

Research Article

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The Effect of Red Ginseng Extract on Inflammatory Cytokines after Chemotherapy in Children

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Ginseng has been used as an herbal medicine, widely used in Asian countries, for long time. Recently, beneficial effects for immune functions of Korean red ginseng (KRG) have been reported in adults. This study was performed to investigate the effects of ginseng on immune functions in children after cessation of chemotherapy or stem cell transplantation for advanced cancer. Thirty patients, who were diagnosed and treated for leukemia and solid cancer at the department of pediatrics and adolescence of the Yeungnam University Hospital from June 2004 to June 2009, were enrolled for the study. The study group consisted of 19 patients who received KRG extract (60 mg/kg/d) for 1 yr and 11 patients who did not receive KRG extract were the control group. Blood samples were collected every 6 mo. Immune assays included circulating lymphocyte subpopulation, serum cytokines (IL-2, IL-10, IL-12, TNF-alpha, and IFN-gamma), and total concentrations of serum IgG, IgA, and IgM subclasses. Age at diagnosis ranged from 2 mo to 15 yr (median 5 yr). Nine patients received stem cell transplantation. The cytokines of the KRG treated group were decreasing more rapidly than that of the control group. Lymphocyte subpopulations (T cell, B cell, NK cell, T4, T8, and T4/T8 ratio) and serum immunoglobulin subclasses (IgG, IgA, and IgM) did not show significant differences between the study and the control groups. This study suggests that KRG extract might have a stabilizing effect on the inflammatory cytokines in children with cancer after chemotherapy.

Keywords: *Panax ginseng*, Korean red ginseng, Cytokine, Childhood cancer

INTRODUCTION

Children with cancer are usually immunosuppressed because of chemotherapy and underlying malignancy [1,2]. After completion of chemotherapy, patients are immunosuppressed for variable follow up periods [3-5]. Specific antibodies against vaccination were deficient at completion of chemotherapy, regardless of the underlying malignancy [6]. Similar results were obtained for T and NK cells [7,8].

Ginseng (the root of *Panax ginseng* Meyer) has been used as a representative herbal medicine and a vital-additive drug in East Asian countries, including Korea,

China, and Japan, for about 2,000 years. Currently, approximately 200 substances, such as ginsenosides, polysaccharides, polyacetylenes, peptides and amino acids have been isolated from ginseng [9]. The Korean red ginseng (KRG) extract is made by steamed and sundried six-year-old ginseng roots.

The biomedical and pharmacological activities of ginseng, regarding the anti-tumor effect, cardiovascular function [10], cognitive function in Alzheimer disease [11], and the improvement of insulin resistance [12] have been reported. Also various studies have shown that

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these ginseng extracts modulate the immune response, *in vitro* and *in vivo*. In clinical trials, ginseng extract treated healthy volunteers had a lower incidence of influenza and colds, high antibody titers, and higher natural killer cell activity [13]. In addition, ginseng extract showed immune-modulatory effects, such as intracellular killing, and phagocytosis in controlled double-blind study [14].

Well-known effects of red ginseng are improving the quality-of-life and immune-modulation. However, there has been no data for the effects of KRG in children with cancer after completion of chemotherapy. The purpose of this study is to investigate the immune-modulatory effects of KRG in children after chemotherapy.

METHODS AND MATERIALS

Patient population

Thirty patients who were diagnosed and successfully completed chemotherapy or hematopoietic stem cell transplantation (HSCT) for leukemia, lymphoma or solid tumor, at the department of pediatrics and adolescence of the Yeungnam University Hospital from June 2004 to June 2009, were enrolled for the study.

Nineteen patients, who received KRG extract for 1 yr, were included in the study group, while the control group consisted of 11 patients who did not receive KRG extract. This study was approved by the institutional review board (IRB) of Yeungnam University Medical Center (IRB no. PCR 09-79). A written informed consent was obtained from the patient's guardian.

Study protocol

KRG extracts were supplied by Korea Ginseng Corporation (Seoul, Korea). Nineteen patients in the study group received KRG extract 60 mg/kg daily for 1 yr. Blood samples were collected every 6 mo. Immune assays included circulating lymphocyte subpopulations, serum cytokines (IL-2, IL-10, IL-12, TNF- α , and IFN- γ), and total concentrations of serum IgG, IgA, and IgM subclasses.

Immunoglobulin assay

Quantitative serum IgG, IgA, and IgM were analyzed by an automated analyzer UniCel DXC 800 (Beckman Coulter, Brea, CA, USA).

Subsets for circulating lymphocyte

Lymphocyte subsets were analyzed, using a two-laser detector FACS Calibur (Becton Dickinson, San Jose, CA, USA) and the Simultest IMK-Lymphocyte reagent

Table 1. Characteristics of the subjects

| | Study | Control |
|-------------------------------------|---------------|---------------|
| Number | 19 | 11 |
| Age at diagnosis (yr) ¹⁾ | 7.2 \pm 5.1 | 6.0 \pm 4.6 |
| Sex (male) | 13 | 5 |
| Study onset after chemotherapy | | |
| <12 mo | 9 | 11 |
| \geq 12 mo | 10 | 0 |
| HSCT | | |
| Yes | 8 | 1 |
| No | 11 | 10 |
| Diagnosis | | |
| Leukemia | 13 | 5 |
| ALL | 9 | 5 |
| AML | 4 | 0 |
| Lymphoma | 4 | 1 |
| Other solid cancer | 2 | 5 |

HSCT, hematopoietic stem cell transplantation; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia.

¹⁾ Values represent the mean \pm SD.

(Becton Dickinson) according to the manufacturer's protocol. Whole blood (100 μ L) and fluorochrome-labeled antibodies (20 μ L each) were mixed and incubated at room temperature for 20 min. The stained blood samples were treated with a lysing solution to remove the red blood cells. The samples were then washed and fixed in 1% paraformaldehyde. Enumeration of lymphocyte subsets was done using FACS Calibur flow cytometer, via Cell Quest Pro software (Becton Dickinson).

Plasma preparation from blood

Whole blood was collected into EDTA-containing Vacutainer tubes (Becton Dickinson). Whole blood 5 mL was diluted with an equal volume of phosphate-buffered saline. Diluted blood was layered onto the surface of the 5 mL Ficoll paque plus (GE healthcare, Tokyo, Japan) in a 50 mL conical tube, and was centrifuged with 2,000 rpm for 30 min at 18°C. The upper layer was centrifuged with 800 rpm for 10 min, and the resulting supernatants were collected and stored at -70°C for an analysis of cytokines by enzyme-linked immunosorbent assay (ELISA).

Enzyme-linked immunosorbent assay

Levels of IL-2, IL-10, IL-12, TNF- α , and IFN- γ in plasma were assessed by using the ELISA kits (Becton Dickinson) according to the manufacturer's instructions. Monoclonal antibodies were coated on a 96-well plate. Standards and samples were added to the

wells, and were incubated at room temperature for 2 h. The plates were then washed with a wash buffer and were incubated with streptavidin antibodies, followed by incubation with biotinylated antibodies. Plates were incubated in the dark with tetramethylbenzidine substrate after washing. Once adequate color was developed, the reaction was stopped by adding a stop buffer. ELISA

plates were read at 450 nm using a Model 680 Microplate Reader (Biorad, Hercules, CA, USA). Cytokine levels were estimated by using the standard recombinant cytokines supplied with the kits as a reference.

Statistical methods

Repeated ANOVA was used to compare the differ-

Table 2. Comparisons of immunoglobulins and lymphocyte subsets between study and control groups

| | | Baseline | 6 mo | 12 mo | <i>p</i> -value | |
|-----------------------------|---------|-----------------|-----------------|-----------------|-----------------|----------------|
| | | | | | Within groups | Between groups |
| IgG (mg/dL) | Study | 953.3±306.4 | 1,074.3±259.9 | 887.9±285.5 | 0.044 | 0.682 |
| | Control | 838.2±328.7 | 838.0±351.1 | 730.4±264.8 | 0.177 | |
| IgA (mg/dL) | Study | 118.2±84.3 | 146.5±59.4 | 131.8±59.7 | 0.002 | 0.048 |
| | Control | 83.2±40.4 | 98.0±56.2 | 111.6±60.3 | 0.007 | |
| IgM (mg/dL) | Study | 80.0±70.2 | 128.8±66.2 | 101.0±38.6 | <0.001 | 0.054 |
| | Control | 65.0±38.8 | 73.4±22.3 | 68.0±19.9 | 0.162 | |
| WBC (/mm ³) | Study | 5,661.1±2,537.0 | 6,765.3±2,363.8 | 7,109.0±2,354.7 | 0.003 | 0.641 |
| | Control | 5,601.8±1,743.5 | 7,000.0±1,501.7 | 6,902.7±1,672.5 | 0.004 | |
| Lymph (/mm ³) | Study | 2,278.7±1,539.2 | 2,645.5±1,218.5 | 2,877.6±1,312.7 | 0.023 | 0.216 |
| | Control | 1,951.6±1,062.5 | 2,865.5±1,089.6 | 2,991.4±821.7 | <0.001 | |
| T cell (/mm ³) | Study | 1,360.0±863.9 | 1,629.1±748.1 | 1,840.0±762.5 | 0.004 | 0.224 |
| | Control | 1,073.2±675.9 | 1,609.9±878.4 | 1,983.9±655.2 | <0.001 | |
| B cell (/mm ³) | Study | 584.9±556.3 | 651.5±524.4 | 651.3±586.3 | 0.755 | 0.566 |
| | Control | 616.7±458.7 | 718.7±487.2 | 623.7±250.1 | 0.665 | |
| T4 (/mm ³) | Study | 539.4±334.0 | 755.1±428.9 | 872.6±414.0 | 0.001 | 0.463 |
| | Control | 516.2±370.5 | 830.1±497.5 | 1,027.8±372.0 | <0.001 | |
| T8 (/mm ³) | Study | 787.5±530.4 | 873.0±427.4 | 966.9±483.5 | 0.043 | 0.118 |
| | Control | 543.5±291.8 | 795.8±431.7 | 971.5±321.8 | 0.002 | |
| T4/T8 ratio | Study | 0.7±0.3 | 0.9±0.3 | 1.0±0.3 | 0.048 | 0.326 |
| | Control | 0.9±0.3 | 1.1±0.2 | 1.1±0.2 | 0.018 | |
| NK cell (/mm ³) | Study | 249.2±294.3 | 259.7±200.5 | 311.5±384.6 | 0.323 | 0.772 |
| | Control | 206.2±112.5 | 262.9±190.8 | 307.1±104.1 | 0.149 | |

Values represent the mean±SD.

WBC, white blood cell; Lymph, lymphocyte.

ences in the laboratory values between the study and the control groups, which were followed by multiple comparisons with the Bonferroni correction. Mann-Whitney *U*-test was used to compare the differences of the serum cytokines within the study group.

A *p*-value of <0.05 was considered to indicate a statistically significant difference between the groups. The analysis was performed using the SPSS ver. 19.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The mean age at diagnosis of the study group was 7.2±5.1 yr, and that of the control group was 6.0±4.6 yr (Table 1). All 11 patients in the control group and 9 of the 19 study group patients were enrolled within 12 mo after their last chemotherapy. Hematopoietic stem cell transplantation was done for 8 patients in the study group. Diagnoses of the subjects were acute leukemia, lymphoma and other solid cancers.

Changes of the serum IgG, IgA, and IgM are summarized in Table 2. There were no statistically significant differences between the study and the control groups, except IgA. White blood cell, lymphocyte, T-cell, T4 cell, and T8 cell counts of both groups were gradually increased for 1 yr (*p*<0.05). But there were no statisti-

cally significant differences compared between the study group and the control group. B-cell counts in both groups were not significantly changed for 1 yr (Table 2).

Serum cytokines, such as IL-2, IL-10, IL-12, TNF- α , and IFN- γ in the study group were decreased over 1 yr (Table 3). IL-2, TNF- α , and IFN- γ in the control group were not significantly changed for 1 yr. **Changes of cytokines were significantly different between the study and the control groups.** The cytokines of red ginseng treated group were decreasing more rapidly than those of the control group.

In the study group, the baseline levels of cytokine IL-2, IL-10, IL-12, and IFN- γ were not significantly different depending on HSCT or time interval from the completion of chemotherapy to the study. Only TNF- α level, at baseline and 6 months' follow up for study group whose time interval was more than 12 mo after completion of chemotherapy, were lower than those within 12 mo (Table 4). In this study, no significant adverse event was observed in the KRG treated group.

DISCUSSION

According to the annual report of cancer statistics in Korea, more than 1,700 children and adolescents under the age of 17 yr are diagnosed with cancer. The number of children and adolescents receiving treatment for can-

Table 3. Comparisons of cytokines between study and control groups

| | Baseline | 6 mo | 12 mo | <i>p</i> -value | |
|-----------------------|------------|------------|------------|-----------------|----------------|
| | | | | Within groups | Between groups |
| IL-2 (pg/mL) | | | | | <0.001 |
| Study | 9.44±1.83 | 7.65±1.35 | 6.40±0.86 | <0.001 | |
| Control | 8.49±0.75 | 8.11±0.87 | 8.00±0.54 | 0.196 | |
| IL-10 (pg/mL) | | | | | <0.001 |
| Study | 11.68±0.74 | 10.26±0.62 | 9.48±0.91 | <0.001 | |
| Control | 11.13±0.50 | 10.67±0.60 | 10.35±0.36 | <0.001 | |
| IL-12 (pg/mL) | | | | | <0.001 |
| Study | 46.37±6.97 | 37.59±5.20 | 30.77±3.83 | <0.001 | |
| Control | 37.39±3.05 | 35.45±1.57 | 33.82±1.76 | 0.011 | |
| TNF- α (pg/mL) | | | | | <0.001 |
| Study | 10.62±1.27 | 9.57±1.20 | 8.88±1.18 | <0.001 | |
| Control | 10.49±0.32 | 10.12±0.57 | 10.34±0.49 | 0.125 | |
| IFN- γ (pg/mL) | | | | | <0.001 |
| Study | 6.70±1.70 | 5.83±1.62 | 5.73±1.73 | <0.001 | |
| Control | 6.65±0.32 | 6.45±0.39 | 6.38±0.25 | 0.064 | |

Values represent the mean±SD.

Table 4. Changes of cytokines in study group depending on HSCT or time interval from completion of chemotherapy to the study

| | Baseline | 6 mo | 12 mo |
|--------------------|------------|------------|------------|
| IL-2 (pg/mL) | | | |
| HSCT | | | |
| No | 9.13±2.01 | 7.74±1.48 | 6.40±1.05 |
| Yes | 9.86±1.56 | 7.51±1.20 | 6.42±0.55 |
| <i>p</i> -value | 0.509 | 0.804 | 0.74 |
| Study onset | | | |
| <12 mo | 10.07±1.14 | 8.00±1.16 | 6.75±0.96 |
| ≥12 mo | 8.87±2.18 | 7.32±1.47 | 6.10±0.65 |
| <i>p</i> -value | 0.369 | 0.165 | 0.128 |
| IL-10 (pg/mL) | | | |
| HSCT | | | |
| No | 11.90±0.76 | 10.31±0.69 | 9.59±0.70 |
| Yes | 11.36±0.60 | 10.18±0.54 | 9.33±1.16 |
| <i>p</i> -value | 0.177 | 0.840 | 0.840 |
| Study onset | | | |
| <12 mo | 11.99±0.65 | 10.55±0.66 | 9.92±0.36 |
| ≥12 mo | 11.39±0.71 | 9.99±0.44 | 9.08±1.07 |
| <i>p</i> -value | 0.06 | 0.054 | 0.057 |
| IL-12 (pg/mL) | | | |
| HSCT | | | |
| No | 45.48±7.62 | 36.81±5.14 | 29.77±4.14 |
| Yes | 47.57±6.24 | 38.64±5.42 | 32.16±3.05 |
| <i>p</i> -value | 0.84 | 0.62 | 0.231 |
| Study onset | | | |
| <12 mo | 47.32±7.39 | 38.72±4.22 | 31.89±2.26 |
| ≥12 mo | 45.50±6.84 | 36.56±5.97 | 29.77±4.72 |
| <i>p</i> -value | 0.414 | 0.391 | 0.462 |
| TNF- alpha (pg/mL) | | | |
| HSCT | | | |
| No | 10.45±1.03 | 9.62±0.91 | 9.35±0.99 |
| Yes | 10.85±1.58 | 9.49±1.57 | 8.23±1.14 |
| <i>p</i> -value | 0.215 | 0.934 | 0.126 |
| Study onset | | | |
| <12 mo | 11.32±0.76 | 10.16±1.00 | 9.44±0.85 |
| ≥12 mo | 9.98±1.33 | 9.03±1.14 | 8.37±1.22 |
| <i>p</i> -value | 0.014 | 0.041 | 0.054 |
| IFN-gamma (pg/mL) | | | |
| HSCT | | | |
| No | 6.85±1.28 | 5.69±1.38 | 5.69±1.67 |
| Yes | 6.49±2.23 | 5.99±1.99 | 5.77±1.91 |
| <i>p</i> -value | 0.741 | 0.039 | 0.247 |
| Study onset | | | |
| <12 mo | 7.24±0.40 | 6.31±0.41 | 6.34±0.41 |
| ≥12 mo | 6.21±2.25 | 5.38±2.15 | 5.17±2.25 |
| <i>p</i> -value | 0.568 | 0.624 | 0.236 |

Values represent the mean±SD.

A *p*-value was calculated by the Mann-Whitney test for comparing cytokines within study group depending on hematopoietic stem cell transplantation (HSCT) or time interval.

cer are about 5,000 [15]. With the improvement of anti-cancer therapy, 80% of children with cancer would be long-term survivors [16]. As the numbers of childhood cancer survivors are increasing, concerns about long-term complications and their life qualities are increasing.

Survivors of childhood cancer have a high rate of chronic medical illness. It is postulated that the damage to the organ systems of children, caused by chemotherapy and radiation therapy, might be the contributing factors. Anti-cancer chemotherapy causes significant adverse effects on multiple organ tissues in cancer patients [17]. Both chemotherapy and radiotherapy cause tissue damage, and consequently, they induce overproduction of cytokines, such as TNF-alpha, IL-1, IL-2, TGF-beta, and IL-6. Cytokines are biologically active proteins that play a key role in immune-regulation. These cytokines can induce apoptosis, fibrosis, tissue damage, and bone marrow suppression [18]. Mazur et al. [19] reported that IL-2 and TNF-alpha were elevated for 12 mo after the completion of chemotherapy in children with acute lymphoblastic leukemia.

Martin et al. [20] reported that healthy persons have lower levels of serum cytokines, serum IL-2 and IL-10 are below the detection limit in a healthy person, and half of a healthy person shows under the detection limit of TNF-alpha. Berdat et al. [21] evaluated 79 children to establish the reference cytokine values in children of different age groups. The mean value of IL-10 is within the range of 3.3 to 5.5 pg/mL, and that of TNF-alpha is within the range of 2.2 to 3.5 pg/mL. In this study, serum cytokines, such as IL-2, IL-10, and TNF-alpha were elevated from the baseline in the study and the control groups. Time intervals from the completion of chemotherapy or HSCT to the study onset did not show a significant difference in the baseline cytokine levels of IL-2 and IL-10. Only TNF-alpha levels of the study group, whose time interval was more than 12 mo after the last chemotherapy, were significantly lower than those of within 12 mo after the completion of chemotherapy. This means that TNF-alpha gradually decreased over time after chemotherapy, and KRG extract contributed to a more rapid decrease in TNF-alpha level.

IL-12 is produced by activated antigen-presenting cells, such as dendritic cells, macrophages, and natural killer cells. IL-12 plays a role in the cell-mediated immunity by regulation of T cell response. IL-12 induces IFN-gamma production, and promotes the differentiation of naïve T cells into Th1 cells. The production of IL-12 is important in the protection of various infections [22]. IFN-gamma is the only type II interferon, and is critical

for an innate and adaptive immunity against a viral and intracellular bacterial infection and for tumor control. IFN-gamma regulates the proliferation and differentiation of leukocyte, and is released from Th1 cell, cytotoxic T cell and NK cell [23]. Release of IFN-gamma occurs in the early stage of immune stimulation. Therefore, IFN-gamma may represent a diagnostic parameter for the immune activation, such as infection and graft-versus-host disease [24].

In this study, IL-12 and IFN-gamma of the study group were more rapidly decreasing over time. We assume that KRG extract may have a role in suppressing the immune activation and stimulation.

Ginsenosides are the major component of KRG [9], and various ginsenosides have been reported to show various biological activities, including anti-inflammatory and anti-tumor effect [25]. Ginsenoside Rg3 is metabolized to ginsenoside Rh2 by a human intestinal microflora. This transformed ginsenoside Rh2 shows an anti-allergic activity by the cell membrane-stabilizing activity [26]. Also, protopanaxadiol ginsenoside, which is composed of ginsenoside Rb1, Rb2, Rc, and compound K, has a potent inhibitory effect against TNF-alpha [26,27].

In an animal study, ginsenoside Rd inhibits transplant rejection by the suppression of cytokine IL-2, IL-12, TNF-alpha, and IFN-gamma [28]. In rats, ginsenosides Rb1 and Rb2 inhibit the expression of TNF-alpha and other cytokines and reduce the infarction volumes [29,30].

Recently, there are some clinical applications of immunosuppressive effect of KRG. Xu et al. [31] reported that refractory acute graft-versus-host disease after a liver transplantation was successfully treated with KRG. Serum levels of TNF-alpha, IFN-gamma, and IL-10 were increased at the initiation of graft-versus-host disease and after the application of KRG, TNF-alpha, IFN-gamma, and IL-10 levels decreased rapidly with amelioration of clinical symptoms. These results are comparable to our study. Suh et al. [32] reported that KRG had immunomodulatory properties associated with IL-2, IL-8, and IL-10 in adult colon cancer patients after surgery. They reported IL-2 was increased and IL-10 was decreased after KRG administration. In contrast, in our study, inflammatory cytokine levels of study and control groups were consistently reduced during follow-up. Serum cytokines, such as IL-2, IL-10, IL-12, TNF-alpha, and IFN-gamma in the red ginseng treated group were decreasing more rapidly and statistically significantly ($p < 0.001$) comparing to the control group.

It is reported that ginseng saponin inhibit proinflam-

matory cytokines [33]. Also, red ginseng protected oxidative stress-induced cell [34]. Taken together, in this study, KRG extract significantly reduced the inflammatory cytokines IL-2, IL-10, IL-12, TNF-alpha, and IFN-gamma in children after the completion of cancer treatment. Administration of KRG extract, after cancer treatment, might help in protecting tissue damage from inflammatory cytokines. Whether the reduced inflammatory cytokines are associated with decreased late complications of childhood, cancer survivors require further long-term follow up.

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