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Basic nutritional investigation

Interleukin-1 genotype-selective inhibition of inflammatory mediators by a botanical: a nutrigenetics proof of concept

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Abstract

Objective: Although observational studies have shown that genotype may influence nutritional effects on target outcomes, there are few reported studies that stratified subjects by genotype before a nutritional intervention. This proof-of-concept trial determined whether specifically formulated botanical mixtures reduced inflammation in individuals with genetic variations that predispose to overexpression of interleukin-1β (IL-1β) and early heart disease.

Methods: Healthy adults with elevated C-reactive protein (CRP) were stratified into genetic groups based on being positive ($IL1^{Pos}$) or negative ($IL1^{Neg}$) for the at-risk IL-1 gene variations. $IL1^{Pos}$ ($n = 39$) and $IL1^{Neg}$ ($n = 40$) subjects were then randomized to the candidate botanical formulation or placebo. The botanical formulation included rose hips, a blueberry and blackberry mixture, and a grapevine extract.

Results: At 12 wk of dosing with the botanical formulation, IL-1β gene expression by stimulated peripheral blood mononuclear cells was significantly lower than at baseline and significantly lower than placebo in $IL1^{Pos}$ and $IL1^{Neg}$ subjects. Mean IL-1β gene expression treatment effect over the 12-wk period was greater in $IL1^{Pos}$ than in $IL1^{Neg}$ subjects. At 12 wk of dosing the botanical mixture produced no mean change in serum CRP levels. However, in $IL1^{Pos}$ subjects, significantly more subjects achieved a reduction in CRP with the botanical mixture than with placebo. No CRP effect was observed in the $IL1^{Neg}$ subjects.

Conclusion: This study represents one of a few prospective clinical trials in which genetic variations were shown to differentially influence nutrient effects on outcomes.

Keywords: Nutrigenetics; Interleukin-1; Genotype; C-reactive protein; Botanical; Rose hips

Introduction

The promise of nutrigenetics is that genetic information may be used to guide individuals to better nutrition. This assumes that some individuals will have health benefits from consumption of certain nutrients and other individuals will have less benefit or adverse reactions. In an effort to develop practical applications of nutrigenetics, we focused on the role of genetic variations in key inflammatory mechanisms as a target for nutrient modulation.

Overexpression of the inflammatory response is associated with various chronic diseases of aging [1–4], including the role of inflammation as a key biological mechanism in cardiovascular disease events [5,6]. Interleukin-1 (IL-1) has been implicated in the development of atherosclerosis, and increased IL-1 biological activity in animal models increases the rate of development of atherosclerosis and leads to spontaneous inflammation in mid to large arteries [7–9]. Recent studies in humans have shown that IL-1 gene variations alter gene expression [10], are associated with higher levels of IL-1β and other inflammatory mediators, and are

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associated with an increased risk of early myocardial infarction [4,11].

This proof-of-concept trial was designed to determine whether individuals with the genetic variations that predispose to overexpression of IL-1 and an early myocardial infarction could be modulated by means of botanical extracts that were selected for this specific IL-1 target. We used cell-based models to screen nutrient extracts that inhibited IL-1β or inhibited effects downstream of IL-1, such as C-reactive protein (CRP). Healthy subjects were selected with elevated CRP and were stratified into genetic groups based on being positive ($IL1^{Pos}$) or negative ($IL1^{Neg}$) for the IL-1 gene variations associated with overexpression of IL-1β. $IL1^{Pos}$ and $IL1^{Neg}$ subjects were then randomized to various mixtures of candidate botanical extracts selected from the screening assays or placebo. In this study consumption of the multi-ingredient botanical mixture for 12 wk inhibited IL-1β gene expression and CRP significantly in $IL1^{Pos}$ subjects compared with placebo, and there was less effect in $IL1^{Neg}$ subjects. This represents one of a few prospective clinical proofs that selected nutrients can differentially influence outcomes based on genetic variations.

Materials and methods

This proof-of-concept, randomized, controlled clinical trial was part of a research program to determine whether botanicals selected on specific screening criteria reduced IL-1β gene expression in participants with IL-1 gene variations associated with overexpression of IL-1 and with increased risk for cardiovascular events.

Clinical trial methodology

The clinical trial was registered (study no. NCT00303238) with ClinicalTrials.gov (http://www.clinicaltrials.gov/ct/show/NCT00303238).

The trial was a randomized, placebo-controlled design approved by the New England Institutional Review Board (Wellesley, MA, USA) and was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines. Participants were recruited from a participant database of 2329 healthy adults collected under a separate protocol approved by the institutional review board (Western IRB, Olympia, WA, USA). The study was conducted from October of 2004 through May of 2005 at eight United States research sites (Access Business Group, Buena Park, CA; Alticor, Ada, MI; East Coast Clinical Research, Salisbury, MA; National Institute for Clinical Research, Los Angeles, CA; Omega Medical Research, Warwick, RI; Providence Clinical Research, Burbank, CA; Sall Research Medical Center, Bellflower, CA; Southbay Pharma Research, Buena Park, CA).

Non-smoking adults $\geq$18 y of age in good general health were eligible to participate. Subjects were excluded if they were on medications for blood pressure, coagulation disorders, or high cholesterol or were taking steroids, including asthma inhalers, non-steroidal anti-inflammatory drugs, or hormone replacement therapy. Women were also excluded if they were pregnant or lactating or were of child-bearing age and unwilling to use birth control.

Those who met the inclusion/exclusion criteria for the trial were screened for serum CRP, vital signs, serum chemistry, hematology, and urinalysis approximately 2 wk before the study initiation. Subjects with CRP levels from 2 to 10 mg/L were qualified for participation. Qualified participants were stratified by IL1 genotype and then randomized to one of three different experimental botanical formulations or a placebo. Each IL1 genotype included sufficient qualified subjects to allow randomization of 20 subjects per treatment group at the start of the study. Data are presented on one of the three botanical formulations and the placebo. One of the other two botanical formulations showed no effect on the primary outcomes and the other formulation achieved results in the same pattern as that reported for the formulation reported below but with less strong results (data not shown).

Experimental formulations

The botanical mixtures were developed to inhibit IL-1 production and to inhibit mediator production downstream of IL-1. Two hundred twenty candidate botanical ingredients were evaluated in vitro for their ability to inhibit IL-1β gene expression in human mononuclear cells (U937 and THP-1) stimulated with lipopolysaccharide. Twenty of the candidate ingredients were also screened for their ability to inhibit mediator production downstream of IL-1 by assaying effects on CRP production by HepG2 cells stimulated with IL-1β and IL-6. The ingredient list was narrowed based on the biological assays and non-biological parameters, such as reliability of sourcing.

The lead inhibitor of IL-1 production was a rose hips extract and four secondary ingredients (blueberry powder, blackberry powder, grapevine extract, and *Aframomum melegueta*) that were selected based on in vitro inhibition of CRP. The rose hips extract was formulated into three botanical mixtures with various combinations of the four secondary ingredients. This report describes the clinical data on the placebo and the formulation that included rose hips extract (1200 mg/d), blackberry powder (165 mg/d), blueberry powder (330 mg/d), and grapevine extract (40 mg/d; RH-B-GV). Tablets were developed with the botanical ingredients plus inert excipients including microcrystalline cellulose, corn starch, dicalcium phosphate, and processing aids such as modified cellulose gum, magnesium stearate and silicon dioxide. The placebo tablets contained a combination of the same inert excipients and matched the botanical tablets in size and appearance. The identity and quality of the individual botanical ingredients were tracked by the following markers, respectively: dehydro-ascorbate, anthocyanins, and all-*trans*-resveratrol. Each participant

was given a box containing the product in packets and instructions.

After 12 wk of supplementation, the participants were given one-half the dose for an additional 8wk to evaluate whether the product could be used as an initial dose regimen followed by a lower "maintenance" dose. The data on the maintenance doses are not presented.

Compliance was evaluated by means of participant interview and counting of study product returned to the clinic at weeks 4, 8, and 12. Non-compliance was defined as consumption of <80% of the scheduled intakes of study product. Participants with continual non-compliance were dropped from the study. The subject retention at 12 wk was 88% for the test formulation group and 72% for the placebo group.

Outcome variables

The primary outcomes for this study were IL-1β gene expression in peripheral blood mononuclear cells (PBMCs) collected from the subjects and inhibition of ex vivo IL-1β protein production by subjects' serum. Secondary outcome parameters included serum levels of CRP as measured by a high-sensitivity analysis, a panel of selected cytokines, fibrinogen, serum amyloid A, and serum intracellular adhesion molecule-1. All outcomes were measured at baseline and after 4, 8, and 12 wk of dosing.

In vivo IL-1β gene expression was assessed in participants' PBMCs using quantitative real-time polymerase chain reaction. The ex vivo IL-1β production was measured by incubating the participants' plasma with a THP-1 human mononuclear cell line in vitro. The cultured cells were then challenged with lipopolysaccharide, and the production of IL-1β protein was evaluated by an enzyme-linked immunosorbent assay methodology.

Serum CRP levels were measured using a high-sensitivity assay (nephelometry; Dade Behring Covance Central Laboratories, Indianapolis, IN, USA). For each time point, two fasting blood samples were taken about 1 wk apart, CRP was measured in both, and the results of the two blood samples were averaged for each time point.

Serum and plasma samples were analyzed for all other parameters using flow cytometric methodology (Covance Central Laboratories). At the interim analysis, it was noted that the chosen method of analysis was not sensitive enough to detect small changes in cytokine levels in generally healthy people. For this reason, frozen plasma samples were analyzed using enzyme-linked immunosorbent assay methodology (Burleson Research Technologies; Morrisville, NC, USA). Due to the small amount of available retained plasma for each participant, only IL-6 and IL-10 could be reanalyzed.

Safety and tolerability were assessed by measuring changes in serum chemistry, hematology, urinalysis, vital signs, body weight, and reported adverse events.

IL1 genotype stratification of subjects

DNA was extracted and IL1 genotyping was performed at Kimball Genetics (Denver, CO, USA) by polymerase chain reaction and restriction fragment length polymorphism analysis, as described previously [12]. All genetic analyses were performed blinded to clinical data. Single nucleotide polymorphisms were genotyped at two loci in the gene for IL-1β (IL1B[−511] C → T; and IL1B[+3954] C → T) and at one locus in the gene for IL-1α (IL1A[+4845] G → T).

Subjects were classified as $IL1^{Pos}$ if they had any of the following three genotypes: 1) were homozygous for the common allele (C) at IL1B(−511); 2) carried two copies of the less common allele (T) at IL1A(+4845); or 3) carried one copy of the less common allele at IL1A(+4845) plus at least one copy of the less common allele (T) at IL1B(+3954). Genotype C/C at IL-1B(−511) has been associated with increased expression of IL-1β protein and with increased risk for cardiovascular events [11]. IL1B(+3954) allele T, alone or in combination with IL1A(+4845) allele T, has been associated with increased levels of IL-1 and CRP [4,13]. All other individuals were designated $IL1^{Neg}$. Approximately 59% of Caucasians have IL1 genotypes that qualify as $IL1^{Pos}$ (data not shown).

Data management and statistical methods

All laboratory data were sent to a third-party data manager (Ockham Development Group; Cary, NC, USA) and entered into a secured database containing no subject-identifying information. Recruitment from the participant database involved a decoding step not associated with the sponsor. All intervention trials were constrained by randomization to include equal numbers of different genotyped subjects such that genotype and other protected information was not revealed by recruitment inclusion/exclusion criteria.

The primary outcome analysis was a two-way analysis of variance test to compare each participant's baseline measurement with that participant's treatment measurements over time and to compare differences between treatments. Tukey's highest significant difference test was used to analyze differences post hoc. In some instances data were analyzed with Fisher's exact test. All tests of significance were performed at $\alpha = 0.05$.

Results

Effects of treatment on IL-1β gene expression and production

There were no significant differences between any of the groups at baseline (Table 1). IL-1β gene expression in PBMCs from treated subjects is shown in Figure 1a for the two IL1 genotype groups. After 12 wk of dosing, PBMCs from subjects receiving the RH-B-GV botanical formulation

Table 1
Primary biomarker outcomes at baseline*

Genotype	Treatment	IL-1 gene expression (copy number/μg RNA)	IL-1 production (units IL-1/mL)	CRP (mg/L)
$IL1^{Pos}$	RH-B-GV	10.78 ± 21.38	0.67 ± 0.13	4.9 ± 1.7
$IL1^{Neg}$	RH-B-GV	11.38 ± 20.78	0.65 ± 0.14	5.4 ± 2.7
$IL1^{Pos}$	Placebo	15.67 ± 42.26	0.63 ± 0.14	5.8 ± 4.4
$IL1^{Neg}$	Placebo	11.23 ± 27.94	0.66 ± 0.12	5.3 ± 2.4

CRP, C-reactive protein; IL-1, interleukin-1; $IL1^{Neg}$, negative for at-risk IL-1 gene variations; $IL1^{Pos}$, positive for at-risk IL-1 gene variations; RH-B-GV, mixture of rose hips, blueberries and blackberries, and grapevine

* Mean ± SD.

had lower IL-1β gene expression. In general, the placebo groups showed great variability in IL-1β gene expression. $IL1^{Pos}$ subjects receiving the RH-B-GV formulation had a mean reduction in expression of 61.2% at week 12 compared with baseline, which was significantly different from expression in the $IL1^{Pos}$ placebo group ($P < 0.001$). $IL1^{Neg}$ subjects receiving RH-B-GV had a mean reduction in expression of 43.8% at week 12, and that group was significantly different from expression in the $IL1^{Neg}$ placebo group ($P < 0.05$).

We also evaluated the IL-1β gene expression response by averaging all measurement periods between 4 and 12 wk of treatment for each subject. The distribution of responses is shown in Figure 1b for $IL1^{Pos}$ and $IL1^{Neg}$ subjects taking

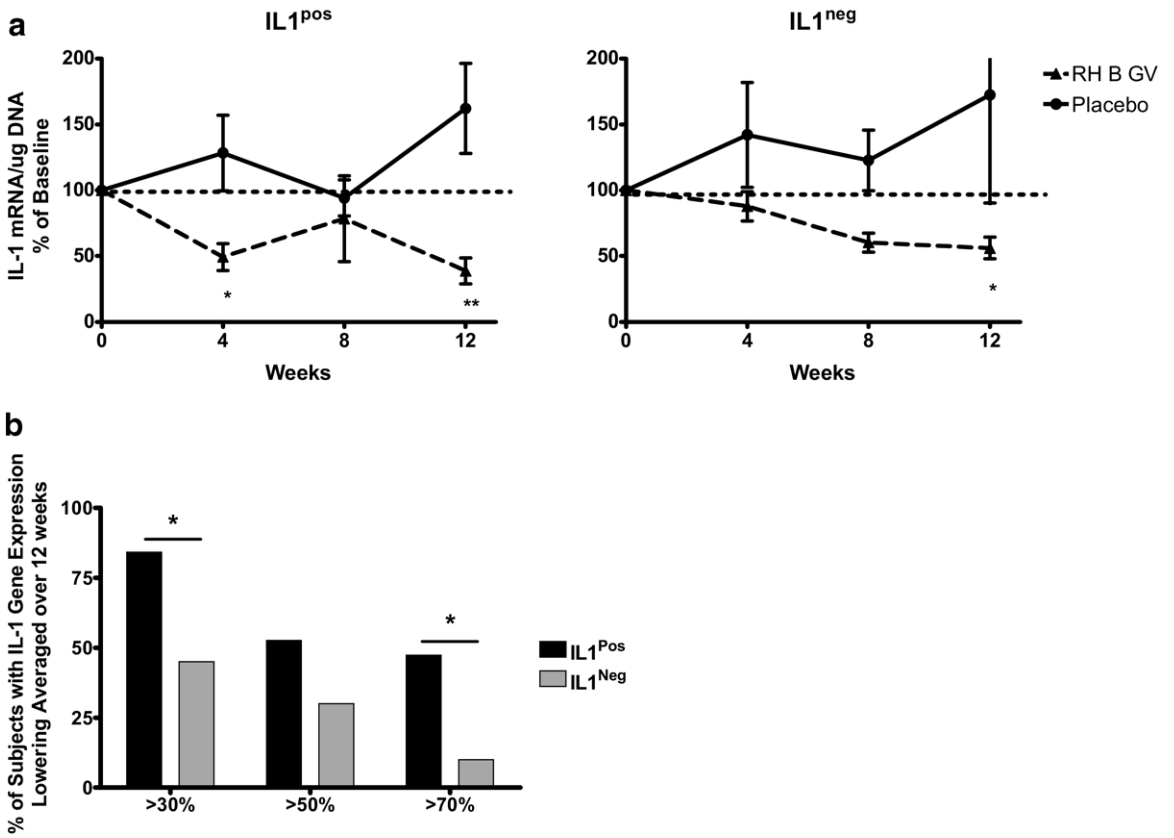

Fig. 1. (a) IL-1β gene expression in subjects consuming botanical or placebo. Fasting blood samples were collected at the indicated times and IL-1β mRNA and total DNA were isolated from peripheral leukocytes and measured as described in MATERIALS AND METHODS. IL-1β mRNA at each time point is expressed as a percentage of baseline. IL-1β gene expression for subjects with $IL1^{Pos}$ and $IL1^{Neg}$ composite genotypes are shown in the left- and right-hand panels, respectively. $^{*}P < 0.05$; $^{**}P < 0.001$ for the botanical mixture RH-B-GV (triangles) versus placebo (circles). The dashed horizontal line at 100% is placed for reference to baseline. (b) Effect of IL1 composite genotype on IL-1β gene expression in subjects consuming the botanical mixture. IL-1β gene expression data from weeks 4, 8, and 12 were averaged relative to baseline for each subject treated with the botanical RH-B-GV formula. A contingency table was constructed with the numbers of subjects with IL-1β gene expression decreases >30%, >50%, and >70% compared with baseline ($^{*}P = 0.012$). IL-1, interleukin; $IL1^{Neg}$, negative for at-risk IL-1 gene variations; $IL1^{Pos}$, positive for at-risk IL-1 gene variations; RH-B-GV, mixture of rose hips, blackberries and blueberries, and grapevine.

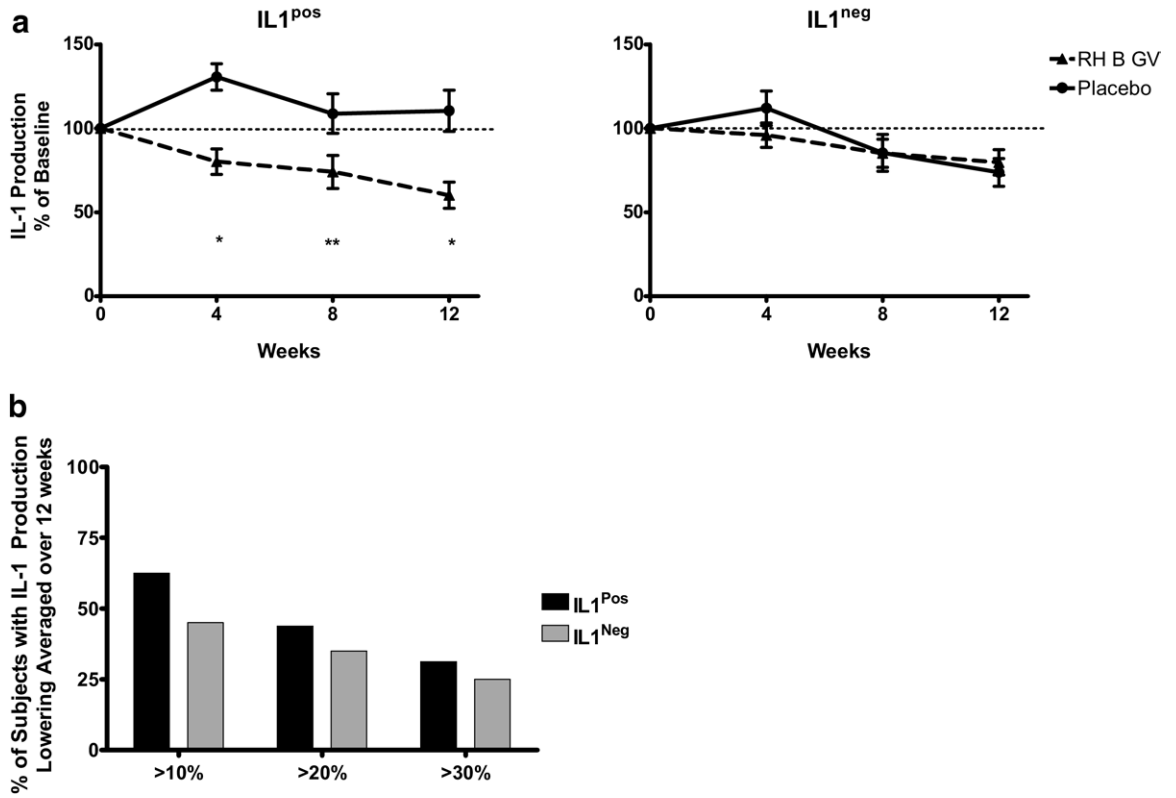

Fig. 2. (a) Ex vivo IL-1β production in subjects consuming botanical or placebo. Fasting blood samples were collected at the indicated times and plasma was prepared for ex vivo assessment of IL-1 production as described in MATERIALS AND METHODS. IL-1β production at each time point is expressed as a percentage of that measured in baseline plasma samples. IL-1β production levels for subjects with $IL1^{Pos}$ and $IL1^{Neg}$ composite genotypes are shown in the left- and right-hand panels, respectively. $^{*}P < 0.05$; $^{**}P < 0.001$ for the botanical RH-B-GV (triangles) versus placebo (circles). The dashed horizontal lines at 100% are placed for reference to baseline. (b) Effect of IL1 composite genotype on ex vivo IL-1β production in subjects consuming the botanical mixture. IL-1β production data from weeks 4, 8, and 12 were averaged relative to baseline for each subject treated with the botanical formula, RH-B-GV. A contingency table was constructed with numbers of subjects with IL-1 production decreases >10%, >20%, and >30% compared with baseline. IL-1, interleukin; $IL1^{Neg}$, negative for at-risk IL-1 gene variations; $IL1^{Pos}$, positive for at-risk IL-1 gene variations; RH-B-GV, mixture of rose hips, blackberries and blueberries, and grapevine.

the RH-B-GV botanical. More $IL1^{Pos}$ than $IL1^{Neg}$ subjects had a >30% reduction ($P = 0.012$) and >70% reduction ($P = 0.012$) in IL-1β gene expression in response to the RH-B-GV botanical.

Ex vivo inhibition of IL-1β protein production from monocyte cell lines treated with plasma from experimental subjects provides an indication of whether the consumed formulations are available in serum in a bioactive form. The ex vivo IL-1β production from treated subjects is shown in Figure 2a for the two IL1 genotype groups. In general, the plasma from placebo groups produced no change relative to baseline. Plasma from $IL1^{Pos}$ subjects receiving the RH-B-GV botanical formulation inhibited IL-1β production significantly and consistently at all monitoring periods, with a mean reduction of 28.2% from baseline at 12 wk (different from $IL1^{Pos}$ placebo, $P < 0.05$). Plasma from $IL1^{Neg}$ subjects receiving the RH-B-GV botanical exhibited variability in IL-1β inhibition over the three monitoring periods and exhibited a mean reduction of 13.0% at 12 wk, which was not significantly different from placebo.

The IL1 genotype influence on ex vivo IL-1β protein production in response to the RH-B-GV treatment is shown in Figure 2b. The reduction in ex vivo IL-1β production for each subject was averaged over all measurement periods between 4 and 12 wk of treatment. In response to the RH-B-GV botanical, there was no significant difference in ex vivo IL-1 production between the two IL1 genotypes.

Effects of treatment on serum CRP levels

When serum CRP levels were expressed as group means relative to baseline, subjects receiving the placebo formulation exhibited great variability in CRP levels in both IL1 genotypes, and mean CRP levels in placebo subjects were increased relative to baseline at some of the time points. In subjects receiving the RH-B-GV botanical formulation, the mean level of CRP remained similar to baseline in both IL1 genotypes (Fig. 3a).

When the distribution of the individual CRP responses was examined, there appeared to be a minimal response or a substantial response to the RH-B-GV botanical, which may be

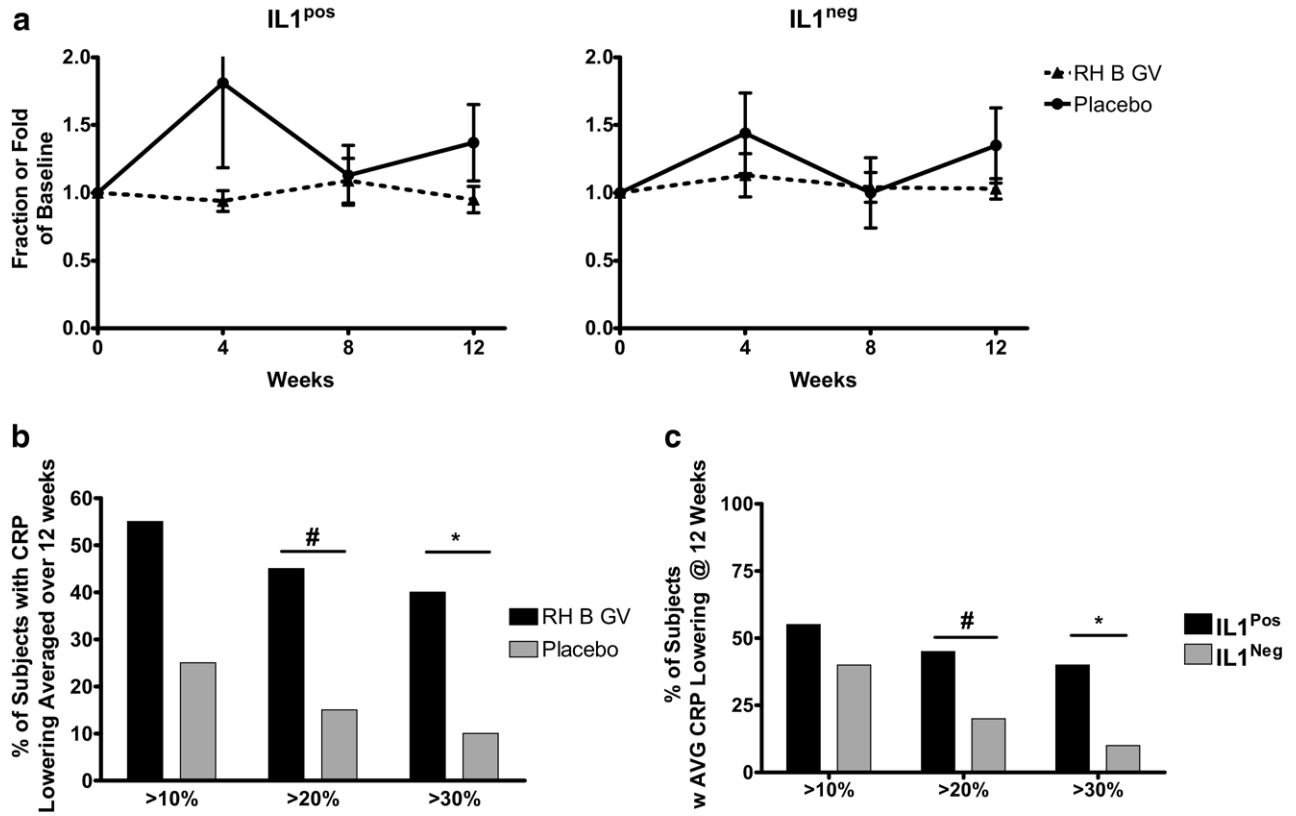

Fig. 3. (a) CRP levels in subjects consuming botanical or placebo. Fasting blood samples were collected at the indicated times and high-sensitivity CRP was measured as described in MATERIALS AND METHODS. CRP at each time point is expressed as a fraction or fold of baseline. CRP for subjects with $IL1^{Pos}$ and $IL1^{Neg}$ composite genotypes are shown in the left- and right-hand panels, respectively, for the botanical RH-B-GV mixture (triangles) versus placebo (circles). (b) CRP treatment response in $IL1^{Pos}$ subjects consuming botanical or placebo. CRP data from weeks 4, 8, and 12 were averaged relative to baseline for $IL1^{Pos}$ subjects treated with the botanical RH-B-GV mixture or placebo. A contingency table was constructed with numbers of subjects with CRP decreases >10%, >20%, and >30% compared with baseline ($^{\#}P = 0.08$; $^{*}P = 0.03$). (c) Effect of IL1 composite genotype on CRP levels in subjects consuming the botanical mixture. CRP data from weeks 4, 8, and 12 were averaged relative to baseline for subjects treated with the botanical formula, RH-B-GV. A contingency table was constructed with the numbers of subjects with CRP decreases >10%, >20%, and >30% compared with baseline for $IL1^{Pos}$ versus $IL1^{Neg}$ ($^{\#}P = 0.08$; $^{*}P = 0.03$). AVG, average; CRP, C-reactive protein; IL-1, interleukin; $IL1^{Neg}$, negative for at-risk IL-1 gene variations; $IL1^{Pos}$, positive for at-risk IL-1 gene variations; RH-B-GV, mixture of rose hips, blackberries and blueberries, and grapevine.

masked in group means. The reduction in serum CRP levels for each subject was averaged over all measurement periods between 4 and 12 wk of treatment and is shown in Figure 3b for $IL1^{Pos}$ subjects treated with placebo or the RH-B-GV botanical. In $IL1^{Pos}$ subjects treated with the botanical, 55% (11 of 20 subjects) showed a reduction of CRP >10% and 40% (8 of 20) had mean reductions >30%. In placebo-treated $IL1^{Pos}$ subjects, 26.3% (5 of 19) had CRP reductions >10% and 10.5% (2 of 19) had reductions >30%.

The IL1 genotype influence on the RH-B-GV treatment response, as measured by serum CRP levels, is shown in Figure 3c. In subjects treated with the botanical, a CRP reduction >10% was observed in 55% of subjects (11 of 20) who were $IL1^{Pos}$ and in 40% (8 of 20) who were $IL1^{Neg}$. A CRP reduction >30% was observed in 40% subjects (8 of 20) who were $IL1^{Pos}$ and in 10% (2 of 20) who were $IL1^{Neg}$.

Serum protein levels of IL-6 and IL-10 exhibited great variability during the 12 wk of the study, and there were no significant differences between test and placebo groups in these parameters (data not shown). Test products were well tolerated with infrequent and minor adverse events, with no differences between placebo and the active formulation. In no instance was an adverse event clearly associated with the study product. No treatment-related changes were observed in any of the serum chemistry, hematology, vital signs, or liver function parameters during the study. Three adverse events were reported in the subjects receiving the botanical formulation, including an intestinal mass, a fracture of the patella, and an osteoarthritis episode. One adverse event, a hypoglycemic reaction, was reported in subjects receiving the placebo formulation. None of these events were considered related to the study product.

Discussion

In this study we clinically evaluated botanical mixtures that may be of benefit in lowering inflammatory mediators in subjects who, because of genetic variations, overexpress IL-1β and are at increased risk for early cardiovascular events. This

nutrigenetics clinical trial demonstrated that a botanical mixture targeting IL-1 production and response was able to significantly reduce IL-1β gene expression and CRP in healthy individuals carrying gene variations that are associated with overexpression of IL-1.

The botanical extracts were selected from in vitro screens to assess modulation of IL-1β overexpression, the biological effect associated with the IL1^{Pos} genotypes used in the clinical study. The study population included healthy adults with CRP levels between 2 and 10 mg/L who were stratified by IL1 genotype before randomization to the botanical formulation or placebo. We believe this is one of the first human trials to demonstrate the clinical potential of nutrigenetics and the successful development of a drug or nutritional product to modulate directly the effects of a genetic variation.

The role of inflammation in cardiovascular disease has been well documented [5,6,14], and low CRP levels have been shown to be as protective against secondary cardiovascular events as low low-density lipoprotein cholesterol levels [6]. The genes encoding the proinflammatory cytokines IL-1α and IL-1β are among the first activated in the course of an inflammatory response. IL-1 activates biological pathways within the arterial wall that are implicated in the development of atherosclerosis [15–17]. Excess IL-1 biological activity in animals leads to spontaneous arterial inflammation with massive inflammatory cell infiltration in mid and large arteries [18,19] and increased atherosclerotic lesion size with enrichment of macrophages within lesions [7,9].

The IL1 genotypes that constitute the IL1^{Pos} group in this study have been shown to increase the risk of early myocardial infarction by more than two-fold [11] and increase the expression of IL-1β [4,11,20,21]. These same polymorphisms have been associated with increased risk or more severe progression in several human conditions, including periodontal disease [4,22], gastric cancer [23,24], Alzheimer's disease [25,26], and others.

The test formulation in this study was composed of two botanical components. The rose hips extract was selected based on potent inhibition of IL-1β gene expression in lipopolysaccharide-stimulated monocytes. A combination of extracts of blueberries, blackberries and grapevine was also included in the formulation based on inhibition of response to IL-1 as measured by CRP release by hepatocytes stimulated with IL-1β plus IL-6.

In the IL1^{Pos} subjects, daily consumption of the botanical produced a mean decrease from baseline in their PBMC IL-1β gene expression by 61.2%. The magnitude of effect was different by genotype, with a mean reduction from baseline of IL-1β gene expression of >30% in 84.2% of the IL1^{Pos} subjects compared with 45% of IL1^{Neg} subjects. Significantly more of the IL1^{Pos} subjects showed a CRP response with the botanical supplement compared with the placebo. In addition, the botanical effect on CRP appeared to be genotype specific. For example, in subjects taking the botanical, a reduction in CRP of >30% was evident in 40% of IL1^{Pos} subjects versus 10% of IL1^{Neg} subjects.

Several investigators have previously reported, in observational or epidemiologic studies, that nutrient associations with health and biomarker outcomes are genotype specific. A few dramatic examples of these nutrient-gene interactions include the differences in association between dietary polyunsaturated fatty acid and serum lipid values based on peroxisome proliferator activated receptor-α gene variations [27] and differences in association between polyunsaturated fatty acid levels and atherosclerosis based on a 5-lipoxygenase genotype [28]. In addition, studies are beginning to emerge that evaluate drugs or nutrients in a population that has been preselected based on one specific genotype [29]; however, such designs may not allow one to assess the influence of genotype on the effect of the drug or nutrient.

We are aware of few reports of nutrigenetics clinical studies that stratified individuals initially by genotype and then evaluated responses to specific nutrients in a randomized controlled clinical trial. McCombs et al. [30] selected 11 subjects who were heterozygous for the uncommon apolipoprotein allele A-IV and 12 who were homozygous for the common allele. All subjects consumed a low-cholesterol diet for 2 wk and then switched to a high-cholesterol diet. After 3 wk of a high challenge diet, the hypercholesterolemia response was significantly less in the apolipoprotein A-IV heterozygotes than in subjects homozygous for the common allele. More recently [31] healthy males were stratified by genotype for the peroxisome proliferator activated receptor-α polymorphism that produces a Leu162Val change in the polypeptide sequence. Ten carriers of the V162 allele and 10 L162 homozygotes were, at different times, fed diets that differed in the ratio of polyunsaturated to saturated fats. A significant genotype-by-diet interaction was observed for total cholesterol and small-particle low-density lipoprotein levels.

Although genetic variations have been reported to explain differential drug/nutrient responses, there are few, if any, examples of the successful intentional development of a drug or nutritional product to modulate directly the effects of a genetic variation. One possible example is the genetic variation for 5-lipoxygenase–activating protein, which is associated with an increased production of leukotriene B_4 and myocardial infarction and the demonstration of the use of a 5-lipoxygenase–activating protein inhibitor to lower not only leukotriene B_4 but also CRP [29].

We can only speculate on why the botanicals were more effective in the IL1^{Pos} subjects. The botanicals for inhibition of IL-1β were screened initially in monocyte cell lines (U937) that carry the IL1^{Pos} genotype, so the screening process may have enriched for effect with that gene variation. One may also postulate that the overexpressing genotype is more sensitive to activation and inhibition than the genotype that is associated with less IL-1β protein release. One of the primary mechanisms by which nutrients may influence biological activity is through direct and indirect effects on transcription factors [32], and multiple nutrients have been shown to modify IL-1 gene expression [33,34]. We recently identified the functional single nucleotide poly-

morphisms in the IL-1β gene and demonstrated that some of these single nucleotide polymorphisms have significant allelic differences in transcription factor binding and in promoter activity [10]. There was no genotype effect on the botanical inhibition of IL-1β in the ex vivo assay, which suggests that the genotype did not influence plasma levels of bioactive components.

In this study, there were no indications of adverse effects on any of the chemistry or blood parameters that were measured in the present trial. Currently, serious adverse events have not been associated with IL-1 antagonist drug therapy [35], and the long-term effects of botanical modulation of IL-1β expression in specific genotypes are unknown.

This study has some limitations. First, we used biomarker outcomes in this short-term study. One cannot determine, based on this study alone, if the botanical preparation that significantly lowers IL-1β and serum CRP levels in an at-risk group translates into altered risk for first cardiovascular events. Decreased inflammatory mediators have been shown to significantly reduce the incidence of cardiovascular events in patients with previous heart disease [6], and a study in progress will determine if lowering CRP in otherwise healthy adults produces a reduction in first cardiovascular events [36]. Second, the study sample was small. The study was designed based on IL-1β gene expression data from a 2-wk pilot clinical study using rose hips in subjects carrying the $IL1^{Pos}$ genotype. In the randomized controlled study, the test formulation in $IL1^{Pos}$ subjects achieved statistical significance for the primary and some secondary outcomes. Previous reports of clinical nutrigenetics studies [30,31] that stratified initially by genotype before the nutritional challenge were able to show significant differences with 10 to 12 subjects per experimental group. In larger populations one may find factors that contribute to the outcome that may not be evident in smaller studies. Large prospective studies of healthy individuals present substantial recruitment and retention challenges, especially if the subject cannot directly measure the outcomes.

This study is one of the first nutrigenetics clinical trials in which subjects were stratified initially by genotype and then randomized to an active botanical mixture or a placebo. A specially formulated botanical mixture, including rose hips, a mixture of berries, and a grapevine extract, was able to reduce inflammatory biomarkers in healthy individuals carrying gene variations that are associated with overexpression of IL-1 production and increased risk for cardiovascular events.

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