Original Article

Combined nattokinase with red yeast rice but not nattokinase alone has potent effects on blood lipids in human subjects with hyperlipidemia

Nae-Cherng Yang PhD[1], Chien-Wen Chou BS[2], Chung-Yin Chen PhD[3], Kai-Lin Hwang MS[4], Yi -Chueh Yang BS[5]

[1]*Department of Nutrition and Health Science, Chungchou Institute of Technology, Changhua, Taiwan*
[2]*Division of Endocrinology, Chi Mei Medical Center, Tainan, Taiwan*
[3]*Division of Cardiology, Kuang Tien General Hospital, Taichung, Taiwan*
[4]*Department of Public Health, Chung Shan Medical University, Taichung, Taiwan*
[5]*Department of Medical Laboratory, Chi Mei Medical Center, Tainan, Taiwan*

The purpose of this randomized, double-blind, placebo-controlled, parallel comparison study was to evaluate the lipid-lowering effect of orally administrated nattokinase and nattokinase combined with red yeast rice (RYR) extract on blood lipids in patients with hyperlipidemia. A total of 47 patients with hyperlipidemia were assigned to one of three groups: 1. nattokinase-mono formula (50 mg/capsule), 2. combined formula of nattokinase with RYR (300 mg of extract/capsule) and 3. placebo. Subjects received a twice daily dose of two capsules for six months. The mono formula showed no effects on blood lipids until month six, while the combined formula ameliorated all of measured lipids starting from month one. In the combined group significant decreases were found with regard to: triglycerides (TG) by 15%, total cholesterol (TC) by 25%, low-density lipoprotein cholesterol (LDL-C) by 41%, TC/high-density lipoprotein cholesterol (HDL-C) ratio by 29.5%, and increases in HDL-C by 7.5%. These changes were sustained until the end of study. After controlling for baseline levels, only the combined group, but not mono group, showed a significant difference ($p<0.0001$) in TC, LDL-C and TC/HDL-C ratio when compared with the placebo group. In summary, this study provides long-term efficacy of nattokinase supplementation and shows that the combined formula has relatively more potent effects than the mono formula on lowering of blood lipids, suggesting that combined nattokinase with RYR will be a better neutraceutical for patients with hyperlipidemia than nattokinase alone.

Key Words: nattokinase, red yeast rice, human trial, hyperlipidemia, blood lipid

INTRODUCTION

Nattokinase, a serine proteinase from *Bacillus subtilis*, has been reported to have potent fibrinolytic activity.[1] The enzyme is composed of 275 amino acids with a molecular weight of 27.7 kDa in its mature form[2] and is considered to be one of the most active functional ingredients found in natto, a traditional Japanese food prepared from fermented soybeans. Hyperlipidemia is a highly predictive risk factor for cardiovascular disease (CVD)[3,4] and regulating hyperlipidemia has proven to be effective in lowering the morbidity and mortality associated with CVD.[3] A study by Iwai *et al*. has shown that natto extracts can lower triglycerides (TG) and total cholesterol (TC) levels in cholesterol-fed rats.[5] However, the effect of nattokinase on blood lipids in patients with hyperlipidemia remains unclear.

In addition, there are currently many products available commercially around the world, most popular of which is a combination of nattokinase and red yeast rice (RYR) extracts. It is well known that RYR contains an inhibitor of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase called monakolin K (also known as mevinollin and lovastatin), which is very effective in reducing plasma levels of TC and low-density lipoprotein cholesterol.[6] In addition, RYR contains other ingredients, such as large quantities of unsaturated fatty acids, flavonoids and sterols, which may also have blood lipid lowering effects.[7] A meta-analysis by Liu *et al.* examined the effects of dietary supplementation with RYR on blood lipids in hyperlipidemia patients for 4-12 weeks.[8] This supplementation significantly reduced blood levels of TC, TG and LDL-C. Although the effects of RYR on high-density lipoprotein cholesterol (HDL-C) are not consistent, they also concluded that HDL-C levels can be increased with RYR supplementation.[8] These results have

Corresponding Author: Dr Nae-Cherng Yang, Department of Nutrition and Health Science, Chungchou Institute of Technology, Changhua 510, Taiwan
Tel: 886-4-8395316; Fax: 886-4-8395316
Email: naeboy@dragon.ccut.edu.tw
Manuscript received 21 January 2009. Initial review completed 29 June 2009. Revision accepted 14 August 2009.

demonstrated RYR's ability to reduce blood lipids in humans. However, little known is about the effect of the-combined formula of nattokinase with RYR on blood lipids in patients with hyperlipidemia. It can be expected that RYR would have an additional effect with nattokinase on lowering blood lipids.

Thus, we conducted this randomized clinical trial to examine the effect of mono- and combined nattokinase formulae on patients with hyperlipidemia, and to investigate the effect of these supplements on blood lipids. We hypothesized that both mono- and combined nattokinase formulae would have lipid-lowering effects in patients with hyperlipidemia. In addition, the combined formula would have a greater effect than that of the mono formula. Although natto has been used for many years without safety concerns, an increase in uric acid concentration after soybean consumption has been reported in some people.[9] In addition, the toxin citrinin, found naturally in RYR, has been associated with hepatotoxicity and nephrotoxicity.[6] We therefore monitored the safety of our subjects during this study by assessing uric acid, creatinine, aspartate transaminase (AST), and alanine transaminase (ALT) concentrations, and noted any adverse effects reported by patients on a self-administered questionnaire.

MATERIAL AND METHODS

Subjects

This was a randomized, double-blind, placebo-controlled, parallel comparison study conducted from May 2007 to March 2008 in Chi Mei Medical Center (CMMC) and Kuang Tien General Hospital (KTGH). The protocol was approved by the Institutional Review Boards (IRBs) of both CMMC and KTGH. Between May 2007 and September 2008, 47 adult men and non-pregnant women, above 40 years of age and presented with untreated hyperlipidemia, had provided written consent and were, randomly assigned to one of the three study groups: nattokinase mono formula (18 subjects; mono group), nattokinase combined formula (19 subjects; combined group) and placebo (10 subjects; placebo group). The enrollment criteria included a blood TC of 200-300 mg/dL, TG of 200-500 mg/dL, or either LDL-C of 130-200 mg/dL or HDL-C <40 mg/dL (male) and <50 mg/dL (female). Patients who had received any lipid-lowering drugs or intervention, had a previous myocardial infarction, major trauma or recent hospitalization, or who underwent surgery requiring anesthesia (including coronary artery bypass graft) within the previous 12 weeks were excluded from the study. In addition, any patients who had a recent or abrupt change (within 1 month) in their usual diet, an unstable medical condition, life expectancy less than 6 months, any known allergies to any components of the study , who were currently receiving antibiotic therapy for acute infection, who were receiving warfarin treatment, had acute diseases, or had a TC >300 mg/dL or TG >500 mg/dL were, in the opinion of investigators, not suitable to participate in this study, and were excluded. All study participants underwent screening evaluations up to 30 days prior to administration of the investigational products.

Investigational products

The nattokinase mono group received enteric coated capsules containing 50 mg nattokinase (1,750 FU), purified by ultrafiltration of natto extract. The nattokinase combined group received capsules containing 50 mg nattokinase (1,750 FU), purified from natto extract, and 300 mg RYR, extracted from *Monascus purpureus*. The placebo capsule contained microcrystalline cellulose and was similarly coated and identical in appearance to the nattokinase capsule. The suggested dose was 2 capsules in the morning and 2 capsules before bed-time each day.

Outcome assessment

After a two- to four-week screening period, the study products were administered orally for six months. Patients were asked to return all of the unused trial products and empty bottles for drug accountability. Treatment compliance was measured by percentage of the trial products consumed. All laboratory samples were collected after an overnight fast from all subjects. Laboratory tests, including TG, TC, LDL-C, HDL-C, and uric acid, as well as vital signs and body weight were evaluated during the screening, and at 1-, 3-, and 6-month intervals after the initiation of treatment. Vital signs and body weight were measured at every visit. Each patients' self-evaluation of tolerance and physical improvement were assessed by a structured patient questionnaire administered at months 1, 3, and 6. Each patient was carefully monitored for the development of any adverse events.

Laboratory methods

Plasma TC was determined by the cholesterol esterase and cholesterol oxidase methods using a commercially available kit (Wako Pure Chemical Co. Ltd, Osaka, Japan). The concentration of TG in the plasma was also determined using a commercial diagnostic kit (Triglyceride E-Test Wako, Wako Pure Chemical Industries, Osaka, Japan). HDL-C and LDL-C were evaluated using Determiner HDL-C/LDL-C kits (Kyowa Medex, Tokyo, Japan). A commercial kit (Shino-Test, Japan) was used for uric acid levels, and creatinine levels were determined using Creatinine-test-Wako (Wako Pure Chemical Industries, Osaka, Japan). Plasma AST and ALT were analyzed by automated Roche/Hitachi analyzer system (Roche Diagnostic, Mannheim, Germany).

Statistical analysis

Data are presented as mean and standard deviation for continuous variables. The Wilcoxon signed-rank test[10] was performed to compare the values at baseline and 6 months after the initiation of product administration to each group. All laboratory and vital evaluations were then compared among three groups using the Kruskal-Wallis test,[11] followed by least significant difference (LSD) multiple comparisons of means.[12] Fisher's exact test was performed for comparison of categorical variables.[13] Analysis of covariance (ANCOVA) with repeated measures was performed with baseline level and treatment group as covariates.[14] Statistical analyses were conducted by the study statistician with SAS v8.02 (SAS Institute Inc., Cary, NC, USA). A p-value less than 0.05 was considered to be statistically significant.

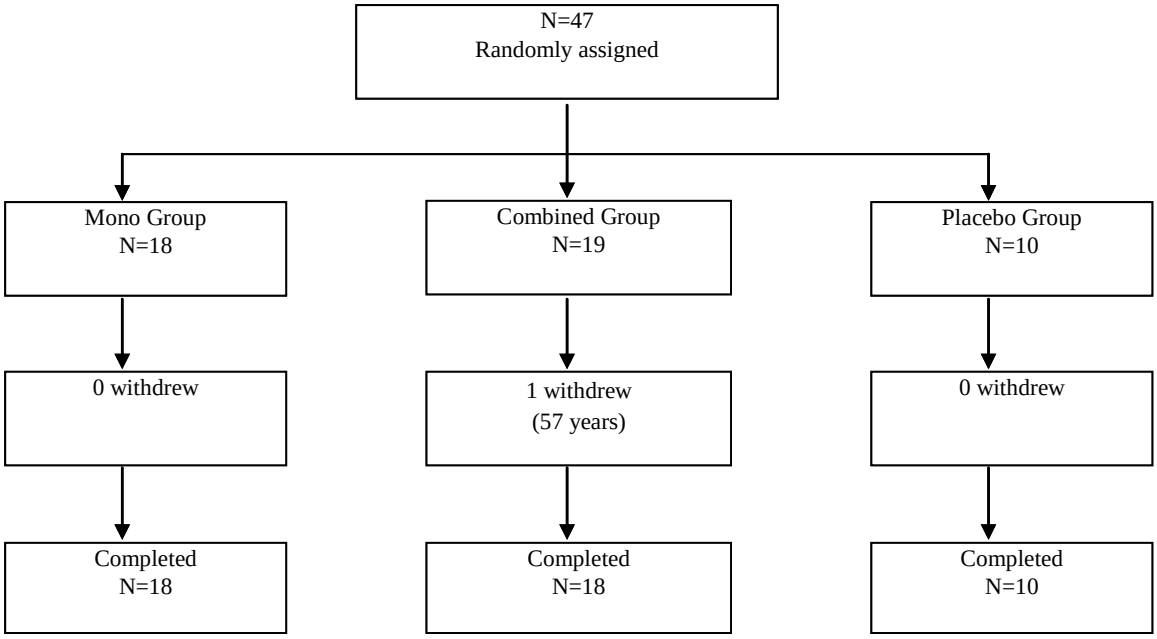

Figure 1. Disposition of trial subjects in three randomized groups. All but one subject in Combined group (57 y, withdrew by Month 1) completed the study.

RESULTS

Demographic characteristics and medical history

Among the 47 subjects who were enrolled, one subject receiving the combined formula refused to return on time before the one-month visit and was then withdrawn from the study (Figure 1). The demographic characteristics and medical history of the 46 subjects who completed the study are summarized in Table 1. There was an equal number (23, or 50.0%) of males and females enrolled, with an average age of 53.7±10.0 years, with no significant differences in all demographic characteristics and medical history in each study group. Hypertension was observed, respectively, in mono, combined, and placebo groups, in 6 (33.3%), 4 (22.2%) and 2 (20%) subjects, diabetes mellitus was observed in 6 (33.3%), 6 (33.3%) and 2 (20%) subjects, lipid disorder was observed in 9 (50%), 9 (50%) and 2 (20%) subjects, and liver disease was observed in 2 (22.2%), 4 (22.2%) and 0 subjects.

Baseline assessments and compliance rate

Most of the baseline assessments were not significantly different among the three groups, using the Kruskal-

Table 1. Demographic characteristics and medical history of patients with hyperlipidemia

Variables	Mono (N=18)	Combined (N=18)	Placebo (N=10)	*p*-value
Gender				0.593
Male, n (%)	11 (61.1)	8 (44.4)	4 (40.0)	
Female, n (%)	7 (38.9)	10 (55.6)	6 (60.0)	
Age (years), mean (SD)	51.6 (8.6)	54.4 (10.4)	56.3 (11.8)	0.496
Cardiovascular Diseases, n (%)	0 (0.0)	1 (5.6)	0 (0.0)	1.000
Stroke, n (%)	0 (0.0)	1 (5.6)	0 (0.0)	1.000
Myocardial Infarction, n (%)	0 (0.0)	1 (5.6)	0 (0.0)	1.000
Hypertension, n (%)	6 (33.3)	4 (22.2)	2 (20.0)	0.753
Physical Inactivity, n (%)	0 (0.0)	0 (0.0)	1 (10.0)	0.217
Diabetes Mellitus, n (%)	6 (33.3)	6 (33.3)	2 (20.0)	0.841
Lipid disorder, n (%)	9 (50.0)	9 (50.0)	2 (20.0)	0.296
Overweight, n (%)	1 (5.6)	1 (5.6)	0 (0.0)	1.000
Chronic Renal Diseases, n (%)	0 (0.0)	1 (5.6)	1 (10.0)	0.691
Liver Diseases, n (%)	2 (11.1)	4 (22.2)	0 (0.0)	0.291
Smoking History				0.909
Current, n (%)	4 (22.2)	3 (16.7)	1 (10.0)	
Stopped, n (%)	2 (11.1)	1 (5.6)	1 (10.0)	
Alcohol History				0.490
Average Consumption, n (%)	7 (38.9)	5 (27.8)	2 (20.0)	
Excessive consumption, n (%)	0 (0.0)	0 (0.0)	1 (10.0)	

p-value for comparison among three groups by Fisher's exact test or Kruskal-Wallis test when appropriate. None of these demographic characteristics and medical history variables were significantly different among the groups.

Table 2. Baseline levels in terms of blood lipids, liver and renal functions, and compliance rates by group in patients with hyperlipidemia

Variables	Mono (N=18)	Combined (N=18)	Placebo (N=10)	*p*-value
Systolic Blood Pressure (mmHg), mean (SD)	129.5 (14.4)	131.3 (13.6)	130.0 (14.5)	0.837
Diastolic Blood Pressure (mmHg), mean (SD)	82.8 (9.9)	82.0 (9.7)	80.7 (12.1)	0.965
Heart Rate (/min), mean (SD)	75.1 (8.3)	73.0 (9.6)	73.0 (7.5)	0.681
Body Weight (kg), mean (SD)	67.3 (13.0)	69.2 (9.9)	68.5 (13.4)	0.707
Body Mass Index (kg/m^2), mean (SD)	24.7 (2.9)	26.7 (3.5)	25.3 (3.1)	0.361
Waist Circumference (cm), mean (SD)	88.8 (10.0)	87.2 (7.7)	90.0 (10.9)	0.824
Triglyceride (mg/dL), mean (SD)	218.5 (125.1)	150.6 (58.3)	213.4 (112.0)	0.245
Total Cholesterol (mg/dL), mean (SD)	220.9 (39.9)	221.2 (32.3)	212.5 (37.8)	0.876
Low-Density Lipoprotein Cholesterol (mg/dL), mean (SD)	125.7 (37.1)	137.7 (30.5)	126.9 (48.9)	0.494
High-Density Lipoprotein Cholesterol (mg/dL), mean (SD)	51.5 (17.1)	54.8 (12.8)	42.9 (9.1)	0.093
Uric Acid (mg/dL), mean (SD)	6.4 (1.7)	5.6† (1.3)	7.1† (1.4)	0.029*
Aspartate transaminase (U/L), mean (SD)	28.2 (12.3)	31.3 (10.3)	27.4 (7.5)	0.403
Alanine transaminase (U/L), mean (SD)	33.4 (13.8)	32.2 (16.8)	33.1 (19.3)	0.842
Creatinine (mg/dL), mean (SD)	0.92 (0.18)	0.98 (0.50)	1.00 (0.19)	0.407
Compliance Rate (%), mean (SD)	93.6 (8.0)	96.7 (3.8)	95.7 (4.5)	0.256

*: $p<0.05$ for comparison among three groups by Kruskal-Wallis test. †Combined group had a significantly lower uric acid level than that of the placebo group (by LSD multiple comparison, $p<0.05$).

Table 3. Baseline levels and changes from baseline for blood lipids at month 1, 3 and 6 by group in patients with hyperlipidemia

Variable	Mono (N=18)		Combined (N=18)		Placebo (N=10)	
	Mean	(SD)	Mean	(SD)	Mean	(SD)
Triglyceride (mg/dL)						
Baseline	218.5	(125.1)	150.6	(58.3)	213.4	(112.0)
Change in Month 1	-14.8	(53.6)	-22.8*	(36.9)	6.3	(117.5)
Change in Month 3	-12.8	(83.5)	-12.5	(42.6)	-14.2	(85.0)
Change in Month 6	-56.9**	(74.3)	-31.9**	(43.4)	-39.4	(99.7)
Group effect vs Placebo†	NS‡		NS			
Total Cholesterol (mg/dL)						
Baseline	220.9	(39.9)	221.2	(32.3)	212.5	(37.8)
Change in Month 1	-2.3	(18.3)	-55.1**	(25.7)	-4.7	(42.8)
Change in Month 3	3.0	(18.2)	-48.4**	(32.4)	9.2	(23.2)
Change in Month 6	-0.4	(17.9)	-41.9**	(30.1)	12.5	(22.7)
Group effect vs Placebo	NS		*p<0.0001*			
Low-Density Lipoprotein Cholesterol (mg/dL)						
Baseline	125.7	(37.1)	137.7	(30.5)	126.9	(48.9)
Change in Month 1	0.1	(17.3)	-56.0**	(21.0)	4.4	(25.2)
Change in Month 3	3.9	(21.4)	-47.4**	(27.4)	10.2	(18.1)
Change in Month 6	6.0	(17.5)	-43.3**	(30.1)	16.5	(31.3)
Group effect vs Placebo	NS		*p<0.0001*			
High-Density Lipoprotein Cholesterol (mg/dL)						
Baseline	51.5	(17.1)	54.8	(12.8)	42.9	(9.1)
Change in Month 1	0.3	(3.9)	4.1*	(8.2)	1.1	(5.7)
Change in Month 3	1.7	(5.3)	-0.0	(6.9)	1.8	(5.4)
Change in Month 6	5.0**	(6.5)	6.5**	(8.5)	3.9	(8.6)
Group effect vs Placebo	NS		NS			
Total Cholesterol/HDL-C Ratio						
Baseline	4.6	(1.2)	4.1	(0.6)	5.1	(1.2)
Change in Month 1	-0.12	(0.52)	-1.21**	(0.41)	-0.32	(1.09)
Change in Month 3	-0.07	(0.63)	-0.91**	(0.55)	-0.14	(0.80)
Change in Month 6	-0.41**	(0.59)	-1.09**	(0.54)	-0.28	(0.84)
Group effect vs Placebo	NS		*p<0.0001*			

*:*p<0.05*, **: *p<0.01* compared values with baseline level within group (by Wilcoxon Signed-Rank test). †: Analysis of covariance (ANCOVA) with repeated measures was performed to examine the group effect with the baseline value as a covariate and placebo group as reference. ‡: NS stands for non-significant.

Table 4. Safety data of participants at baseline, month 1, 3 and 6 by group

Variable	Mono (N=18)		Combined (N=18)		Placebo (N=10)	
	Mean	(SD)	Mean	(SD)	Mean	(SD)
Uric Acid (mg/dL)						
Baseline	6.4	(1.7)	5.6	(1.3)	7.1	(1.43)
Change in Month 1	-0.14	(0.97)	-0.29	(0.67)	-0.26	(1.03)
Change in Month 3	-0.37	(0.79)	-0.29*	(0.52)	0.22	(1.07)
Change in Month 6	-0.53	(1.18)	-0.61**	(0.80)	-0.66	(1.29)
Aspartate transaminase (U/L)						
Baseline	28.2	(12.3)	31.3	(10.3)	27.4	(7.5)
Change in Month 6	3.0	(17.9)	-2.9	(7.7)	-1.7	(4.5)
Alanine transaminase (U/L)						
Baseline	33.4	(13.8)	32.2	(16.8)	33.1	(19.3)
Change in Month 6	11.9	(36.7)	-2.0	(12.9)	-2.7	(6.6)
Creatinine (mg/dL)						
Baseline	0.92	(0.18)	0.98	(0.50)	1.00	(0.19)
Change in Month 6	-0.04	(0.13)	0.02	(0.12)	0.00	(0.12)

*:$p<0.05$, **:$p<0.01$ compared values with baseline level within group. Only subjects in the Combined group showed a significant decrease in uric acid in Months 3 and 6 (by Wilcoxon Signed-Rank test, $p<0.05$). No other safety measures in all groups had significant changes during the study period.

Wallis test, except that the level of uric acid was significantly different between the combined formula and placebo groups (p<0.05) (Table 2). At the conclusion of the study, the average compliance rate was 95.3±6.0% for all subjects with 93.6±8.0%, 96.7±3.8%, and 95.7±4.5% for the mono, combined, and placebo group, respectively, with no significant difference among these groups.

Effects of nattokinase on blood lipids

The changes from baseline and the comparison within groups are presented in Table 3. Triglycerides decreased by 56.9±74.3 mg/dL (26%, p<0.01) and HDL-C increased by 5.0±6.5 mg/dL (9.7%, p<0.01) in the subjects who took the mono formula for six months. All of the measurements of blood lipids were changed significantly in the subjects taking the combined formula for one month, including: TG decreasing by 22.8±36.9 mg/dL (15%, p=0.023), TC by 55.1±25.7 mg/dL (25%, p<0.01), LDL-C by 56.0±21.0 mg/dL (41%, p<0.01) and HDL-C increasing by 4.1±8.2 mg/dL (7.5%, p=0.021) at month 1; these changes were sustained until the end of study. In particular, TG and HDL-C changed continuously, as shown in Table3, where TG decreased 31.9±43.4 mg/dL (21%, p<0.01) and HDL-C increased 6.5±8.5 mg/dL (11.9%, p<0.01) at month six. The ratio of TC to HDL-C (TC/HDL-C ratio) also significantly decreased by 0.41± 0.59 (8.9%, p<0.01) at month six in the mono group, while it decreased by 1.21±0.41 (29.5%, p<0.01) at month one and was sustained throughout month six (decreasing by 1.09±0.54, % reduction of 26.6%, p<0.01) in the combined group. No significant changes in the placebo group were observed for any of these blood lipids.

When comparing the changes in the three groups, the combined group had a significantly larger reduction than the placebo group in terms of TC, LDL-C and TC/HDL-C ratio (p<0.0001) after one month of product administration and throughout the study (Table 3), after controlling for the baseline values. The placebo group was used as reference. However, no differences were found between the mono group and the placebo group with respect to changes in blood lipids.

Safety and self-evaluations

The levels of uric acid, AST, ALT, and creatinine remained stable during the entire period (Table 4), except that the combined group had a significantly decreased uric acid levels after 3 months of intake, with a 10.8% reduction (p<0.01) at month six. No notable adverse events were reported among all three groups.

DISCUSSION

There are many commercial nattokinase products promoted as nutritional supplements available in the market worldwide, especially in East Asia. However, data from long-term consumption of nattokinase products in humans are limited in literature. This study is the first randomized clinical trial to assess the efficacy of the oral intake of nattokinase products with a consumption period of as long as six months. In this trial, the nattokinase mono formula significantly reduced TG by 26% and the TC/HDL-C ratios by 8.9%, as well as increasing the HDL-C levels by 10% after a six-month administration period. However, ANCOVA analysis with repeated measures showed that no differences were found between the mono group and the placebo group in all of measured lipids. Although this might be due to a small sample size, nattokinase-mono formula seems to have no significant effect on lowering blood lipids after 6 months of intake in this study. In addition, the effect of the combined formula of nattokinase on blood lipids was more potent than that of the mono formula; the levels of TG, TC, LDL-C, HDL-C and TC/HDL-C ratio were all affected much profoundly by the administration of the combined-formula. For example, the TC/HDL-C ratio in the combined group was 2.7-fold lower than that in the mono group after six months of administration. The results have suggested that long-term intake of combined nattokinase with RYR for-

mula has relatively more potent effects than the nattokinase-mono formula on patients with hyperlipidemia.

It is well established that elevated TC, LDL-C, TC/HDL-C ratio and low HDL-C are strongly predictive of cardiovascular events.[3,4,15] In humans, high levels of TG in the bloodstream have been linked to atherosclerosis, and, by extension, to the risk of heart diseases and stroke. After controlling for baseline levels, only the combined group showed a significant difference in terms of TC and LDL-C levels as well as the TC/HDL-C ratio, when compared with the placebo group. This suggests that the combined formula can decrease CVD risk factors and be considered as a nutraceutical for patients with hyperlipidemia. Although dietary supplementation of RYR alone was not included in this trial, several studies[7,8,16] have reported its lipids-lowering abilities on patients with hyperlipidemia. For example, two reports have proposed that dietary supplementation of RYR in hyperlipidemic patients for two months could significantly reduce LDL-C by 22-28%, TG by 11-22%, and TC by 16-17%,[7,16], which were comparable to our results of LDL-C, TG and TC, respectively, with a percent reduction of 31%, 21% and 19% at month six for the combined group. In this study, the mono formula showed no effects on blood lipids until month six, while the combined formula decreased all lipid indicators from month one. In addition, the combined group had a significantly larger reduction in TC, LDL-C and TC/HDL-C ratio from month one than both mono and placebo groups, while no significant difference was observed between mono and placebo groups. Based on these observations, we have concluded that the beneficial effects of dietary supplementation with the combined formula in patients with hyperlipidemia were mainly due to the effects of RYR on blood lipids.

In the literature, one animal study has shown that natto extracts have a lowering effect on TG and TC in cholesterol-fed rats.[5] In this study, TG was significantly reduced by 26% but not TC after the administration of an oral nattokinase-mono formula for six months in patients with hyperlipidemia, which is not wholly consistent with previous animal studies. One possible explanation is that our investigational nattokinase product has been purified by ultra filtration, whereas the pervious animal study used crude natto extracts. Natto extracts have been reported to contain large amounts of isoflavones that are known to affect blood cholesterol.[17,18] Thus, the purified nattokinase product contained fewer additional ingredients and thus has less effective on blood lipids. On the other hand, if nattokinase itself is involved in the regulation of lipid metabolism, the authors believe that the most probable mechanism is via its proteolytic activity on certain protein targets involved in lipids metabolism. Since enzymes are biocatalysts for reactions, any metabolic reaction would be profoundly affected if enzyme associated with such a reaction were inactivated. For example, monkonlin K inhibited HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis, and thus had a potent effect on decreasing cholesterol levels.[6] In this study, dietary supplementation of nattokinase for six months did not show a remarkable effect on blood lipids, which suggests that the possibility of nattokinase having a direct involvement in the proteolysis of lipid metabolism-associated enzymes is relatively low.

Furthermore, nattokinase is extracted from a traditional Japanese food called natto, which is produced from the fermentation of soybeans by *Bacillus subtilis*.[1] However, soybean-protein consumption may be harmful by increasing plasma levels of uric acid.[9] Moreover, citrinin, a toxin found in RYR, has been associated with hepatotoxicity and nephrotoxicity.[6] The present study has shown that a daily intake of 200 mg of a nattokinase product or 200 mg of nattokianse combined with 1,200 mg RYR for six months did not result in toxicity. All of the measurements used to indicate product safety remained stable throughout the investigation period, including AST, ALT, creatinine, uric acid and vital signs. Moreover, the uric acid increase was ameliorated in patients taking the combined formula after three months, suggesting an advantage of the combined use of nattokianse with RYR in commercial markets. Questionnaire evaluations also revealed no notable adverse events caused by the mono- or combined nattokinase administration. These results support the safety of the nattokinase mono and combined products.

In summary, we found that dietary supplementation of the combined formula of nattokinase with RYR had a much greater effect on lowering lipids than the mono formula. The result suggested that the combined formula would be a better neutraceutical to decrease CVD risk factors for patients with hyperlipidemia compared to the nattokinase-mono formula.

ACKNOWLEDGEMENT

This study was supported by the Industry-Academy Cooperation Project of the Ministry of Education, Taiwan (Project No. F-13-008). The subject care was provided by Drs. Chwen-Yi Yang, Shih-Chung Huang, Bien-Hsien Huang, Shih-Ting Tseng, Chung-Shing Wu, Chia-Chang Yao, Ms Ying-Ching Chung, Mei-Pi Sung and Wan-Ting Lin. We thank GeneFerm Biotechnology Co., Ltd. for providing the investigational products. Clinical trial registration no: NCT00749801.

AUTHOR DISCLOSURES

Nae-Cherng Yang, Chien-Wen Chou, Chung-Yin Chen, Kai-Lin Hwang, and Yi -Chueh Yang had no conflicts of interest.

REFERENCES

1. Tai MW, Sweet BV. Nattokinase for prevention of thrombosis. Am J Health Syst Pharm. 2006;63:1121-3.
2. Urano T, Ihara H, Umemura K, Suzuki Y, Oike M, Akita S, Tsukamoto Y, Suzuki I, Takada A. The profibrinolytic enzyme subtilisin NAT purified from Bacillus subtilis Cleaves and inactivates plasminogen activator inhibitor type 1. J Biol Chem. 2001;276:24690-6.
3. Preiss D, Sattar N. Lipids, lipid modifying agents and cardiovascular risk: a review of the evidence. Clin Endocrinol. 2009;70:815-28.
4. Ferns G, Keti V, Griffin B. Investigation and management of hypertriglyceridaemia. J Clin Pathol. 2008;61:1174-83.
5. Iwai K, Nakaya N, Kawasaki Y, Matsue H. Antioxidative functions of natto, a kind of fermented soybeans: effect on LDL oxidation and lipid metabolism in cholesterol-fed rats. J Agric Food Chem. 2002;50:3597-601.

6. Lin YL, Wang TH, Lee MH, Su NW. Biologically active components and nutraceuticals in the Monascus-fermented rice: a review. Appl Microbiol Biotechnol. 2008;77:965-73.
7. Lin CC, Li TC, Lai MM. Efficacy and safety of Monascus purpureus Went rice in subjects with hyperlipidemia. Eur J Endocrinol. 2005;153:679-86.
8. Liu J, Zhang J, Shi Y, Grimsgaard S, Alraek T, Fønnebø V. Chinese red yeast rice (Monascus purpureus) for primary hyperlipidemia: a meta-analysis of randomized controlled trials. Chin Med. 2006;1:4.
9. Garrel DR, Verdy M, PetitClerc C, Martin C, Brule D, Hamet P. Milk- and soy-protein ingestion: acute effect on serum uric acid concentration. Am J Clin Nutr. 1991;53: 665-9.
10. Rice JA. A nonparametric method – the signed rank test. In: Mathematical statistics and data analysis. Belmont: Wadsworth & Brooks; 1988. p.374-6.
11. Rice JA. A nonparametric method – the Kruskal-Wallis test. In: Mathematical statistics and data analysis. Belmont: Wadsworth & Brooks; 1988. p.406-8.
12. Dawson B, Trapp RG. Multiple-comparison procedures. In: Basic & Clinical Biostatistics. Thrid Edition. Singapore: McGraw-Hill; 2001. p.169-72.
13. Dawson B, Trapp RG. Small expected frequencies & Fisher's exact test. In: Basic & Clinical Biostatistics. Thrid Edition. Singapore: McGraw-Hill; 2001. p.149-51.
14. Kleinbaum DG, Kupper LL, Muller KE. Analysis of covariance and other methods for adjusting continuous data. In: Payne M, editor. Applied regression analysis and other multivariable methods. Boston: PWS-KENT Publishing Company; 1988. p.297-307.
15. Huang CF, Li TC, Lin CC, Liu CS, Shih HC, Lai MM. Efficacy of Monascus purpureus Went rice on lowering lipid ratios in hypercholesterolemic patients. Eur J Cardiovasc Prev Rehabil. 2007;14:438-40.
16. Heber D, Yip I, Ashley JM, Elashoff DA, Elashoff RM, Go VL. Cholesterol-lowering effects of a proprietary Chinese red-yeast-rice dietary supplement. Am J Clin Nutr. 1999;69: 231-6.
17. Taku K, Umegaki K, Sato Y, Taki Y, Endoh K, Watanabe S. Soy isoflavones lower serum total and LDL cholesterol in humans: a meta-analysis of 11 randomized controlled trials. Am J Clin Nutr. 2007;85:1148-56.
18. Xiao CW, Mei J, Wood CM. Effect of soy proteins and isoflavones on lipid metabolism and involved gene expression. Front Biosci. 2008;13:2660-73.

Original Article

Combined nattokinase with red yeast rice but not nattokinase alone has potent effects on blood lipids in human subjects with hyperlipidemia

Nae-Cherng Yang PhD[1], Chien-Wen Chou BS[2], Chung-Yin Chen PhD[3], Kai-Lin Hwang MS[4], Yi -Chueh Yang BS[5]

[1]*Department of Nutrition and Health Science, Chungchou Institute of Technology, Changhua, Taiwan*
[2]*Division of Endocrinology, Chi Mei Medical Center, Tainan, Taiwan*
[3]*Division of Cardiology, Kuang Tien General Hospital, Taichung, Taiwan*
[4]*Department of Public Health, Chung Shan Medical University, Taichung, Taiwan*
[5]*Department of Medical Laboratory, Chi Mei Medical Center, Tainan, Taiwan*

納豆激酶合併紅麴複方而非單方在高血脂病患中有明顯的降血脂效果

此一隨機分配、雙盲、安慰劑對照、平行比較之臨床試驗的目的在評估口服納豆激酶及納豆激酶合併紅麴萃取物對高血脂病患降低血脂的效果。總計有 47 名高血脂病患被分配到三組中的其中一組：1. 納豆激酶單方(50 毫克/膠囊)，2. 納豆激酶合併紅麴萃取物(300 毫克萃取物/膠囊)複方，3. 安慰劑。受試者接受每日兩次、每次兩顆為期六個月的口服劑量。單方組在六個月之前並未顯示有降血脂的效果，而複方組從第一個月開始在各項血脂測量上皆有改善。複方組在以下幾項檢測結果有顯著改變：三酸甘油脂(TG)降低 15%，總膽固醇(TC)降低 25%，低密度膽固醇(LDL-C)降低 41%，TC/高密度膽固醇(HDL-C)比值降低 29.5%，HDL-C 上升 7.5%；且這些改變持續維持到試驗結束。在控制了基礎值(baseline)後，只有複方組在 TC、LDL-C 及 TC/HDL-C 與安慰劑組相較有顯著差異($p<0.0001$)，而單方組則未顯著與安慰劑組不同 ($p>0.05$)。總結而言，本研究提供長期補充納豆激酶的效益評估結果，並顯示納豆激酶複方相較於單方有更明顯的降血脂效果，顯示對於高血脂患者來說，納豆激酶合併紅麴之複方產品相對於納豆激酶單方而言，是更佳的營養療品(neutraceutical)。

關鍵字：納豆激酶、紅麴、人體試驗、高血脂、血脂