Lycopene supplementation decreases oxidative stress in hemodialysis patients receiving intravenous iron therapy: An open-label, randomized controlled clinical trial

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Abstract
The aim of our study was to evaluate the effect of lycopene on the antioxidant status and the level of homocysteine (HCY) in dialysis patients receiving intravenous iron therapy. A total of 60 hemodialysis patients receiving intravenous iron therapy were randomly assigned to the treatment group and the control group. Patients in the treatment group (n = 30) received oral lycopene and intravenous iron, while patients in the control group (n = 30) only received intravenous iron therapy. At the initiation of the study, oxidant indexes and HCY concentration were tested. After 8 weeks, all of the laboratory variables were repeatedly evaluated. At the initiation of the study, no significant differences were found in the level of oxidant stress and the level of HCY between two groups. After 8 weeks, the levels of superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) decreased, while the levels of malondialdehyde (MDA) and homocystinuria (HCY) increased in both the groups. Besides, the levels of SOD and GSH-px were higher and the level of MDA was lower in the treatment group than in the control group (P < 0.05, respectively). The level of HCY in the treatment group was relatively low, but there was no significant difference between the two groups. In conclusion, we found that 8-week lycopene supplementation attenuated oxidative stress in hemodialysis patients receiving intravenous iron therapy.

Keywords
hemodialysis, inflammation, lycopene, oxidative stress

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Introduction
Oxidative stress might contribute to increased incidence of cardiovascular events in patients with chronic kidney disease (CKD). Mitochondrial oxidative phosphorylation provides >95% of adenosine triphosphate (ATP) with the remainder derived from glycolysis or tricarboxylic acid cycle. Acetyl-CoA (coenzyme A) is synthesized from the β-oxidation of free fatty acids and the oxidation of pyruvate. Pyruvate is synthesized from glycolysis and can be submitted either for decarboxylation to acetyl-CoA or for dehydrogenation to lactate. Antioxidant agents such as vitamin C, vitamin E, and carotenoids might play a crucial role in antioxidant therapy in dialysis patients. In clinical researches, nephrologists have used antioxidant agent supplementation to reduce oxidative stress in patients undergoing hemodialysis. However, no conclusive evidence supports...
that routine supplementation with antioxidant agents is beneficial for dialysis patients.

Compared to healthy individuals, the plasma levels of lycopene and blood glutathione peroxidase (GSH-px) activity significantly decreased in patients undergoing hemodialysis. The level of lycopene was correlated with low-density lipoprotein. Lycopene might represent an additional factor which contributes to reduce oxidative stress in patients undergoing hemodialysis. Compared to natural foods, lycopene can be used as an antioxidant supplementation which does not enhance potassium load.

Intravenous iron therapy required by hemodialysis patients might initiate lipid peroxidation and disturb the tissue and organ functions. Previous study has shown that given vitamin E attenuated lipid peroxidant in dialysis patients receiving intravenous iron, which suggested that antioxidant therapy might be a reasonable therapeutic approach for dialysis patients. The aim of this study is to evaluate the antioxidant effect of lycopene on dialysis patients receiving intravenous iron therapy. We also evaluated the effect of lycopene on the level of homocysteine (HCY) in dialysis patients receiving intravenous iron.

Methods

Subjects

A total of 60 hemodialysis patients treated in the Department of Nephrology of the 2nd Affiliated Hospital of Wenzhou Medical College from 1 November 2014 to 1 November 2015 participated in this study and were randomly assigned to the treatment group and the control group. Patients in the treatment group (n = 30) received oral lycopene and intravenous iron, while patients in the control group (n = 30) only received intravenous iron therapy. Inclusion criteria were as follows: (1) maintenance dialysis patients on regular hemodialysis for at least 3 months; (2) adults (>18 years); (3) serum ferritin <500 μg/L and transferrin saturation (SAT) <30%; (4) hemoglobin <110 g/L; (5) without receiving intravenous iron therapy in 2 weeks before the clinical trial; and (6) without receiving blood transfer in 8 weeks preceding the clinical trial. Exclusion criteria were as follows: (1) history of hypersensitivity to intravenous iron or lycopene; (2) other contraindications to intravenous iron; (3) myocardial infarction or cerebrovascular accident in the year preceding the clinical trial; (4) infectious disease in 1 month preceding the clinical trial; (5) evidence of uncooperative attitude; (6) malignant tumor; (7) severe chronic liver disease; (8) active peptic ulcer; (9) history of asthma; (10) autoimmune diseases including systemic lupus erythematosus, and antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis; (11) the level of serum albumin <25 g/L; (12) hemorrhagic diseases; (13) pregnancy or lactating; (14) individuals receiving antioxidant agents including vitamin C, vitamin E, and carotenoids; (15) uncontrolled heart failure; and (16) patients receiving hemofiltration or hemoperfusion therapy.

This study was approved by the ethics committee of the 2nd Affiliated Hospital of Wenzhou Medical College. All participants gave their informed consent.

Treatment

Lycopene

A single lycopene capsule (500 mg) contains 39.06 mg of lycopene (Healthy Nature Resource USA, Inc., production no. J31020414612). Patients assigned in the treatment group were treated for 8 weeks with one lycopene capsule twice daily.

Intravenous iron

A solution of iron hydroxide sucrose complex (Venofer, Nanjing Henger Pharmacy, product with code no. approved by SFDA H20046043, production no. 080702) was used to supply iron. An ampoule containing 100 mg of iron was diluted with sterile saline to a total of 100-mL solution. The iron solution was infused via venous line of the extracorporeal circuit 2 h after initiation of hemodialysis. The infusion time was 30–60 min. A dose of 100 mg of iron was administrated twice a week, and a total dose of 1000 mg of iron was infused. During infusion of iron, all of the patients were monitored carefully.

Hemodialysis

All patients received hemodialysis therapy three times per week (12–15 h) with a blood flow rate of 250 mL/min and dialysate flow rate of 500 mL/min.
Polyflux L dialyzers (Gambro, Sweden) were used for dialysis therapy. Dialysate A was supplied by Shandong Weihai Pharmacy (D204 or 2480) and dialysate B was supplied by Gambro (BiCart 720 g). Gambro Central Water Treatment System (GB19706.1-1995) was used to produce dialysis water. The quality of dialysis water adhered to current AAMI (Association for the Advancement of Medical Instrumentation) standards (Composition of dialysate: sodium 140mmol/L, Chlorine 110mmol/L, Calcium 1.5mmol/L, Magnesium 0.75mmol/L, HCO3- 32mmol/L, potassium 2.0mmol/L).

Blood sample and laboratory variables
At the initiation of the study, all blood specimens were collected after an overnight fast of at least 10 h in the 2nd Affiliated Hospital of Wenzhou Medical College. All blood specimens were collected in the morning of dialysis day. Superoxide dismutase (SOD), GSH-px, and malondialdehyde (MDA) were used as oxidative stress indexes. The levels of SOD, GSH-px, and MDA were tested using commercially available kits. Diagnostic reagents for determination of oxidative stress were provided by Nanjing Jiancheng Bioengineering Institute. Xanthine oxidase method was used to detect the SOD activity in serum. Dithio-bis-nitrobenzoic acid was used to detect the GSH-px activity. The level of MDA in the serum was measured using thiobarbituric acid method. After 8 weeks, the levels of SOD, GSH-px, and MDA were repeatedly evaluated.

Other laboratory variables were determined in the central laboratory of the 2nd Affiliated Hospital of Wenzhou Medical College. Enzymatic cycling method was used to determine the concentration of HCY.

Outcomes
The primary outcome was the level of oxidative stress after 8 weeks of supplementation of lycopene. Oxidative stress was indicated by the levels of SOD, GSH-px, and MDA. The secondary outcome was the level of HCY after 8 weeks of lycopene supplementation.

Statistical analysis
Data were analyzed using Stata. Continuous variables were shown as mean ± standard deviation or median and interquartile range, appropriately. A two-tailed \( P \)-value <0.05 was considered significant. Student's t-test or rank-sum test was used to compare data between two groups. Categorical variables were indicated as frequency, and chi-square test was used to assess the differences.

Results
Comparison of characteristics of study population in two groups
A total of 60 patients with mean age of 53 years were recruited for this study. There were no significant differences in age, gender ratio, duration of hemodialysis, and dry weight between two groups. No differences were found between two groups in the levels of serum creatinine, serum albumin, hemoglobin, and other laboratory variables, as shown in Table 1.

Comparison of oxidant stress indexes and the level of HCY in two groups
No differences were found between two groups in the levels of SOD, GSH-px, MDA, and HCY at the initiation of clinical trial, as shown in Table 2. After 8 weeks, the levels of SOD and GSH-px decreased, while the levels of MDA and HCY increased in both the groups. Besides, the levels of SOD and GSH-px were higher and the level of MDA was lower in the treatment group than in the control group \( (P < 0.05, \text{respectively}) \). The level of HCY in the treatment group was relatively low, but there was no significant difference between the two groups, as shown in Table 3.

Discussion
Lipid abnormality and antioxidant deficiency could exist in patients with chronic renal failure and hemodialysis. Intravenous iron is a common therapy for dialysis patients. Previous studies have shown that iron-initiated perioxidant might lead to disturbance of tissue function. The normal antioxidant system depends on the balance between oxidant and protective antioxidant. Human being has complex antioxidant systems including enzymic and non-enzymic antioxidant, which protect the cells and organ systems of the body against free radical damage. The antioxidants can be endogenous or obtain from exogenous resources. GSH-px and SOD are
effective enzymatic antioxidant agents, and MDA is used to assess the degree of lipid peroxidation. Patients undergoing hemodialysis had a low level of GSH-px and SOD as well as a high level of MDA, which indicated an increase in oxidative stress in patients with dialysis. Non-enzymatic antioxidant agents include vitamin C, vitamin E, carotenoids, and thiol antioxidants. Among these antioxidant agents, lycopene exhibits the highest physical quenching rate constant with oxidant stress. In fact, patients with chronic renal failure had significant deficiency of lycopene which is the most active antioxidant carotenoid in vitro. The antioxidant agents can be supplemented from dietary; however, fruits and vegetables rich in antioxidants might be restricted in some of CKD patients. Though strict

table 1. Baseline characteristics of two groups.a

|                        | Treatment group (n = 30) | Control group (n = 30) | P-value |
|------------------------|-------------------------|------------------------|---------|
| Age (years)            | 53.89 ± 14.36           | 52.43 ± 13.35          | 0.68    |
| Male/female            | 17/13                   | 16/14                  | 0.80    |
| Duration of dialysis (months) | 14.31 ± 12.56   | 12.44 ± 10.37          | 0.53    |
| Serum creatinine (µmol/L) | 982.11 ± 264.07 | 957.26 ± 286.81        | 0.73    |
| Blood urea nitrogen (mmol/L) | 24.68 ± 6.78    | 23.68 ± 6.47           | 0.73    |
| Uric acid (µmol/L)     | 438.27 ± 101.93        | 433.27 ± 82.17         | 0.84    |
| Hemoglobin (g/L)       | 89.17 ± 10.37          | 90.08 ± 10.02          | 0.73    |
| Serum albumin (g/L)    | 32.49 ± 3.00           | 32.78 ± 3.15           | 0.72    |
| Alanine transaminase (µ/L) | 15.13 ± 7.66      | 21.92 ± 17.71          | 0.06    |
| Aspartate aminotransferase (µ/L) | 17.23 ± 6.39    | 21.77 ± 11.00          | 0.06    |
| Lactate dehydrogenase (mmol/L) | 145.8 ± 23.00   | 161.76 ± 39.65         | 0.06    |
| Total cholesterol (mmol/L) | 4.32 ± 1.39        | 4.40 ± 0.94            | 0.79    |
| Low-density lipoprotein (mmol/L) | 2.29 ± 0.92   | 2.12 ± 0.72            | 0.43    |
| High-density lipoprotein (mmol/L) | 0.90 ± 0.21     | 0.96 ± 0.41            | 0.48    |
| Serum triglyceride (mmol/L) | 1.40 (1.10–2.63) | 1.68 (1.18–2.49)       | 0.54    |
| Creatinine phosphokinase (µ/L) | 83.03 ± 45.63 | 83.84 ± 46.67          | 0.95    |
| Creatinine phosphokinase-MB (µ/L) | 3.4 (2.2–4.3)  | 2.7 (1.9–3.9)          | 0.23    |
| Serum iron (µmol/L)    | 11.2 (8.21–14.68)     | 10.43 (8.13–16.11)     | 0.90    |
| Transferrin saturation (%) | 17.26 ± 6.68    | 17.80 ± 6.37           | 0.75    |
| Ferritin (µg/L)        | 241.30 ± 90.96        | 234.29 ± 112.15        | 0.79    |
| C-reactive protein (mg/L) | 2.94 (1.37–6.19) | 2.79 (1.59–6.27)       | 0.42    |
| Erythropoietin (U/w)   | 7000.00 ± 1463.85     | 7200.00 ± 1521.28      | 0.61    |

aMean ± SD or median (25th–75th percentiles) for continuous variables.

Table 2. Oxidative stress and homocysteine at the initiation of clinical trial.

|                        | Treatment group (n = 30) | Control group (n = 30) | P-value |
|------------------------|-------------------------|------------------------|---------|
| SOD (nU/mL)            | 78.85 ± 16.11           | 79.83 ± 15.72          | 0.81    |
| GSH-px (U/mg)          | 62.92 ± 16.21           | 62.89 ± 17.94          | 0.99    |
| MDA (µmol/L)           | 5.97 ± 1.33             | 5.96 ± 1.37            | 0.98    |
| Homocysteine (µmol/L)  | 40.47 ± 23.72           | 39.97 ± 19.02          | 0.94    |

SOD: superoxide dismutase; GSH-px: glutathione peroxidase; MDA: malondialdehyde.

Table 3. Oxidative stress and homocysteine after 8 weeks of intravenous iron therapy.

|                        | Treatment group (n = 30) | Control group (n = 30) | P-value |
|------------------------|-------------------------|------------------------|---------|
| SOD (nU/mL)            | 68.32 ± 15.14           | 55.58 ± 14.64          | 0.002   |
| GSH-px (U/mg)          | 55.43 ± 14.90           | 44.74 ± 15.11          | 0.008   |
| MDA (nmol/L)           | 11.51 ± 3.52            | 22.38 ± 4.65           | <0.0001 |
| Homocysteine (µmol/L)  | 40.76 ± 19.94           | 45.04 ± 27.94          | 0.50    |

SOD: superoxide dismutase; GSH-px: glutathione peroxidase; MDA: malondialdehyde.
dietary restrictions in CKD are difficult to fulfill, dietary restriction might be a potential explanation for the deficiency of antioxidant agents. In the previous study, in the similar level of consumption of carotenes, the level of lycopene was significantly lower in patients with chronic renal failure. Theoretically, lycopene, a non-toxic natural substance, could be used as an antioxidant agent in hemodialysis patients.

In this study, we used lycopene as an antioxidant agent and supplemented it to dialysis patients receiving intravenous iron. After 8-week iron hydroxide sucrose, the concentrations of SOD and GSH-px decreased and the level of MDA was enhanced in patients undergoing hemodialysis. However, lycopene attenuated oxidative stress in hemodialysis patients receiving intravenous iron. Considering diet control for dialysis patients, lycopene supplementation might be an alternative method to decrease the oxidative stress in patients undergoing dialysis.

Hyperhomocysteinemia is common in maintenance dialysis patients and HCY is also a detectable marker in inflammation and pathogenesis of cardiovascular disease (CVD). Apart from dietary restriction and metabolic abnormality, some treatments for CKD patients might exacerbate the deficiency of antioxidant agents in patients undergoing dialysis. Hemodialysis therapy can clear water-soluble vitamins including antioxidants. In this study, we also did not find any substantial effect of lycopene on the concentration of HCY in dialysis patients receiving intravenous iron therapy.

In conclusion, we found that 8-week lycopene supplementation decreases oxidative stress in hemodialysis patients receiving intravenous iron therapy. Lycopene supplementation might be beneficial for hemodialysis patients.

Declaration of conflicting interests
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