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Yogurt containing *Lactobacillus gasseri* PA-3 alleviates increases in serum uric acid concentration induced by purine ingestion: a randomized, double-blind, placebo-controlled study

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Abstract

Objective: Ingestion of yogurt containing *Lactobacillus gasseri* PA-3 (PA-3, Accession No: NITE BP-224) (PA-3Y) has been shown to reduce serum uric acid (SUA) levels by interfering with the intestinal absorption of food-derived purines in animal studies. To confirm this mechanism in humans, the ability of PA-3 intake to alleviate purine ingestion-induced increases in SUA levels was analyzed.

Research Methods and Procedures: In this randomized, double-blind, placebo-controlled crossover study, 16 healthy adults were randomized to groups ingesting of 112 g of PA-3Y or yogurt without PA-3 (control yogurt) in addition to standardized meals for 3 days. Purine-loading tests, in which subjects ingested 112 g of PA-3Y or control yogurt followed immediately by 498 mg of a mixture of purine nucleotides, were performed on the fourth day of each test period. Blood and urine samples were collected before and after the purine-loading tests.

Results: The increase in the SUA concentration

from the baseline was significantly lower following the ingestion of PA-3Y than of control yogurt alone, especially at 30 ($P=0.033$) and 60 ($P=0.028$) minutes. In addition, the area under the curve for the increase in the SUA concentration from the baseline to 150 minutes was also significantly lower ($P=0.041$) in the PA-3Y than in the control yogurt group. However, urinary and fractional excretions of uric acid were not different between the two groups.

Conclusion: The ingestion of PA-3 before purine intake alleviates the increase in SUA levels, probably by reducing purine absorption in the intestine, and not by enhancing urinary excretion of uric acid.

Introduction

Hyperuricemia is defined as a serum uric acid (SUA) concentration over 7.0 mg dL^{-1} . Persistent hyperuricemia can lead to the deposition of urate crystals in the joints and kidneys, resulting in acute arthritis or kidney injury such as nephrolithiasis and nephropathy¹⁻³⁾. Hyperuricemia has also been associated

受付：2017年11月10日，受理：2018年3月2日

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Key words: serum uric acid, nucleotide, nucleoside, purine base, urine, absorption of purine, lactic acid bacteria

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with other diseases, including hypertension, diabetes mellitus, chronic kidney disease, and arteriosclerosis⁴⁻⁶⁾. Because the routine ingestion of purine-rich foods can lead to elevated SUA concentrations and increase the risk of gout⁷⁻⁹⁾, Japanese guidelines for the management of gout and hyperuricemia recommend a dietary purine intake ≤ 400 mg/day¹⁰⁾. As most foods contain purines, restricting purine ingestion is achievable only by restricting the total food intake. In addition, purines frequently add flavor to foods, and flavorful foods usually contain high quantities of purines¹¹⁾, thus, making the restriction of purine-rich foods even more difficult¹²⁾. Since it is not easy to maintain a purine-restricted diet for long periods of time, foods that alleviate absorption of dietary purines are therefore desirable.

The consumption of yogurt, produced by the fermentation of milk by lactic acid bacteria, was found to result in lower SUA levels⁸⁾ and a lower risk of gout⁷⁾, although the precise mechanism remains unclear. These bacteria have been reported to absorb and metabolize exogenous nucleosides and purine bases¹³⁾, with several strains of *Lactobacillus*, a genus of lactic acid bacteria, are able to convert purine nucleosides to purine bases¹⁴⁾. Furthermore, purine bases were not as easily absorbed as their corresponding nucleosides in the intestines of rats^{15,16)}. Analysis of hundreds of strains of lactobacilli showed that *Lactobacillus gasseri* PA-3 (PA-3) had the strongest ability to degrade nucleosides¹⁷⁾. PA-3 has also been shown to take up exogenous AMP (adenosine 5'-monophosphate), adenosine, and adenine *in vitro*¹⁷⁾, and the oral administration of PA-3 to rats, along with AMP, adenosine, hypoxanthine, inosine, or IMP reduced the absorption of these purines by the rat intestines¹⁸⁾. Moreover, studies in humans found that SUA levels were lower in subjects who ingested yogurt containing PA-3 (PA-3Y) than in those who ingested yogurt without PA-3 (control yogurt)¹⁹⁾. We therefore hypothesized that the ingestion of PA-3 along with

food reduces the absorption of dietary purines by intestinal cells. The present study investigated whether PA-3 alleviates the increased SUA levels induced by purine ingestion in humans.

Subjects and Methods

Yogurt containing PA-3 (PA-3Y)

The yogurt used in this study, made from milk fermented with strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*, was provided by Meiji Co., Ltd. (Tokyo, Japan). Subjects were administered 112 g of yogurt containing $0.5-3 \times 10^8$ cfu g⁻¹ *Lactobacillus gasseri* PA-3 (Accession No: NITE BP-224) or yogurt without PA-3 (control yogurt). The ingredients, composition, and quality standards of yogurt containing PA-3 (PA-3Y) were identical to those of commercially available Meiji probio yogurt PA-3. PA-3Y and control yogurt were indistinguishable from each other in appearance and taste.

Participants

Volunteers were recruited by HUMA R&D Co., Ltd. (Tokyo, Japan) from February to March 2016. Sixteen healthy male subjects, aged ≥ 20 years and with fasting serum uric acid levels between 4.0 and 7.0 mg dL⁻¹ were enrolled. Subjects were excluded if they had: (1) a history of hyperuricemia or gout, (2) a severe or progressive disease, (3) food allergies, or (4) lactose intolerance. Subjects were also excluded if they (5) routinely took dietary supplements for hyperuricemia, or (6) had any condition that would preclude participation, as determined by a health examination or for other reasons. All subjects provided written informed consent.

Study design and protocol

The present randomized, double-blind, placebo-controlled crossover trial was conducted from March to May 2016 with washout periods of >2 weeks. Sixteen

subjects were randomized, based on a computer-generated allocation, into two groups with comparable SUA levels and age. Allocation, performed by an individual unrelated to this study, involved placing randomization numbers into sealed envelopes, with allocation not disclosed until analyses were complete. After a 14-day or longer washout period, subjects were crossed over to the other yogurt (Figure. 1).

Participants were instructed to avoid alcohol consumption and snacking during the 24 hours prior to each test period and to fast, except for the consumption of water, starting at 9 pm the evening before. To control total food ingestion, all participants took standardized meals (Nichirei Foods Co., Ltd., Tokyo, Japan) for 3 days before the purine-loading test. Morning meals contained, on average, 605 kcals, 111 g carbohydrate, 8.9 g fat, and 21 g protein; noon meals contained, on average, 616 kcals, 114 g carbohydrate, 11 g fat, and 17 g protein; and evening meals contained, on average, 632 kcals, 112 g carbohydrate, 12 g fat, and 20 g protein. Participants were also instructed to take a cup of allocated PA-3Y or control yogurt for 3 days before morning meals.

This study was registered with the University Hospital Medical Information Network (UMIN-CTR) as UMIN000022264 and was performed according to the guidelines outlined in the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Hyogo College of Medicine (approval No. 948).

Purine-loading test

Purine-loading tests were performed at the Hyogo College of Medicine from 7:30 am until 1:00 pm. Subjects took a cup of PA-3Y or control yogurt and immediately ingested 498 mg of a mixture of purine nucleotides dissolved in 10 mL of water, followed by drinking 50 mL of water. The mixture of purine nucleotides included equal weights of adenosine 5'-monophosphate, disodium 5'-inosinate, and disodium 5'-guanylate.

Using an intravenous cannula (Covidien Japan, Tokyo, Japan), blood was collected from each subject 30 minutes before and 30, 60, 90, and 150 minutes after purine loading (Figure.2). Serum was obtained by centrifuging blood samples at 2,000 x g for 5 minutes at room temperature. Urine samples were collected by complete voiding 60 minutes before purine loading, and every hour for the first 3 hours after purine loading. To ensure urine production, subjects ingested 200 mL of water every hour. The volume of collected urine was measured. Concentrations of uric acid and creatinine in blood and urine were analyzed by SRL, Inc. (Tokyo, Japan), using the Uricase-POD and enzymatic methods, respectively.

Adverse events

All subjects were interviewed face-to-face by medical doctors about their physical condition and sleep quality before purine-loading tests.

Figure 1: Study design.

PA-3Y, yogurt containing *Lactobacillus gasseri* PA-3.

Figure 2: Flow diagram showing times of blood and urine collection during individual purine-loading tests.

PA-3Y, yogurt containing *Lactobacillus gasseri* PA-3.

Outcomes

The objective of the present study was to evaluate the effects of PA-3. Primary outcomes were SUA levels and changes in SUA from the baseline during the purine-loading tests. Changes in SUA of each subject were calculated as: $(\text{SUA}_{30} - \text{SUA}_{\text{pre}})$, $(\text{SUA}_{60} - \text{SUA}_{\text{pre}})$, $(\text{SUA}_{90} - \text{SUA}_{\text{pre}})$, or $(\text{SUA}_{150} - \text{SUA}_{\text{pre}})$, where SUA_{pre} denotes the SUA concentration at 30 minutes before purine loading, and SUA_{30} , SUA_{60} , SUA_{90} , and SUA_{150} correspond to SUA concentrations at 30, 60, 90, and 150 minutes, respectively, after purine loading. Secondary outcomes included urate excretion into the urine and changes in urate excretion.

Statistical analyses

Results are reported as the mean $\pm$ standard error (SE). All analyses were performed using Microsoft Excel software. Blood sample parameters were compared using two-sided Wilcoxon's signed-rank tests, and urine sample parameters were compared using two-sided Mann-Whitney U tests because some subjects lacked corresponding data. P values < 0.05 were considered significant.

Results

Participant flow and baseline characteristics

A flow chart of the 16 recruited subjects is shown in Figure 3. One subject dropped-out prior to the second test for personal reasons. Thus, 15 participants completed both purine-loading tests. Baseline characteristics were similar in the two groups (Table 1). Mean serum uric acid concentrations were 5.8 mg dL⁻¹ in both groups, ranging from 4.5–6.9 mg dL⁻¹ in subjects initially randomized to PA-3Y and from 4.3–6.9 mg dL⁻¹ in subjects initially randomized to control yogurt. Of the 15 participants, one was excluded because of a history of hyperuricemia. The per-protocol set (PPS) therefore consisted of 14 subjects.

SUA levels and changes in SUA from baseline during purine loading

SUA concentrations of individual subjects during purine-loading tests are shown in Fig. 4. SUA concentrations were not significantly different between PA-3Y and control yogurt groups (Figure 5). However, SUA concentrations tended to be lower PA-3Y than control yogurt group at all time points, especially 30 minutes after purine loading ($P = 0.075$). Moreover, the changes in SUA concentrations from the

Table 1 Baseline characteristics of participants

	All subjects (n=22)	Control yogurt/PA-3Y (n=11)	PA-3Y/Control yogurt (n=11)
Age, years	34.5 ± 1.9	32.9 ± 2.6	36.1 ± 2.7
Height (cm)	171 ± 1.3	170 ± 0.9	173 ± 2.5
Body weight (kg)	68.7 ± 2.5	67.9 ± 2.5	69.5 ± 4.6
BMI [kg (m ²) ⁻¹]	23.4 ± 0.7	23.6 ± 0.8	23.1 ± 1.1
SUA level (mg dL ⁻¹)	5.8 ± 0.2	5.8 ± 0.2	5.8 ± 0.2

Data are presented as mean ± SE.

Control yogurt/PA-3Y, subjects ingested control yogurt, followed by washout, and then ingested PA-3Y. PA-3Y/control yogurt, subjects ingested PA-3Y, followed by washout, and then ingested control yogurt. Abbreviations: BMI, body mass index; SUA, serum uric acid.

Figure 3: Participant flow diagram.

PA-3Y, yogurt containing *Lactobacillus gasseri* PA-3.

Figure 4: SUA concentrations of individual subjects in the PA-3Y group (A) and in the control yogurt group (B) during purine-loading tests.

SUA, serum uric acid; PA-3Y, yogurt containing *Lactobacillus gasseri* PA-3.

Figure 5: SUA concentrations during purine-loading tests.

Results are expressed as the mean $\pm$ SE. SUA, serum uric acid; PA-3Y, yogurt containing *Lactobacillus gasseri* PA-3.

Figure 6: SUA changes in SUA concentrations from the baseline during purine-loading tests.

Results are expressed as the mean $\pm$ SE. SUA, serum uric acid; PA-3Y, yogurt containing *Lactobacillus gasseri* PA-3.

*P < 0.05 compared with control yogurt.

Figure 7: Areas under the curve (AUC) for changes from the baseline in SUA concentrations during purine-loading tests.

Results are expressed as the mean $\pm$ SE. SUA, serum uric acid; PA-3Y, yogurt containing *Lactobacillus gasseri* PA-3.

*P < 0.05 compared with control yogurt.

Table 2 Urinary and fractional excretion of uric acid and clearance of creatinine

	Period 1	Period 2	Period 3	Period 4
Urinary excretion (mg kg ⁻¹ h ⁻¹)				
Control yogurt	0.36 ± 0.06	0.33 ± 0.05	0.31 ± 0.03	0.25 ± 0.02
PA-3Y	0.35 ± 0.04	0.31 ± 0.04	0.32 ± 0.04	0.28 ± 0.03
Changes in urinary excretion from baseline (mg kg ⁻¹ h ⁻¹)				
Control yogurt	–	–0.02 ± 0.06	–0.05 ± 0.04	–0.11 ± 0.06
PA-3Y	–	0.02 ± 0.06	–0.03 ± 0.06	–0.06 ± 0.06
Fractional excretion (%)				
Control yogurt	4.7 ± 0.5	4.8 ± 0.5	5.9 ± 0.6	5.4 ± 0.5
PA-3Y	4.6 ± 0.6	4.3 ± 0.5	5.2 ± 0.4	4.7 ± 0.4
Ccr (mL min ⁻¹)				
Control yogurt	168 ± 20	130 ± 15	100 ± 14	93 ± 11
PA-3Y	171 ± 20	140 ± 13	113 ± 13	109 ± 13

Data are presented as mean ± SE.

PA-3Y, yogurt containing *Lactobacillus gasseri* PA-3; Ccr, creatinine clearance.

baseline were significantly lower in the PA-3Y than control yogurt group, especially at 30 (P=0.033) and 60 (P=0.028) minutes after purine loading (Figure. 6). The area under the curve (AUC) for changes in the SUA concentration from the baseline to 150 minutes after purine loading was significantly lower (P=0.041) in the PA-3Y than control yogurt group (Figure. 7).

Urinary urate excretion and change in urate excretion

We also evaluated whether PA-3Y affects the urinary excretion of uric acid. During both study periods, there were no significant between-group differences in urinary excretion of uric acid, changes in the urinary excretion of uric acid from the baseline, or clearances of uric acid and creatinine (Table 4).

Adverse events

None of the subjects experienced an adverse event (AE) or serious AE (SAE) during the study.

Discussion

PA-3 has been shown to degrade nucleosides and incorporate nucleotides, nucleosides, and purine bases

*in vitro*²⁰). Moreover, PA-3 was shown to reduce the *in vivo* absorption of orally-ingested purines by rat intestines^{17, 18}). The present study showed that PA-3Y reduced the increase in the SUA concentration observed during purine-loading tests without increasing urinary uric acid excretion. Taken together, these findings showed that, in humans, PA-3Y alleviated the rise in the SUA concentration, probably by scavenging food-derived purines in the intestines, resulting in decreased absorption, and not by augmentation of the urinary excretion of uric acid, being similar to previous *in vitro* and *in vivo* findings^{17, 18, 20}). It has been reported that *Lactobacillus gasseri* exhibits resistance to gastric acid and bile salts²¹). Therefore, it is likely that PA-3 remains active on reaching the intestine.

Although PA-3Y did not reduce SUA levels to the baseline following purine-loading tests, the AUC for changes in the SUA concentration was significantly lower in the PA-3Y than in the control yogurt group. Ingestion of PA-3Y for 8 weeks reduced SUA concentrations¹⁹). Together, these results strongly indicate that PA-3Y reduces the total absorption over time of dietary purines by intestinal cells, not merely

at one time point. Therefore, the administration of PA-3Y, in addition to restricting the intake of dietary purines, may additively contribute to alleviating the increase in SUA. The effects of PA-3Y on the SUA concentration by PA-3Y intake in this trial were similar to those previously reported¹⁹⁾, indicating that the level of reduction of SUA elevation was significant to achieve SUA homeostasis.

Ingestion of milk protein has been reported to reduce the SUA concentration by promoting the urinary excretion of uric acid^{22, 23)}. In this study, it remains unclear whether PA-3Y promoted the excretion of uric acids because we did not include a control group that ingested water rather than the control yogurt without PA-3. The amount of milk protein that lowered SUA levels was 80 g²³⁾, while the amount of milk protein contained in PA-3Y was about 7–8-fold lower than in previous studies^{22, 23)}. Therefore, it is unlikely that milk protein in the test yogurts enhanced the excretion of uric acid. However, the ingestion of large amounts of PA-3Y or that over long periods of time, may additively contribute to reducing the increase in SUA by promoting the urinary excretion of uric acid.

In addition to PA-3, several food items, such as *Prunus mume* fruits and chrysanthemum flower oil, have been shown to exhibit anti-hyperuricemic effects^{24, 25)}, probably by inhibiting xanthine oxidase activity^{26, 27)}. Chrysanthemum flower oil alleviates the rise in SUA during purine-loading tests, probably by inhibiting the metabolism of nucleic acids, with stronger hypouricemic effects on subjects with SUA levels > 7.1 mg dL⁻¹ than those with SUA levels > 6.5 mg dL⁻¹²⁸⁾. In contrast, the mechanism by which PA-3 reduces the SUA concentration is different, as it alleviates purine absorption in the intestines. Moreover, the present study found that PA-3 inhibited the increased SUA levels induced by the purine-loading tests, even in subjects with normal SUA levels (<7.0 mg dL⁻¹). Because SUA levels are less easily regulated in hyperuricemic persons with a decreased

ability to excrete uric acid, PA-3 may be useful to reduce elevated SUA in hyperuricemic subjects with a decreased capacity of urinary excretion. Therefore, combinations of PA-3 with other food ingredients that inhibit xanthine oxidase activity might exert greater hypouricemic effects.

Reducing dietary purines is extremely important for improving hyperuricemia, although it is difficult. In that respect, PA-3 is the first strain of lactic acid bacteria confirmed to lower SUA levels by reducing the absorption of purines in both animals and humans^{17–19)}. Therefore, PA-3 may be a useful supplementary food for subjects who ingest large amounts of dietary purines.

An estimate showed that, for a crossover trial, a sample size of 16 subjects would show a difference of 0.2 mg dL⁻¹ in the change in SUA levels after the ingestion of purines and PA-3Y or control yogurt, assuming a maximum standard deviation of 0.2 mg dL⁻¹, a type I error of 0.05, and a power of 80%²⁹⁾. Although we calculated a minimal sample size of 16 subjects, the number finally analyzed was lower than 16. The effects of PA-3 may be clearer in studies involving a larger number of subjects.

Conclusion

The ingestion of PA-3Y before purine intake alleviates the increase in SUA levels by reducing purine absorption in the intestine, and not by enhancing the urinary excretion of uric acid.

Conflict of interest

This study was funded by Meiji Co., Ltd. (Tokyo, Japan) and was approved by the committee on Conflicts of Interest at Hyogo College of Medicine. This study was supported by Grants-in-Aid for Scientific Research from the Gout Research Foundation to MK. HT, HM, NY, CS, HK, and YA are employed by Meiji Co., Ltd., which sells dairy products including those using *L. gasseri* PA-3.

MK and YM designed the study, performed experiments, analyzed the data, performed the statistical analyses, and wrote the manuscript. HK and TY contributed to the conception and design of the study. HT, HM, NY, CS, HK, and YA performed the experiments.

Acknowledgements

The authors are grateful for the technical assistance of Drs. Manabu Kadoya, Kae Hamamoto, Miki Kakutani-Hatakeyama, Akio Miyoshi, and Akiko Morimoto.

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