urinary concentrations of uric acid were determined using Wako Uric Acid kit (Wako Pure Chemical, Osaka, Japan), whereas those of hypoxanthine, xanthine, and oxypurinol were measured by high-performance liquid chromatography as described previously [10]. The concentrations of lactic acid in blood and noradrenaline in plasma were also determined as described previously [1,10].

2.2. Analysis of statistical data

Data are shown as the mean ± SD. Significance ($P < .05$) was determined using analysis of variance.

3. Results

3.1. Plasma concentrations of purine bases and oxypurinol

In the combination and exercise-alone experiments, exercise markedly increased the plasma concentrations of hypoxanthine by 13.8- and 21.4-fold, respectively, at period 2, as compared with those values at period 1, and then decreased gradually at periods 3 and 4 in both (Table 1). However, in the combination experiment, the plasma concentration of hypoxanthine was 2.9- ($P < .01$), 1.9- ($P < .05$), 2.6- ($P < .01$), and 4.7-fold ($P < .01$) higher at periods 1, 2, 3, and 4, respectively, as compared with the respective values in the exercise-alone experiment (Table 1), indicating that oxypurinol significantly inhibited xanthine dehydrogenase activity. In the allopurinol-alone experiment, the plasma concentration of hypoxanthine did not change

Table 1
Plasma concentrations of purine bases and oxypurinol

	Period 1	Period 2	Period 3	Period 4
Hypoxanthine				
Combination	1.12 ± 0.43****	15.48 ± 5.72**,***	10.78 ± 3.74**,***	7.75 ± 3.02**,***
Exercise alone	0.38 ± 0.18	8.14 ± 2.86**	4.22 ± 1.61**	1.66 ± 0.38**
Xanthine				
Combination	3.77 ± 1.78****	5.15 ± 2.07**,***	7.69 ± 2.23**,***	8.56 ± 2.93**,***
Exercise alone	0.35 ± 0.19	0.91 ± 0.45*	1.07 ± 0.21**	0.99 ± 0.48*
Urate				
Combination	280 ± 37	292 ± 40	299 ± 42	304 ± 40*
Exercise alone	305 ± 36	313 ± 33	349 ± 37	341 ± 29*
Oxypurinol				
Combination	30.7 ± 5.3	30.9 ± 6.4	29.4 ± 5.4	30.8 ± 5.6

Values are shown as mean ± SD (μmol/L).
* $P < .05$, as compared with each value in period 1.
** $P < .01$, as compared with each value in period 1.
*** $P < .05$, as compared with each value at the same period in the exercise-alone experiment.
**** $P < .01$, as compared with each value at the same period in the exercise-alone experiment.

at any time during the experiment (data not shown). However, at period 1, it was 2.8-fold ($P < .01$) higher in the allopurinol-alone experiment, as compared with that in the exercise-alone experiment, indicating the effect of oxypurinol on plasma hypoxanthine.

In the combination and exercise-alone experiments, exercise also increased the plasma concentrations of xanthine by 1.4- ($P < .01$) and 2.5-fold ($P < .05$), respectively, at period 2, as compared with those values at period 1 (Table 1). Furthermore, at periods 1, 2, 3, and 4 in the combination experiment, the plasma concentration of xanthine was higher by 10.8- ($P < .01$), 5.7- ($P < .01$), 7.2- ($P < .01$), and 8.6-fold ($P < .01$), respectively, as compared with the respective values in the exercise-alone experiment, indicating that oxypurinol considerably inhibited xanthine dehydrogenase activity. In the allopurinol-alone experiment, the plasma concentration of xanthine did not change at any time during the experiment (data not shown). However, at period 1, it was 10.0-fold higher than that in the exercise experiment, indicating the effect of oxypurinol on plasma xanthine.

In the combination and exercise-alone experiments, exercise increased the plasma concentrations of urate by 1.09- ($P < .05$) and 1.12-fold ($P < .05$), respectively, at period 4, as compared with those values at period 1 (Table 1). In contrast, in the allopurinol-alone experiment, the plasma concentration of xanthine did not change at any time during the experiment (data not shown).

Furthermore, in the combination (Table 1) and allopurinol-alone (data not shown) experiments, the plasma concentrations of oxypurinol were not changed.

3.2. Urinary excretion of purine bases and oxypurinol

In the combination and exercise-alone experiments, exercise increased the urinary excretion of hypoxanthine by 7.3- ($P < .05$) and 17.5-fold ($P < .01$) at period 2 and by 10.7- ($P < .01$) and 19.2-fold ($P < .01$) at period 3, respectively, as compared with those values at period 1 (Table 2). These results indicate that adenine nucleotide degradation was enhanced in muscles by exercise in those experiments, in contrast with the allopurinol-alone experiment, during which the urinary excretion of hypoxanthine did not change and was significantly low at periods 2 and 3, as compared with the respective values in the exercise-alone experiment (data not shown). At period 1, the urinary excretion of hypoxanthine was increased by 3.75- ($P < .05$) (Table 2) and 3.67-fold ($P < .05$) in the combination and allopurinol experiments, respectively, as compared with that obtained in the exercise experiment. In addition, the urinary excretion of hypoxanthine was increased by 2.1-fold in the combination experiment at period 3, as compared with that in the exercise-alone experiment (Table 2). These findings demonstrate the effect of oxypurinol on urinary hypoxanthine.

In the combination experiment, the urinary excretion of xanthine was decreased by 0.2-fold ($P < .05$) at period 2 and then increased by 2.4- ($P < .01$) and 2.5-fold ($P < .01$) at periods 3 and 4, respectively, whereas in the exercise-alone study at period 3 the urinary excretion of xanthine was increased by 5.2-fold as compared with that at period 1 (Table 2). On the other hand, in the allopurinol-alone experiment, the urinary excretion of xanthine did not change at any time during the experiment (data not shown). In the combination and allopurinol-alone experiments, the urinary excretion of xanthine was increased by 19.8- ($P < .01$) and 17.6-fold ($P < .01$), respectively, at period 1; by 12.1- ($P < .01$) and 13.0-fold ($P < .01$), respectively, at period 2; by 9.3- ($P < .01$) and 3.4-fold ($P < .01$), respectively, at period 3; and by 13.0- ($P < .01$) and 4.3-fold ($P < .01$), respectively,

Table 2
Urinary excretion of purine bases and oxypurinol

	Period 1	Period 2	Period 3	Period 4
Hypoxanthine				
Combination	8.9 ± 4.4***	58.7 ± 49.9*	86.7 ± 47.4**·***	55.4 ± 35.0*
Exercise alone	2.16 ± 0.5	37.9 ± 18.6**	41.5 ± 16.7**	17.2 ± 15.3
Xanthine				
Combination	18.6 ± 9.8****	14.7 ± 6.7*·****	45.3 ± 21.8**·****	47.1 ± 18.7**·****
Exercise alone	0.9 ± 0.6	1.2 ± 0.5	4.9 ± 1.8**	3.6 ± 1.8*
Uric acid				
Combination	139 ± 36	54 ± 23**	155 ± 45	153 ± 35
Exercise alone	181 ± 40	87 ± 12*	206 ± 45	229 ± 39*
Oxypurinol				
Combination	38.0 ± 8.6	19.7 ± 3.6**	42.2 ± 8.9	41.5 ± 7.2
Allopurinol alone	38.6 ± 6.1	40.2 ± 9.9	39.2 ± 6.1	35.7 ± 6.9

Values are shown as mean ± SD (μmol/h).

* $P < .05$, as compared with each value in period 1.

** $P < .01$, as compared with each value in period 1.

*** $P < .05$, as compared with each value at the same period in the exercise-alone experiment.

**** $P < .01$, as compared with each value at the same period in the exercise-alone experiment.

at period 4, as compared with each of those values in the exercise-alone experiment, indicating the effect of oxypurinol on urinary xanthine.

In the combination and exercise-alone experiments, exercise decreased the urinary excretion of uric acid by 0.6- ($P < .05$) and 0.5-fold ($P < .05$) at period 2, as compared with those values at period 1. However, in the exercise-alone experiment, exercise increased the urinary excretion of uric acid by 1.3-fold ($P < .05$) at period 4, as compared with that at period 1, suggesting an increase in plasma concentration of urate at period 4, whereas the urinary excretion of uric acid was not increased at that time point in the combination experiment (Table 2). In the allopurinol experiment, the urinary excretion of uric acid did not change at any time during the experiment (data not shown). In the combination experiment, exercise decreased the urinary excretion of oxypurinol by 0.48-fold ($P < .01$) at period 2, whereas during the allopurinol-alone experiment, the urinary excretion of oxypurinol did not change (data not shown).

3.3. Fractional excretion of purine bases and oxypurinol (clearance of purine bases and oxypurinol/creatinine clearance × 100)

In the combination and exercise-alone experiments, exercise decreased the fractional excretion of hypoxanthine by 0.4- ($P < .01$) and 0.1-fold ($P < .01$), respectively, at period 2, as compared with those values at period 1. These results suggest that the near peak of plasma concentration of hypoxanthine was at the midpoint of period 2 in those experiments, whereas during the allopurinol-alone experiment, the fractional excretion of hypoxanthine did not change (data not shown).

In the combination and exercise-alone experiments, exercise decreased the fractional excretion of xanthine by 0.3- ($P < .01$) and 0.4-fold ($P < .01$), respectively, at period 2, as compared with those values at period 1. Furthermore, it also decreased the fractional excretion of uric acid by 0.6- ($P < .01$) and 0.4-fold ($P < .01$), respectively. In contrast, during the allopurinol-alone experiment the fractional excretion of xanthine and uric acid values were not changed (data not shown).

In the combination experiment, exercise decreased the fractional excretion of oxypurinol by 0.4-fold ($P < .01$) at period 2 (Table 3), whereas in the allopurinol experiment, the value did not change during the experiment (data not shown).

Table 3
Fractional excretion of purine bases and oxypurinol (clearance of purine bases and oxypurinol/creatinine clearance × 100)

	Period 1	Period 2	Period 3	Period 4
Hypoxanthine				
Combination	100 ± 31	56 ± 37**	104 ± 38	99 ± 41
Exercise alone	88 ± 22	78 ± 38**	108 ± 58	126 ± 56
Xanthine				
Combination	72.3 ± 22.2	48.1 ± 12.9**	77.4 ± 23.6	79.5 ± 23.7
Exercise alone	39.8 ± 10.8	22.8 ± 8.9**	60.2 ± 27.3	59.9 ± 36.8
Uric acid				
Combination	11.6 ± 2.5	4.9 ± 1.5**	11.9 ± 2.4	11.9 ± 2.4
Exercise alone	14.0 ± 3.5	7.8 ± 1.4**	12.7 ± 3.4	15.7 ± 2.4
Oxypurinol				
Combination	17.5 ± 3.0	10.9 ± 3.1**	19.2 ± 2.1	19.1 ± 1.6

Values are shown as mean ± SD.
** $P < .01$, as compared with each value in period 1.

3.4. Clearance of creatinine

In the combination and exercise-alone experiments, exercise decreased creatinine clearance from 111 ± 11 and 111 ± 7 mL/min, respectively, at period 1, to 96 ± 12 ($P < .05$) and 94 ± 10 ($P < .05$) mL/min, respectively, at period 2. There was no decrease in creatinine clearance at periods 3 and 4 (data not shown).

Furthermore, during the allopurinol-alone experiment, creatinine clearance did not change (data not shown).

3.5. Blood concentration of lactic acid

In the combination and exercise-alone experiments, exercise increased the concentrations of lactic acid in blood from 0.73 ± 0.16 and 0.78 ± 0.13 mmol/L, respectively, at period 1, to 5.9 ± 1.7 ($P < .01$) and 6.4 ± 2.0 ($P < .01$) mmol/L, respectively, at period 2, whereas the concentration of lactic acid in blood at periods 3 and 4 was not changed, as compared with those values in period 1 (data not shown).

In the allopurinol-alone experiment, the concentration of lactic acid in blood did not change throughout the experiment (data not shown).

3.6. Plasma concentration of noradrenaline

In the combination and exercise-alone experiments, exercise increased the plasma concentrations of noradrenaline from 155 ± 35 and 187 ± 48 pg/mL, respectively, at period 1, to 934 ± 375 ($P < .01$) and 1184 ± 607 ($P < .05$) pg/mL, respectively, at period 2, and then they decreased to 215 ± 64 and 235 ± 112, respectively, at period 4. The plasma concentration of noradrenaline was not determined at period 3. In those experiments, the plasma concentrations of noradrenaline at period 4 were not changed, as compared with the respective values at period 1.

4. Discussion

One of the intriguing findings in the present study is that exercise decreased the urinary excretion of oxypurinol.

Rigorous muscular exercise consumes adenosine triphosphate at a greater rate than can be produced and resupplied to exercising muscles, leading to adenine nucleotide degradation, followed by increases in the plasma concentrations of hypoxanthine, xanthine, and urate. Furthermore, such physical activity also increases the concentrations of lactic acid in blood and noradrenaline in plasma and also decreases

creatinine clearance, leading to a decrease in the urinary excretion of uric acid, xanthine, and oxypurinol [4,5,10]. Therefore, exercise may cause decreases in the urinary excretion of uric acid, xanthine, and oxypurinol.

Previous studies have suggested that hypoxanthine is excreted via the renal transport pathway, which is different from that of uric acid, although the two may also share that transport pathway [10-13]. Recently, Enomoto et al [14] reported a urate transporter (URAT1), by which the secretion of lactic acid was found to be coupled with the reabsorption of uric acid, but not with that of xanthine in vitro. Those findings strongly suggest that the enhanced secretion of lactic acid caused by an increase in blood concentration of lactic acid accelerates the reabsorption of uric acid via URAT1, whereas it does not accelerate that of xanthine. In addition, our previous study showed that lactate infusion to obtain a blood concentration of approximately 3.0 mmol/L decreased the urinary excretion of uric acid, but did not decrease that of xanthine, hypoxanthine, or oxypurinol [4]. Therefore, the urinary excretion of xanthine and oxypurinol may not depend on URAT1.

In the present study, exercise decreased the fractional excretion of uric acid, xanthine, and oxypurinol. These results strongly suggest that an increase in the plasma concentration of noradrenaline by exercise contributes to a decreased urinary excretion of xanthine and oxypurinol as well as that of uric acid because a previous study demonstrated that noradrenaline infusion decreased the fractional excretion of uric acid, xanthine, and oxypurinol [10].

Another intriguing finding is that oxypurinol did not adequately inhibit the exercise-induced increase in plasma concentration of urate.

Clinically, it is important to understand the effects of oxypurinol because a rapid increase in the plasma level of uric acid due to rigorous muscular exercise can lead to gouty attack in patients with gout. We concluded that oxypurinol does not inhibit the production of uric acid due to adenosine triphosphate degradation enough to cancel out the decrease in urinary excretion of uric acid caused by exercise; thus, rigorous exercise will sometimes induce gouty attack in patients with gout despite their intake of allopurinol. However, it remains undetermined whether administration of more than 300 mg of allopurinol can inhibit the increase in plasma concentration of urate due to rigorous exercise or whether the timing of allopurinol intake has a significant effect. Therefore, additional examinations are needed.

References

[1] Ka T, Yamamoto T, Moriwaki Y, Kaya M, Tsujita J, Takahashi S, et al. Effect of exercise and beer on the plasma concentration and urinary excretion of purine bases. J Rheumatol 2003;30:1036-42.

[2] Yamanaka H, Kawagoe Y, Taniguchi A, Kaneko N, Kimata S, Hosoda S, et al. Accelerated purine nucleotide degradation by anaerobic but not by aerobic ergometer muscle exercise. Metabolism 1992;41:364-9.

[3] Yamamoto T, Moriwaki Y, Takahashi S, Ishizashi H, Higashino K. Effect of muscular exercise by bicycle ergometer on erythrocyte purine nucleotides. Horm Metab Res 1994;26:504-8.

[4] Yamamoto T, Moriwaki Y, Takahashi S, Nasako Y, Higashino K. Effect of lactate infusion on renal transport of purine bases and oxypurinol. Nephron 1993;65:73-6.

[5] Yu TF, Spirota TH, Halpern BM, Gutman AB. Effect of sodium lactate infusion on urate clearance in man. Proc Soc Exp Biol Med 1957;96:809-13.

[6] Elion GB, Kovensky A, Hitchings GH. Metabolic studies of allopurinol, an inhibitor of xanthine oxidase. Biochem Pharmacol 1966;15:863-80.

[7] Elion GB, Yu TF, Gutman AB, Hitchings GH. Renal clearance of oxypurinol, the chief metabolite of allopurinol. Am J Med 1968;45:69-77.

[8] Hande K, Reed E, Chabner B. Allopurinol kinetics. Clin Pharmacol Ther 1978;23:598-605.

[9] Appelbaum SJ, Mayersohn M, Dorr RT, Perrier D. Allopurinol kinetics and bioavailability. Intravenous, oral and rectal administration. Cancer Chemother Pharmacol 1982;8:93-8.

[10] Yamamoto T, Moriwaki Y, Takahashi S, Tsutsumi Z, Hada T. Effect of norepinephrine on the urinary excretion of purine bases and oxypurinol. Metabolism 2001;50:1230-3.

[11] Auscher C, Pasquier C, Pehuet P, Delbarre F. Study of urinary pyrazinamide metabolites and their action on the renal excretion of xanthine and hypoxanthine in a xanthinuric patient. Biomedicine 1978;28:129-33.

[12] Yamamoto T, Moriwaki Y, Takahashi S, Tsutsumi Z, Hiroishi K, Yamakita J, et al. Effect of glucagon on renal excretion of oxypurinol and purine bases. J Rheumatol 1997;24:708-13.

[13] Yamamoto T, Moriwaki Y, Takahashi S, Tsutsumi Z, Hada T. Effect of losartan potassium, an angiotensin II receptor antagonist, on renal excretion of oxypurinol and purine bases. J Rheumatol 2000;27:2232-6.

[14] Enomoto A, Kimura H, Chairoungdua A, Shigeta Y, Jutabha P, Cha SH, et al. Molecular identification of a renal urate anion exchanger that regulates blood urate levels. Nature 2002;417:447-52.