Lifeceramics-treated water reduces serum uric acid levels and improves hemorheological activity in hyperuricemic rats

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Abstract. Lifeceramics (LC) is made of zeolite and oyster shell and is hypothesized to act as an anti-oxidative agent. In the present study, the effects of LC-treated water (LC water) on the concentration of serum uric acid (SUA) and the hemorheological parameters in male rats with hyperuricemia (HUA) was assessed. To prepare LC water, distilled water was mixed with LC particles. HUA was induced in rats by daily potassium oxonate (PO) injection (250 mg/kg). The PO-injected rats were separated into three different groups and were administered distilled water (PO rats), allopurinol [a xanthine oxidase (XOD) inhibitor] solution [PO + allopurinol (AP) rats] or LC water (PO+LC rats) by gavage. Control rats were intraperitoneally injected with sodium carboxymethyl cellulose solution and administered untreated distilled water by gavage. After injection and gavage for 5 weeks, the SUA concentration, hemorheology index and antioxidant index were measured. The SUA concentration and blood deformation index of the PO rats were significantly higher and lower, respectively, compared with the control rats. However, in the PO+LC rats, the SUA concentration and blood deformation index decreased and increased, respectively, to a level similar to that of the control as well as that in the PO+AP rats. Furthermore, the PO-induced increase in XOD activity was suppressed by combined treatment with LC water, resulting in a decrease in malondialdehyde concentration. These results suggest that LC water can reduce the SUA concentration, increase serum antioxidant activity and improve hemorheological activity in hyperuricemic rats.

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Abbreviations: LC, lifeceramics; HUA, hyperuricemia; PO, potassium oxonate; AP, allopurinol; XOD, xanthine oxidase; MDA, malondialdehyde; SUA, serum uric acid; GSH, glutathione

Key words: lifeceramics, hyperuricemia, serum uric acid, hemorheology, oxidative stress

Introduction

Hyperuricemia (HUA) is a precursor of gout and is characterized by a chronic increase in serum uric acid (SUA) levels (1-3). HUA is implicated in various systemic disorders and is often included amongst the diagnostic criteria for metabolic syndrome, which is a complex disorder of the cardiometabolic system with potentially lethal systemic and hemodynamic consequences (1-3). Lifestyle changes, such as a reduction in alcohol consumption and increase in water intake, as well as drug therapy, including administration of xanthine oxidase (XOD) inhibitors, such as allopurinol (AP), can be applied to reduce SUA levels (1,2). However, lifestyle changes alone are often inadequate to reduce the SUA levels to physiological levels. Furthermore, drug therapy may cause various adverse reactions, including severe hypersensitivity to AP, agranulocytosis and aggravated renal toxicity due to impaired pyrimidine metabolism (2,4,5). Therefore, there is an urgent need to develop safe and effective anti-HUA agents.

Daily oral treatment with natural vanadium-containing Mt. Fuji ground water has been reported to regulate blood glucose levels in hyperglycemic humans and rats without severe diabetes mellitus (6). Therefore, a new kind of water-based formulation to reduce SUA levels may potentially benefit patients with HUA. In the present study, the effect of lifeceramics (LC)-treated water (LC water) on SUA concentration and hemorheology in hyperuricemic rats was assessed. LC is formulated from zeolite and oyster shells under high temperature and pressure conditions, and has been reported to possess antibacterial activity (7,8). In our previous studies, it was shown that sake (alcohol) administration can induce liver damage and alcoholic hepatitis in rats; however, the combined treatment with sake and LC did not result in damage (9,10). To the best of our knowledge, it has not been determined whether

LC water can protect against various disorders, such as the metabolic syndrome.

In our previous studies, it was shown that asymptomatic HUA enhances oxidative stress, deteriorated hemorheological parameters of red blood cells and increased blood viscosity and coagulation status (3,11). Zhou *et al* (12) reported on oxidative stress and microinflammatory responses in asymptomatic young patients with primary HUA and suggested that the inflammatory response is associated with oxidative stress induced by high uric acid (UA) levels. Furthermore, the protective effects of LC water against oxidative stress in cultured human cells was reported in our previous study (13). The aim of the present study was to examine the effects of LC water on SUA levels and hemorheological parameters in hyperuricemic rats and provide a potentially novel lifestyle intervention for the treatment of patients with HUA.

Materials and methods

Reagents. Potassium oxonate (PO) was purchased from Shanghai Jinsui Bio-Technology Co., Ltd. The LC particle powder was obtained from Wedge Co., Ltd. AP was purchased from Hefei Jiulian Pharmaceutical Co., Ltd. The measurement kits for SUA (cat. no. 20171024SUA) and malondialdehyde (MDA) (cat. no. 20171024MDA) concentration, and for XOD (cat. no. 20161105XOD) and glutathione (GSH) (cat. no. 20171024GSH) activities were obtained from Nanjing Jiancheng Clinical Reagent Co., Ltd. All other reagents were purchased from Beijing BD Bio-Tech Co., Ltd. (Beijing, China).

Animals and treatment. To prepare LC water, distilled water was mixed with LC particles and the solution was allowed to stand for 18 h at room temperature (22±1˚C) to precipitate the large particles of LC (8). All animals were treated according to the protocols for animal care approved by the Animal Ethics Committee of Chengde Medical University (Chengde, China). These protocols have been described previously (3,11). Briefly, 60 male Sprague Dawley rats (6-week-old: 180-220 g weight), purchased from Beijing Vital River Lab Animal Technology Co. Ltd., were housed in plastic cages of 40x60x80 cm in size (5 rats per cage) and maintained under standard laboratory conditions on a 12-h light/dark cycle, at a constant ambient temperature of 22±1˚C and relative humidity of 55%. After one week of feeding all the rats with distilled water, rats were randomly divided into 6 independent groups; Control, PO alone, PO+AP, PO+LC (low), PO+LC (medium) and PO+LC (high); with 10 rats per a group (Table I).

PO was dissolved in PBS and injected intraperitoneally every day (250 mg/kg/day) for 5 weeks in all rats, except for the control group. AP was dissolved in distilled water. The AP solution and the LC water were administered as drinking water. Control rats were injected intraperitoneally with sodium carboxymethyl cellulose dissolved in PBS and administered distilled water via gavage. The daily consumption of drinking water was recorded and replaced with freshly prepared water. The mean value of the consumption volume of drinking water per rat, per day was 20 ml regardless of the type of water provided (distilled or LC water) (data not shown).

The treatment regimen of all groups including the 3 groups that received different doses of LC [low (L), medium (M), high (H)] and with the exception of the group given LC water only, is shown in Table I and in Fig. S1. Blood was collected from the abdominal aorta at the end of the 5-week treatment. Serum was extracted from the blood samples and cryopreserved until use.

Biochemical assays. SUA concentration was measured using the phosphotungstic acid method; the XOD activity and GSH levels were determined using colorimetry; and serum MDA concentration was measured using the thiobarbituric acid method, as previously described (3,11). All biochemical assays were performed in our laboratory at a constant ambient temperature of 22±1˚C and relative humidity of 55%, according to the manufacturer's protocol.

Measurement of hemorheological parameters. Blood samples were anticoagulated using 2% heparin, and the whole blood viscosity of the samples at high (150 s^{-1}), medium (60 s^{-1}) and low (20 s^{-1}) shear rates were measured using a viscometer, as described previously (3). The viscosity of the plasma, separated from the anticoagulated blood by centrifuging at 1,000 x g for 10 min at room temperature (22±1˚C), was also measured. The erythrocyte deformability index and the aggregation index were measured using an automatic rheometer, as described previously (3).

Statistical analysis. Data are expressed as the mean ± standard deviation. Comparisons between multiple groups were performed using a one-way ANOVA with a post-hoc Tukey's test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

SUA concentration. The concentration of SUA in PO-treated rats (PO rats) was ~2-fold higher compared with the control rats. However, these levels were reduced when the PO rats were administered AP (PO+AP) (Fig. 1). Furthermore, the concentration of SUA in the rats administered with a combination of PO and varying doses of LC water (PO+LC) was slightly higher compared with the control rats and lower compared with the PO rats (Fig. 1). LC (M) and LC (H) exhibited similar effects compared with that of AP in reducing the SUA concentration (Fig. 1).

Hemorheological parameters. Plasma and whole blood viscosity of the PO rats were significantly higher compared with the control rats, and the viscosity did not differ significantly between rats treated with PO, and PO with AP or LC combination (Table II). The erythrocyte deformability index of the PO rats decreased to ~75% of that observed in the control rats. However, the erythrocyte deformability index of the PO+LC rats, as well as that of the PO+AP rats, did not decrease as much and was significantly higher compared with the PO rats (Fig. 2). There was no significant difference observed in the aggregation index among all the treatment groups (Table II).

Antioxidant capacity. The serum XOD activity in the PO rats was higher compared with the control rats. The

Table I. Treatment groups used in the present study. n=10 per group.

				PO+LC		
Reagents[a]	Control	PO	PO+AP	Low	Medium	High
PO	-	250	250	250	250	250
AP	-	-	25	-	-	-
LC	-	-	-	25	50	100

[a]mg/kg/day. PO, potassium oxonate; AP, allopurinol; LC, lifeceramics.

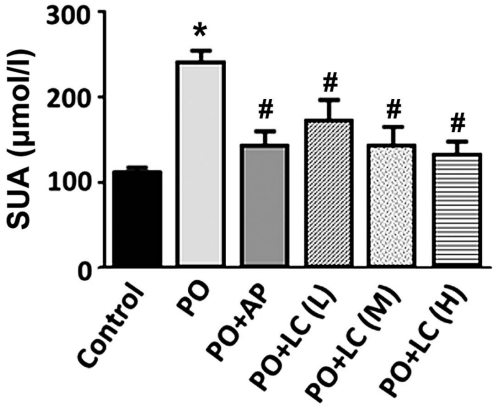

Figure 1. SUA concentrations in the hyperuricemia rat model with various treatments. n=10 per group. *P<0.05 vs. control, #P<0.05 compared with PO. SUA, serum uric acid; PO, potassium oxonate; AP, allopurinol; LC, lifeceramics; L, low; M, medium; H, high.

MDA concentration and GSH activity in the PO rats was slightly but not significantly higher and lower, respectively, than those in the control rats (Table III). However, the serum XOD activity in the PO+LC (M) and PO+LC (H) rats decreased to that of the control rats as did the XOD activity in the PO+AP rats (Table III). The MDA concentration in all PO+LC rats was lower compared with the PO rats, but the GSH activity in the PO+LC (M) rats was not notably higher compared with the PO rats (Table III).

Discussion

In the present study, the increase of SUA in PO rats was significantly suppressed by administration of LC water as well as by AP gavage. The PO-untreated and LC water-treated rats did not show any significant differences in the SUA levels from the control rats (data not shown). Thus, the results of the present study suggest that the LC water may have effectively inhibited the increase in SUA concentration in hyperuricemic rats.

LC is marketed as a food which promotes a healthy status in humans (8). For a person weighing 60 kg, daily consumption of LC as food should be 1.6-6.0 g according to the manufacturer. In the present study, rats consumed 25-100 mg/kg/day (Table I). The applied PO dosage was in accordance with our previous study (3). The potential effects of PO toxicity remains to be determined; however, LC toxicity was previously evaluated (8). The median lethal dose of LC administered orally in rats is estimated to be 4.1 g per rat (8). It is possible that lethality is the result of ileus caused by LC overdose. However, no other significant side effects have been reported (8).

UA is an important endogenous antioxidant in the human body (1,2,14). However, the ability of UA to scavenge free radicals is limited, and high concentrations of SUA can affect the redox balance system and promote oxidative stress injury (1,2,12,15). In the present study, serum XOD activity in the rats treated with a combination of PO and LC water was lower compared with PO rats without LC. XOD is a key enzyme involved in the formation of UA, and a rate-limiting enzyme for converting hypoxanthine to xanthine, which is subsequently converted to UA (1,14). Therefore, the LC water may potentially reduce the concentration of UA by regulating the activity of XOD.

Recent studies have suggested that HUA may be directly associated with the hemorheological characteristics (3,11). A high concentration of SUA can result in a decrease in erythrocyte deformability, and can increase whole blood viscosity at different shear rates (3,11). In the present study, PO-induced HUA significantly decreased erythrocyte deformation, as previously reported (3,11). However, the combined administration of PO with LC water significantly suppressed the decrease of erythrocyte deformation. Furthermore, serum MDA concentration decreased in the PO+LC rats, suggesting high oxidative stress conditions in the PO rats and the possibility of its suppression by LC water. There is the possibility that the LC water may decrease SUA concentration, enhance anti-oxidative activity and improve the hemorheological characteristics.

In our previous study, the anti-oxidative effects of LC water in cultured human cells were reported (13). Additionally, in the liver of rats with alcoholism, LC administration reduced MDA content and increased GSH activity, suggesting the anti-oxidative effects of LC on alcoholic hepatic injury (9). Hwang *et al* (16) also reported the anti-oxidative properties of water mixed with a hydrophilic ceramic powder in plant and mammal cell lines. It has been previously reported that drinking hot spring water is effective against HUA, possibly due to the pharmacological effects of the chemicals involved in purine and UA metabolism (17). Thus, drinking LC water may provide protection against HUA due to the increased anti-oxidative activity.

The present LC study is based on our established theory of the 'human SOS response', which attempts to explains the

Table II. Hemorheological properties of the rats in each group. n=10 per group.

				PO+LC		
Hemorheological property	Control	PO	PO+AP	Low	Medium	High
Plasma viscosity, mPa·s	1.14±0.11	2.32±0.65[a]	2.25±0.13	2.10±0.19	1.93±0.24	2.13±0.26
Whole blood viscosity, mPa·s						
150 s^{-1}	4.01±0.27	5.59±0.78[a]	5.93±0.61	6.39±0.91	5.49±0.72	5.84±0.51
60 s^{-1}	5.55±0.43	7.42±1.04[a]	8.18±1.06	9.18±1.58	7.50±0.93	7.77±0.48
20 s^{-1}	8.12±0.79	10.72±1.69[a]	12.33±1.97	14.82±3.61	11.09±1.66	11.31±0.61
Aggregation index	2.02±0.06	1.91±0.10	2.06±0.12	2.30±0.33	2.01±0.12	1.94±0.11

[a]P<0.05 vs. control. PO, potassium oxonate; AP, allopurinol; LC, lifeceramics.

Table III. Effects of AP and LC water on oxidative status in serum. n=10 per group.

				PO+LC		
Factor	Control	PO	PO+AP	Low	Medium	High
Xanthine oxidase, U/l	54.41±3.12	63.46±9.14[a]	52.11±5.92[b]	59.96±3.80	54.13±6.85[b]	53.32±4.39[b]
Malondialdehyde, nmol/ml	7.89±0.76	8.43±0.92	7.22±0.402	6.36±0.59[b]	5.43±0.44[b]	5.40±0.57[b]
Glutathione, U/ml	27.96±7.86	24.36±9.87	28.14±16.57	34.24±9.90	36.44±6.23[b]	38.98±10.00[b]

[a]P<0.01 vs. control; [b]P<0.01 vs. PO. PO, potassium oxonate; AP, allopurinol; LC, lifeceramics.

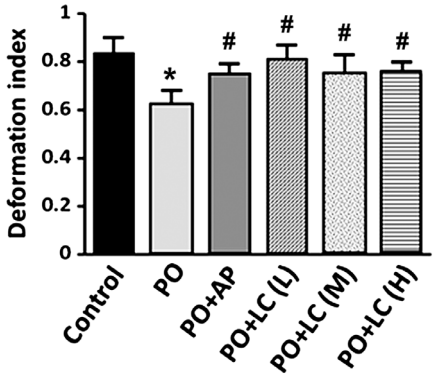

Figure 2. Erythrocyte deformation in the hyperuricemia rat model with various treatments. n=10 per group. *P<0.05 vs. control, #P<0.05 compared with PO. PO, potassium oxonate; AP, allopurinol; LC, lifeceramics; L, low; M, medium; H, high.

biophysiological mechanism supervising metabolic reactions against various stressors such as oxidative stress (8), and on the findings regarding the LC-induced response, which includes regulation of physiological response to various agents, such as metabolites from foods (8). The effectiveness of LC has been explored in various metabolic disorders by using the same treatment scheme as that of the present study (9,10). In addition, in hyperglycemic rats, the onset of cataracts is suppressed by drinking LC water (unpublished results). Similarly, in hyperlipidemic rats, a decrease in serum triglycerides and low density lipoprotein is detected in response to drinking LC water (unpublished results). Interestingly, the levels of triglycerides in human blood serum tended to decrease after drinking LC water for 30 days (8). Therefore, LC may be a potential therapeutic for clinical treatment of various diseases.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

KL, XG and JY performed the experiments. FG and XL prepared and analyzed the data. KL, XT and NS designed the study. AS and KK analyzed and interpreted the data, and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments were approved by the Animal Ethics Committee of Chengde Medical University (Chengde, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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