Safety And Efficacy of Korean red ginseng Intervention (SAEKI) Trial: Rationale, Design, and Expected Findings

John L Sievenpiper, Marco Di Buono, P. Mark Stavro, Alexandra L Jenkins, Ki Yeul Nam, Melody Choi, Asima Naeem, Lawrence A Leiter, Mi-Kyung Sung, Vladimir Vuksan

From the Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario (JLS, MD, PMS, ALJ, LAL, VV); Clinical Nutrition and Risk Factor Modification Centre (JLS, MD, PMS, ALJ, MC, AN, LAL, VV) St. Michaels Hospital, Toronto, Ontario; Korean Ginseng and Tobacco Research Institute, Daejeon, South Korea (KYN); Department of Food and Nutrition, Sookmyung Womens University, Seoul, South Korea (MKS)

Abstract

Diabetes mellitus is reaching epidemic proportions worldwide. The insufficiency of medication to cope with this burden has coincided with a dramatic rise in the prevalence of use of complementary and alternative therapies, especially herbal treatments. This surge in demand presents a challenge to prove the safety and efficacy of these treatments in diabetes. Korean red ginseng (steam treated *Panax ginseng* C.A. Meyer) is a strong candidate to succeed. It has been shown to possess a multitude of hypoglycemic effects and improve metabolic disturbances related to diabetes in in vitro and animal models. Data in humans is also emerging to support these benefits. Whether these results can be replicated in a rigorous clinical testing program is unclear. We therefore investigated the antidiabetic effects of Korean red ginseng in a series of 2 acute and 1 longterm randomized, double-blinded, placebo-controlled clinical trials. This paper provides the rationale for this program of study, expanding on the problem of diabetes, its management, and the possible role for Korean red ginseng. It then describes the design and expected findings.

Correspondance (Dr. Vladimir Vuksan)
Tel : 416-867-7450 Fax : 416-867-7442
E-mail : v.vuksan@utoronto.ca

Introduction

Historical accounts of diabetes mellitus first appeared in the medical texts of several ancient cultures. Symptoms that included polyuria and polydypsia were described in the Egyptian *Ebers papyri* ~3000y ago, Greek *Epidemics Book III* of Hippocrates ~2400y ago, and the Chinese *Nei Ching* ~2300y ago [1,2,3]. Hindu writings in the *Ayurvedic* texts ~2100-2700y ago used these same symptoms and others including glucosuria and the smell of breath acetone to differentiate two main types of diabetes mellitus: one inherited and another acquired through obesity [1,3]. This represented one of the first recorded etiologic classifications of type 1 and type 2 diabetes. Treatments described in the writings from these cultures included largely diet and plant based remedies [1,2,3]. Ethnopharmacological investigations have since revealed thousands of plants including various ginseng species that are implicated in the treatment of diabetes. A comprehensive review of data for 1000 of these plants reported that >80% have demonstrated some antidiabetic activity in studies [4]. There is, nevertheless, only one example of an approved antidiabetes drug that was developed from a plant with a long history of use for diabetes: Metformin from French lilac [3]. Numerous plants remain candidates for antidiabetic drug development. Korean red ginseng (steam treated *Panax ginseng* CA Meyer) may hold promise in this regard. This paper will provide a rationale for studying the antidiabetic effects of Korean red ginseng in a series of 2 acute and 1 longterm clinical trials, by expanding on the problem and management of diabetes, the resulting opportunity for complementary and alternative medicine (CAM), and the evidence supporting the use of Korean red ginseng in the treatment of type 2 diabetes. It will then describe the conceptual framework, design, and expected findings of the studies that were conducted.

Rationale

The problem of diabetes

Diabetes is emerging as a global health epidemic affecting people in both developing and developed countries. Between 1995 and 2025 the prevalence of adult diabetes is predicted to increase by 27% in developed countries and 48% in developing countries [5]. The global prediction is an increase in prevalence from 1.6% to 3.3%. In the U.S. and Canada, where the prevalence is estimated at 7.8% [6] and 5.8% [7] respectively in adults, the trends may already be

outpacing the predictions. The most recent data from the centers for disease control (CDC) show that from 1990-2000, diabetes increased by 33% [8], while one of its most important precipitating factors, obesity, increased by 50% [9]. Alarmingly, the greatest increases (70%) came in the younger adult cohorts that typically have the lowest prevalence: those aged 30-39y for diabetes [8] and 18-29y for obesity [9].

The human and economic costs of this high and increasing prevalence are considerable. Microvasuclar complications include diabetic nephropathy, the leading cause of endstage renal disease in the west [10]; retinopathy, the leading cause of blindness in the west [11]; and neuropathy, the cause of half of all nontraumatic lower limb amputations in the U.S. [12]. Macrovascular complications include coronary, and cerebral vascular and peripheral vascular disease. People with diabetes are at 3- to 8-fold greater risk of death from myocardial infarction (MI) and stroke than those without diabetes and at the same risk for an MI as those who have already had a previous MI [13]. The predisposing risk is so high that ~75% with diabetes will die prematurely from CVD [14]. Trend analysis confirms this increased CVD burden. Recent National Health and Nutrition Examination Survey data report that men with diabetes have benefited far less from declines in CHD mortality, while women with diabetes have experienced an increase in risk compared with their nondiabetic counterparts [15]. Taken together, the microvascular and macrovascular complications contribute to a higher mortality rate, lower survival rate, and lower life expectancy for this cohort [16].

Management of type 2 diabetes

Diabetes is comprised of a heterogeneous group of metabolic diseases, characterized by hyperglycemia. The most prevalent of these diseases, occurring in >90% of patients, is classified as type 2 diabetes [17]. It is caused by a combination of insulin resistance and an insulin secretory defect that in >80% clusters with obesity, low HDL-cholesterol, high triglycerides, elevated blood pressure, and impaired fibrinolysis [18]. This constellation of features defines the dysbolism of insulin resistance and is termed the metabolic syndrome [19,20]. Because of this clustering of risk factors and the increased CVD burden that accompanies it, diabetes is now treated as a risk equivalent to established CVD in new therapeutic guidelines [20,21]. In addition to the insulin resistance and/or insulin secretory defect underlying the hyperglycemia, guidelines for pharmacological and nutritional medical therapy of diabetes aggressively target the related risk factors: obesity, dyslipidemia, hypertension, and impaired fibrinoloysis [20-23]. Despite the

numerous preventative strategies and armories of medication employed by this approach, prevention and treatment of type 2 diabetes remain grossly unsatisfactory. The increasing trends in the prevalence of diabetes and its complications described above are testament [8]. That treatment goals for glycemic control continue to be unmet is also an example of the insufficiency of treatment. The largest prospective intervention study in type 2 diabetes, the *United Kingdom Prevention Diabetes Study* (UKPDS), showed that after 3y of intensive treatment policy only 50% attained the glycemic control target of HbA_{1c} <7%. By 9y this proportion decreased to 24% [24]. A compelling argument can be made for more effective prevention and treatment modalities.

An Opportunity for CAM

CAM approaches that include ginseng may hold promise for the development of better therapeutic strategies. A compelling argument has been made that the random in vitro "high-throughput" screening for new drug therapies preferred by pharmaceutical companies has less practical merit than an ethnopharmacological approach that involves ethnobotany and traditional medicine screening for candidate therapies [3]. This is especially true for diseases such as diabetes that are complex metabolic disorders, as certain metabolic targets of these approaches may be unrelated or secondary to effects on more proximal defects [3]. For example, targeting glycogen synthase activity may result in missing a primary effect on glucose transport activity or targeting phosphoinositide 3-kinase in the insulin-signaling cascade may miss a primary effect on an upstream step. Isolated downstream effects might also be discounted. CAM treatments not identified by conventional in vitro screening systems might therefore still be proven to have clinical efficacy and potential for development.

The public in their actions already endorse this approach. The insufficiency of treatment coincides with a surge in the demand for CAM treatments, especially herbs. The use of herbs increased ~4-fold from 1990-1997 in the U.S [25]. The most recent estimates suggest that 75% of general medical patients in the U.S. are using CAM therapies [26]. One of the strongest independent determinants of this behavior is the use of CAM to treat diabetes [27]. Among people with diabetes, it has been reported that 37% use CAM [28]. This increased demand has occurred in the absence of safety and efficacy evidence, adequate regulatory standards, patient disclosures to physicians, and physician education. The result has been a series of articles [28], commentaries [29], editorials [31-35], and letters [36-38] calling for randomized controlled clinical trials to evaluate CAM treatments.

Ginseng and type 2 diabetes

Ginseng research in diabetes is beginning to answer the call for more rigorously conducted studies. The American Diabetes Association (ADA) in its most recent evidence-based nutrition recommendations [39] recognized ginseng as one of the most studied herbs in the area of diabetes. Evidence is accumulating that the whole root of various panax species including Asian (*Panax ginseng* C.A. Meyer) [40-46], American (*Panax quinquefolius* L.) [46], and Sanchi (Panax notoginseng [Burk.] F.H. Chen.) [46] ginsengs and various subtypes including Korean Red (steam treated Panax ginseng C.A. Meyer) ginseng [46] at doses from 100-1000mg/kg possess significant hypoglycemic action in assorted rodent models. The same is true for different plant parts (rootlets and berries) [40,47,48], glycosidal saponins (total ginsensoides, total and isolated protopanaxadiol [PPD] ginsenosides, Rb_1, Rb_2, Rc, Rd, Rg_3, and total and isolated protopanaxatriol [PPT] ginsenosides, Rg_1, Re, Rf, Rg_2, Rh_1) [45,48-56], peptidoglycans (panaxans A-U for the Asian ginseng and quiquefolans A-C for American ginseng) [57-61], and water (DPG-1, DPG-3, DPG-3-2) and methanol (Fraction 3 and 4, EPG-3-2) extracted fractions of Asian ginseng [62-67]. Together these different species of whole roots, their extracted fractions, and individual compounds have shown effects on glucose clearance [42-45,51] and disposal [40,52-54] and insulin secretion [55,64,66] and signaling [56].

There is a growing database of human studies that supports these effects. We showed consistent postprandial glycemia-lowering efficacy in a series of acute dosing (1-9 g) and timing (120 min to 0min before oral glucose) response studies in people with and without type 2 diabetes with a first batch of American ginseng, using a 25g-oral glucose tolerance test (OGTT) protocol [67-70]. Our subsequent clinical studies have begun to implicate the role of ginsenosides in these effects. We showed that while 6g of this same American ginseng also lowered postprandial glycemia using a higher 75 g-OGTT protocol, 6 g of a second batch with a depressed ginsenoside profile was ineffective [71]. The lack of effect of the 2nd batch was attributed largely to a decrement in PPD ginsenosides. In another study, we confirmed the postprandial glycemia-lowering efficacy of 3 g of a 3rd American ginseng batch with a ginsenoside profile similar to our 1st batch, while an Asian ginseng batch, with an apparently inverted profile, was found to increase glycemia during a 75g-OGTT. Regression models assessed the PPD:PPT ginsenoside ratio as the sole independent predictor of these effects [72]. Taken together, our observations suggested that American ginseng with a profile similar to our efficacious batches might have long-term thera-

peutic value. To investigate this possibility, we studied the longterm effects of a proprietary extract, *CNT 2000* (*Chai-Na-Ta Corp.,* Langley, BC), designed to have a PPD:PPT ginsenoside ratio similar to that of our first efficacious American ginseng batch. We found that 3g/d (1g before each meal TID) of the extract decreased fasting plasma glucose and HbA_{1c} significantly compared with placebo in an 8-week double-blinded, crossover trial of 24 subjects with type 2 diabetes [73]. Two other studies of uncharacterized ginseng batches have replicated this longterm efficacy in diabetes. The first study found that 100 to 200 mg/day of an unspecified ginseng decreased fasting plasma glucose, the area under curve during a 75 g-OGTT, and HbA_{1c} after 8 weeks of treatment in a double-blind placebo-controlled, parallel study of 36 type 2 diabetic subjects [74]. The second study found that 3-4.5 g of a Korean red ginseng extract decreased HbA_{1c} after 24 months of treatment compared with controls in an unblinded, parallel study of 67 type 2 diabetic subjects [75].

Complementary effects on several related features of the metabolic syndrome have also been observed in animal models. First, an extract of Asian ginseng berries with high Re improved obesity, inducing weight loss in db/db [47] and ob/ob mice [48]. Second, Korean red ginseng and its total ginsenosides [76,77], fractions 3 and 4 [78,79], a lipophillic ginseng fraction [80], Rb_1 [81], and Rb_2 [82,83] improved the dyslipidemia that characterizes the syndrome, decreasing serum triglycerides while increasing serum HDL-cholesterol in the rat. This is in addition to related effects that included decreasing serum cholesterol [78,79,82-85], plasma free fatty acids [78], and liver total cholesterol [81,84,85] and increasing clearance of ^{14}C-labelled cholesterol from plasma and excretion of C^{14}-labelled cholesterol and its metabolites (bile acids, saponifiable material, sterols) from the liver [79]. Third, different ginseng species and their fractions improved hypertension. Sanchi ginseng decreased both systolic and diastolic blood pressure [86] and total ginsenosides from Asian ginseng decreased mean blood pressure in a dose dependent manner in the rat [87]. An aqueous Asian ginseng extract [88], Korean red ginseng preparations with increasing concentrations of Rg_3 [89], total ginsenosides [87], PPTs, Rg_1 [90,91], Rg_3 [90,91], Re [92,93], and Rb_1 [93] also produced related increases in endothelium-dependent relaxation mediated by nitric oxide and cGMP [87-90,92,93] and endothelium-independent relaxation mediated by potassium channels [91] in studies in animal aorta, cerebral arteries, and ventricular myocytes. Fourth, various ginseng species and their fractions improved impaired fibrinolysis. Japanese ginseng (*Panax japonicus* C.A. Meyer) [94], notoginsenoside-R1 [95], Rg_1[96,97], Rg_2, Rg_3, Re, Rb_1, Rb_2, Rc [97], and Ro [97,98] increased fibrinolysis mediated by

increases in tissue plasminogen activator (tPA) [95,96] and urokinase [94,97] and decreases in plasminogen activator inhibitor-1 (PAI-1) [95]. An Asian ginseng extract [99], Korean red ginseng [100], the lipophilic fraction of Asian ginseng [80,101], Rb_1, Rb_2, Rc, Re, Rg_1, Rg_2, Ro [97], and Rg_3 [102] have also produced related inhibitory effects on platelet aggregation and adhesion in rabbit, rat, and human platelets [97,99,101,102] and coagulation, assessed by increased activated partial prothrombin time (APPT), prothrombin time (PT), and thrombin time (TT) in platelet-poor rat plasma [80,100,102].

These effects on 4 main features of the metabolic syndrome are again supported by results in humans. In the clinical diabetes trial mentioned above, 100 and 200mg/d of the unspecified ginseng improved obesity, decreasing weight after 8 weeks of treatment compared with baseline in the double-blind placebo-controlled, parallel study of 36 type 2 diabetic subjects [74]. Korean red ginseng at a dose of 4.5g/d (1.5g before each meal TID) improved dyslipidemia, decreasing triglycerides and increasing HDL cholesterol after 7 days in an uncontrolled pilot study of 5 normal and 6 hyperlipidemic men [76]. In our longterm randomized, double-blind, crossover study with the American ginseng extract *CNT2000,* Total-cholesterol, LDL-cholesterol, and the Total-/HDL-cholesterol ratio were reduced compared with placebo, with an observable but insignificant increase in HDL cholesterol [103]. Different ginseng preparations improved aspects of hypertension. Korean red ginseng at a dose of 4.5 g/d (1.5 g before each meal TID) decreased 24h mean systolic blood pressure compared with placebo after 8 weeks in 26 subjects with essential hypertension in a nonrandomized, unblinded, crossover study with a shortened placebo phase (4 vs. 8 weeks) [104]. The Asian ginseng extract *Ginsana G115* (Pharmaton, Ridgefield, CT, USA) significantly decreased acute blood pressure 2h after ingestion compared with baseline [105]. Korean red ginseng significantly increased forearm blood flow responses, consistent with mediation by nitric oxide, in 7 hypertensive test subjects compared with 10 untreated hypertensive control subjects [106]. In our longterm study with the American ginseng extract *CNT2000,* both systolic and diastolic blood pressures were significantly reduced compared with placebo [107]. Finally, again in our longterm study with the American ginseng extract *CNT2000,* impaired fibrinolysis was improved, as indicated by a significant reduction in PAI-1 from baseline. But the comparison with placebo was only approaching significance [108].

Korean red ginseng and type 2 diabetes

The experimental and clinical data is persuasive that, among the ginseng preparations studied,

Table 1. Comparison of the ginsenoside profile of Korean red ginseng (72,109-118)* with an effecacious American ginseng (67-70)

Ginsenosides	Content (% wt/wt) and ratios		Δ (%)
	Korean red ginseng	Amerincan ginseng	
Protopanaxadiols (PPD)			
Pb_1	0.73	1.53	-52.29
Pb_2	0.46	0.06	666.67
Rc	0.47	0.24	-5.833
Rd	0.24	0.44	-45.45
Rg_3		-	-
Subtotal	2.10	2.27	-7.489
Protopanaxatriols (PPT)			
Rg_1	0.38	0.1	280
Re	0.16	0.83	-80.72
Rf	0.42	0	-
Rg_2	0.09	-	-
Subtotal	1.02	0.93	10
Total	3.38	3.20	5.625
Ratios			
PPD:PPT	2.14	2.44	-12.33
Rb_1:Rg_1	2.67	15.30	-82.55
Rg_2:Rc	1.27	0.25	408
Rg_1:Re	1.66	0.12	1277.8

*Mean calculated for 36 distinct batches reported in 11 studies

Korean red ginseng has significant antidiabetic benefits. All of the glycemic and metabolic syndrome parameters investigated in animal and in vitro studies were improved by Korean red ginseng and its principal PPD ginsenosides Rb_1, Rb_2, Rc, Rd, and Rg_3 and PPT ginsenoside Rg_1, Re, and Rg_2. Obesity was the only exception. The same consistency was true in the human studies. Other than American ginseng, Korean red ginseng was the only type of ginseng confirmed to improve longterm glycemic control and at least 2 features of the metabolic syndrome. Moreover, its ginsenoside profile shares important similarities with other efficacious types of ginseng. If we pool the ginsenoside concentrations for 36 different batches of Korean red ginseng reported in 11

separate studies [72,109-118] and compare their mean with the ginsenoside concentrations of our 1st efficacious batch of American ginseng [67-70], then the profiles are remarkably similar (Table 1). Although there are marked species-specific differences in some ginsenosides, the PPD content and the PPD:PPT ratio, implicated in our acute studies as predictors of glycemia-lowering efficacy, are within 8-12% in Korean red ginseng.

Despite the apparent strength of this evidence for the efficacy of Korean red ginseng, there remains uncertainty regarding the reproducibility of its results. The batch-to-batch variability in the composition of commercial ginseng can be high [113-119]. If we again consider the pooled ginsenoside concentration for the 36 batches of Korean red ginseng [72,109-118], the calculated coefficient-of-variation ranges from 37% for the PPD:PPT ratio to 177% for Rd. The implication is that its safety and efficacy could be equally highly variable. The poor quality (lack of randomization, blinding, placebo-control, etc.) of the clinical research conducted to date on Korean red ginseng is also a concern. Whether in our randomized, double-blinded, placebo-controlled testing system the results we have observed with American ginseng in humans will apply to Korean red ginseng is unclear. We therefore undertook a series of rigorously conducted acute and long-term trials to assess the safety and efficacy of Korean red ginseng in the management of diabetes. These are described in detail in the sections that follow.

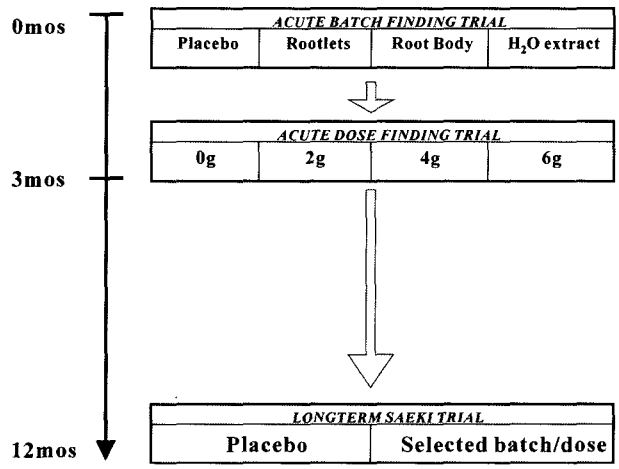

Fig. 1. Conceptual Framework of the clinical research investigating Korean red ginseng. In each step the most efficacious fraction/ dose is assessed by comparison with placebo in healthy participants, from where it proceeds to the next step for further study. This process continues in a stepwise fashion until the final step, in which the most efficacies fraction of Korean red ginseng at the most efficacious dose is will have its longterm efficacy and safety assessed in people with type 2 diabetes mellitus.

Conceptual Framework

The research was designed to follow the *World Health Organization (WHO) Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines* [120]. It included 2 acute "phase 1" type trials in healthy subjects and 1 longterm "phase 2" safety and efficacy trial in type 2 diabetic subjects (Fig. 1). Each of the studies proceeded in a stepwise fashion, deriving from the last. This type of "acute-to-chronic" model was used to success in our American ginseng-testing program, in which a series of acute OGTT trials were used to determine the batch, dosing, and timing of American ginseng that resulted in longterm safety and efficacy. The first acute trial, the *Acute Batch Finding Trial*, identified an efficacious batch of Korean red ginseng from three root fractions using an OGTT protocol. The most efficacious fraction advanced to the next step, the *Acute Dose Finding Trial*. This second acute trial identified the optimal dose of the most efficacious fraction identified in the previous trial again using an OGTT protocol. The resulting batch and dosing information was then applied to the final step, the *Longterm Safety And Efficacy of Korean red ginseng Intervention* (*SAEKI*) *Trial*. This trial investigated the safety and efficacy of the most efficacious Korean red ginseng fraction dose in subjects with type 2 diabetes over 3 months using a randomized, placebo controlled, double-blinded, crossover design. The first two acute trials took 3 months to complete and the final longterm trial an additional 9 months.

Design: Acute Trials

Participants

Healthy participants were recruited for the 2 acute studies from newspaper advertisements, our outpatient database, and postings. Interested volunteers were briefed on the nature of the study, treatments, and procedures. They were also questioned as to their medical history, lifestyle, exercise, smoking and dietary habits, and use of medication, supplements, and alcohol. Those candidates that met the inclusion criteria described in Table 2 were invited for screening where they signed an approved consent form and had their inclusion criteria confirmed.

Power analysis

Twelve healthy participants were needed per study. We assumed that tests would be conducted

Table 2. Subject inclusion criteria

1. Euglycemic (FPG 4-6mmol/L)
2. Nonpregnant 18-65 years old
3. Not taking herbs or supplements that affect glycemia
4. Clinically euthyroid
5. Normal renal and hepatic function
6. No major illness/ disease
7. No gastro-intestinal disorders
8. Normo-tensive
9. No heavy alcohol use (>3 drinks/day)
10. No heavy cigarette use (>10cigarettes/day)

at a significance level of á=0.05 and 1-â=80% in a repeated measures design that has 1 group of subjects that undergoes 4 tests per study. Based on the literature, it was also assumed that the SD in postprandial plasma glucose would be 1 mmol [121] and the expected difference for these parameters would be 2mmol. These proposed differences are similar to those seen in our acute trials with American ginseng [67-70]. They are also similar to those seen when comparing a placebo with a solfonylurea, which operates through an insulinotropic mechanism as we speculate that ginseng might [122]. From this set of assumptions, it was determined that a minimum of 10 subjects was required producing a within-subject SD of 0.88 for glucose. This design was calculated to achieve 81% power for glucose when an F test was used to test the ginseng factor at a 5% significance level and the actual SD among the appropriate means is 0.56 (an effect size of 0.63) for glucose. Allowing for a ~20% attrition rate, the sample size for recruitment was set at 12 people.

Treatments

Two treatment sets derived from a single batch of Korean red ginseng were investigated (Fig. 2). The ginseng was selected by the *Korean Ginseng and Tobacco Research Institute* (Daejeon, South Korea) to be a representative efficacious batch of Korean red ginseng. In the first acute trial, the following 3 derived fractions were tested at a high dose of 6g: (1) whole root water extract, (2) root body, and (3) rootlets. The whole root water extract fraction was prepared by extraction of ground whole root by distilled water at a ratio of 1:10 at 85°C 3 times, followed by cooling, centrifuging at 1500rpm, and spray drying. The root body and rootlets fractions were prepared mechanically by separating the lateral roots and root hairs from the main body of the root. These fractions were selected to provide a wide range of composition that would allow for a partitioning of effects. The rootlets of *Panax ginseng* have been shown to contain >3-fold higher

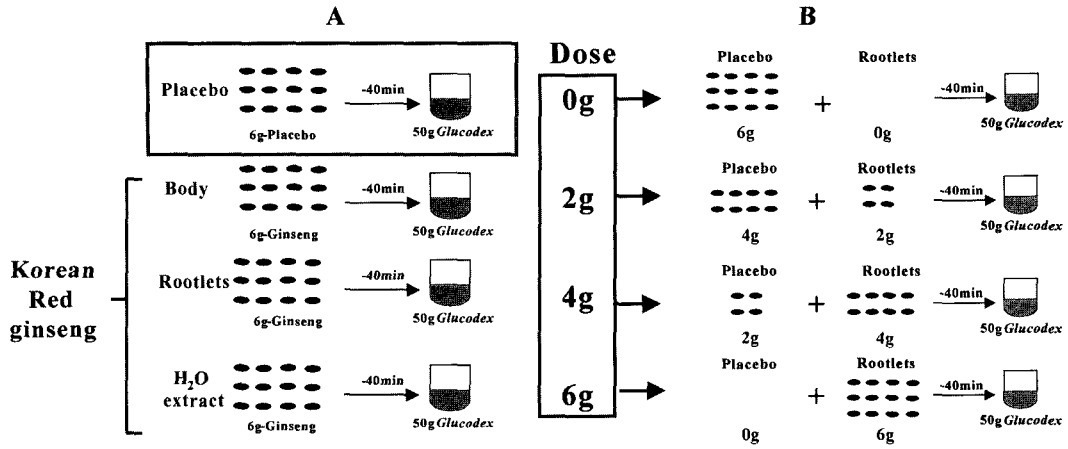

Fig. 2. Schematic of the treatments for (A) the acute batch and (B) dose finding trials. Capsules were taken 40 minutes before a 50 g oral glucose tolerance test (50 g-OGTT) in both studies. In the dose finding trial the number of capsules was kept equal among the doses by adding placebo capsules to the lower doses to keep the capsule count at 12 for each treatment.

total ginsenosides than the main root body, a >2-fold higher PPD: PPT ratio and >6-fold higher Rb_1:Rg_1 ratio [123]. In the second acute trial, 3 scaled-down doses of the most efficacious fraction identified in the preceding study were tested: 2 g, 4 g, and 6 g. All treatments were administered in the form of dried ground powder encapsulated in 500 mg capsules, taken with 300 ml of water, 40 min before a 50 g-*Glucodex*® (*TechniLab*, Rougier, Quebec). The placebo treatments consisted of identical placebo capsules containing cornstarch. These capsules were matched with the active treatments for appearance, energy, and carbohydrate content. The number of capsules was also kept equal among the doses in the second acute trial by adding placebo capsules to the lower doses to keep the capsule count at 12 for each treatment.

Study Design, blinding, and monitoring

Each of the 4 studies proceeded in a stepwise fashion with the results of the preceding studies dictating the doses and extracts to be used in subsequent studies. Randomized, double-blinded, placebo-controlled, multiple-crossover designs were used. Only the blinder knew the identity of the treatments. She performed and maintained the blinding of packages, labels, and randomization of the treatments while not having contact with patients or data. Randomization was done using a random number table. Monitoring of all patient records, data sheets, sample handling, laboratory sample storage, and sample inventory for completeness and adherence to the protocol

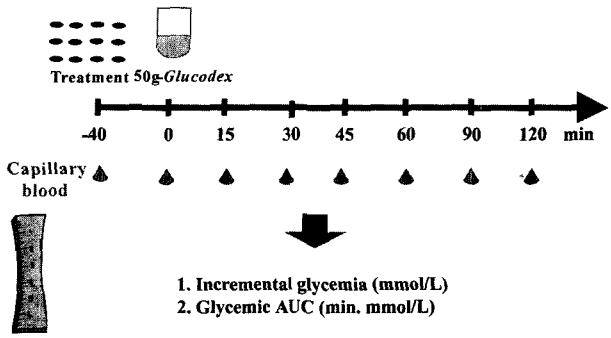

Fig. 3. Schematic of the protocol for the acute batch and dose finding trials with derived plasma glucose endpoint measures.

were conducted by the study monitor.

Protocol

The protocol was designed to follow the *World Health Organization* (WHO) [124] guidelines for the OGTT (Fig. 3). Participants attended St. Michaels hospital (Toronto, ON, Canada) following a 10-12h overnight fast. They were instructed to maintain the same dietary and exercise patterns the evening before each test and consume a minimum of 150g of carbohydrate per day for the 3 days prior to the test. Compliance was assessed by an OGTT questionnaire. A minimum 3-day "washout" separated visits. This was considered sufficient, as ginseng has been shown to have a half-life less than 8 hours [125] with a time course of effects not lasting beyond 24 hours [126]. We have also used this duration of washout in our previous acute American ginseng studies without observing any carryover effects [67-70]. At the start of each test, subjects had a catheter inserted into a forearm vein and kept patent by saline. From this device, a 7 ml-blood sample was obtained by a registered intravenous nurse. One of the randomly selected treatments was then administered with 300 ml of water. Another blood sample was collected 40 min later. A 75g-Glucodex® was then consumed over exactly 5min. Additional blood samples were drawn at 15, 30, 45, 60, 90, and 120 min after the start. Participants also indicated any adverse symptoms during each test and the intervening "washout" days between clinic visits using visual analogue 7-point bipolar scales.

Ginsenoside Analyses

Analysis of 9 common ginsenosides (Rb_1, Rb_2, Rc, Rd, Rg_3 Rg_1, Rf, Re, Rg_2) in each of the 3

fractions was done using HPLC-ELSD analyses at the *Korean Ginseng and Tobacco Research Institute* (Daejeon, South Korea). Samples were prepared by a multiple step MeOH, water, hexane, and butanol extraction using HPLC grade solvents. The HPLC conditions included: chromatograph-(Beckman Instruments); column Lichrosorb-NH_2 5 μm 250 × 4.6 column (Merck Co.); mobile phase (A) acetonitrile/water/isopropanol (80:5:15) and (B) acetonitrile/water/isopropanol (80:20:15); flow rate-1 ml/min; ELSD detection-an ELSD 2000 detector (Altech co.) at an operating temperature of 92°C with nebulizing gas nitrogen, 2.0 L/min; and injection volume - 5-20 μL. The institute provided all ginsenoside standards.

Blood Glucose Analyses

All samples were analyzed within three days of collection. The glucose concentration of each was determined by the glucose oxidase method using a YSI 2300 Stat glucose/L-lactate analyzer, model 115 (Yellow Springs, Ohio, U.S.). The inter-assay coefficient of variation of this method for two sample pools was 3.3% (n=91, 3.99±0.13 mmol/L) and 1.8% (n=89, 14.35±0.26 mmol/L).

Data Analyses

Blood glucose curves were plotted as the incremental change over time and the positive incremental area under each curve (AUC) was calculated geometrically for each participant, ignoring areas below the fasting value [127]. Incremental blood glucose was used to control for baseline differences. Statistical analyses were then performed using the *Number Cruncher Statistical System (NCSS) 2000 software* (NCSS statistical software, Kaysville, Utah). Repeated measures two-way ANOVA assessed the interactive and independent effects of batch/dose and sampling time on incremental changes in blood glucose. If the interaction terms were significant, then pairwise comparisons with placebo were done at individual time points using repeated measures one-way ANOVA adjusted by the Tukey Kramer test for multiple pairwise comparisons. All results were expressed as mean±SEM and significant at $p<0.05$.

Design: Longterm Trial

Participants

Healthy type 2 diabetic adult participants of mixed gender and ethnicity were recruited from our patient database, postings at St. Michaels Hospital, and print advertisements. Interested vol-

Table 3. Subject inclusion criteria

1. Nonpregnant 18-65 years old
2. Confirmed diabetes > 6mos
3. No insulin use
4. HBA_{1c} 6.0-8.5%
5. FPG 6.4-8.5mmol/L
6. Not taking herbs or supplements that affect glycemia
7. Clinically euthyroid
8. Normal renal and hepatic function
9. No major illness/diabetes complications free
10. No gastro-intestinal disorders
11. Normo-tensive
12. No heavy alcohol use (>3 drinks/day)
13. No
14. heavy
15. cigarette use (>10cigarettes/day)

unteers were briefed on the nature of the study, treatments, and procedures to be followed. They were also questioned as to their medical history, lifestyle, exercise, smoking and dietary habits and use of medication, drugs, vitamins, herbs, supplements, and alcohol. Those candidates that met the inclusion criteria found in Table 3 were invited for screening, at which time informed consent was acquired from all prospective subjects and their inclusion criteria was confirmed. Any person with abnormal results was invited back for a second screening for confirmation.

Power Analysis

A total of 40 type 2 diabetic participants were needed. We assumed that tests would be conducted at a significance level of alpha=0.05 and 1-β=80% in a randomized block design with one group measured 6 times in which each subject is a block with two factors: treatment at 2 levels (placebo, Korean red ginseng fraction) and time at 3 levels (weeks 0, 6, 12). Based on our previous longterm diabetes trial with the American ginseng extract *CNT2000* [73], it was also assumed that the primary endpoint with the greatest variability, HbA_{1c}, had a within block SD of 0.8%. The minimum detectable difference in HbA_{1c} was assumed to be equivalent to the minimum 1% reduction expected when using a first-line oral agent such as a sulfonylurea or metformin [128]. This reduction represents a clinically significant difference. An epidemiological analysis of the data from UKPDS 35 indicated that for every 1% reduction in HbA_{1c}, there is a 21% decrease in any diabetes related endpoint and death, a 14% decrease in myocardial infarc-

tion, and 37% reduction in microvascular complications, without a threshold of risk [129]. From this set of assumptions, it was calculated that this design would achieve 94%, 84%, and 100% power with 25 subjects (blocks) when an F test is used to test the treatment groups factor, the time factor, and their interaction respectively at a 5% significance level with the actual SD among the appropriate means 0.20 (an effect size of 0.29), 0.19 (an effect size of 0.27), and 0.30 (an effect size of 0.43) respectively. Allowing for a 35% rate of attrition to account principally for frequent background medication changes, the sample size for recruitment was set at 40 people.

Treatment Protocol

Applying the treatment data from the acute testing, the most efficacious Korean red ginseng fraction at its most efficacious dose had its longterm efficacy and safety investigated compared to an identical cornstarch placebo. Treatments were administered as a prandial agent given 40 minutes before breakfast, lunch, and dinner (Fig. 4). The meals were part of a *Canadian Diabetes Association* (*CDA*) diet implemented and maintained by a registered dietitian and documented by 7-day food records. It consisted of a high-carbohydrate low-fat diet designed for weight maintenance consisting of a macronutrient profile of 55%:30%:15% of calories from carbohydrate:fat:protein, with <10% of calories from saturated fat, >25 g of dietary fiber and an emphasis on low glycemic index foods.

Study Design, Blinding, Monitoring

The study employed a randomized placebo-controlled, double-blinded, crossover design (Fig.

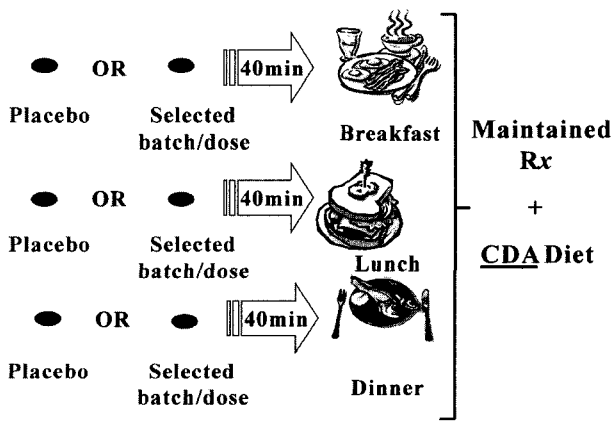

Fig. 4. Schematic of the treatment protocol for the Longterm SAEKI Trial.

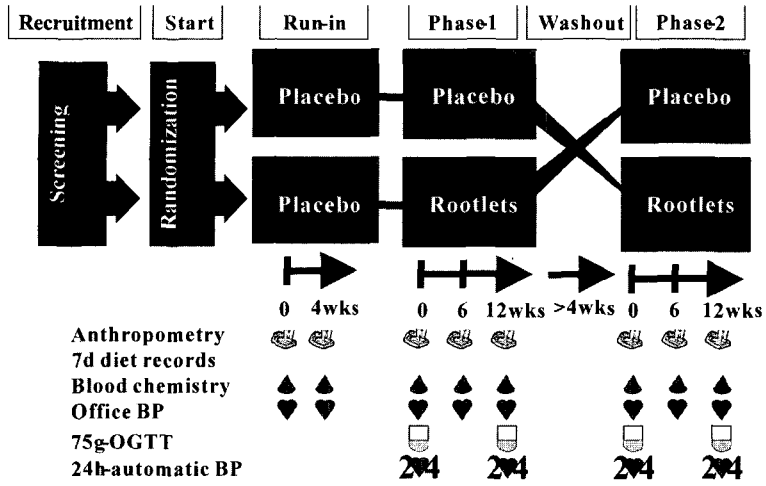

Fig. 5. Schematic of the Longterm SAEKI Trial randomized, placebo-controlled, double-blinded, crossover design.

5). This design consisted of 4 distinct periods: placebo-run-in, phase-1 treatment, placebo-washout, and phase-2 treatment, with the two treatment phases identical except for the treatment assignments. Participants began on the placebo run-in phase for 4wks to habituate to the CDA diet, treatment protocol, and trial procedures and stabilize baseline parameters. After this period, 50% of the participants were randomized to placebo and 50% to the Korean red ginseng fraction. Participants continued on their respective treatments for 12 weeks, attending the clinic every 6wks to have their blood drawn and blood pressure, weight, height, and body fat measured; receive a new treatment batch; return unused pills; submit 7d food records done the week preceding the visit and a 24h urine collection collected the day before the visit; and interview with the principal investigator. At the beginning and end of the treatment period, a 5-point 75 g-OGTT was performed and a 24h ambulatory blood pressure monitoring study was conducted. This first treatment period was followed by a 4-6 week placebo-washout phase, over which participants maintained the CDA diet, treatment protocol, visit schedule, and measurement procedures. This was intended to mitigate carryover effects. In the second treatment phase, participants were crossed over to the alternate treatment for an identical 12-week treatment period. The same methods for the randomization and blinding described above for the two acute trials were applied to the longterm trial.

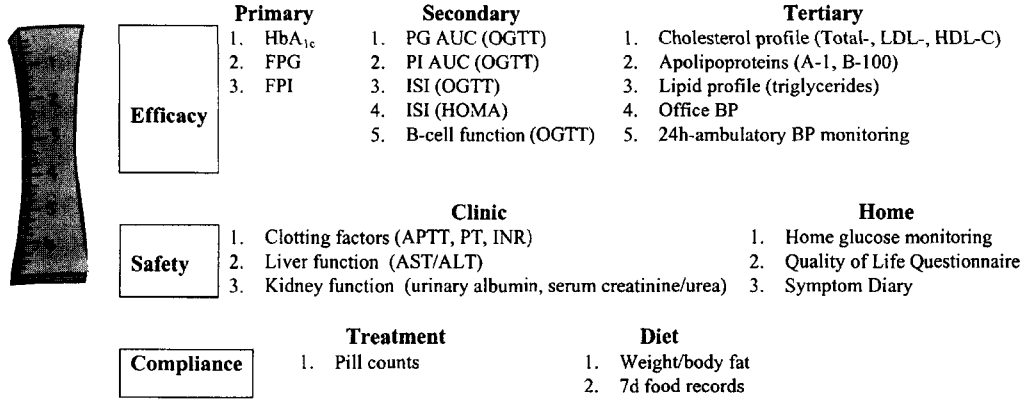

Fig. 6. Schematic of SAEKI Trial Outcome measures. FPG denotes fasting plasma glucose; FPI, fasting plasma insulin; PG AUC, plasma glucose area under the curve; PI AUC, plasma insulin area under the curve; OGTT, oral glucose tolerance test; ISI, insulin sensitivity index, HOMA, the Homeostasis Model Assessment.

Outcome Measures

There were three levels of outcome measures: efficacy, safety, and compliance (Fig. 6). Efficacy measures were further subdivided into three levels. Primary efficacy measures included the gold standard marker of longterm glycemic control, HbA_{1c} [128], as well as fasting plasma glucose and insulin. Secondary efficacy measures included various fasting and postprandial plasma indices of glycemic control and insulin sensitivity. And tertiary efficacy measures featured a full serum lipid, cholesterol, and apolipoprotein profiles, office BP, and 24h-blood pressure (BP) monitoring using an ambulatory BP monitor (ABPM 90207 *Spacelabs*, Seattle WA) to assess the remaining aspects of metabolic control. Apolipoprotein-B was included as part of this profile because of its strong link with the metabolic syndrome [130]. Safety measures included standard clinical assessments of kidney, liver, and haemostatic function; home glucose monitoring for hypoglycemic events; and subjective ratings of symptoms. Finally, compliance measures included measurements of both treatment compliance and dietary/lifestyle compliance.

Plasma/serum Analyses

Samples were frozen at 72°C pending analysis at the completion of the study. Banting and Best Diabetes Centre (BBDC) Core Laboratory, Toronto, Canada again performed all plasma glucose and insulin analyses at the end of the study. Glucose was assessed using the glucose oxidase

method [131] and insulin using double antibody radioimmunoassay method [132]. St. Michaels Hospital Core laboratory did the analyses of serum lipids and cholesterol. Analysis of total cholesterol, HDL, and triglycerides were done enzymatically [133,134]. LDL content was estimated by the formula of Friedewald et al. [135]. Apolipoprotein (Apo) A1 and B were determined by rocket immunoelectrophoresis [136]. St. Michaels Hospital Core laboratory also did the analyses of all safety measures at each patient visit using standard techniques.

Data Analyses

Indices of glucose and insulin were assessed. Plasma glucose (PG), insulin (PI) curves from the 75 g-OGTTs done at the beginning and end of each phase were plotted as the incremental change over time and the positive incremental AUCs were calculated geometrically for each participant, ignoring areas below the fasting value [127]. Again incremental values were used to control for baseline differences. The Homeostasis Model Assessment (HOMA) and 75g-OGTT derived insulin sensitivity indices (ISI) for whole body insulin sensitivity and the 75g-OGTT derived β-cell function index were calculated using standard formulas. Fasting PG and PI values were used to assess HOMA ISI according to the formula: 22.5/FPG×FPI [137]. 75g-OGTT ISI was calculated according to the formula by Matsuda et al. [137]: 10 000 divided by the square root of ([fasting-PG×fasting-PI] × ([mean-PG × mean-PI]), where PG is expressed in mg/dl (0.0551 mmol/L) and PI in μU/ml (6 pmol/L). Finally, 75-OGTT β-cell function was assessed according to the formula: Δ30-PI/Δ30-PG [138]. Statistical analyses were then performed using *NCSS 2000* (NCSS statistical software, Kaysville, Utah). Comparisons of change-from-baseline, intermediate-differences, and end-differences in all efficacy, safety, and compliance parameters were assessed between the treatments. Repeated measures two-way analysis of variance (ANOVA) adjusted for multiple pairwise comparisons with the Tukey Kramer test assessed interactive and independent effects of treatment and time on these parameters. Pairwise comparisons at each study time interval were done using repeated measures GLM ANCOVA adjusted for starting value, sex, and age. Repeated measures one-way ANOVA assessed absolute and % change differences from start to end. All results were expressed as mean±SEM and considered statistically significant if $p < 0.05$.

Expected Findings

We expected that the findings with Korean red ginseng would be consistent with those we

reported for American ginseng. Because rootlets have been shown to have a higher PPD:PPT ratio than either whole root or root body [123] and a higher ratio has been shown to be a significant positive predictor of glycaemic lowering efficacy [72], we hypothesized that the Korean red ginseng rootlets would advance as the most efficacious fraction from the first testing step, *Acute batch finding trial.* As we have found that doses of American ginseng from 1-9g were equally efficacious in our acute testing [67-70], we further hypothesized that this fraction would lower postprandial glycemia equally at doses from 2-6 g in the second testing step, *Acute dose finding trial.* Taken together, Korean red ginseng rootlets at the lowest 2 g dose would advance to the final testing step, the longterm *SAEKI Trial.* It was hypothesized that as a successful prandial agent in the 2 acute studies, the "acute-to-chronic" safety and efficacy seen with American ginseng and established prandial oral agents would hold true for the Korean red ginseng rootlets. Longterm glycemic control (HbA_{1c}, FPG, FPI, ISI[HOMA and OGTT], and PPG and PPI AUC) and other related parameters of metabolic control (blood pressure, total-, LDL-, and HDL-cholesterol, and triglycerides) would be improved.

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