Ginseng in the Treatment of AIDS

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ABSTRACT

Despite introduction of highly active antiretroviral therapy, the AIDS pandemic continues to spread across the world. Although the development of an effective vaccine is urgently required, we still do not have any vaccine. In this regard, we need to look back towards alternative ways based on history and the recent scientific literature. Immunotherapy is currently receiving great attention as supporting treatment modalities in the management of cancer and AIDS patients whose immune function is compromised. Ginseng has long been used to maintain the vitality of man in the Orient. Recent studies have shown that ginseng has significant potential as an immune modulator and adjuvant. We have reported the beneficial effects of Korean red ginseng (KRG) in HIV-1-infected individuals since 1991. Several patients have remained healthy for up to 23 years in the absence of HAART. Of note, most patients treated with KRG reveal significantly high frequency of genetic defects in HIV-1 genes of as well as attenuation of chronic immune activation. A series of our data and literature show the possibility that ginseng could be a safe and effective medicine for treating AIDS patients.

Keywords: genetic defects, HIV-1, Korean red ginseng

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INTRODUCTION

The introduction of a highly active antiretroviral drug therapy (HAART) has proven effective in the treatment of human immunodeficiency virus (HIV)-1-infected patients (Ho et al. 1995; Wei et al. 1995). HAART alone, however, cannot eradicate the virus in the reservoirs (Chun et al. 1997; Finzi et al. 1997; Hendel et al. 1999). With regard to immunopathogenesis, production of Th1 cytokines is gradually reduced in HIV-1-infected patients (Chun et al. 1998, 1999). Thus, the key immune modulator in cell-mediated immunity, interleukin (IL)-2 has recently been tried in combination with HAART (Chun et al. 1999). Although IL-2 therapy significantly increases the number of circulating CD4 T cells, it has also many limitations because of severe adverse effects. Thus, a new modality with safety is required for more effective therapy of AIDS. Despite the discouraging news of expanding epidemics and the many biological changes imposed by HIV, there is no report on an effective vaccine. Considering the low transmission rate of HIV in settings of sexual transmission or needle stick injuries, a vaccine that induces even a modest improvement in antiviral defenses may have a profound impact on the transmissibility of HIV (Womack et al. 2004). Regarding pathogenesis, HIV infection is a kind of chronic wasting disease accompanied by a chronic generalized immune activation state that is significantly attenuated in simian immunodeficiency virus (Macaca mulatta) infected macaques (Muranyi et al. 2004). Ginseng, a medicinal herb, has long been used to maintain the vitality of man in the Orient because its medicinal properties have been recognized through experience for thousands of years. Famous books on Oriental medicine (Shen Nong Ben Cao Jing in China; Dongeuibogam in Korea, 1610) say that long-term intake of ginseng prolongs life. We have treated HIV-infected individuals with Korean red ginseng (KRG) since late 1991. Our data shows that long-term intake of KRG might attenuate the HIV-1 gene as well as its clinical usefulness.

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In this context, our clinical experience over 18 years indicate that KRG could be a good alternative medicine in the treatment of HIV individuals and related scientific findings also show an immunological basis for its usefulness.

**HOW DOES GINSENG WORK IN HUMANS?**

In the Orient, ginseng (Panax ginseng C. A. Meyer) especially has been used as a medicine for more than 2000 years (Li et al. 1973). At present, ginseng ranks as having the second highest annual sales of any herbal medicine in the USA market place and is used world-wide (O’Hara et al. 1998). Since the late 1960s, many studies have been performed to identify the active ingredients of ginseng and their functions. Ginseng is considered an adaptogenic agent, which enhances physical performance, promotes vitality and increases resistance to stress and aging, and possesses immunomodulatory activity (Singh et al. 1984; Scaglione et al. 1990; See et al. 1997). The adaptogenic properties of ginseng are believed to be due to its effects on the hypothalamic-pituitary-adrenal axis (Hiai et al. 1979; Fulder et al. 1981; Nocerno et al. 2000). Its immunomodulatory activity means to improve defense systems that can overcome tumor and microbial infection.

As the front line of host defense, the importance of innate immunity in AIDS pathogenesis is being highly recognized recently as we realize that adaptive immunity alone such as cytotoxic T lymphocyte activity or a neutralizing antibody against HIV cannot control HIV infection. It is well known that ginseng augments innate immunity, especially on natural killer cell activity as well as adaptive immunity. NK function (15 ± 7) in AIDS patients with a CD4 count < 200/μl is significantly lower (113 ± 20) than in normal controls (p<0.001). The presence of ginseng extract significantly enhanced NK-function by peripheral blood mononuclear cells from normal controls at concentrations of >10 μg/ml and AIDS at 1 μg/ml in a dose-dependent manner (See et al. 1997).

P. ginseng extract also increased cell-mediated immune functions, such as CD4 T helper subset, in man (Scaglione et al. 1990). In the multicentre, two-arm, randomized, placebo-controlled, double-blind investigation related to effect of ginseng on common cold morbidity, ginseng also induced a higher immune response in vaccination against influenza, and raised antibody titres, compared to the placebo group (Scaglione et al. 1996). NK activity levels 2-3 months after vaccination with ginseng treatment were nearly 2-fold higher than the placebo group. P. ginseng also stimulated basal NK cell activity and helped recovery of NK function in cyclophosphamide-immunosuppressed mice (Kim et al. 1990). Ginseng was a safe and potent adjuvant, when used for immunisation against vaccines injection in mice (Biondo et al. 1997), and in cyclophosphamide-immunosuppressed mice (Kim et al. 2000; Kwon et al. 2001). P. ginseng is the most commonly used and extensively researched species. Approximately 200 substances, such as ginsenoside, polysaccharides, polyacetylenes, peptides and amino acids, have been isolated from Korean ginseng (Attele et al. 1999). Nevertheless, its major components are ginseng saponin (such as about 40 ginsenosides) and polysaccharides (such as ginsans, pana-xans). The major components of raw ginseng or dried ginseng (named as a white ginseng) are ginsenosides Rb1, Rb2, Rf and Rg, and macromolecule polysaccharides such as pana-xans B (1800 KDa) and K (130 KDa). However, those of red ginseng are ginsenosides Rg3 and Rh2, and acidic polysaccharides, such as panaxans M (800KDa) and T (11 KDa), which are produced by heat process. The heat process in raw ginseng increased acidic polysaccharides activity (Konno et al. 1984; Lee et al. 1997; Belogortseva et al. 2000; Kim et al. 2003).

Investigating the efficacy of ginseng therapy is a complex process, because ginseng contains many constituents. Although pharmacological activities of all components were not clarified, the bioactive constituents of ginseng are considered to be ginseng saponin and polysaccharides. The ginsenosides have been reported to show anti-tumor (Wakabayashi et al. 1998; Chang et al. 2003; Helms et al. 2004), anti-diabetic (Yokozawa et al. 1985; Xie et al. 2005), anti-inflammatory activity (Park et al. 2004), anti-allergic (Choo et al. 2003; Park et al. 2003), endothelium-independent aorta relaxation (Kim et al. 1999) adjuvant-like (Wu et al. 1992), and immunomodulating effects (Rivera et al. 2003; Lee et al. 2004). The polysaccharides have been reported to show anti-inflammatory (Ahn et al. 2006), anti-diabetic (Ng and Yeung 1985; Belogortseva et al. 2000), antitumor (Shin et al. 2004), and immunostimulating effects (Konno et al. 1984), etc. Recently, the importance of inflammation in association with AIDS pathogenesis has highly been recognized. We also would like to put a high value of KRG intake in regard to anti-inflammation-effect of ginseng.

**GINSENG SAPONIN AS AN IMMUNOMODULATING AGENT**

Ginseng saponins, ginsenosides, are triterpene glycosides (Tanaka et al. 1972). These ginsenosides can be categorized into three groups depending on their aglycones: proto-panaxadiol, protopanaxatriol and oleanolic acid. P. notoginseng saponin is consisted of some ginsenosides and notogignosides (Zhu et al. 2004). Ginsenosides have been reported to exhibit potent biological activities such as immunomodulating, anti-diabetic, anti-tumor effects, etc. In detail, saponins from P. ginseng and P. notoginseng potently stimulate cellular and humoral immune responses (Song et al. 2007; Sun et al. 2009). Ginsenoside Rb1, which is the richest constituent in P. ginseng, induced serum-detectable amounts of IL-4 and IL-10 as early as 24 h after primary vaccine injection in mice (Biondo et al. 2008). Five weeks after booster, immune lymphocytes was still producing large amounts of cytokines including IFN-γ, IL-2, IL-4, IL-
10 and TNF-α. The Rb1 adjuvanted vaccines stimulated similar titres of antigen specific IgG. The ginsenoside Rb1 also had the strongest adjuvant effects, when used for immunisation against S. aureus (Hu et al. 2003). Ginsenosides Rb1 as well as Rg1 are potent adjuvants inducing higher or similar antibody titres than the vaccine adjuvanted with alum hydroxide alone (Rivera et al. 2003, 2005). Ginsenoside Rg1 increases the proportion of TIL cells among the total T-cell subsets and promotes IL-2 gene expression in murine splenocytes (Lee et al. 2004). Ginsenoside Rg1 had no mitogenic effects on unstimulated CD4 T cells. The ginsenoside Rg1 is a desirable agent for enhancing CD4 T-cell activity, as well as the correction of Th1-dominant pathological disorders. P. ginseng intake increases the immune response by induction of interleukin-12 production (Larsen et al. 2004).

In a cellular and humoral immunity study of ginsenosides-Rb1, Rb1, and Rb2 against S. aureus, the NO production from stimulated macrophages was significantly enhanced against the phospho-JNK1/2, phospho-p38 MAPK, and NF-κB was also decreased in the same culture system (Ahn et al. 2006). However, ginsan markedly down-regulated the production of proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, IFN-γ, IL-12, and IL-18, in macrophages (Lee et al. 2004).

The polysaccharide fraction from P. quinquefolius also enhances immune responses: it increased immunoglobulin and cytokines production (Wang et al. 2004). In addition, it significantly increased IL-2 and IFN-γ productions in Con-A-induced spleen cells in a dose-dependent manner in the murine model.

Red ginseng acidic polysaccharide (RGAP), having B cell-specific mitogenic activity, induced the secretion of IL-6 in spleen cells in a concentration-dependent manner. RGAP also restored the immune responses, such as splenocyte proliferation and NK cell activity, suppressed by pacitaxel or cyclophosphamide (Shin et al. 2004; Du et al. 2008). Interestingly, acid polysaccharides of P. ginseng induce the expression of IL-2, IFN-γ, IL-1α, and GM-CSF, as well as LAK cells and CD8+ T cells more than other ginseng polysaccharides (Cho et al. 1994; Kim et al. 1998; Wang et al. 2001). The immunomodulating activity of polysaccharides is a common feature of several species of ginseng (Chinese ginseng P. notoginseng, American ginseng P. quinquefolium, and Korean ginseng P. ginseng) although the structure of ginseng polysaccharides has not been clarified and their activity is significantly different with regard to HIV pathogenesis.

### Ginseng and Antiviral Activity

Several proteins isolated from ginseng revealed anti-reverse transcriptase (RT) or anti-protease of HIV-1 (Wang et al. 2000; Zhang et al. 2002; Wei et al. 2009). Quinqueginsin has been isolated from the roots of P. quinquefolius. This protein displays a variety of biological activities. It possessed ribonucleolytic activity toward yeast tRNA and specific activity toward poly C. An inhibitory action was expressed toward HIV-1 RT. This action was potentiated after chemical modification with succinic anhydride (Lam et al. 2002). A xylanase with a molecular weight of 15 kDa inhibits HIV-1 RT (McElhaney et al. 2004).

### Effects of KRG in AIDS Patients

Except for our experience, there is no clinical study with ginseng for HIV-1 infected individuals although American ginseng does not alter ZDV pharmacokinetics but reduces oxidative stress markers (Lee et al. 2008). Our clinical studies in HIV-1-infected individuals have been done with KRG which was manufactured by steaming under pressure. Studies with KRG in AIDS patients have been done with KRG which was manufactured by steaming under pressure and drying. During the process of manufacturing, there are great changes in the constituents; 12 specific ginsenosides including Rg3 and maltol belong to red ginseng only, and red ginseng has more antioxidants, and an increase in the Browning reaction, and a high content of acid polysaccharides (4-7%) compared to 0.6-0.8% in white ginseng (Nam et al. 1996). It is generally accepted that the effects of red ginseng are superior to white ginseng (Matsuda et al. 1987). The KRG used in our studies is a commercial product which was prepared from 6-year-old roots by Korea Ginseng Corp. A daily dose for our studies was 5.4 g and patients were told to take six capsules (300 mg/capsule) orally, three times daily. Daily 5.4 g is double the recommended dose. For females, we strongly recommend that the dose must be lessened to around 2.7 g by our long-term experience.
Six-month pilot study: KRG attenuates chronic immune activation

We have treated HIV-1-infected patients with KRG alone or in combination with zidovudine (ZDV; the first approved antiretroviral drug) from late 1991 (Cho et al., 1994, 1996, 2002). A 6 month pilot study consisting of 4 arms showed a significant increase in CD4 T cell percentage in KRG (n = 23), ZDV (n = 29), combination of KRG and ZDV arms (n = 16), whereas significant decrease was observed in control group (n = 24) (Fig. 1A). Contrary to our expectation, CD8+ T cell percentage also significantly increased in both KRG (p < 0.01) and combination (p < 0.05) arms compared with mild increases in the other 2 arms (Fig. 1B). The increase in CD4 T cells by ginseng intake in the healthy group was also previously reported by another group although CD8 T cell change was not shown (Scaglione et al., 1990). We measured soluble CD4 (sCD4) and CD8 antigen (sCD8) in sera by 3 months. Interestingly, sCD8 significantly decreased in both KRG and combination arms, whereas it mildly increased in the other 2 arms. However, sCD4 did not show any significant change in the 4 arms. With regard to its antiviral effect, p24 antigen decreased a little in both arms of the KRG treatment in contrast to a rebound phenomenon in ZDV. The decrease in sCD8 was compatible for the increase in CD8 T cells in arms with KRG treatment (Fig. 2). We thought the decrease in sCD8 in sera is very important because it is physiologically released from CD8+ T lymphocytes (CTL) and a marker indicating the immune activation state. A decrease in sCD8 was consistently observed and maintained over 6 years up to the final measure, whereas in the ZDV arm, it showed a rebound phenomenon. Thus, the study has been continuously conducted although there were several interruptions of 4-5 months between the pilot study and the second term, between the second and third, and between the third and fourth terms, and so on. However, some patients who had felt the effect of KRG intake took it following personal purchase during the lack of KRG supply. Patients 90-05 and 88-17 belonged to this example (Cho et al., 2006, 2009).

Ginseng might affect the HIV-1 env gene

Statistical analysis on the changes in CD4 T cells in 1996 showed that KRG intake has beneficial effects in HIV-1-infected patients. Thus, we questioned whether KRG intake could affect the HIV gene in vivo if our data were true over 5 years. At that time, there was no information on the HIV-1 gene in Korean patients. As in other countries, as the first target gene, we determined the env gene which shows the highest variation rate. We determined it in 65 patients (Cho et al., 1997). Among them, 40 patients were followed-up over 60 months by CD4 T cell measurement. Data analysis showed a significant inverse correlation between the decrease in CD4 T cell and the duration of KRG intake whereas there was no such correlation between CD4 T cells and ZDV. Above all, intrapatient variation of amino acids in 44 patients showed a significant inverse correlation with the duration of KRG intake (Fig. 3). In other words, we could interpret that long-term intake of KRG slows the variation rate in the env gene in patients treated with KRG for a prolonged period. We thought that this phenomenon is very implicit with regard to HIV’s intrinsic nature.

Delayed development of resistance mutation to ZDV

In Korea, ZDV monotherapy was introduced in early 1991 to treat HIV-1-infected patients and was the only antiretroviral therapy until early 1997 (Cho et al., 1993, 1996, 2002). It is well known that the effects of ZDV monotherapy were not maintained up to 12 months because of the development of resistance mutations in reverse transcriptase of the pol gene of HIV (Larder et al., 1989; St. Clair et al., 1991; Kellam et al., 1992). Surprisingly, CD4 T cells did not fall in the patients treated with ZDV and KRG compared to ZDV monotherapy. Thus, we investigated whether there is a delay in the development of mutations along with maintenance of CD4 T cells. Nine patients treated with ZDV and
KRG maintained the CD4 T cell count steadily over 75 ± 24 months, whereas 9 patients with ZDV monotherapy revealed a significant decrease in CD4 T cells. In addition, the frequency of 6 resistance mutations (M41L, D67N, K70R, L210W, T215Y/F, and K219Q) was 21.7% in the former group and 56.3% in the latter group (p < 0.01). Interestingly, the frequency of the first resistance mutation, K70R was significantly higher in the former than in the latter because second mutation, T215Y/F was nearly not developed in the former (Cho et al. 2001). Interestingly, we could not find multinucleoside drug resistance (MDR) mutations in our cohort treated with KRG (Cho et al. 1996, 2002) although the frequency of Q151M and related mutations has been reported to be 3.5 to >19% for patients treated with multiple nucleoside reverse transcriptase inhibitors for > 1 year (Kavlick et al. 1998; Maeda et al. 1999).

KRG slows depletion of CD4 T cell irrespective of HLA class I

We previously showed that long-term intake of KRG delayed disease progression in HIV-1-infected patients. To investigate whether this slow progression was affected by KRG-intake alone or in combination with HLA factor, we analyzed clinical data in 68 HIV-1-infected patients who lived for more than 5 years without antiretroviral therapy (Sung et al. 2005). The average KRG-intake over 112 ± 31 months was 4082 ± 3928 g, and an annual decrease in CD4 T cells was 35.0 ± 29/μL. Data analysis showed that there are significant inverse correlations between the HLA prognostic score and annual decrease in CD4 T cells (r = -0.379) (p < 0.01) as well as between the amount of KRG-intake and annual decrease in CD4 T cells (r = -0.347) (p < 0.01) (Cho et al. 2004). In conclusion, these data show that KRG intake independently and significantly affected the slow depletion of CD4 T cells, irrespective of HLA class I (Sung et al. 2005).

Moreover, when we focused the same analysis method on the 31 patients who have been living for more than 10 years without any antiretroviral therapy, we found that there is an association between the duration of KRG intake and the occurrence of gΔnef (p < 0.01) (Cho et al. 2006). The detection of gΔnef was significantly inhibited by HAART (Cho et al. 2009). Recently, we found that the median time for first detection of gΔnef was 13 months (Cho et al. 2010). In conclusion, our data show that gΔnef is inducible by KRG intake and its proportion is dependent on the duration of KRG intake and dose. This response is a noble concept in AIDS therapy because there is no report that any antiretroviral drug induces deletion in the HIV-1 genes.

Effect on 5LTR and gag region

Now, to investigate the relationship whether there are genetic defects in other genes of HIV-1 besides the nef gene, we determined the full sequences of HIV-1 in the 10 LTSP. Five out of the 10 were included in a pilot study consisting of a KRG-only group (n = 23) in 1991 (Cho et al. 1994). In addition to beneficial clinical findings, we obtained several important genetic defects in HIV-1. As a novel finding, we obtained significantly frequent gross deletion in the 5’LTR and gag region irrespective of the HIV-1 subtype (Cho et al. 2008, 2009). In addition, there were many premature stop codons or gross deletions in the pol gene in 7 (unpublished data) out of the same 10 patients who revealed gΔnef (Cho et al. 2010). These findings suggest that frequent genetic defects might be related to slow progression by long-term therapy of KRG.

Beneficial effects of a combination of KRG and HAART

To determine whether KRG has beneficial effects on HIV-1-infected patients administered HAART, we analyzed the CD4 T cell count, viral load, and resistance mutations to HAART in 46 individuals. The study population was divided into two groups, specifically, a combination of HAART plus KRG (n = 23) and HAART alone (n = 23). The annual increase in CD4 T cell count in the combination group was significantly higher than that in the HAART alone group (p < 0.05). High-level resistance mutations were significantly lower in the combination group than those in HAART alone (Sung et al. 2009).

FUTURE PERSPECTIVES

We think that our clinical trial with KRG is the longest follow-up study in the literature although there were several interruptions. Almost all patients revealed significant genetic defects in HIV-1. We anticipate that a patient who was diagnosed in 1988 can survive 30 years in the absence of...
HAART. KRG is an herb of choice in patients in early stage, not indicated for HAART. KRG could be a good alternative in patients intolerable for HAART. Combination therapy such as HAART plus immune therapy like KRG could be considered as best regimen in the future. Further well designed studies are needed whether what is the most effective component and how long the potency of HAART plus KRG is maintained.

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