INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality in industrialized nations. Likewise, diabetes, a major risk factor in CVD, is escalating to pandemic proportions. From 1995 to 2025 the prevalence of adult diabetes was predicted to increase by 27% in developed countries and 48% in developing countries [1].

Despite numerous preventative strategies and the growing armamentarium of medications available, the prevalence of impaired glucose tolerance, diabetes and its associated vascular complications continue to grow. Although oral anti-hyperglycemic agents have been shown to reduce microvascular complications, they have failed to deliver the anticipated macrovascular benefits in people with diabetes [2-6]. A meta-analysis showed that the insulin sensitizers, rosiglitazone, although effective at improving glycemic control, as assed by HbA1c, increased the risk of cardiovascular mortality by 40% in type 2 diabetes, a group already predisposed to increased premature cardiovascular death [5]. Three landmark clinical trials also recently demonstrated that intensive glycemic control by oral agents or insulin was of no benefit [3,6] or even increased all cause mortality by 22% and cardiovascular mortality by 35% in people with poorly controlled type 2 diabetes [2]. These data indicate a clear and present need for more effective treatment strategies.

Evidence is emerging to support ginseng as a new approach to treat diabetes and its vascular complications. Prior to 2000, there was only a small group of flawed and poorly reported studies in humans to support the antidiabetic efficacy of ginseng [7]. The limited data prompted us to conduct a series of human trials to address systematically whether the glycemic lowering
effects of ginseng were reproducible across different protocols and ginseng sources and whether ginsenosides, for which the most compelling evidence existed for biological activity, were mediating the glycemic effects. We also set out to investigate the discrepancy in available literature on ginseng effects on vascular parameters. We showed that ginseng had poor reproducibility across different source parameters and that ginsenosides were only weak predictors [8-11]. In the absence of a clear connection between its composition and efficacy, clinical screening was the only way to assure reliable benefit. This led us to develop an efficacy-based clinical screening model, in which the most efficacious ginseng sources, doses, and modes of administration were selected from sequential, “phase I”, acute, single-bolus, randomized controlled trials and then applied in “phase II”, longterm, randomized controlled trials to demonstrate the sustainability of efficacy and safety [12,13]. An example of this systemic evaluation concept was demonstrated for American ginseng use in diabetes [12-20].

The Korean Red Ginseng Clinical Testing Program has both a diabetes arm and a vascular function arm. This review discusses the current state of the clinical research in each arm of this Toronto based program.

**KOREAN RED GINSENG CLINICAL DIABETES PROGRAM**

The clinical screening model is used to test whether the batch, preparation, dosing, and timing of a Korean red ginseng (KRG) source could be selected to have long-term efficacy using the same acute postprandial testing program [13]. This species was considered to be a good candidate for efficacy screening, as it is the only other type that has been reported to decrease glycemia in humans [21]. It also shares a similar protopanaxadol (PPD):protopanaxatriol (PPT) ginsenoside ratio with the selected batch of American ginseng used in our American Ginseng Clinical Testing Program [8,12]. We hypothesized that a batch of KRG selected will lower post-prandial glycemia. Fractionation and extraction were applied to a single batch of KRG (Korea Ginseng and Tobacco Research Institute, Daejeon, Korea) to produce different preparations with a wide range in ginsenoside profiles. These included 3 preparations: KRG-rootlets, -root body, and -whole root H₂O extract. We used this starting material to initiate a KRG testing program consisting of an acute and a longterm phase. A depiction is provided in Fig. 1. Sequential, “phase I”, acute, single bolus, double-blind, randomized, placebo-controlled, multiple crossover, clinical trials were conducted, to identify an efficacious KRG preparation, dose, mode, and timing of administration. The dose range was from 2 to 6g, typically recommended by traditional Chinese medicine and practitioners, the mode of administration was a periprandial oral agent and timing was -40 min prior to a meal, based on our prior clinical experience. The studies consisted of a preparation-finding trial (KRG-rootlets, -root body, and -whole root H₂O extract), followed by a dose-finding trial (0[placebo], 2, 4, and 6 g) of the most efficacious fraction. A 50 g-oral glucose tolerance test (OGTT) protocol was used in which single boluses of the selected KRG preparations and doses were given as an oral agent -40 min preprandially to normoglycemic subjects. The preparation and treatment protocol gained from these acute studies was then applied to the safety and efficacy of KRG intervention (SAEKI) trial, a longterm, double-blind, “phase II” randomized controlled trial, conducted by our group in subjects with type 2 diabetes.

**Sequential acute randomized controlled trials**

**Acute trial 1**

The first, acute, preparation-finding, “phase I”, randomized controlled trial [22] assessed the efficacy of single 6 g doses of the Korean red ginseng-rootlets, -root body, and -whole root H₂O-extract given as an oral agent -40 min preprandially in lowering the glycemic response to a 50 g-OGTT relative to placebo in 7 normoglycemic subjects (Fig. 1, panel 1). A wide variation in the ginsenoside profiles was achieved across the 3 root fractions. This variation coincided with differential effects, although the effects did not appear to be related to differences in the PPD:PPT ginsenoside ratio. Korean red ginseng-rootlets decreased the glycemic response to the 50 g-OGTT at 90-min compared with placebo, while neither the KRG-root body nor -whole root H₂O extract affected glycemia significantly. This was reflected in a significant 29% reduction in area under the curve (AUC) by the KRG-rootlets compared with placebo. We concluded that KRG rootlets, as the most efficacious preparation, would advance to the next step in the acute phase of the clinical testing program.

**Acute trial 2**

In the second acute, dose-finding, “phase I”, randomized controlled trial [22], 12 normoglycemic subjects received single doses of 0 g (placebo), 2 g, 4 g, and 6 g of the selected KRG-rootlets following the same 50 g-OGTT protocol described for the preparation-finding tri...
al (Fig. 1, panel 2). A significant effect of KRG rootlets treatment (mean of 3 doses) was found. The mean of all 3 doses decreased mean postprandial incremental glycemia by ��% compared with placebo. This was reflected in the AUC, in which there was a tendency for the mean of all 3 doses of the KRG-rootlets to decrease the AUC by 14% compared with placebo. There was, however, no effect of any of the 3 doses individually versus placebo, the mean of the 3 doses reduced the glycemic response to the 50 g-OGTT significantly compared with placebo, implying that the 3 doses were equally efficacious with the lowest dose advanced. Asterisk indicates significant differences (p<0.05). A p-value is for independent and interactive effects assessed by 2-way ANOVA. RCT â€” Randomized Controlled Trial; TID, administered three times/day. Sievenpiper et al. [33] and Vuksan et al. [23]

Long term randomized controlled trial (SAEKI trial)

The treatment protocol identified by the sequential acute preparation- and dose-finding studies was applied to the longterm, “phase II”, double-blind, randomized, placebo-controlled, crossover SAEKI trial (Fig. 1, panel 3) [23]. Nineteen type 2 diabetic subjects received 2 g placebo or the selected KRG rootlets as an oral agent at -40 min preprandially three times/day (6 g/day) for 12 weeks, while maintained on their conventional diabetes treatment. Fasting plasma insulin and 75g-OGTT derived AUC plasma insulin were significantly decreased on the selected KRG rootlets treatment compared with
placebo. This occurred while fasting plasma glucose was unchanged and 75 g-OGTT derived AUC plasma glucose was significantly decreased. The combination was reflected in an identical 33% increase in both the homeostasis model assessment (HOMA) and the 75 g-OGTT derived insulin sensitivity indices (75 g-OGTT insulin sensitivity index) on the selected KRG treatment compared with placebo. These benefits occurred without increasing adverse events or altering hepatic, renal, haemostatic, or blood pressure function. We concluded that our KRG clinical testing program successfully identified a KRG preparation (rootlets), dose (2 g), and mode of administration (oral agent given -40 min preprandially) that resulted in sustainable improvements in longterm glucose and insulin regulation safely beyond conventional treatment in type 2 diabetes.

KOREAN RED GINSENG CLINICAL VASCULAR PROGRAM

There is convincing pre-clinical evidence that KRG may benefit vascular function. Fractions of KRG have shown hemodynamic effects in animal and aortic ring studies [24-32], including vasodilatation, platelet inhibition and nitric oxide release stimulation by ginsenosides, especially Rg, [25,28,29,31], and enhancement of production of nitric oxide in vitro by polysaccharides [33,34]. Corresponding clinical evaluations of KRG and its components, however, are limited. Clinical evidence on the hemodynamic properties of ginseng has demonstrated either neutral or moderate blood pressure (BP) lowering effects [32,35-37]. Even in the SAEK trial our selected KRG rootlets showed a neutral effect on blood pressure as assessed by office BP and 24h-ambulatory BP measurements in people with type 2 diabetes [23]. Therefore there is much discrepancy in the literature on blood pressure effects of Korean ginseng despite stronger preclinical evidence on its vasodilatory effects. A vascular benefit, however, can occur without a detectable effect on blood pressure. Vasodilating agents have generally been implicated as having little direct effect on elastic arteries and reduction in initial stroke pressure (systolic pressure) but can markedly lower ventricular after load by decreasing muscular artery stiffness and wave reflection amplitude [38,39]. These beneficial effects on arterial waves can occur with or without a reduction in conventional office blood pressure [39]. To determine whether KRG has clinical vasoactive effects beyond brachial BP, augmentation index (Al), a non invasive measure of arterial wave reflection constructed from pulse wave analysis, flow mediated dilatation (FMD) - a measure of blood flow, and central BP, all independent markers of increased total and cardiovascular mortality [40,41], would provide additional clinical and prognostic information concerning hemodynamic effects. We therefore undertook the KRG clinical testing program for vascular function assessed both by conventional BP measurements and by FMD, Al and central BP. We systematically investigated effects of KRG from an efficacy based as well as component based perspective.

Sequential acute randomized controlled trials: blood pressure studies

The KRG clinical testing program for vascular function mirrored the corresponding program for diabetes.

Acute trial 1

In the first vascular function trial, two of the same preparations from the same batch of KRG (rootlets and body) were studied using a “phase 1”, acute, single-bolus, randomized, crossover, double-blind, placebo-controlled, clinical, BP protocol [42]. The rootlets and body of the same ginseng batch were selected due to a difference in the total ginsenoside content between the rootlets and the root (84% higher in rootlets) while minimizing the environmental and genetic differences between test samples. Seventeen nondiabetic, non hypertensive individuals arrived at our clinic after a 10 to 12-h fast on 3 different mornings without taking their antihypertensive drugs that morning. Individuals first had office BP measures taken until three consecutive systolic and diastolic readings differed by <5 mmHg. Subjects were then fitted with a SpaceLabs ambulatory BP monitor (ABPM), which measured BP every 5-min for the first 30 min – the average of these 7 measures constituted the baseline BP reading. Individuals then consumed 3 g of encapsulated cornstarch (placebo) or KRG-body or rootlets in random order on separate days. BP was recorded with the ABPM every 10-min for 160 min. At 60-min, a standardized breakfast was consumed to simulate typical physiological conditions. The change in systolic and diastolic BP at each time-point relative to baseline was determined for each individual and averaged. We found that KRG rootlets significantly reduced systolic BP at 120-min compared with placebo. KRG body, containing less total ginsenosides, showed no effect on BP. We concluded that, similar to the data from the diabetes program, the data from the vascular function program demonstrated that the KRG rootlets were the more efficacious root fraction, by effectively lowering BP in

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healthy individuals. Ginsenosides were implicated in these effects.

**Acute trial 2**
In order to explore further the role of ginsenosides in BP effects, the second vascular function trial [43] was designed. The ginsenoside Rg1 was selected for study, as it had been directly implicated as the most potent vasodilator among ginsenosides supported by the most robust evidence [25,28,29,31]. Using a “phase I”, acute, single bolus, randomized, placebo-controlled, 4-period crossover clinical trial design, the effect of KRG extracts with escalating levels of ginsenoside Rg1 on BP. After a 12-h fast on 4 separate days, 10 drug-treated subjects with hypertension and 3 drug-naive subjects with high-normal BP randomly consumed 500 mg of placebo (0 mg Rg1) or KRG extract with low (0.27 mg), medium (0.81 mg), or high (2.43 mg) doses of ginsenoside Rg1 in identical capsules at time 0-min. Drug-treated individuals refrained from taking antihypertensive agents on test days. At 60-min, a 356-mL (380-calorie) Ensure breakfast was consumed. BP was measured with ABPM (Accutrack II, Suntech Medical Instruments, Raleigh, NC, USA) every 5 min for the first 20 min before time 0-min (the mean of this represented baseline BP) and every 15 minutes for 180 min after the consumption of placebo or KRG extract. We found that the KRG extract with the medium Rg1 dose resulted in large systolic and diastolic BP reductions relative to baseline at 135-min. Consumption of the low and high dose Rg1 extracts showed no significant effect on BP. It was concluded that escalating the dose of ginsenoside Rg1 up to 0.84mg in KRG extracts can result in profound acute blood pressure effects.

**Acute trial 3: flow mediated dilatation study**
While mild or neutral blood pressure lowering effects were observed in the first trial, the subsequent objective was to evaluate the effect of ginseng treatment on more sensitive vascular markers to provide additional information on potential waveform modulation beyond systolic and diastolic blood pressure parameters. Our next step therefore was to explore further the effects of the KRG rootlets identified in the first acute blood pressure trial on these more sensitive markers of vascular function, starting with FMD. Our further objective was also to investigate the direct contribution of its extracted ginsenoside and polysaccharide fractions [44]. The ginsenoside and polysaccharide fractions were extracted at a dose bioequivalent to that provided by 3 g of the KRG rootlets. This approach allowed evaluation of the direct influence of major ginseng fractions on FMD, as well as the comparison of their effects with those of the unprocessed rootlets from which they had been fractionated. FMD studies of the effects of the selected KRG rootlets and its ginsenoside and polysaccharide fractions were undertaken. Using a “phase I”, acute, single-bolus, randomized, crossover design, 17 healthy subjects received the selected KRG rootlets (3 g), ginsenoside extract (0.21 g), polysaccharide extract (0.22 g), or placebo (cornstarch) in random sequence. Extracted polysaccharides and ginsenosides were delivered at doses identical to those found in 3 g of root. Flow mediated dilatation at the brachial artery was assessed at baseline and at 90- and 180-min post treatment. We found that the selected KRG significantly improved FMD at 180 min compared to placebo. The ginsenoside extract produced a comparable shear stress-induced vasodilation compared to control, whereas the polysaccharide fraction did not (Fig. 2A). The ginsenoside extract and whole rootlets did not significantly differ in the changes induced on the vascular endothelium. It was concluded that the selected KRG root acutely improved vascular endothelial function in healthy individuals compared to control, an effect partly attributed to its ginsenoside containing fraction which was high in ginsenoside Rg1.
Acute randomized controlled trial 4: augmentation index study

In the same population of 17 healthy subjects, our subsequent objective was to further investigate vasoactive efficacy of the selected KRG rootlets on pulse wave reflection [44], and compare the effects of rootlets to its two major isolated fractions: ginsenosides and polysaccharides. Pulse wave reflection is a marker of arterial stiffness and provides additional information on the workload of the heart, coronary blood flow, and mechanical integrity of arteries. Radial AI, which characterizes pulse wave reflection, was measured by applanation tonometry (HEM-9000AI; Omron Healthcare Co., Kyoto, Japan) at baseline and at 1h, 2h, and 3h post treatment. The ginsenoside fraction significantly lowered radial AI indicating a decrease in pulse wave reflection, whereas the polysaccharide fraction again did not (Fig. 2B). We concluded that the selected KRG rootlets and their ginsenoside fraction significantly improved arterial stiffness, implicating the ginsenosides (noted to be high in ginsenoside Rg3) as the principal pharmacologically active components of the root. Taken together with the FMD study, these data support further investigation into KRG’s microcomponent fractions, especially its ginsenoside fraction and provides a better basis for ginseng standardization for vascular function related to improved endothelial function and arterial stiffness.

Acute randomized controlled trial 5: Rg3 ginseng vascular effects

Subsequent to observed favorable vasoactive effects of KRG rootlets and its ginsenoside fraction, our further aim in the stepwise assessment was to concentrate and further isolate the ginsenoside that may possess vasodilating properties. As ginsenoside Rg3 was supported by pre-clinical evidence to be the most effective vasoactive component of ginseng and was previously found to have a mild effect on brachial blood pressure, we set to investigate vascular effects of a 10% Rg3-enriched KRG fraction. The sample used in this trial was manufactured by the BTGin Co. (Okcheon, Korea), using chemo-enzymatic process (in situ) and it has a significantly higher concentration of ginsenoside Rg3 (20 times higher) compared to the sample previously used in our program. Our specific objective therefore, was to evaluate the acute effects of 10% Rg3-enriched KRG on arterial stiffness, peripheral and central systolic BP (SBP) and diastolic BP (DBP) in healthy adult volunteers.

Using a double-blind, randomized, crossover design, 23 individuals (SBP, 113±3 mmHg; DBP, 70±2 mmHg) were administered 400 mg 10% Rg3-enriched KRG extract or 400 mg corn starch placebo. Aortic augmentation index (Alx) and central BP were measured using applanation tonometry by radial pulse wave analysis and peripheral BP was evaluated oscillometrically. Measurements were taken at baseline and 1.2 and 3hrs post-treatment. Compared to placebo, there was a significant reduction in peripheral mean arterial pressure, peripheral DBP, and central SBP and DBP 3hrs post-treatment (Fig. 3). Central DBP and central mean arterial pressure were significantly lower at 1h (p<0.05) and 3h (p<0.05) post-treatment. Compared to placebo, Alx was significantly decreased post-treatment, with over 9% absolute reduction at 3hrs time point (Fig. 4). This study is the first to investigate the efficacy of Rg3-enriched KRG extract and its effects on arterial stiffness and central and peripheral blood pressure. This study provide preliminary evidence of acute efficacy of an Rg3 KRG extract on indexes of peripheral and central vascular function in healthy, normotensive, young adults. Further clinical testing is still required as this extract may be more potent in higher-risk subjects.

As with the KRG testing program for diabetes, confirmation of these findings and their sustainability needs to be established by "phase II", long term randomized clinical trials. This exciting new prospect of applying Rg3-enriched ginseng in the long-term clinical trial, together
with selected American ginseng extract in patients with diabetes and concomitant hypertension is underway.

**COMBINATION THERAPY: LONG TERM CLINICAL TRIAL**

In light of the evidence of the KRG and American ginseng clinical evaluation programs for diabetes and vascular function, our ensuing intention was to consider a combination therapy with aim of advancing ginseng evaluation using a multi-targeted approach. Combination therapy has proven to be a popular treatment strategy for tighter diabetes control. Although the exact mechanism of action is unknown, based on preliminary evidence and our American and KRG screening programs, both species of ginseng act through a different, but complementary mechanism of action; American ginseng may act by increasing insulin secretion, whereas, KRG may act by improving insulin sensitivity. Therefore a long term trial was designed to investigate the therapeutic potential of American ginseng and KRG in combination to address both glycemic control via complementary action as well vascular activity potential.

The study followed a randomized, double-blind, placebo-controlled, two-centre, two-arm parallel design in individuals with type 2 diabetes. Effects on glycemic control are assessed by examining biochemical markers and the glycemic response to an oral glucose tolerance test. Additional metabolic parameters and blood pressure parameters are also evaluated as markers of CVD risk. The combined use of American ginseng and KRG for 12 wk demonstrated a significant improvement in long term marker of glycemic control, HbA1c, by 0.4% as well as a significant improvement in systolic blood pressure by 5 mmHg compared to placebo. The combination treatment also demonstrated to be safe, but did not significantly affect blood lipids. These findings encourage further investigation of the mechanism and roles of Korean and American ginseng combined and continuation of systematic evaluation of their effective components.

**CONCLUSION**

While our clinical evidence, along with traditional indication for generic ginseng is largely aligned in supporting a diabetes and possibly vascular indication, the safety and efficacy of specific ginseng sources in improving glycemic and vascular outcomes in humans remains questionable. We applied the robust systematic, “acute-to-chronic”, stepwise clinical screening model to our KRG via two efficacy arms of the program. We were successful in identifying KRG sources with reproducible and sustainable glycemic benefit in diabetes as assessed
by markers of glucose and insulin regulation. We were also successful in identifying the fractions of ginseng that have contributed to the vasoactive potential as assessed by markers of endothelial function, BP and arterial stiffness. Based on our and other laboratories, preliminary clinical studies indicate that a mechanism of action for KRG to improve diabetes control is through increase in insulin sensitivity, and to improve vascular function is through vaso-motor dilatory modification based on reflected arterial waveforms. Although our clinical screening model may provide a basis for component based “clinical standardization” of KRG, there remains a clear need to develop a better basis for standardization that ties composition directly to efficacy. More clinical work is needed to expand upon the connections that have been drawn between aspects of the ginsenoside profile, for example Rg3, of KRG and efficacy. There is also a requirement for more “phase II” trials of effectiveness are required to support clinical indications for KRG and its fractions in diabetes and vascular disease. Progress in these areas may bring us closer to establishing standardization parameters of ginseng leading to more consistent predictions of KRG efficacy.

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