Effects of Mulberry Leaf Extract Rich in 1-Deoxynojirimycin on Blood Lipid Profiles in Humans

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Received 10 May, 2010; Accepted 26 May, 2010; Published online 6 August, 2010

Summary Mulberry leaves are rich in 1-deoxynojirimycin (DNJ), an inhibitor of α-glucosidase. We previously showed that DNJ-rich mulberry leaf extract suppressed elevation of postprandial blood glucose in humans. The objective of this study was to evaluate the effects of DNJ-rich mulberry leaf extract on plasma lipid profiles in humans. An open-label, single-group study was conducted in 10 subjects with initial serum triglyceride (TG) level ≥200 mg/dl. Subjects ingested capsules containing DNJ-rich mulberry leaf extract at 12 mg three times daily before meals for 12 weeks. Our findings showed a modest decrease in serum TG level and beneficial changes in the lipoprotein profile following 12-week administration of DNJ-rich mulberry leaf extract. No significant changes in hematological or biochemical parameters were observed during the study period; no adverse events associated with DNJ-rich mulberry leaf extract occurred.

Key Words: 1-deoxynojirimycin, mulberry leaf extract, serum triglyceride, serum lipid profile

Introduction

Intake of food from animal sources, especially those with a higher ratio of lipid energy, increases risk of hyperlipemia, which, in turn may cause arteriosclerosis and even myocardial infarction and cerebral infarction in the long run. It is therefore very important to endeavor to control serum lipids in daily life.

Mulberry (Morus alba L. and other plants of the genus Morus) has traditionally been cultivated in China, Korea, and Japan to use its leaves to feed silkworms (Bombyx mori L.) or as Chinese herbal tea based on folklore. In the modern era, health benefits from mulberry products have been scientifically verified, and naturally occurring 1-deoxynojirimycin (DNJ), a kind of azasugar, was first isolated from its roots by Yagi et al. [1] in 1976. DNJ is a glucose analogue with a secondary amine group instead of an oxygen atom in the pyranose ring of glucose. DNJ potently inhibits α-glucosidase in the small intestine by binding to the active center of α-glucosidase [2]. More recently, DNJ has also been found in the leaves and fruits of M. alba [3, 4], suggesting that the legendary antidiabetic effect of mulberry leaves may be attributed to DNJ, which inhibits postprandial hyperglycemia [2, 4] by inhibiting α-glucosidase in the small intestine. Since then, the preventive effect of mulberry leaves on diabetes has been extensively studied. There have been findings from animal studies sup-
porting the hypothesis that mulberry leaves delay the onset of diabetes, as indicated by the fact that a rapid increase in the postprandial blood glucose level was inhibited [5–7].

We have established a new assay method of DNJ in mulberry leaves [8–10] and developed a method for cultivating and processing mulberry leaves as well as food containing DNJ-rich mulberry leaves [11]. A study of the mulberry leaf food was conducted in healthy and marginally diabetic subjects, showing that it inhibited postprandial increases in the blood glucose and insulin levels [11]. In a long-term treatment study, intake of mulberry leaf DNJ was not associated with abnormalities [11]. A study in rats showed that DNJ and mulberry leaf extract increased the serum adiponectin level and stimulated AMP-activated protein kinase to activate the β-oxidation and thereby inhibit lipid accumulation in the liver [12]. The present study was designed to evaluate the effect of the mulberry extract on serum lipids in humans with relatively high triglyceride (TG) level.

Materials and Methods

Study design

To evaluate the effect of DNJ-rich mulberry leaf extract on serum lipids in humans, a 12-week long-term treatment study was conducted in subjects with relatively high serum TG level (≥200 mg/dl). This was an open-label study designed to carry out investigations before the start of the intake of test food and at weeks 6 and 12. The pretreatment tests were performed twice, and the results from the second test (performed ~2 weeks before the start of the investigation) were regarded as the baseline levels. The study was conducted from January to June 2008. After being reviewed and approved by the institutional review board of the Medical Corporation Nishi Inn (Osaka, Japan), the study was conducted in the Medical Corporation Koshikai Kinki Kenshin Center (Osaka, Japan). It was conducted in accordance with the Declaration of Helsinki (as revised in Tokyo 2004), the Ethical Guidelines for Epidemiological Research (Notification No. 1 of Ministry of Education, Culture, Sports, Science and Technology/Ministry of Health, Labour and Welfare, 2004), and the study protocol.

DNJ-rich mulberry leaf extract

The DNJ-rich mulberry leaf extract was provided in an aluminum pouch packed with 3 hard capsules containing 12 mg of DNJ in total (supplied by Minato Pharmaceutical Co., Ltd., Tokyo, Japan). The extract was given at a dose of 3 capsules three times daily before each meal (9 capsules, equivalent to 36 mg of DNJ, per day) for 12 weeks. Three capsules of the test food contained 5.1 kcal of energy, 0.29 g of protein, 0 g of lipid, 0.97 g of carbohydrate, and 0.44 mg of sodium.

The dose of the test food was determined based on the data from the previous investigations. Briefly, after the intake of mulberry leaf extract or placebo, post-sucrose challenge blood glucose was measured in healthy subjects. By the intake of mulberry leaf extract equivalent to 12 or 18 mg of DNJ, the increase in blood glucose was significantly suppressed 30 and 60 min after the administration of sucrose (50 g) [11], suggesting that the single effective dose of mulberry leaf extract may be equivalent to 12 mg of DNJ. In addition, there were no adverse effects in a 38-day continuous treatment study, in which mulberry leaf extract at a DNJ dose of 18 mg three times daily (equivalent to 54 mg of DNJ per day) was given to healthy subjects [11].

Subjects

Subjects were recruited in accordance with the regulations of the Site Management Organization to enroll those who met all of the inclusion criteria and none of the exclusion criteria. The inclusion criteria were as follows: males between 20 and 64 years of age; Japanese nationality; and serum TG level ≥200 mg/dl. The exclusion criteria were as follows: regular use of pharmaceutical product(s) and/or specified health food to improve serum TG; allergy to study-related food or drug; participation in another clinical study or trial at the start of the present study; continuous pharmacotherapy for any disease; and considered by the investigator to be ineligible for the study based on the laboratory values, etc. As a result, 10 subjects were included in the study. Subjects were instructed to confirm the intake of test food and record any subjective symptom and treatment with pharmaceutical product(s) in a diary, which was used in an interview by a physician for reference.

Test parameters

Alcohol intake was restricted from two days prior to the tests, and no foods or beverages were allowed from the end of dinner on the day before tests to the completion of tests. On each test day, blood and urine were collected in the morning.

In hematologic tests, white blood cell count, red blood cell count, hematocrit, and platelet count were measured. In blood biochemical tests, total TG, chylomicron (CM)-TG, very low density lipoprotein (VLDL)-TG, total cholesterol, low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol, aspartate amino transferase (AST), alanine amino transferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), total bilirubin, albumin, total protein, blood urea nitrogen, creatinine, blood glucose, insulin, hemoglobin, hemoglobin A1c, Na, K, and Cl were measured. In special tests, adiponectin, leptin, apolipoprotein B (ApoB), and lipoprotein fractions were measured [13–15]. Urinalysis, protein and glucose were measured. Plasma
lipoproteins were separated to 20 fractions according to the particle size [CM (2 fractions), large VLDL (3 fractions), medium VLDL, small VLDL, large LDL, medium LDL, small LDL, very small LDL (3 fractions), very large HDL (2 fractions), large HDL, medium HDL, small HDL, and very small HDL (2 fractions)]. Of the 20 fractions, the level of cholesterol in LDL fractions was assessed according to the sub-class particle size: large, medium, small, and very small. In addition, the mean particle size of VLDL, LDL, and HDL was calculated from the particle size of each of the 20 fractions.

Statistical analysis
All test values are expressed as mean ± standard deviation. For each parameter, 1-sample t-test was performed to compare the value at week 6 or 12 with the baseline value (obtained from the second pretreatment test). No multiplicity was taken into consideration. All statistical analyses were performed at a two-sided significance level of 5%.

Results

Subject characteristics
Subject characteristics are shown in Table 1. All subjects were male, with an age of 43.6 ± 7.7 years, a baseline body weight of 79.2 ± 10.8 kg, and a body mass index (BMI) of 28.0 ± 3.7. During intake of test food, no significant change was observed in systolic or diastolic blood pressure or pulse rate, with good compliance as shown by a mean intake rate of 95.7%. No deviation from the protocol was noted.

Analysis population
At week 6, a blood sample from 1 subject was chylous. Also at reexamination performed 1 week later, the blood sample from the same subject was chylous. At the discretion of the investigator, this subject was excluded from the efficacy analysis because the chylous samples were not appropriate for measurement of serum lipids.

Efficacy evaluation
Changes in serum TG and cholesterol over time. The changes in serum TG and cholesterol over time are shown in Table 2. The mean serum TG level decreased from 312 ± 90 mg/dl at baseline to 269 ± 66 mg/dl at week 6 and to 252 ± 78 mg/dl at week 12, but the differences were not statistically significant (week 6, p = 0.200; week 12, p = 0.058). There was also no significant change from baseline at week 6 or 12 in total cholesterol, LDL-cholesterol, or HDL-cholesterol.

Lipoprotein fractions. The changes in CM-TG and VLDL-TG over time are shown in Table 3. The mean CM-TG level was 9.0 ± 5.6 mg/dl at week 12, significantly decreased from baseline (18.6 ± 9.1 mg/dl) (p = 0.031). At week 6, it was 10.5 ± 6.8 mg/dl, decreased from baseline, but with no significant difference (p = 0.068). The mean VLDL-TG level was 139.6 ± 45.9 mg/dl at week 12, increased from baseline (110.3 ± 23.3 mg/dl), but with no significant difference (p = 0.089). At week 6, it was 162.7 ± 46.7 mg/dl, significantly increased from baseline (p = 0.007).

The changes in blood LDL-cholesterol level over time according to the particle size are shown in Table 4. The

Table 1. Subject characteristics

| Parameter          | Unit     | Baseline (Week 0) | Week 6       | Week 12       |
|--------------------|----------|-------------------|--------------|--------------|
| Height             | cm       | 168.3 ± 5.8       | —            | —            |
| Body weight        | kg       | 79.2 ± 10.8       | 78.1 ± 10.5  | 77.1 ± 11.0  |
| BMI                | kg/m²    | 28.0 ± 3.7        | 27.6 ± 3.4   | 27.2 ± 3.5   |
| Systolic blood pressure | mmHg | 129 ± 19        | 128 ± 15     | 131 ± 14    |
| Diastolic blood pressure | mmHg | 84 ± 13         | 82 ± 11      | 81 ± 12     |
| Pulse rate         | beat/min | 85 ± 15         | 77 ± 10      | 79 ± 12     |

Mean ± SD (n = 10). No significant difference was observed, compared with week 0.

Table 2. Changes in serum TG and cholesterol over time

| Parameter         | Reference range | Unit  | Baseline (Week 0) | Week 6       | Week 12       |
|-------------------|-----------------|-------|-------------------|--------------|--------------|
| Serum TG          | 30–149          | mg/dl | 312 ± 90          | 269 ± 66     | 252 ± 78     |
| Total cholesterol | 130–220         | mg/dl | 232 ± 25          | 243 ± 20     | 230 ± 21     |
| LDL-cholesterol   | 70–139          | mg/dl | 139 ± 32          | 149 ± 25     | 145 ± 23     |
| HDL-cholesterol   | 40–90           | mg/dl | 42 ± 6            | 44 ± 6       | 43 ± 5       |

Mean ± SD (n = 9). No significant difference was observed, compared with week 0.
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The changes in the mean particle size of VLDL, LDL, and HDL over time are shown in Table 5. The mean particle size of VLDL was 47.03 ± 0.91 nm at baseline, significantly decreased at week 6 to 45.92 ± 1.42 nm (p = 0.007), and further decreased to 45.87 ± 1.19 nm at week 12 (p = 0.001). The mean particle size of LDL was 24.12 ± 0.38 nm at baseline, significantly increased at week 6 to 24.58 ± 0.29 nm (p = 0.001), and further increased to 24.48 ± 0.29 nm at week 12 (p = 0.014). The mean particle size of HDL was 10.40 ± 0.18 nm at baseline, significantly increased at week 6 to 10.51 ± 0.13 nm (p = 0.007), and further increased to 10.54 ± 0.19 nm at week 12 (p = 0.014).

Changes in adiponectin, leptin, and ApoB over time. The changes in adiponectin, leptin, and ApoB over time are shown in Table 6. The mean adiponectin level was 6.3 ± 2.1 μg/ml at baseline, significantly decreased at week 6 to 4.9 ± 1.5 μg/ml (p = 0.001) and further decreased to 4.7 ± 1.4 μg/ml at week 12 (p = 0.014). The mean leptin level was 5.2 ± 3.9 mg/dl at baseline, significantly increased at week 6 to 6.1 ± 4.4 mg/dl (p = 0.001) and further increased to 6.7 ± 5.2 mg/dl at week 12 (p = 0.014).

Table 3. Changes in CM-TG and VLDL-TG over time

| Parameter | Unit | Baseline (Week 0) | Week 6 | Week 12 |
|-----------|------|------------------|--------|---------|
| CM-TG     | mg/dl| 18.6 ± 9.1       | 10.5 ± 6.8 | 9.0 ± 5.6* |
| VLDL-TG   | mg/dl| 110.3 ± 23.3     | 162.7 ± 46.7** | 139.6 ± 45.9 |

Mean ± SD (n = 9). * p<0.05, ** p<0.01, compared with week 0.

Table 4. Changes in the blood LDL-cholesterol level over time according to the particle size

| Parameter       | Unit | Baseline (Week 0) | Week 6 | Week 12 |
|-----------------|------|------------------|--------|---------|
| Large LDL       | mg/dl| 18.1 ± 5.2       | 22.2 ± 5.6 | 20.7 ± 4.7* |
| Medium LDL      | mg/dl| 32.8 ± 8.9       | 42.4 ± 10.3** | 43.4 ± 9.3** |
| Small LDL       | mg/dl| 28.7 ± 7.1       | 34.7 ± 7.3** | 36.2 ± 6.9** |
| Very small LDL  | mg/dl| 21.0 ± 4.4       | 16.0 ± 2.7** | 16.9 ± 3.4* |

Mean ± SD (n = 9). * p<0.05, ** p<0.01, compared with week 0. Numbers in parentheses represent percent distribution among the for LDL subfractions.

Table 5. Changes in the mean particle size of VLDL, LDL, and HDL over time

| Parameter | Unit | Baseline (Week 0) | Week 6 | Week 12 |
|-----------|------|------------------|--------|---------|
| VLDL      | nm   | 47.03 ± 0.91     | 45.92 ± 1.42* | 45.87 ± 1.19** |
| LDL       | nm   | 24.12 ± 0.38     | 24.58 ± 0.29** | 24.48 ± 0.29* |
| HDL       | nm   | 10.40 ± 0.18     | 10.51 ± 0.13 | 10.54 ± 0.19** |

Mean ± SD (n = 9). * p<0.05, ** p<0.01, compared with week 0.
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Table 6. Changes in adiponectin, leptin, and ApoB over time

| Parameter | Unit     | Baseline (Week 0) | Week 6 | Week 12 |
|-----------|----------|-------------------|--------|--------|
| Adiponectin | μg/ml    | 6.3 ± 2.1         | 4.9 ± 1.5** | 4.6 ± 1.4** |
| Leptin    | ng/ml    | 6.3 ± 4.1         | 5.2 ± 3.9** | 4.7 ± 4.4** |
| ApoB      | mg/dl    | 99 ± 9            | 104 ± 13 | 90 ± 12 |

Mean ± SD (n = 9). ** p<0.01, compared with week 0.

Table 7. Hematologic tests

| Parameter                  | Reference range | Unit | Baseline (Week 0) | Week 6 | Week 12 |
|----------------------------|-----------------|------|-------------------|--------|--------|
| White blood cell count     | 3.9–9.8         | 10³/μl | 5.8 ± 1.1         | 6.3 ± 1.7 | 6.2 ± 2.3 |
| Red blood cell count       | 431–565         | 10³/μl | 506 ± 38          | 505 ± 23 | 501 ± 21 |
| Hemoglobin                | 13.7–17.4       | g/dl  | 15.6 ± 1.0        | 15.4 ± 0.6 | 15.5 ± 0.7 |
| Hematocrit                | 40.2–51.5       | %     | 45.5 ± 2.7        | 45.3 ± 2.1 | 45.2 ± 1.9 |
| Platelet count            | 13.4–34.9       | 10³/μl | 26.1 ± 5.1        | 25.5 ± 5.8 | 25.6 ± 4.7 |

Mean ± SD (n = 10). No significant difference was observed, compared with week 0.

tively, significantly decreased from baseline (6.3 ± 4.1 ng/ml) (week 6, p = 0.002; week 12, p = 0.001). The mean ApoB level was 104 ± 13 and 90 ± 12 mg/dl at weeks 6 and 12, respectively, showing no significant difference from baseline (99 ± 9 mg/dl).

Safety evaluation

The safety was evaluated in 10 male subjects who ingested the test food.

Laboratory parameters. The changes in the laboratory parameters other than serum lipids over time are shown in Tables 7 and 8. There was no change indicative of an adverse event in any subject. The mean blood urea nitrogen level was significantly increased at week 12 (baseline, 11.6 mg/dl; week 12, 12.9 mg/dl), and the mean Na level was significantly increased at weeks 6 and 12 (baseline, 141 mEq/l; week 6, 143 mEq/L; week 12, 143 Eq/l). However, all of these were slight changes within the reference range, with no clinical significance.

Adverse events. A list of reported events is presented in Table 9. A total of 5 events were reported in 2 subjects: soft stools, nasal congestion/headache, myalgia, pharyngeal pain/cough, and fever (1 event each). All of these events were mild in severity and considered by the investigator to be unrelated to the test food.

Discussion

Elevation of serum TG, like elevated LDL-cholesterol level, is a risk factor for arteriosclerosis [16]. Hypertriglyceridemia may be attributed to excessively elevated serum VLDL level, which occurs because of increased VLDL synthesis and decreased lipoprotein lipase, a process that degrades VLDL to LDL (abnormal metabolism of VLDL) [17].

We previously investigated the metabolic effects of DNJ-enriched mulberry extract for the prevention of diabetes [11, 12]. In rats, administration of DNJ strongly attenuated lipid accumulation through β-oxidation, which was promoted by increased serum adiponectin level and activation of AMP-activated protein kinase (AMPK) [12]. We therefore conducted the present study to evaluate the effects of DNJ in humans with high serum TG level.

We found that serum TG level was decreased in 7 of 9 subjects at week 12 following administration of DNJ. Mean serum TG level was decreased by 43 mg/dl at week 6, and by 60 mg/dl at week 12 (Table 2). In two subjects, serum TG level was decreased below 150 mg/dl, the upper limit of the reference range. Overall, there were no significant differences at week 12 when compared to baseline. The lack of statistical significance may have been attributed to markedly increased serum TG level in two other subjects. Future studies with larger sample sizes are required to confirm the effects of DNJ on serum TG level in humans.

Mean CM-TG level, which reflects the transport of exogenous lipids (primarily derived from food), was 50% lower at week 12, when compared to baseline. Mean VLDL-TG level, which reflects the transport of endogenous lipids, was transiently higher at week 6, but did not differ significantly from baseline at week 12 (Table 3). Since blood samples were collected after restricting intake of high-fat foods in the day preceding the blood test, and after
a 12 h fast, serum chylomicron level was not significantly confounded by foods ingested within the past two days.

No significant differences were seen for cholesterol level at week 12. We assessed changes in serum lipoprotein levels and size of lipoprotein particles. LDL, derived from VLDL, is commonly referred to as “bad cholesterol”, because excess LDL-cholesterol in blood results in vascular cholesterol deposition. Smaller, or very-low-density LDL particles (small and very small LDL fractions) are called “very bad cholesterol” because they are often oxidized and frequently induce adverse effects, including atherosclerosis. Administration of DNJ resulted in a significant decrease in very-small LDL fraction and a significant increase in medium LDL fraction (Table 4). Although we observed a significant increase in small LDL fraction, the increase was modest after small LDL fraction was normalized by the total of the four LDL subfractions; which increased by 15–17% at week 12 following administration of DNJ-rich mulberry leaf extract.

Thus, based on percentage distribution, we found that use of DNJ-rich mulberry leaf extract increased medium LDL fraction, and decreased very small LDL fraction. The mean particle size of VLDL was significantly decreased, but mean particle sizes of LDL and HDL were significantly increased (Table 5). Recent studies have linked abnormalities in LDL structure to risks of cardiovascular disease [18]. Our findings suggest that use of DNJ-rich mulberry leaf extract may be beneficial by improving plasma lipoprotein profile.

Adiponectin and leptin—hormones involved in stimulation of fat burning, were significantly decreased (Table 6). It is generally accepted that stimulation of fat burning improves dyslipidemia. In the present study, changes seen in the plasma lipid profiles of subjects who took DNJ-rich mulberry leaf extract appeared to be uncorrelated with changes in adiponectin and leptin levels.

In conclusion, use of DNJ-rich mulberry leaf extract modestly decreased TG level after 12-week intake, although the decrease was not statistically significant. DNJ-rich
mulberry leaf extract may be potentially used to decrease very bad cholesterol. Additional studies with larger sample sizes are warranted. In terms of the safety of oral DNJ-rich mulberry leaf extract, there were changes in some laboratory parameters in the long-term treatment study. No clinically significant changes in laboratory parameters were seen during the study; there were also no reports of subjective symptoms or adverse events related to use of DNJ-rich mulberry leaf extract. Our findings confirm that short-term use of DNJ-rich mulberry leaf extract is safe.

Acknowledgments

This study was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan for the project “Development of Evaluation and Management Methods for Supply of Safe, Reliable and Functional Food and Farm Produce”.

Abbreviations

DNJ, 1-deoxynojirimycin; TG, triglyceride; CM, chylomicron; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; AST, aspartate amino transferase; ALT, alanine amino transferase; γ-GTP, γ-glutamyl transpeptidase; Apo B, apolipoprotein B; BMI, body mass index; AMPK, AMP-activated protein kinase.

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