Effect of mulberry leaf extract with enriched 1-deoxynojirimycin content on postprandial glycemic control in subjects with impaired glucose metabolism

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ABSTRACT

Aims/Introduction: The glucose analogue, 1-deoxynojirimycin (DNJ), found in mulberry (*Morus alba*) leaves, is a promising α-glucosidase inhibitor. We evaluated the effect of the ingestion of mulberry leaf extract with enriched DNJ content on postprandial hyperglycemia in subjects with impaired glucose metabolism.

Materials and Methods: In study 1, we carried out a randomized, double-blind, crossover trial to assess the effects of single ingestion of mulberry leaf extract (3, 6 or 9 mg DNJ) or placebo on blood glucose and insulin concentrations during 2 h after a carbohydrate (200 g boiled white rice) challenge in 12 subjects with fasting plasma glucose (FPG) in the range of 100–140 mg/dL. Study 2 was a randomized, double-blind, placebo-controlled trial to assess the efficacy of 12-week extract supplementation (6 mg DNJ, t.i.d.) for long-term glycemic control in 76 subjects with FPG in the range of 110–140 mg/dL.

Results: In study 1, ingestion of the mulberry leaf extract led to attenuated postchallenge acute glycemia in a dose-dependent manner ($P = 0.006$, group × time interaction, two-way ANOVA). In study 2, the serum 1,5-anhydroglucitol concentration, a sensitive indicator of postprandial glycemic control, in the extract group increased and was higher than that in the placebo group over the 12-week treatment period ($P < 0.001$, group × time interaction, two-way ANOVA); no differences in FPG, glycated hemoglobin and glycated albumin concentrations were observed between the groups.

Conclusions: Long-term ingestion of mulberry leaf extract with enriched DNJ content could result in improved postprandial glycemic control in individuals with impaired glucose metabolism. These trials were registered with UMIN (no. UMIN000003154 and UMIN000003155). (J Diabetes Invest, doi: 10.1111/j.2040-1124.2011.00101.x, 2011)

KEY WORDS: 1-Deoxynojirimycin, α-Glucosidase inhibitor, Postprandial hyperglycemia

INTRODUCTION

Epidemiological evidence indicates that postprandial hyperglycemia is an independent risk factor for cardiovascular disease[1]. Several experimental results also suggest that postprandial hyperglycemic spikes contribute to the pathophysiology of diabetic cardiovascular complications[2]. Improvement of postprandial glycemic control is therefore the most promising approach to decrease the morbidity and mortality associated with cardiovascular disease in individuals with prediabetes and diabetes. To date, several intervention trials have shown that intensive diet and exercise programs[3], and pharmacological interventions[4–8] help to prevent or delay the progression to type 2 diabetes in subjects with impaired glucose metabolism, although not all pharmacological interventions are effective[4,5,9,10]. Among the trials, the STOP-NIDDM Trial[11,12] showed that the treatment with acarbose, an α-glucosidase inhibitor (αGI), in subjects with impaired glucose tolerance is associated with a significant risk reduction not only in the progression to type 2 diabetes, but also in the development of cardiovascular events. Much attention has therefore been focused on αGI as preventive and therapeutic agents for type 2 diabetes and its complications.

Mulberry (*Morus alba* L.) leaves have been widely cultivated for sericulture from ancient times. The leaves are also consumed as herbal tea in Asian countries. Mulberry leaves are rich in amino group-containing sugar analogs, termed iminosugars[13]. The glucose analog 1-deoxynojirimycin (DNJ), the most abundant iminosugar in mulberry leaves, is reportedly a potent αGI[13–16]. Consequently, mulberry leaves have recently received much interest as a herbal supplement, especially for individuals with prediabetes. At present, various mulberry leaf products are

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available on the market as food supplements; however, there have been few assessments of the effect of such products on postprandial glycemic control.

Previously, we found that the DNJ content in most commercially available mulberry leaf products is too low (approximately 0.1% of dry matter) to exert an αGI effect[17]. Thus, we developed a procedure to enrich the DNJ content (approximately 1.5%) in mulberry leaf extract, and observed that ingestion of this extract resulted in reduced post-sucrose challenge glycemia in healthy subjects[18]. In the present study, we first evaluated the effective dose of DNJ on postprandial glycemic control in subjects with impaired glucose metabolism by using a carbohydrate (200 g boiled white rice) tolerance test (study 1). Furthermore, we carried out a 12-week supplementation trial (study 2) to ascertain the long-term efficacy of the ingestion of mulberry leaf extract with enriched DNJ content.

MATERIALS AND METHODS

Subjects and Materials

The studies were approved by the responsible ethics review boards, and written informed consent was obtained from all subjects. All the subjects were recruited from the Soiken Inc., volunteer database (Osaka, Japan). Exclusion criteria included pregnancy, lactation, severe medical illness and concurrent use of any agent for blood glucose control. The preparation of mulberry leaf extract and quantification of the DNJ content were carried out as reported previously[18].

Carbohydrate Tolerance Test (Study 1)

Study 1 was a randomized, double-blind, four-period, crossover trial. Twelve subjects (9 men and 3 women, aged 49.7 ± 10.3 years) with fasting plasma glucose (FPG) in the range of 100–140 mg/dL (at the screening visit within 2 months before the first tolerance test day) were recruited and randomly assigned to one of four treatment sequences: A-D-C-B, B-A-D-C, C-B-A-D and D-C-B-A. Here, A = 0 + 3, B = 1 + 2, C = 2 + 1 and D = 3 + 0 of mulberry leaf extract capsule(s) plus identical placebo capsule(s), corresponding to 0, 3, 6 and 9 mg DNJ, respectively. On each test day (each test separated by 2 weeks), after overnight fasting, the subjects ingested one of the four DNJ doses (A, B, C or D) with 100 mL of water. The capsules were packaged in a coded aluminum pouch with three capsules per dose so that neither the staff nor the subject was aware of the type of treatment. A total of 15 min after the capsule ingestion, all subjects consumed 200 g of boiled white rice (Sato-no-gohan; Satosyokuhin, Niigata, Japan) with 2 g of dry seasoning (Ajidoraku Furikake; Marumiya, Tokyo, Japan). The test meal provided 311 kcal, with 70.0 g carbohydrate, 4.8 g protein and 1.3 g fat, and was consumed within 8 min. Blood samples were drawn before the capsule ingestion and 30, 60, 90 and 120 min after starting the meal. All subjects were instructed to maintain their usual physical activities and dietary habits throughout the study period. Dietary intake and alcohol consumption during the 3 days before each tolerance test were assessed by self-reports.

Long-term Supplementation Trial (Study 2)

Study 2 was a 12-week randomized, double-blind, placebo-controlled trial followed by a 4-week post-treatment observation period. A total of 76 subjects (50 men and 26 women, aged 53.7 ± 6.7 years) with FPG in the range of 110–140 mg/dL (at the screening visit in the 4 or 5 weeks before the start of the intervention) were recruited and randomized to receive either the mulberry leaf extract or the placebo. During the 12-week trial, mulberry leaf extract (6 mg DNJ) or identical placebo tablets were ingested t.i.d. before meals. The tablets were packaged in an aluminum pouch with three tablets per dose as described in the preceding subsection. Every 4 weeks of the supplementation period (week 0, 4, 8 and 12) and 4 weeks after withdrawal of the supplementation (week 16), anthropometric data and blood samples were collected from the subjects after they had fasted overnight, adverse effects were assessed by interview and self-reports, and compliance was checked by self-report and returned tablet count. Urine samples were collected at week 0, 12 and 16. Throughout the study period (from screening to post-treatment), the subjects were instructed to maintain their usual lifestyle (avoid excessive eating and drinking, intense exercise and lack of sleep). Dietary intake, alcohol consumption and physical activity (pedometer count) during the 3 days before each test visit were also assessed by self-reports.

Clinical Measurements

In both studies, bodyweight, blood pressure and heart rate were measured at every visit (both screening and test visits), but height was measured once at the screening visit. Waist and hip circumferences were measured at every visit in study 2. Blood and urine samples were analyzed at Kishimoto Clinical Laboratory (Tomakomai, Japan) and SRL (Tachikawa, Japan). Plasma glucose concentrations were measured by using a glucose oxidase method. Insulin concentrations were measured by using radioimmunoassay. Glycated hemoglobin (HbA_{1c}) was measured by using a latex agglutination method. The HbA_{1c} values are expressed in National Glycohemoglobin Standardization Program units (%) according to the guideline of the Japanese Diabetes Society[19]. Glycated albumin (GA) and 1,5-anhydroglucitol (1,5AG) concentrations were measured by using enzymatic methods. The other blood measurements and urinalysis were analyzed by using the standardized methods. The mean values at the screening visit and week 0 were used as the baseline values of the anthropometric and blood measurements in study 2.

Statistical Analysis

The data are expressed as the mean ± SD. Statistical analyses were carried out by using SPSS (version 11.5; SPSS, Chicago, IL, USA), and the significance was defined as $P < 0.05$. In study 1, two-way ANOVA for repeated measurements was carried out to assess the changes over time in plasma glucose and insulin among the four groups. The differences among the group means at each time-point were assessed by using paired *t*-tests with the Bonferroni correction. In study 2, two-way ANOVA for repeated

measurements was carried out to assess the changes over time in the glycemic control parameters (FPG, insulin, HbA_{1c}, GA and 1,5AG) between the groups. The changes in other biochemical measurements, anthropometric data, dietary habits and physical activity were also assessed by repeated-measures ANOVA. The Bonferroni correction was applied for multiple comparisons. The differences between the groups in the values at each time-point were assessed by using unpaired *t*-tests.

RESULTS

Effect of Mulberry Leaf Extract Ingestion on Carbohydrate Tolerance

Of the 12 subjects enrolled, one was withdrawn because of the difficulty in multiple blood collections within 120 min. Data from another subject were excluded before disclosure of treatment assignments as a result of major lifestyle changes (overseas business trips, twice) during the four test visits. Finally, data from 10 subjects (8 men and 2 women) were included in the statistical analysis. The mean age of these subjects was 50.0 ± 10.6 years and the mean body mass index (BMI) was 24.3 ± 1.7 kg/m^2. There were no significant differences in anthropometric data, biochemical measurements and reported dietary habits (total energy, carbohydrate, protein, fat, cholesterol, fiber and alcohol) among the four groups. Furthermore, no adverse events were observed over the study period.

Ingestion of mulberry leaf extract with enriched DNJ content tended to attenuate postprandial acute glycemia in a dose-dependent manner ($P = 0.006$, group × time interaction; Table 1). The plasma glucose concentration at 30 min was significantly lower in the 6 and 9 mg DNJ groups (treatments C and D, respectively) than in the placebo group (0 mg DNJ, treatment A). The serum insulin concentration at 30 min was also lower in the three groups that were given mulberry leaf extract (3, 6 and 9 mg DNJ) than in the placebo group; however, the overall change in the insulin concentration over 120 min did not differ statistically among the four groups.

Efficacy of Mulberry Leaf Extract Supplementation for Long-term Glycemic Control

Of the 76 subjects enrolled, eight withdrew for personal reasons during the trial. Data from three subjects were excluded before disclosure of treatment assignments as a result of major lifestyle changes (two subjects) and exclusion criteria (one subject, disclosed after enrolment). Finally, data from 65 subjects ($n = 33$ in the mulberry leaf extract group [21 men and 12 women, aged 53.6 ± 5.8 years] and $n = 32$ in the placebo group [22 men and 10 women, aged 53.5 ± 7.5 years]) were included in the statistical analysis. There were no significant differences in baseline characteristics, including the glycemic control parameters (Table 2), anthropometric measurements and serum lipid profiles (Supporting Information Table S1), between the mulberry leaf extract and the placebo groups. The average energy intake during the 3 days before the initial test visit (at week 0), as calculated from the self-reports, was statistically different between the groups; however, the difference was not observed at any other time-point (Supporting Information Table S2). Furthermore, no significant differences were observed between the groups in intake of each type of nutrient (carbohydrate, protein, fat, cholesterol and fiber), alcohol consumption and pedometer counts. Hence, the effects of dietary habits and physical activity were few, if any, in the study. The mean compliance to the prescribed dose of mulberry leaf extract and placebo was 99.4 ± 1.4% and 99.0 ± 1.8%, respectively.

Among the glycemic control parameters, the change in the serum 1,5AG concentration over the study period was significantly different between the groups ($P < 0.001$, group × time interaction; Table 2). The 1,5AG concentration of the mulberry leaf extract group increased from the baseline through to week 4, 8 and 12, and was higher than that of the placebo group at week 8 and 12. The increased 1,5AG concentration in the extract group returned to the baseline level after the 4-week post-treatment observation period (at week 16). FPG and insulin concentrations were not significantly changed over the study

Table 1 | Plasma glucose and insulin concentrations after a carbohydrate (200-g boiled white rice) challenge in the four 1-deoxynojirimycin (DNJ) dose groups (study 1)

Parameter/Group	Baseline	30 min	60 min	90 min	120 min	*P**
Glucose (mg/dL)						
0 mg DNJ (Placebo)	110 ± 10	145 ± 28[a]	171 ± 40	162 ± 37	158 ± 37	0.006
3 mg DNJ	117 ± 13	131 ± 21[a,b]	171 ± 36	174 ± 38	157 ± 29	
6 mg DNJ	116 ± 19	121 ± 19[b]	166 ± 28	178 ± 34	146 ± 41	
9 mg DNJ	108 ± 9	114 ± 12[b]	147 ± 23	160 ± 37	151 ± 45	
Insulin (μU/mL)						
0 mg DNJ (Placebo)	8.0 ± 2.2	15.8 ± 6.8[a]	26.0 ± 17.2	27.7 ± 19.7	28.8 ± 14.5	0.395
3 mg DNJ	9.5 ± 6.8	11.1 ± 4.5[b]	18.9 ± 9.7	29.0 ± 17.7	26.2 ± 13.0	
6 mg DNJ	8.1 ± 3.9	9.2 ± 1.9[b]	18.7 ± 7.8	25.3 ± 11.5	25.2 ± 10.8	
9 mg DNJ	7.3 ± 1.4	10.0 ± 3.1[b]	17.5 ± 7.4	21.9 ± 7.0	24.7 ± 13.6	

Data are mean ± SD ($n = 10$). *Two-way ANOVA, group × time interaction.
Values with different superscript letters at a time point are significantly different at $P < 0.05$ by paired *t*-test with Bonferroni correction.

Table 2 | Changes in the concentrations of fasting plasma glucose (FPG), insulin, glycated hemoglobin (HbA_{1c}), glycated albumin (GA), and 1,5-anhydroglucitol (1,5AG) during the 12-week supplementation with mulberry leaf extract (6 mg 1-deoxynojirimycin, thrice daily) or placebo followed by 4-week observation without supplementation (study 2)

Parameter/Group	Baseline	Week 4	Week 8	Week 12	Posttreatment observation (week 16)	*P**
FPG (mg/dL)						
Mulberry leaf extract	114 ± 7	115 ± 10	112 ± 10	114 ± 11	117 ± 10	0.326
Placebo	115 ± 6	114 ± 9	114 ± 8	112 ± 12	115 ± 9	
Insulin (μU/mL)						
Mulberry leaf extract	7.2 ± 3.7	7.4 ± 3.6	6.8 ± 2.9	7.3 ± 5.1	8.3 ± 7.5	0.740
Placebo	7.0 ± 3.4	7.0 ± 3.4	6.8 ± 2.9	6.7 ± 3.3	7.0 ± 3.9	
HbA_{1c} (%)						
Mulberry leaf extract	6.0 ± 0.4	5.9 ± 0.3†	5.9 ± 0.3†	5.8 ± 0.4†	5.8 ± 0.4†	0.687
Placebo	5.9 ± 0.4	5.8 ± 0.4†	5.9 ± 0.4	5.8 ± 0.4†	5.8 ± 0.4†	
GA (%)						
Mulberry leaf extract	16.9 ± 1.6	16.8 ± 1.5	16.5 ± 1.5†	16.5 ± 1.5†	16.2 ± 1.5†	0.608
Placebo	17.1 ± 1.8	17.1 ± 1.8	16.8 ± 1.7†	16.7 ± 1.8	16.5 ± 1.8†	
1,5AG (μg/mL)						
Mulberry leaf extract	17.5 ± 6.0	19.6 ± 6.4†	21.0 ± 6.2†,‡	20.5 ± 6.7†,‡	18.5 ± 6.6	<0.001
Placebo	17.0 ± 5.6	17.2 ± 5.3	17.7 ± 5.5	17.4 ± 5.6	17.1 ± 5.5	

Data are mean ± SD (n = 33 in the extract group, n = 32 in the placebo group). *Two-way ANOVA, group × time interaction. †Significantly different from the baseline value ($P < 0.05$ with Bonferroni correction). ‡Significantly different compared with the placebo value at each time point ($P < 0.05$ by unpaired t-test).

period in both groups. HbA_{1c} and GA concentrations were reduced during the study period in both groups; however, there were no significant differences at any time-point between the groups.

No serious adverse events were observed over the study period (including in the withdrawn and excluded subjects). Although the cause of two adverse events (abdominal distension and modest proteinuria) could not be ascertained, both events were observed in the placebo subjects. Several anthropometric and blood measurements changed during the study period in both groups; however, no significant differences were observed between the groups at any time-point (Supporting Information Table S1).

DISCUSSION

Although much attention has been focused on the αGI effect of DNJ in mulberry leaves, the effective dose of DNJ to improve postprandial glycemic control has not been well-defined. Furthermore, there are few data regarding the long-term efficacy of mulberry leaf extract supplementation in individuals with prediabetes. Recently, we showed that ingestion of mulberry leaf extract with enriched DNJ content (12 mg DNJ) effectively reduced hyperglycemia after a 50-mg sucrose challenge in healthy subjects[18]. The results of the carbohydrate tolerance test (study 1) confirm the αGI effect of such extracts (3–9 mg DNJ) on postprandial hyperglycemia in individuals with prediabetes and mild diabetes. Levitt and coworkers[20,21] have recently reported similar effects of a mulberry leaf-containing extract on postchallenge hyperglycemia. Although the DNJ content of their extract was not determined accurately, they claimed that the extract contained 5 mg of 'deoxynojirimycin-type compounds' per dose[20].

On the basis of the positive results of the tolerance test (study 1), we analyzed the efficacy of the extract for long-term glycemic control (study 2). Over the 12-week supplementation period, the serum 1,5AG concentration increased in the extract group, in which subjects ingested 6 mg of DNJ thrice daily before each meal. The serum 1,5AG concentration is reportedly maintained at a constant level under euglycemic conditions; however, it decreases as a result of competitive inhibition of renal tubular reabsorption by glycosuria under hyperglycemic conditions[22,23]. Serum 1,5AG concentrations therefore respond sensitively to blood glucose fluctuations within a few days, reflecting even transient elevations as a result of postprandial hyperglycemic spikes[24–26]. Hence, the increased 1,5AG concentration could reflect continuous reduction in postprandial hyperglycemic spikes over the 12-week supplementation period. Indeed, the increased 1,5AG concentration returned to the baseline level after the 4-week withdrawal of extract supplementation.

In contrast, no significant differences in FPG, HbA_{1c} and GA concentrations were observed between the groups. Whereas the 1,5AG concentration responded to glycemic fluctuations within a few days, HbA_{1c} and GA concentrations reflected time-averaged glycemia in the past 2–3 months and past 2–3 weeks, respectively. In contrast to the present results, a meta-analysis of αGI

drug trials in patients with type 2 diabetes showed that FPG and HbA_{1c} concentrations are lowered by acarbose and miglitol[27]. Because the subjects in those trials had diabetes, their baseline HbA_{1c} and FPG concentrations were much higher than those in the present trial. In addition, most of the trials were carried out for much longer durations (≥12-week trials were enrolled in the meta-analysis). In the meta-analysis, the HbA_{1c}-lowering efficacy of acarbose was observed to have a positive correlation with the basal HbA_{1c} concentrations. Thus, the absence of significant effects on the FPG and HbA_{1c} concentrations in the present trial might be a result of, at least in part, the lower baseline concentrations of the subjects. In addition, the dose and duration of extract supplementation might not be enough to decrease the longitudinal average of blood glucose effectively, even though postprandial hyperglycemic spikes were continuously suppressed over the 12-week supplementation period.

Although we measured post-carbohydrate challenge blood glucose concentration only within 120 min in study 1, Mudra *et al.*[21] reported that mulberry leaf extract ingestion in subjects with type 2 diabetes delayed post-sucrose challenge blood glucose response and therefore led to higher blood glucose concentrations than placebo during 120–240 min after the challenge. Thus, no significant differences in HbA_{1c} between the groups in study 2 might be the result of little change in total increment of postprandial blood glucose (extrapolated to infinite time). Despite the little effect on HbA_{1c}, the inhibitory effect of mulberry leaf extract on blood glucose fluctuations, as shown by increased 1,5AG concentrations, might support the beneficial effect of the extract supplementation on cardiovascular outcomes, because postprandial hyperglycemic spikes are postulated to play important roles in the pathogenesis of diabetic cardiovascular complications[1,2].

Gastrointestinal symptoms, such as abdominal distension, diarrhea and flatulence, are the most frequent adverse events of αGI agents. These symptoms contributed to the high withdrawal rate of acarbose in the STOP-NIDDM Trial[11]. The lack of such symptoms in the present trial therefore suggests the excellent safety and tolerability of mulberry leaf extract; however, additional trials with higher doses, in subjects with higher postprandial glucose and for longer durations are necessary to confirm the efficacy (including cardiovascular outcomes), safety and tolerability.

In study 1 and study 2, we studied subjects with FPG 100–140 and 110–140 mg/dL, respectively. Thus, the subjects should include the categories of diabetes and prediabetes (study 1 and study 2) as well as normal glucose metabolism (study 1) based on the classification of the Japanese Diabetes Society[19]. The heterogeneity might limit the strength of our conclusions, as the efficacy of mulberry leaf extract might differ by the status of carbohydrate metabolism impairment. In addition, because the participants in study 2 were free-living and self-selecting their foods, there might be some bias, such as some participants encouraged lifestyle modifications during the study period by themselves. The bias might affect the changes in bodyweight, BMI, HbA_{1c} and GA in both groups. The lifestyle modification bias (in particular, food selection) and seasonal variations might also contribute some changes in lipid profile; nonetheless, no significant differences were observed between the groups.

Increasing evidence strongly supports the efficiency of interventions for preventing or delaying the onset of diabetes in individuals with prediabetes. Accordingly, many expert committees now recommend that such individuals should be counseled regarding lifestyle changes. Furthermore, some pharmacological approaches are assumed to help such individuals, especially those who are at very high risk for progression to diabetes or who are unable to achieve adequate glycemic control by lifestyle modification alone. However, few medications that prevent or delay the onset of diabetes in individuals with impaired glucose metabolism are presently covered by health insurance. Meanwhile, although there is no internationally agreed definition, numerous 'functional foods' with approved health claims are now available worldwide[28]. With regard to glucose metabolism, for example, several foods that possibly decrease the rate of glucose absorption (e.g. foods containing indigestible dextrins) are currently on the market under the approved category 'Foods for Specified Health Use' in Japan[29]. The present results might warrant further investigation into the efficacy of mulberry leaf extract and its active ingredient DNJ as a dietary supplement or a pharmaceutical agent for improving postprandial glycemic control in individuals with impaired glucose metabolism.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 | Changes in anthropometric measurements, alanine aminotransferase, serum creatinine and serum lipid profiles in study 2
Table S2 | Dietary intake, alcohol consumption and pedometer count in study 2

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