Immune Modulating Effects of Daily Supplementation of COLD-fX (a Proprietary Extract of North American Ginseng) in Healthy Adults

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Received 20 April, 2006; Accepted 15 June, 2006

Summary  In a previous study, COLD-fX (CVT-E002), a proprietary extract of the root of North American ginseng (\textit{Panax quinquefolium}), was found effective in the prevention of upper respiratory tract infections (URIs) in healthy adults. The underlying mechanisms of its action, however, were not determined. The present study was carried out to explore if the effects observed could be due to COLD-fX-mediated changes in the distribution of peripheral blood leukocytes, changes in the relative distribution of lymphocyte subsets or IgA levels in plasma. At the onset of an influenza season, a total of 323 subjects between 18 and 65 years with a history of more than 2 colds in the previous year, participated in a randomized double-blind placebo-controlled study. The participants were instructed to take 2 capsules/day of either COLD-fX or placebo for a period of 4 months. Two blood samples were collected from 42 (COLD-fX = 21; placebo = 21) of the 323 recruited volunteers. These samples were obtained before and after the treatment, and were used for determination of white blood cell differential counts, enumeration of lymphocyte subsets and measurement of plasma levels of immunoglobulin A (IgA). The distribution of colds over the 4 month period was found to be similar in both treatment and placebo groups. However although non-significant, the severity of the colds in the COLD-fX group was found to decrease over time. COLD-fX intervention increased the proportion of T-helper and natural killer (NK) cells, and decreased IgA levels in plasma to a greater extent than the placebo. It is possible, therefore, that these cells acted synergistically to reduce the severity and duration of URIs in the COLD-fX group. Further studies are warranted to determine the effects of daily supplementation of COLD-fX on the activities of T-helper and NK cells in plasma.

Key Words: COLD-fX, NK cells, common cold

Introduction

COLD-fX, a patented extract from the roots of North American ginseng (\textit{Panax quinquefolium}), containing mainly poly-furanosyl-pyranosyl-saccharides, has been found effective in the prevention of upper respiratory tract infections (URIs). In an earlier clinical trial, daily administration of COLD-fX reduced the number of acute respiratory illnesses due to influenza and respiratory syncytial virus in institutionalized seniors \cite{1}. In another study daily dosing of COLD-fX during an early cold & flu season reduced the number of
COLD-fX and Immune Function

Methods

The study was conducted as a randomized, double blind, placebo controlled trial during September 2003 to April 2004 at the University of Alberta, Edmonton, Canada.

Preparation and assignment of COLD-fX / Placebo

COLD-fX, a proprietary product of CV Technologies Inc. (Edmonton, Alberta, Canada) was formulated from the roots of North American ginseng (Panax quinquefolium L., Fam. Araliaceae). It contains 80% polysaccharides and oligosaccharides, and 10% protein. The freeze-dried extract was encapsulated to contain 200 mg/capsule. The placebo used in this study was rice powder and was encapsulated identically to the active treatment. The subjects were randomly assigned to receive either COLD-fX or placebo.

Study design

Following approval by the Human Ethics Committee of the University of Alberta, subjects were recruited through media advertisements from Edmonton and the surrounding areas. Volunteers were required to be between the ages of 18 to 65 years and to have contracted at least 2 infections of a cold in the past year. Subjects were excluded if they had been vaccinated against influenza in the past 6 months. Subjects with medical conditions such as multiple sclerosis, tuberculosis, diabetes, cancer, lupus, HIV/AIDS, cardiovascular disease, hypertension, neurological or psychiatric disease, and renal, pulmonary and hepatic abnormalities were also excluded. Other exclusion factors employed in the study included those on medications such as immunosuppressive drugs, corticosteroids, warfarin, phenalzine, pentobarbital, haloperidol or cyclosporine, as well as pregnant and lactating women, and heavy smokers.

The treatment consisted of taking 2 capsules/day (200 mg/capsule) for a period of 4 months following the onset of an influenza season. Subjects were asked to take their medication daily, in the mornings, after breakfast with a glass of water. Subjects were contacted (by e-mail or phone) every month to assure adherence to the study protocol. Compliance was verified by weighing the returned bottles.

Daily assessments

Participants were asked to complete a daily log documenting the severity of their cold related symptoms throughout the study. This self-assessment was determined on a 4 point scale as follows, 0 = no symptom, 1 = a mild symptom, 2 = a moderate symptom, and 3 = a severe symptom. The following 10 cold symptoms were assessed daily: sore throat, runny nose, sneeze, nasal congestion, malaise, fever, headache, hoarseness, earaches and cough. Total Symptom Score (TSS) for a cold was calculated by summing the daily scores for all symptoms. Daily TSS exceeding 4 was included in

URIs in community-dwelling adults [2]. In a more recent double-blind placebo controlled trial involving 323 healthy volunteers, daily supplementation of COLD-fX for 4 months during an influenza season reduced the absolute risk of recurrent colds by 12.8% (95% CI 4.3–21.3). This moderate dosing of COLD-fX also reduced the severity and duration of URIs by 15 and 20%, respectively [3]. Despite the fact that the COLD-fX treatment employed in the study was found effective in reducing the incidence and severity of colds, the underlying mechanism(s) was not determined. The present study, therefore, was carried out to determine if the effects observed could potentially be due to COLD-fX mediated changes in the distribution of peripheral blood leukocytes, changes in the relative distribution of lymphocyte subsets or, by changes in plasma levels of immunoglobulin A (IgA).

A number of preclinical studies have indicated that COLD-fX has immunomodulatory effects. It enhanced the proliferation of mouse spleen lymphocytes and increasing the production of interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α) and nitric oxide from peritoneal macrophages in vitro [4]. In a recent study, COLD-fX was also found to increase the proportion of macrophages and natural killer (NK) cells in bone marrow and spleen of leukemic mice [5]. It has also been found to increase the release of IL-2 and interferon-gamma (IFN-γ) from murine spleen cells [6]. Furthermore, in a more recent study on human peripheral blood mononuclear cells (PBMC) cultured with live influenza virus, COLD-fX was shown to be effective in enhancing the production of IL-2 and IFN-γ (Unpublished data). IL-2 and IFN-γ are the primary cytokines produced by CD4+ and NK cells [7]. These cytokines are thought to comprise one of the principal host defense mechanisms against many intracellular pathogens including respiratory viruses [8–10]. Studies on children and adults, and CD4+–/− mouse models have demonstrated that an attenuated production of these cytokines relates with a diminished cell mediated immune response to respiratory virus, loss of cytolytic activity of CD8+ lymphocytes, reactivation of latent infection and consequently recurrence of respiratory viral infections [11–13]. In addition, in a study relating stress levels to the frequency of URIs, both low NK cell number and cytotoxicity of NK cells was found to be associated with increased risk of URIs [14]. Other defense mechanisms that may be involved in protection from respiratory pathogens include immunoglobulins, particularly secretory IgA. For example, in a study on patients with Down’s syndrome, lower levels of salivary IgA were found in those reporting frequent URIs [15]. The present study, therefore, was carried out to determine if the effects of COLD-fX in the prevention and treatment of URIs could be related to changes in the distribution of CD4+ and NK cells as well as in plasma levels of IgA.
Blood collection and analysis

The study also involved collection of two blood samples from each subject, however, this blood donation was voluntary. The first sample was collected at recruitment for the establishment of baseline values, and the second, collected on completion of the study. Both blood samples were obtained at approximately the same time of day.

The blood samples were used for white blood cell differential counts and identification of lymphocyte subsets by immunofluorescence. White blood cell differential counts were made by automated analysis. Plasma IgA levels were also measured using an ELISA kit from Bethyl Laboratories Inc. (BET-E80-102, Montgomery, TX, USA).

Immunophenotyping

Lymphocyte subsets in blood samples were characterized by immunofluorescence using supernatants from hybridomas secreting mouse monoclonal antibodies (mAB) specific for the different human immune cell subsets [16]. In 96 well V-bottom plates, red blood cells in whole blood (100 μL) were lysed. The remaining cells were washed with phosphate buffered saline containing 4% fetal calf serum, and incubated with 10 μL of PE-, Biotin- or FITC-labeled mAB for 30 minutes, at 4°C in dark. Two or three color combinations were used to identify the following subsets: CD3 FITC, CD4 PE and CD8 Biotin, to estimate T-helper, T-cytotoxic lymphocytes; CD16 FITC and CD56 PE, to determine NK cells. Streptavidin Quantum red-biotin conjugate was used as a third fluorochrome. Isotype-matched fluorescence controls were also included. The plates were then washed and incubated for another 30 minutes at 4°C with the streptavidin conjugate (10 μL) in wells containing biotinylated mAb. Cells were washed twice, fixed in phosphate buffered saline containing paraformaldehyde (10 g/L with Na azide), and analyzed by flow cytometry on a FACSscan (Becton-Dickenson, San Jose, CA) according to the relative fluorescence intensity using Cell Quest software correcting for background fluorescence.

Statistical analysis

The two treatment groups were compared with respect to all available baseline information. Between group comparisons on pre-treatment and post-treatment white blood cell differential counts, lymphocyte phenotypes, and plasma IgA levels were performed using unpaired t-tests. Paired t-tests were also performed to estimate within group change in these variables.

Results

A total of 323 volunteers were enrolled and randomized. The results of this trial on incidence and severity of colds in the two treatment groups are presented elsewhere [3]. Of the 323 randomized subjects, 42 volunteers (21 COLD-fX and 21 placebo) donated blood samples taken before and after the intervention. Demographic characteristics of these volunteers in the two groups were virtually identical (Table 1). The effects of the treatments on the incidence and severity of URIs in this subgroup during the 4 month treatment period are presented in Table 2. Over the 4 month period, the distribution of colds was found to be similar in both groups. However, the severity of the colds in the placebo group remained constant throughout while in the COLD-fX group the severity of the colds was found to decrease over time.

Table 3 indicates WBC differential count before and after 4 months of treatment with either COLD-fX or placebo. Unpaired comparisons between the two groups found a

<p>| Table 1. Demographic characteristics of the volunteers donating blood samples |</p>
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo (n = 21)</th>
<th>COLD-fX (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr. (SD)</td>
<td>44.6 (10.5)</td>
<td>36.2 (13.4)</td>
</tr>
<tr>
<td>Gender (m, f)</td>
<td>13, 8</td>
<td>9, 12</td>
</tr>
<tr>
<td>Smokers, n</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Colds/yr., (SD)</td>
<td>2.6 (1.2)</td>
<td>3.0 (1.7)</td>
</tr>
</tbody>
</table>

Table 2. Effect of COLD-fX/Placebo treatment on the number, severity and duration of colds

<table>
<thead>
<tr>
<th></th>
<th>15-Nov</th>
<th>December</th>
<th>January</th>
<th>February</th>
<th>15-Mar</th>
</tr>
</thead>
<tbody>
<tr>
<td># of Colds/month</td>
<td>P 1</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>C 2</td>
<td>6</td>
<td>8</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Severity/cold (TSS)</td>
<td>P 18</td>
<td>61.1 (35.1)</td>
<td>69.3 (30.2)</td>
<td>60.8 (45.1)</td>
<td>69 (18.4)</td>
</tr>
<tr>
<td></td>
<td>C 18</td>
<td>(11.3)</td>
<td>89.8 (93.5)</td>
<td>49.6 (30.2)</td>
<td>40 (28.3)</td>
</tr>
<tr>
<td>Duration/cold (days)</td>
<td>P 5</td>
<td>8.1 (6.4)</td>
<td>9.7 (8.8)</td>
<td>9.2 (5.0)</td>
<td>10 (0)</td>
</tr>
<tr>
<td></td>
<td>C 2.5</td>
<td>12.8 (11.3)</td>
<td>9.1 (6.6)</td>
<td>5.7 (2.4)</td>
<td>6 (0)</td>
</tr>
</tbody>
</table>

P: Placebo, n = 21; C: COLD-fX, n = 21
Values are means (SD)
TSS: total symptom score
non-significant at p<0.05
significant difference in the pretreatment counts of lymphocytes and neutrophils. However, post-treatment values and paired comparisons between the groups showed that the treatments did not have any significant effects on any of the leukocytes measured.

Table 4 illustrates the effects of the treatments on changes in distribution of lymphocyte subsets and plasma IgA concentrations. Paired t-tests revealed that in the placebo group, the proportion of T lymphocytes (CD3<sup>+</sup>), T Helper (CD4<sup>+</sup>), and T cytotoxic cells (CD8<sup>+</sup>) remained relatively unchanged, while in the COLD-fX group T lymphocytes and T Helper cells increased. NK cells also increased in both groups however, the magnitude of increase was greater in the COLD-fX group. Plasma IgA levels decreased in both groups. The decrease, however, was more pronounced in the COLD-fX group.

**Discussion**

In agreement with the previous findings [3], the effect of COLD-fX treatment in reducing the severity and duration of colds was also evident in this sub-population. Although not evident initially, the efficacy of COLD-fX in reducing the severity of symptoms appeared to increase over time. However, the efficacy of COLD-fX in reducing recurrent URIs observed in the earlier study was not observed in this subgroup. The difference in sample size may account for this difference between the two populations. In the present study,
despite the sample size being small [21] subjects in each group, it appeared adequate to demonstrate some beneficial effects of COLD-fX in the prevention and treatment of URIs.

The present study was conducted to determine possible mechanisms of action of COLD-fX. The results indicate that COLD-fX treatment does not affect the proportion of leukocytes; however, it appears to affect the proportion of lymphocyte subsets, particularly T-helper (CD4+) and NK cells. Following 4 months of intervention, CD4+ cells were increased by 20% (p<.04) and 7% in the COLD-fX and placebo groups, respectively. The effects on NK cells were more profound, being 63% and 40% in the COLD-fX and placebo group, respectively. This response was not unexpected in either group as the study was conducted during the peak cold/flu season. Cold related increases in these parameters have been reported by others [17]. However, the differences in severity of the colds, and T-helper and NK cell number between the groups suggest an independent mechanism of COLD-fX-induced action that was sustained throughout the latter months of the intervention.

In accordance with an earlier study demonstrating COLD-fX-induced proliferation of T-lymphocytes [4], COLD-fX treatment in the present study also increased the proportion of CD3+ cells in healthy adults. The increase was particularly evident in the proportion of CD4+ cells. Studies on animal models, deficient in B or NK cells, have shown that CD4+ cells are able to confer protection from respiratory pathogens through their ability to secrete IL-2 and IFN-γ [18–20]. Another mechanism of COLD-fX action may be an increased proportion of NK cells. COLD-fX-related increases in the proportion of NK cells in bone marrow and spleen of leukemic mice have also been demonstrated earlier [5]. NK cells comprise an important component of innate immune responses and are known to kill intracellular pathogens either through their cytolytic activity or by releasing IL-2 and IFN-γ [7]. Although the activity of NK cells was not measured in the present study, decreased plasma levels of IgA indicate that perhaps COLD-fX treatment also increased the activity of NK cells, thus increasing IFN-γ secretion [21]. Increased levels of IFN-γ have also been shown to enhance macrophage function and secretion of IgG2a, thus propagating and sustaining immune modulating signals [22, 23]. Reduced plasma levels of IFN-γ have been found in children with recurrent infections of the upper respiratory tract and in animals lacking the transcription factor T-bet, which are known to be highly susceptible to respiratory infections [11, 12, 24, 25]. Release of these cytokines during a respiratory viral infection is also known to affect the duration of the disease. While promoting inflammation initially, the sustained increase of IL-2 and IFN-γ has been found to promote faster resolution of the symptoms and also to prevent the development of secondary complications [26].

In the COLD-fX group, the decreased plasma level of IgA was an intriguing finding. This response may be due, in part, to enhanced transcytosis of serum IgA to nasal and bronchial fluids thus possibly enhancing secretory IgA and viral shredding at these surfaces [21, 27, 28]. It is also possible that enhanced production of IFN-γ by CD4+ or NK cells contributed to the lower levels of plasma IgA as observed in the present study. IFN-γ is known to suppress plasma levels of IgA [21, 29].

In conclusion, a modest dose of COLD-fX (2 capsules/day) during an influenza season for 4 months was effective in increasing T-helper and NK cell number, and diminishing IgA levels in plasma. It is possible that these cells acted synergistically to reduce the severity of URIs in the COLD-fX group. Further studies are warranted to determine the effects of daily supplementation of COLD-fX on the activities of T-helper and NK cells.

Acknowledgments

This study was supported by ‘CV Technologies Inc.’, Edmonton, AB, Canada.

References


