原著 1

Clinical studies on changes in purine compounds in blood and urine by the simultaneous administration of febuxostat and inosine, or by single administration of each

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Abstract

Objective: To examine whether concomitant oral administration of febuxostat, a xanthine dehydrogenase/xanthine oxidase inhibitor, and inosine augments ATP in human blood.

Methods: We performed Stage 1, Stage 2 and Stage 3 studies. In Stage 1 study, one adult healthy male was administered febuxostat 40 mg/day and inosine 1 g/day for 14 days to examine the safety of the combined therapy. In Stage 2, 21 healthy male adults were allocated to groups A-G, each with 3 subjects, and were treated with febuxostat alone, inosine alone or both for 14 days. In Stage 3, febuxostat 40 mg/day and inosine 1 g/day were administered to 5 healthy adult males for 14 days. Purine compounds in blood were compared between before and after the treatments in Stage 2 and Stage 3.

Results: Combined use of febuxostat and inosine was relatively safe at doses of febuxostat 60 mg/day and inosine 3 g/day or less in a 2 week continuous treatment. Serum uric acid levels were markedly decreased by administration of febuxostat alone, and increased by the administration of inosine alone, but milder decreases were seen in combination

therapy compared with febuxostat alone. In Stage 2, concomitant treatment with febuxostat 40 mg/day and inosine 1-2 g/day increased blood hypoxanthine and ATP, no increase occurred with inosine alone, and febuxostat alone slightly increased those values at the higher dose (60 mg/day). Increase in ATP in blood was confirmed by the results of Stage 3 where concomitant use of febuxostat 40 mg/day and inosine 1 g/day increased ATP by 13.8% ($P = 0.031$).

Conclusions: Concomitant oral administration of febuxostat 40 mg/day and inosine 1-2 g/day was shown to safely increase blood ATP. Since cellular ATP deficiency may be involved in various disorders, the present method may be useful for the treatment of those disorders.

Background

ATP (adenosine triphosphate) (hereinafter sometimes referred to simply as ATP) is the most important compound for storing energy in living organisms and supplying it when needed, and it is considered that ATP reduction is related to the pathology of various diseases. For example, as a cause of hereditary hemolytic anemia, reduction of ATP in erythrocytes is

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considered to be a mechanism of hemolysis. Examples of hereditary hemolytic anemia include sickle cell disease¹⁾, pyruvate kinase deficiency²⁾, spherocytosis³⁾, elliptocytosis³⁾, stomatocytosis⁴⁾ and thalassemia⁵⁾.

In addition, intracellular ATP reduction is suggested as a mechanism of myocardial damage due to ischemic heart disease⁶⁾. It has been reported that symptoms were suppressed by the high dose administration of allopurinol, which is a xanthine oxidase/xanthine dehydrogenase inhibitor, to chronic stable angina pectoris patients⁷⁾. The authors and others suggested that allopurinol had a positive effect on ischemic heart disease by increasing ATP⁷⁾. By strengthening ATP in this manner, it can be expected that for diseases in which ATP reduction is associated, the disease state would be improved.

In addition, there are reports that inosine enhances muscle motility during exercise, with the expectation that the action of muscle movement would be enhanced by increased ATP through administration of inosine, but there is also a recent report that denies that effect⁸⁾. However, the reason why inosine does not elicit an effect on enhancing muscle motility may be due to incomplete ATP enhancing action with inosine alone.

Nishino et al. have found that administering xanthine oxidase/xanthine dehydrogenase inhibitors such as febuxostat to amyotrophic lateral sclerosis (hereinafter sometimes referred to as ALS) model mice suppresses disease progression⁹⁾. Allopurinol did not inhibit disease progression. Nishino et al. speculated that increasing ATP of neurons by administration of febuxostat suppresses disease progression⁹⁾. The reason why allopurinol is ineffective is that it consumes PRPP and instead inhibits ATP synthesis⁹⁾. In fact, it has been reported that knock-down of Na/K-ATPase in ALS model mice suppresses degeneration of nerve cells¹⁰⁾. It has also been reported that Na/K-ATPase activity is up-regulated in ALS patients¹⁰⁾. That is, activation of Na/K-ATPase, which decreases ATP, promotes the onset or progression of ALS, and inhibition of Na/

K-ATPase suppresses ATP reduction and thereby suppresses the progress of ALS.

Furthermore, there are reports that symptoms of Parkinson's disease¹¹⁾ and multiple sclerosis¹²⁾ are alleviated by administration of inosine. It is believed that serum uric acid levels are related to the diseases. Clinical trials are underway to increase serum uric acid level by administering inosine and to exert therapeutic effects. However, the reported effect does not currently appear to be sufficient.

It has been reported that intracellular ATP increases somewhat by inosine administration alone. In fact, Ogasawara et al. reported that ATP increased in erythrocytes, whose ATP had been depleted after being left at low temperature for 20–30 days, by adding inosine and leaving for 1 hour¹³⁾. However, in the human body, inosine is rapidly metabolized to uric acid via hypoxanthine and xanthine. Therefore, inosine alone was insufficient to exert sufficient ATP enhancing action in vivo. In addition, even if febuxostat is administered alone, some enhancement of intracellular ATP would be expected, but there is a possibility that this alone would be insufficient.

Methods

Administration test

This study was approved by the institutional review board (IRB of Tsukuba International Clinical Pharmacology Clinic). This study was an open study. Informed consent was obtained from each of the subjects.

The subjects were healthy Japanese males with ages of 20 to 40 years old without histories or present illnesses of renal disorder, hyperuricemia, gout or urolithiasis. The test was conducted in three stages. In Stage 1, a single subject was treated with febuxostat 20 mg and inosine 500 mg twice (after breakfast and dinner) daily for 14 days. After confirming the safety in Stage 1, 21 Japanese healthy adult males were allocated in Stage 2 to Groups A–G, each with 3 subjects, and the

following prescriptions were administered for 14 days:

- Group A: febuxostat 20 mg, twice daily for 14 days
- Group B: inosine 500 mg, twice daily for 14 days
- Group C: febuxostat 20 mg + inosine 500 mg, twice daily for 14 days
- Group D: febuxostat 20 mg + inosine 1000 mg, twice daily for 14 days
- Group E: febuxostat 30 mg, twice daily for 14 days
- Group F: febuxostat 30 mg + inosine 1000 mg, twice daily for 14 days
- Group G: febuxostat 30 mg + inosine 1500 mg, twice daily for 14 days

In Stage 3, 5 subjects were treated with febuxostat 20 mg and inosine 500 mg twice (after breakfast and dinner) daily for 14 days.

Measurement method of purines

Measurement of various purines in peripheral blood was performed according to the literature¹⁴⁾. Briefly, peripheral blood was collected in a test tube containing EDTA-2Na and 500 μ L blood was mixed with 500 μ L ice cold 8% PCA, and the mixture was immediately vortexed. Thereafter, the mixture was centrifuged at $12,000 \times g$ for 5 seconds at 4 °C, and the supernatant was stored at -80 °C. 40 μ L of 2 M K_2CO_3 in 6 M KOH was added to 650 μ L of the lysate to simultaneously precipitate PCA and neutralize the solution. This was centrifuged at $12,000 \times g$ for 10 minutes at 4 °C, and then 160 μ L of mobile phase was added to 40 μ L of the supernatant and subjected to HPLC. The conditions of HPLC are the same as described previously¹⁴⁾. The amount of purine was expressed by the molar amount contained in the solution after removing PCA.

Measurement of purines in urine was the same except that 500 μ L urine but not blood was mixed with 500 μ L ice cold 8% PCA. The subsequent procedures are the same as the method for blood samples.

Other clinical laboratory tests including uric acid measurement

Blood test and urine test were performed by standard

methods. Uric acid was determined by uricase peroxidase method.

Results

Administration study

Stage 1 study

In Stage 1, a healthy volunteer was administered febuxostat 40 mg and 1 g inosine per day for 14 days. Since no side effects occurred in Stage 1, Stage 2 study was started.

Stage 2 study

In Stage 2, 21 healthy subjects allocated to Groups A-G were administered either febuxostat, inosine, or febuxostat + inosine for 14 days as described in the Methods.

As background data, there was no significant difference between groups in age, height, weight (mean = 62.7, SD = 9.5 kg), BMI, systolic blood pressure, diastolic blood pressure, pulse rate or body temperature. For subject ID 9 (Group C), the pulse rates at days 0, 8, and 15 were 70, 92 and 107 beats/min, and a maximum increase of 37 beats/min was observed. There was nothing special to mention except for that observation.

Adverse events in physical examination are as follows.

Skin rash in 2 persons (Group D, Group E); mild, probably related to the treatment.

Tachycardia in 1 person (Group C); maybe related to the treatment.

Erythema in face and hands (Group D); probably not related to the treatment.

By the intervention, there was no particularly noticeable change in clinical laboratory tests other than serum uric acid value. Hyperuricemia occurred in 2 persons (Group B) that were judged to be related to the treatment.

Changes in uric acid

Graphs of the changes of the uric acid levels of individuals are shown for each Group A-G in Figures

1 and 2. There were marked increases in serum uric acid levels (maximum 8.1 mg/dL) in Group B administered with inosine alone. Serum uric acid levels decreased in Group A and Groups C–G. There was no case where serum uric acid level decreased to less than 2 mg/dL in the case of febuxostat 40 mg/day, but serum uric acid levels decreased to lower than 2 mg/dL in some persons in Groups E–G to which febuxostat 60 mg/day was administered.

The mean serum uric acid values for each week in each group and the changes from the first week are shown in Table 1. Serum uric acid level decreased by 2.53 mg/dL by administration of febuxostat 40 mg/dL alone (Group A), while it decreased by 2.23 mg/dL when 1g per day inosine was simultaneously

administered (Group C) and by 1.47 mg/dL when 2 g per day inosine was administered (Group D) (Table 1).

Serum uric acid level decreased by 3.93 mg/dL by administration of febuxostat 60 mg/dL (Group E), while it decreased by 3.03 mg/dL when 2g per day inosine was simultaneously administered (Group F) and it decreased by 2.37 mg/dL when 3 g per day inosine was administered (Group G).

Serum uric acid levels increased by an average of 2.57 mg/dL by 1 g of inosine per day (Group B). Comparing this with the uric acid increasing effect of inosine under administration of febuxostat, serum uric acid level increased by 0.3 mg/dL when 1 g inosine was administered and increased by 1.06 mg/dL when 2 g/day inosine was administered along with 40 mg/

Figure 1: Changes in serum uric acid levels by drug administration. Group A-D

Figure 2: Changes in serum uric acid levels by drug administration. Group E-G

day of febuxostat. In addition, under administration of febuxostat 60 mg/day, serum uric acid level increased by 0.9 mg/dL when 2 g/day inosine was administered and increased by 1.56 mg/dL when 3 g/day inosine was administered. As described above, serum uric acid level increased by 2.57 mg/dL by administration of 1 g/day of inosine without administration of febuxostat, so the serum uric acid raising effect by inosine was greatly suppressed under febuxostat administration.

Urine uric acid/creatinine

Since urinary uric acid concentration varies with urine volume, urinary uric acid content was evaluated using urinary uric acid/creatinine value. Urinary uric

acid/creatinine was markedly increased only in Group B by administration of inosine (Data not shown). Urinary uric acid/creatinine decreased for all samples in Group A and Groups C–G (Data not shown). These changes in urinary uric acid/creatinine are almost the same as the patterns of change in serum uric acid values.

Blood concentrations of purines

Changes of the concentrations of purines in the blood from days 1 to 15 (the values are the concentrations in the solutions obtained after the removal of PCA) are shown in Figure 3–6 for each group. Figures 3 and 4 show the concentrations of ATP and ADP in blood for

Table 1 Average serum uric acid values for different weeks in each group and mean serum uric acid level decreases from week 0 to week 1 and 2.

Group	week 0*	week 1*	week 2*	1-0**	2-0***
A	5.47	2.87	2.93	-2.60	-2.53
B	4.53	7.27	7.10	2.73	2.57
C	4.73	2.53	2.50	-2.20	-2.23
D	5.10	3.30	3.63	-1.80	-1.47
E	6.30	2.33	2.37	-3.97	-3.93
F	5.30	2.53	2.27	-2.77	-3.03
G	4.23	1.63	1.87	-2.60	-2.37

*unit is mg/dL

** The value obtained by subtracting the value for week 0 from that for week 1.

*** The value obtained by subtracting the value for week 0 from that for week 2.

Figure 3: Comparison of blood ATP and ADP between day 1 and day 15. Group A-D In all the figures below, the amount of purine was shown as the concentration in the solution after removal of PCA.

Figure 4: Comparison of blood ATP and ADP between day 1 and day 15. Group E-G

Figure 5: Comparison of blood Hx (hypoxanthine) and X (xanthine) between day 1 and day 15. Group A-D

Figure 6: Comparison of blood Hx (hypoxanthine) and X (xanthine) between day 1 and day 15. Group E-G

each group A–G, which suggest that ATP concentration does not change in Groups A and B, and ATP increases in Groups C and D. There is no consistent trend in Groups E–G. That is, no increase in ATP was observed with febuxostat or inosine alone, but increases in ATP were observed in combination examples, especially in the cases of febuxostat 40 mg/day and inosine 1–2 g/day. A definite tendency was not observed for the combination of febuxostat and inosine exceeding these amounts.

Figures 5 and 6 show concentrations of hypoxanthine (Hx) and xanthine (X) in the blood for each group A–G. Hx concentrations were unchanged in the group treated with only febuxostat 40 mg/day (Group A), but X increased markedly. Neither Hx nor X changed in the administration group of inosine 1 g/day only (Group B). The inosine concentration in the blood was also measured, but no increase in the concentration of inosine was observed in any group (Group A–G) including the group administered with inosine alone (Group B). Since the concentration of purine nucleoside phosphorylase (PNP), an enzyme that converts inosine to Hx, is extremely high in the blood, we think that inosine was quickly degraded into Hx and further degraded into X and uric acid. Significant increases in both Hx and X were observed in the cases where 1–2 g/day of inosine was used in combination with febuxostat 40 mg/day. That is, the effect of "elevation of Hx in blood" which was not observed with febuxostat alone or inosine alone was seen for the combination treatment (Figure 5).

In the administration of febuxostat 60 mg/day alone, a slight rise in Hx was observed along with increasing X (Figure 6E). In the example in which febuxostat 60 mg/day and inosine 2–3 g/day were used in combination, further marked increases in Hx and X were observed (Figures 6F–G). That is, with inosine alone, Hx or X did not change at all, while febuxostat alone increased X and slightly increased Hx at the higher dose.

Concentrations of urinary purines

Table 2 shows the concentrations of inosine, Hx, X, and uric acid in urine on the first day and the fifteenth day. Urinary Hx showed a slight increase when febuxostat alone was given, but a marked increase in those treated with both febuxostat and inosine (Table 2). When inosine alone was administered, no increase in Hx or X was observed. The concentration of X increased most markedly for the combined administration of febuxostat and inosine, but also showed a marked increase for administration with febuxostat alone.

The mean concentrations of X in urine on the fifteenth day were compared between Group A (febuxostat 40 mg) and Group C (febuxostat 40 mg + inosine 1 g), and between Group E (febuxostat 60 mg) and Group F (febuxostat 60 mg + inosine 2 g). As a result, the average X concentration of group C was 2.6 times that of group A, and the average X concentration of group F was 2.4 times that of group E. Although febuxostat has been extensively used in patients for years, reports of xanthine stones and other complications are rare. Therefore, although the urinary concentration of X due to the combined use of febuxostat and inosine was a few times higher than for febuxostat alone, we do not expect the risk of xanthine stones to be greatly increased. However, attention will be necessary in the future.

Stage 3 study

Results from Stage 2 study suggested that blood ATP increased when 40 mg febuxostat and 1.0 – 2.0 g inosine were administered per day while increases in ATP were not clear at higher doses. To confirm an ATP augmentation effect by a concomitant administration of febuxostat and inosine, we administered 20 mg febuxostat and 0.5 g inosine twice a day for 14 days to 5 healthy adult males.

Fig 7 shows the changes of ATP and hypoxanthine in the blood. In all 5 subjects, both ATP and hypoxanthine increased from before the administration to after the

Table 2 Urinary concentrations of purines (μM)

Subject ID	2	3	4	5	6	7	8	9	10
Group	A			B			C		
Day 1									
Ino (μM)	1.3	15.3	38.9	6.4	58.4	43.1	6.6	2.5	0.8
HX (μM)	13.6	53.5	279.4	24.0	243.1	158.8	66.2	15.3	5.9
X (μM)	13.6	37.4	302.3	12.1	258.8	78.3	27.4	7.9	6.6
UA (μM)	218.9	1373.6	4529.8	597.6	3994.8	4194.0	1764.6	459.6	358.4
Day 15									
Ino (μM)	1.9	1.9	2.8	5.1	27.6	3.0	22.3	28.4	20.0
HX (μM)	166.9	183.6	439.0	23.8	101.3	154.8	1465.5	2826.8	4791.0
X (μM)	680.8	1149.6	1180.4	24.8	131.4	71.5	1046.9	4295.5	2527.2
UA (μM)	446.9	1543.2	1547.0	2168.0	3279.2	1085.5	622.9	2630.6	2219.8

Subject ID	11	12	13	14	15	16	17	18	19
Group	D			E			F		
Day 1									
Ino (μM)	52.9	5.1	8.9	1.7	62.0	15.9	3.4	2.8	1.3
HX (μM)	133.1	106.2	15.1	6.8	100.6	248.8	107.4	7.0	84.7
X (μM)	70.1	55.0	8.9	14.2	106.8	171.1	52.9	8.3	62.6
UA (μM)	3432.9	3240.3	1099.7	599.3	5602.6	4667.4	3990.8	435.4	3697.4
Day 15									
Ino (μM)	61.8	6.6	36.9	1.3	1.7	12.7	83.4	11.5	9.3
HX (μM)	4171.9	279.8	2016.6	23.8	163.9	666.6	5141.1	1374.0	1002.5
X (μM)	3131.0	48.2	2258.0	173.4	579.8	1842.1	4690.3	689.3	806.5
UA (μM)	1378.3	3419.5	1398.4	48.4	846.9	614.2	2352.1	275.4	1598.6

Subject ID	20	21	22
Group	G		
Day 1			
Ino (μM)	16.3	24.0	44.6
HX (μM)	41.4	48.2	117.2
X (μM)	22.3	53.3	44.8
UA (μM)	2205.2	2911.1	2304.5
Day 15			
Ino (μM)	24.6	4.5	122.1
HX (μM)	1774.6	526.7	8145.3
X (μM)	1018.6	558.6	4717.9
UA (μM)	201.5	186.6	2044.4

Discussion

With regard to purine metabolism, effects and adverse events of drugs in humans are hard to predict from the data of non-human animals. Humans are genetically deficient in the urate degrading enzyme urate oxidase while most other animals are not¹⁵⁾, the activity of xanthine dehydrogenase/xanthine oxidase in mice is about 100 fold larger in mice than humans¹⁶⁾ the activity of hypoxanthine phosphoribosyltransferase

Discussion

With regard to purine metabolism, effects and adverse events of drugs in humans are hard to predict from the data of non-human animals. Humans are genetically deficient in the urate degrading enzyme urate oxidase while most other animals are not¹⁵⁾, the activity of xanthine dehydrogenase/xanthine oxidase in mice is about 100 fold larger in mice than humans¹⁶⁾, the activity of hypoxanthine phosphoribosyltransferase is about 10 and 30 times higher in humans as compared with mice and rats, respectively¹⁷⁾, and the rate of urate production is about 25 fold larger in mice as compared with humans¹⁸⁾. Xanthine dehydrogenase/xanthine oxidase inhibitors are known to cause severe crystalluria and subsequent bladder tumors in mice and rats while in humans such adverse events do not occur¹⁹⁾.

administration. Average ATP increased from 315.4 to 358.8 μM (13.8% increase) and it was significant (paired t test, $P = 0.031$). Average hypoxanthine increased from 1.80 to 10.92 μM (6.07 fold increase) and it was significant (paired t test, $P = 0.011$).

Figure 7: Comparison of blood ATP and hypoxanthine between day 1 and day 15 in 5 subjects in Stage 3 study.

We therefore examined the effects of concomitant administration of febuxostat and inosine in clinical trials. Combined use of febuxostat and inosine was relatively safe at doses of febuxostat 60 mg/day and inosine 3 g/day or less in a 2 week continuous test. Serum uric acid level was markedly decreased by the administration of febuxostat alone, and increased by the administration of inosine alone, but a milder decrease was seen in combination therapy compared with febuxostat alone. Our Stage 2 study suggested that concomitant treatment with febuxostat 40 mg/day and inosine 1 – 2 g/day increases blood hypoxanthine and ATP *in vivo* in healthy subjects. This was confirmed by the subsequent Stage 3 study in which 5 subjects were treated with febuxostat 40 mg/day and inosine 1 g/day.

No increase in hypoxanthine, xanthine or ATP was observed in the blood when inosine alone was administered. When febuxostat alone was administered, xanthine increased and hypoxanthine and ATP increased slightly at the higher dose.

In conclusion, it was confirmed that a combination of febuxostat and inosine was safe and resulted in a

marked increase in hypoxanthine not observed with febuxostat alone or inosine alone. In addition, the combination of febuxostat and inosine significantly increased ATP in blood while inosine alone did not increase ATP, and febuxostat alone increased ATP slightly at a higher dose. Since cellular ATP deficiency may be involved in various disorders, the present method may be useful for the treatment of those disorders.

Limitations are as follows.

Since the numbers of the subjects in our studies were not so large, further studies with larger sample sizes are necessary to confirm the results of the present study. The subjects in the present study were all healthy male volunteers. To extend the conclusions of the present study to the general population or to patients, further studies are necessary. Furthermore, we showed the augmentation of ATP only in blood. Since ATP is stably present only in cells, blood ATP probably reflects the amount in erythrocytes. Further studies are necessary to examine whether the same effects occur in other cells like neuronal cells and muscle cells *in vivo* in which ATP deficiency is likely to have more

serious consequences. In addition, long term effects of this therapy on blood ATP should be examined in further studies. It should be also explained in later studies why higher febuxostat and inosine than 40 mg and 1.0 – 2.0 g, respectively, per day did not constantly increase ATP although increases in hypoxanthine were evident.

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Conflicts of interest

Naoyuki Kamatani is paid from StaGen Co. Ltd and hold stocks of the same company that is the sponsor of this study.

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