

Lipid-Lowering Effect of Nattokinase in Patients with Primary Hypercholesterolemia

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Background: Nattokinase is a fibrinolytic enzyme isolated from the vegetable cheese natto. Although research suggests that nattokinase has beneficial effects on lipid metabolism, there is no clinical data on its effects in human patients with hypercholesterolemia.

Methods: Thirty patients with primary hypercholesterolemia given instruction on a low cholesterol diet and treated with 400 mg (4000FU) nattokinase or placebo twice daily for 8 weeks were selected.

Results: No significant changes in serum triglyceride, non-HDL cholesterol, total cholesterol/HDL ratio, and LDL/HDL ratio were found in either treatment group. There was a tendency of greater reduction of total serum cholesterol in nattokinase group than placebo group (-6.15% and -6.8% after 4 and 8 weeks treatment, respectively). There were also greater reduction of HDL-C and LDL-C after 8 weeks in nattokinase group (-10.85%, -6.3% respectively) but no statistically significant was noted.

Conclusion: In adults with primary hypercholesterolemia, nattokinase is well tolerated and in combination with low cholesterol diet may have effects on the serum cholesterol profile.

Key Words: Nattokinase • Hypercholesterolemia • LDL cholesterol • HDL cholesterol

INTRODUCTION

Hypercholesterolemia is one of the risk factors of atherosclerosis and coronary artery disease.¹⁻³ Current guidelines for statin therapy called for reductions in LDL-C levels to within normal range, and further reductions in LDL-C < 70 mg/dl are called for very high-risk patients with a history of myocardial infarction or acute coronary syndrome.⁴ Guidelines set forth by the advisory panel of the National Cholesterol Education Pro-

gram recommend that in the primary prevention of coronary artery disease, diet therapy is the initial recommended intervention for lowering LDL-C.¹ However, diet modification is not always effective, thus there is a need to identify additional non-pharmacological therapeutic options if the LDL-C goal has not been achieved with dietary therapy alone or by conventional pharmacologic treatment with statins or fibrates.

Natto is a traditional Japanese food made from fermented soybeans, has attracted attention as a nutritious food. *Bacillus subtilis natto*, a bacterium isolated from natto, secretes several proteases during the fermentation process.⁵ In an experiment by Sumi et al., natto was applied directly to a fibrin plate. Nattokinase was extracted from 300 gm of natto with 220 mL of saline for 15 minutes with stirring at 4 °C. The material was filtered through gauze and then centrifuged at 3000 rpm for 10 minutes.⁶ Nattokinase capsule, an enterically coated capsule, containing plasmin, isoflavones (20.5%), fat (4.6%), carbohydrate, fiber (9.2%), mineral and vitamin K,⁵⁻⁷ has been investigated for possible clinical use.⁸⁻¹² It

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has been found to be effective in preventing bone fractures in premenopausal women^{8,9} and have anti-tumor effects,¹⁰ and to be an antihypertensive agent with angiotensin-converting enzyme inhibiting activities.^{11,12} Interestingly, it also has effects on LDL-oxidation and lipid metabolism in cholesterol-fed rabbits and rats.^{13,14} There was no data regarding the lipid-lowering effects of nattokinase in humans, to our knowledge. Soy protein and isoflavone intakes have effects on human blood cholesterol and lipoprotein levels.^{15,16} Therefore, in this study, we analyzed the lipid-lowering effects of nattokinase in adult patients with primary hypercholesterolemia without any other metabolic disorders.

METHODS

This study was a randomized, double-blind, placebo-controlled, parallel-group study approved by the ethics committee of our hospital. Signed, informed consent was obtained from each patient. Patients with serum cholesterol > 200 mg/dl in spite of restricted dietary control for one month, not on lipid-lowering medications before, and with an overall Framingham heart risk score of $\leq 10\%$ were selected.¹⁷ Patients were excluded if their serum triglyceride level was > 350 mg/dl; blood pressure > 180/110 mmHg; serum creatinine > 2.0 mg/dl; liver dysfunction, fasting and 2 hours postprandial plasma sugar > 126 mg/dl; body mass index > 35, and/or creatinine phosphokinase level more than the double normal value. Patients receiving oral contraceptives or hormone therapy, or having severe liver, lung, kidney, gastrointestinal or hematological disorders and other malignant disorders were also excluded. Thirty adults (M/F: 16/14), ranging in age from 36 to 74 (mean = 53.2 ± 9.87) were randomized to receive either an enteric capsule containing 400 mg (4000 FU) nattokinase or placebo twice daily for 8 weeks in a double-blind fashion. An FU is a fibrin unit or activity unit that quantifies the enzyme's ability to lyse fibrin in vitro. This is approved as a safe health product by our government.¹⁸ Clinic visits were scheduled at weeks 2, 4, 6 and 8. Body weight was measured at each visit. Subjects were asked to record study capsules taken, concomitant medications, and any adverse events in diaries. A registered dietician instructed subjects to adhere to a low-cholesterol diet. It means that

the total caloric intake was 25-30 kcal/kg/day, with fat intake less than 30% (7% from saturated fat), protein intake about 15-20% and the remaining from carbohydrate, and the cholesterol intake less than 300 mg/day. The content of diet was assessed using a dietary record. Lipid and lipoprotein measurements were taken at baseline, week 4 and week 8 after an 8-hour fasting period. The primary efficacy end point was the percent reduction from baseline in total cholesterol, LDL-C and HDL-C levels. The lipoproteins were measured automatically using standard laboratory enzymatic assay. Serum chemistry tests were performed at baseline, week 4 and week 8 to monitor for safety. These tests included measurement of blood glucose, creatinine, transaminase (ALT, AST), creatinine phosphokinase, uric acid and hemoglobin as well as red blood cell and white blood cell counts.

Statistical analysis

Primary statistical hypotheses were the comparison of percent reductions in total cholesterol, HDL-C, LDL-C and triglyceride, non-HDL cholesterol, total cholesterol/ HDL ratio, and LDL/HDL ratio after 4 and 8 weeks of treatment. Changes in lipoprotein at weeks 4 and 8 were analyzed in comparison with baseline at week 0. Data are expressed as mean \pm SD. Comparisons were done by the independent t-test and paired t-test (2-tailed) using SPSS 14 and repeated measure ANOVA. P-values less than 0.05 were considered statistically significant.

RESULTS

Of the 30 randomized subjects, 29 subjects completed the trial. One subject in the placebo group withdrew from the study at week 4. The baseline characteristics are presented in Table 1. There were no significant differences in age, body mass index, baseline serum triglycerides, cholesterol, LDL-C, HDL-C, non-HDL cholesterol, total cholesterol/ HDL ratio, and LDL/HDL ratio between the study and placebo groups, although there were more female subjects in the nattokinase treatment group compared to the placebo group (73.4% vs. 33.3%). The mean percentage changes in lipid parameters in the individual patient from baseline to week 4 and week 8 are presented in Table 2. In the placebo group, the mean changes of

Table 1. Baseline characteristics of subjects in both treatment groups

| | Nattokinase | Placebo | p |
|-----------------------------|--------------|--------------|-------|
| No | 15 | 15 | |
| Age (y/o) | 54.8 ± 9.6 | 51.6 ± 10.1 | 0.374 |
| Sex (% female) | 73.4 | 33.3 | 0.066 |
| Height (cm) | 154.6 ± 6.2 | 163.2 ± 10.3 | |
| Weight (Kg) | 56.9 ± 7.2 | 68.7 ± 13.8 | |
| BMI | 24.2 ± 2.3 | 26.3 ± 3.7 | 0.157 |
| Systolic pressure (mmHg) | 132 ± 12 | 126 ± 13 | 0.208 |
| Diastolic pressure (mmHg) | 81 ± 7 | 79 ± 11 | 0.575 |
| Sugar (mg/dl) | 102 ± 16.0 | 94.9 ± 4.6 | 0.35 |
| ALT (U/L) | 23.3 ± 7.5 | 23.0 ± 9.1 | 0.894 |
| BUN (mg/dl) | 13.9 ± 3.9 | 14.6 ± 3.2 | 0.591 |
| Cr (mg/dl) | 0.8 ± 0.14 | 0.89 ± 0.15 | 0.138 |
| UA (mg/dl) | 6.09 ± 1.53 | 5.91 ± 1.94 | 0.284 |
| Triglycerides (mg/dl) | 160.1 ± 62.7 | 167.9 ± 78.1 | 0.728 |
| Cholesterol (mg/dl) | 239.1 ± 31.7 | 227.0 ± 22.6 | 0.240 |
| LDL-C (mg/dl) | 155.9 ± 25.5 | 152.2 ± 26.2 | 0.701 |
| HDL-C (mg/dl) | 54.9 ± 8.8 | 53.5 ± 9.7 | 0.682 |
| Non-HDL cholesterol (mg/dl) | 184.2 ± 32.1 | 173.5 ± 48.7 | 0.331 |
| Total cholesterol/HDL (%) | 4.46 ± 0.92 | 4.40 ± 1.01 | 0.858 |
| LDL/HDL (%) | 2.90 ± 0.56 | 2.97 ± 0.86 | 0.785 |

Table 2. Percentage changes of lipid parameters from baseline to week 4 and week 8 in both groups

| Mean ± SD | time | Nattokinase group | Placebo group | P |
|--------------|--------|-------------------|---------------|-------|
| Triglyceride | Week 4 | 3.48 ± 38.25 | 7.21 ± 21.76 | 0.698 |
| | Week 8 | 7.92 ± 21.76 | -3.19 ± 33.73 | 0.294 |
| Cholesterol | Week 4 | -6.15 ± 10.3 | -0.71 ± 9.67 | 0.147 |
| | Week 8 | -6.8 ± 13.27 | 1.94 ± 20.65 | 0.183 |
| HDL-C | Week 4 | -5.89 ± 14.51 | -1.53 ± 11.45 | 0.334 |
| | Week 8 | -10.85 ± 12.49 | -3.47 ± 10.28 | 0.105 |
| LDL-C | Week 4 | -1.9 ± 8.0 | -2.5 ± 13.1 | 0.880 |
| | Week 8 | -6.3 ± 14.65 | -3.9 ± 12.2 | 0.597 |

lipid profile levels from baseline were not significant both at weeks 4 and 8. There was tendency of greater reduction of total serum cholesterol in nattokinase group than placebo group (-6.15% and -0.71%, respectively after 4 weeks, and -6.8% and +1.94%, respectively after 8 weeks, power = 0.25). A total of 6 of the 15 (40%) patients in the nattokinase treatment group achieved a serum cholesterol level below 200 mg/dl after 8 weeks (meeting the criteria of the NCEP and EAS). In contrast, only 2 of the 14 (14%) patients in the placebo group achieved a serum cholesterol level below 200 mg/dl. There were also greater reduction of HDL-C and LDL-C after 8 weeks in nattokinase group (-10.85%, -6.3% re-

spectively) but no statistically significant was noted. Time-course changes of total cholesterol, LDL-C and HDL-C levels in both groups also show tendency of greater reduction in nattokinase group but there was no statistically significant by repeated measure ANOVA (Figure 1). The main effects of group and the interaction effect were followings: group P = 0.924, time P = 0.382, interaction effect P = 0.253 in total cholesterol, group P = 0.930, time P = 0.01, interaction effect P = 0.167 in HDL-C, group P = 0.766, time P = 0.06, interaction effect P = 0.235 in LDL-C, respectively. There were no significant changes in triglycerides, non-HDL-C, cholesterol /HDL-C and LDL-C/ HDL-C levels. Furthermore, there were not any adverse events and no other significant differences in routine hematologic and biochemical

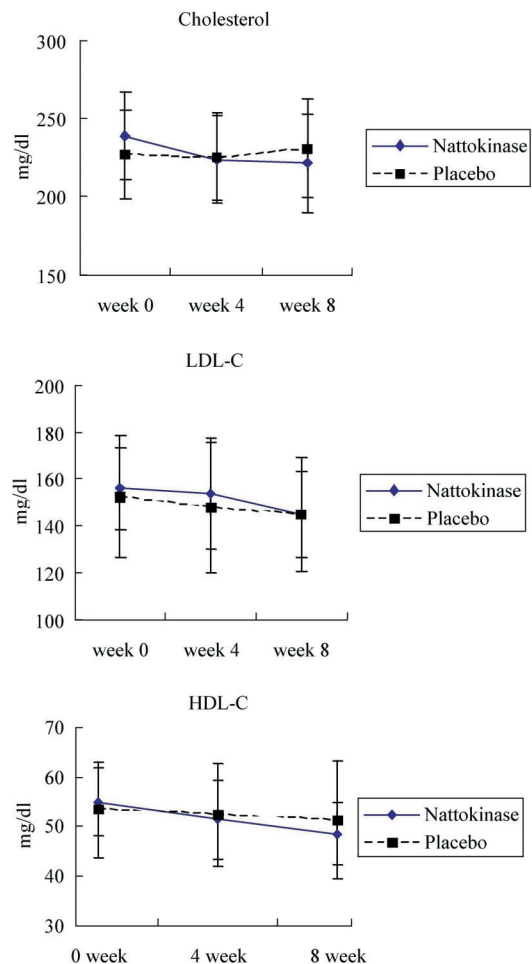


Figure 1. Time-course changes of total cholesterol, LDL-C and HDL-C in the nattokinase treatment and placebo groups. Note there was a tendency of reduction of total cholesterol, LDL-C and HDL-C after treatment with nattokinase but there was no statistically significant.

tests between the treatment and placebo groups. Also, no statistically significant changes in body mass index were found in either group during the study.

DISCUSSION

This is a pilot study about the lipid-lowering efficacy of nattokinase in a small number of low risk patients with hypercholesterolemia. Although there was no statistically significant, serum cholesterol, LDL-C and HDL-C were more decreased in the nattokinase treatment group at week 8. However, there were no statistically significant changes in triglyceride and other lipid profile levels after eight weeks upon study completion. These results suggest that the lipid-lowering effect of nattokinase in humans is quite different from that of statins, fibrates, or ezetimibe. However, it is well known that serum cholesterol can be affected by many factors, such as sex, age, hormonal state, diet, medication, and physiologic condition. Further studies on defining a mechanism for hypolipidemic potential of nattokinase are needed.

In observational studies as well as in clinical trials with statins, each 1% reduction in LDL-C results in approximately a 1.0% to 1.5% reduction and each increase of HDL-C by 1mg/dl is associated with 2-3% decrease in the relative risk of major cardiovascular events.^{2,19} Our findings indicated that nattokinase reduced LDL-C by 6% after treatment for 8 weeks. By extrapolation, such a reduction in LDL-C would result in a 6.0% to 9.0% reduction in the risk of the major cardiovascular events. However, the HDL-C was also decreased by nattokinase. So from the standpoint of primary prevention of CAD, nattokinase treatment may not be preferred as a candidate for clinical application and a longer observation period is needed.

Animal studies using cholesterol-fed rats and rabbits suggest that nattokinase has effects on LDL oxidation.^{13,14} In the experiments, the triglyceride levels were also decreased but HDL-C levels were not. Isoflavones present in soy protein were demonstrated to have effects on blood and liver lipid by the bile-acid binding in rats fed with high-cholesterol diets.²⁰ However their effects on triglyceride were not significant. There are several different mechanisms concerning the LDL-C-lowering

and HDL-increasing actions of soy and isoflavones in humans. Their effects were individualized (3-5% in LDL-C-lowering effect) and did not have a dose-dependent effect.^{15,16} We did not measure content of equol (an isoflavonoid that is well absorbed and possesses significant oestrogenic activity and is directly related with LDL-C-lowering effect in humans). The HDL-C was also decrease in this study. So, the effect of nattokinase on serum lipids in humans of our result is quite different from those of soy protein and isoflavones and that in animal models.

The dietary dose of wet natto is recommended from 100 to 200 grams. The therapeutic dosage recommendation for nattokinase is 500 mg per day. This is the standard dietary amount ingested by many Japanese people for years without side effects.²¹ In our study, 400 mg of nattokinase was administered twice daily (equivalent to the nattokinase in about 200 gm of wet natto) without any adverse effects. However, we did not perform dose-dependency and duration trials.

Study limitations: This was a randomized, double-blind, placebo control study with a relatively small sample size and short duration. Notably, there were considerably more postmenopausal female subjects in the nattokinase treatment group compared to the placebo group. We therefore need to be cautious if there is a gender difference in the response to nattokinase in humans. To differentiate the lipid-lowering effect of nattokinase and isoflavone in humans, we should have an additional control group taking the same amount of pure isoflavones. Indeed, dose optimization studies as well as studies investigating the potential additive effects by combining known lipid-lowering agents with nattokinase are warranted.

In conclusion, the results of this study demonstrate that nattokinase is safe, but both the HDL-C and LDL-C are reduced in patients with primary hypercholesterolemia. The effect of nattokinase in primary prevention of cardiovascular disease needs to be studied further.

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