



## **Angelica sinensis may provide protection against human immunodeficiency virus infection**

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### **Abstract**

Increased oxidative stress and disturbed glutathione redox system play an important role in the pathogenesis of human immunodeficiency virus (HIV) infection. Depletion in intracellular levels of reduced glutathione (GSH) contributes to an increment in tumor necrosis factor  $\alpha$  (TNF- $\alpha$ )-stimulated-HIV-1-transcription, activation of HIV-1-replication, sensitivity to TNF- $\alpha$ -induced cell death, and impairment of CD4+ cell function and survival. Therefore, several studies have investigated the effect of GSH-enhancer agents such as N-acetyl cystein in the treatment of patients with HIV infection. With regard to the beneficial effects of *Angelica sinensis*, a Chinese medicinal herb, on GSH redox system and the pathogenic role of GSH depletion in HIV infection and the immunomodulator effects of active ingredients of this herb, we postulated that *Angelica sinensis* may be of value in the treatment of HIV-infected patients.

### **Keywords**

*Oxidative stress, Human immunodeficiency virus infection, Glutathione, N-acetyl cystein, Angelica sinensis*

### **Introduction**

Human immunodeficiency virus (HIV) infection is a worldwide problem. The HIV/AIDS patients suffer from several opportunistic infections as a result of poor immune system function; therefore, increasing deals of studies have focused on finding

the underlying mechanisms beyond HIV-induced immunosuppression and possible strategies to overcome the related disorders(1).

Oxidative stress has been considered to play an important role in the pathogenesis of different kinds of diseases including HIV-1 infection. Glutathione, a major intracellular redox buffering

compound, is involved in a variety of normal intracellular reactions as well as in a variety of immune functions including T lymphocyte proliferation, T and B-lymphocyte differentiation, natural killer cell activation, and T-lymphocyte-mediated cytotoxicity(2). Several studies have mentioned the important effects of glutathione redox disturbances in the immunopathogenesis of HIV-1 infection . Significantly decreased levels of reduced glutathione (GSH) in both CD4+ and CD8+ lymphocytes together with increased levels of oxidized glutathione(GSSG) in CD4+ lymphocytes have been found in asymptomatic as well as in symptomatic HIV-1-infected patients. However, in patients with the most pronounced immunodeficiency and advanced clinical disease, these abnormalities in glutathione redox system are more common (3). Depletion in intracellular levels of GSH contributes to an increase in tumor necrosis factor  $\alpha$  (TNF- $\alpha$ )-stimulated-HIV-1-transcription (4). Increased oxidative stress has also correlated with activation of HIV-1-replication through activation of nuclear factor kappa  $\beta$  (NF $\kappa$ B) transcriptor factor (5).

Described GSH deficiency in HIV infection along with the inhibitory effect of GSH on HIV and other retroviruses, have prompted researchers to investigate the effect of pro-GSH molecules in the treatment of HIV infection .Administration of high doses of GSH in murin AIDS was previously found to reduce viral replication and disease propagation. Until now, N-acetyl cystein (NAC), an excellent source of sulphhydryl (SH) groups for GSH synthesis, has been studied in the treatment of many oxidative conditions and it has been the only therapeutic strategy for treating oxidative stress-associated disorders in HIV infection. Also, studies reported the preventive effect of NAC on the activation of NF $\kappa$ B and the replication of HIV (6). Recently, Smietana et al has reported various NAC analogues which display equal or better anti-HIV activities than already reported cystein derivatives. However, the proposed mechanism for their anti viral activity is their antioxidant effects not their pro-GSH properties (7).In addition to antioxidant and pro-GSH properties, NAC also appears to enhance T cell function in HIV-infected patients suggesting that the administration of this molecule in HIV infection might be advantageous as both an antiviral agent and an immunomodulator agent. This can be described through the effect of NAC and GSH on T helper 1(Th1) cells. It has been demonstrated that NAC and GSH restore intra- and extra-cellular GSH levels in Tcells and antigen presenting cells (APC) leading to Th1 cytokine production . This finding seems particularly important in the infectious disease in which Th2 predominance is an important aspect in the pathology of disease as seen in acquired immunodeficiency virus (AIDS) (6).

### *Hypothesis*

In view of the fact that GSH augmentation exerts beneficial effects in the treatment of HIV infection, today more attention has been paid to the application of GSH-replenishing agents in these patients. Among these, Radix Angelica sinensis, a root of traditional Chinese medical herb named Angelica sinensis, has several therapeutic applications such as immunomodulation, antioxidant and antiapoptetic properties. The beneficial effects of Angelica sinensis on glutathione redox system are due to its two different active ingredients, the lipophilic component Z-ligustilid (Z-LIG) and the Angelica sinensis polysaccharide (AP). Z-LIG has been considered as the primary lipophilic compound of many medicinal plants including Angelica sinensis .The significant neuroprotective effect of Z-LIG has been demonstrated in previous studies and it has been postulated that this effect is associated with its antioxidant and anti-apoptotic properties. It could penetrate the blood brain barrier (BBB) and ameliorate brain damages following oxidative stress conditions (8). It significantly attenuated increased lipid peroxidation and restored the activities of the antioxidant enzymes, glutathione peroxidase (GPx) and superoxide dismutase (SOD) (9).These properties may make this compound good candidate for preventing and treating neuropathological disorders accompanied with HIV infection.

In recent years, it has been shown that natural polysaccharides derived from different kinds of plants exhibit antiviral activities. The enhancement of host immune responses by polysaccharides has been introduced as a possible means for inhibiting microorganism invasion and tumor growth without harming the host. Angelica sinensis polysaccharide (AP) is such a natural polysaccharide, which has immunomodulatory effect (10).AP directly activates T cells and causes a high level of proliferation of T cells. One study showed that the percentage of CD4+ T cell was remarkably increased by AP while that of CD8+ T cell was slightly decreased.This increase indicated that Th cells was activated by AP. In addition, AP exerts different effect on two general subsets of Th, Th1 and Th2, which are different in cytokine production. While AP significantly increases the production of Th1 related cytokines, IL-2 and IFN $\gamma$  , it decreases the production of Th2 related cytokine, IL-4.According to this, AP might be useful for the correction of Th2 dominant pathological disorders such as AIDS (11). Most of studies have reported the immunomodulatory effect of AP; however, recently, new experiments have focused on antioxidant activity of this agent. It was found that AP not only inhibited malanilaldehyde(MDA) formation, an indicator of

lipid peroxidation, but also enhanced GSH levels and SOD activity in H<sub>2</sub>O<sub>2</sub>-induced macrophages as well as in t-BHP-induced macrophages. The strong antioxidant ability of AP in the immune cells, which exert their biofunctions through free radicals and suffer more from oxidative stress, may be of value in preserving the immune function (12). The underlying antioxidant mechanism of AP seems to be through its effect on nitric oxide (NO) which has antioxidant effect at low concentrations but cytotoxic activity at high concentrations (13).

Taken together, administration of *Angelica sinensis* extract may have beneficial effects in the treatment of HIV infection by both antioxidant and immunomodulatory effects.

### ***Evaluating the hypothesis***

To evaluate the postulated effects of *Angelica sinensis* extract in the treatment of HIV infection, several *in vitro* and *in vivo* studies should be done. *In vitro* models include using cell cultures such as human monocyte derived macrophages infected *in vitro*. *In vivo* models include experimental animal models such as C57BL/6 mice infected with the LP-BMS viral complex and HIV infected patients. In order to calculate the antioxidant activity of AP and Z-LIG, several methods can be used including determination of GSH and MDA levels, determination of GSH/GSSG ratios, determination of SOD and GPx activities (14). To assess the antiviral effect of these two active ingredients, antiviral assay may be used as previously reported by Oiry et al (15). Besides to *in vitro* experiments, *in vivo* tests are needed. For example, the effect of *Angelica sinensis* extract on the numbers of CD4<sup>+</sup> and CD8<sup>+</sup> lymphocyte in peripheral blood can be determined by immunomagnetic qualification. Assessment of plasma HIV-RNA levels which exhibits the extent of HIV transcription is an effective method for calculating the antiviral activity of *Angelica sinensis* extract. To measure this, Quantitative reverse polymerase chain reaction is used (16). In addition, Th1 and Th2 related cytokines, which are important in the pathogenesis of HIV infection, are measurable using commercially available ELISA kits (11).

The remaining major question is that what concentration of extract should be used? Assessing several studies, we found that AP exerts significant antioxidant and pro-GSH activity as well as immunomodulatory effect at the concentration of 100 µg/ml (11, 17). On the other hand, Z-LIG possesses significant antioxidant effect at the concentrations of 2.5-5 µg/ml (9). Therefore, we propose these concentrations for future investigations. In order to find the adequate dose in whole animals, performing pharmacokinetic studies is needed; however, studies, investigated other pharmacological effects of *Angelica sinensis*

extract in whole animal or human, may be valuable in the estimation of appropriate dose.

### ***Discussion***

HIV infection causes chronic inflammation exhibited by high plasma levels of inflammatory cytokines and overproduction of reactive oxygen species (ROS) in seropositive individuals. The exact cause of progressive CD4<sup>+</sup> T lymphocyte depletion, a hall mark of HIV infection, is unknown; however, the most widely-accepted hypothesis is proposed to be apoptotic cell death induced by viral proteins. The GSH deficiency described in HIV infection along with the inhibitory effect of GSH and radical scavenger agents on HIV, raised this hypothesis in our mind that agents such as *Angelica sinensis* extract, which has both GSH replenishing and radical scavenging activity, would be of value in the treatment of HIV.

In addition, by its immunomodulator effect it could prevent CD4<sup>+</sup> T cell depletion and also suppresses the Th2 cytokine profile which is increased in patients with AIDS. One of the most important complications of HIV infection is that AIDS is often accompanied with neurological disorders. About third of adults and half of children with AIDS suffer from AIDS dementia complex (14). The high prevalence of dementia in AIDS patients showed the importance of introducing agents, which are able to reduce this serious and disabling complication. Brain microvessel endothelial cells are a main target of oxidative stress since they are rich in polyunsaturated fatty acids (PUFA). GSH level in the microvessels plays a critical role in the maintaining of Blood brain barrier (BBB) integrity since it acts as a major factor in preserving antioxidant defense system against oxidative stress. Recently, it has been revealed that HIV-1 proteins (gp120 and Tat) may result in BBB changes by ROS overproduction (18). A mild disruption of the BBB has been found in AIDS patients with dementia which is more frequent compared to AIDS patients without dementia or seronegative controls (19). This problem may be associated with the trafficking of immune cells across the BBB. With regard to the critical role of GSH in preserving the proper functioning of the BBB; it seems that GSH augmenting agents such as *Angelica sinensis* extract may have beneficial effect against BBB dysfunction induced by HIV-1 proteins. Therefore, we proposed that *Angelica sinensis* extract might prevent HIV-induced dementia not only through scavenging free radicals but also through preserving BBB integrity.

Today, the treatment of HIV is based on the application of highly active antiretroviral therapy (HAART) (20). Although HAART causes a rise in

circulating CD4+ T cells in most patients, the HIV-associated immunodeficiency seems to persist to a variable degree (21). Unfortunately, HAART are not able to eradicate a pool of chronically infected T cells, even after several years of treatment. This fact along with undetectable HIV-RNA levels in plasma after antiviral treatment may result in rapid virologic rebound following withdrawal of HAART. Besides, increasingly reported serious side effects of HAART such as mitochondrial toxicity and oxidative stress and emergence of drug-resistant strains make several questions in our mind about the safety and efficacy of HAART and show the urgent need for introducing additional modalities in the treatment of HIV infection (22). Combination therapy with GSH supplement and HAART increases in vitro T cell proliferation and suppresses the release of TNF- $\alpha$  from peripheral blood mononuclear cells in HIV-infected patients (23). However, because of lack of adequate and systematic studies, GSH-replenishing agents have received less attention in the treatment of these patients.

Moreover, it has been hypothesized that body's antioxidant defense system, especially the selenoenzyme GSH-Px, acts as an initial defense

against viral infection. In addition, one study reported the greatest difficulty of HIV infection in infecting those with high GSH-Px activity and selenium-enriched diet (24). With regard to the effect of *Angelica sinensis* on GSH content and GSH-relating enzymes including GSH-Px, it also would be of value in the prevention of HIV transmission. This fact may open a new insight in decreasing the prevalence of HIV infection especially in high-risk groups.

### Conclusion

Finding a new agent, which is able to prevent CD4+T cell deletion and dysfunction, HIV replication, and HIV-associated immunodeficiency may contribute to the revolution in the treatment of HIV infection. If the beneficial effects of GSH enhancer agents such as *Angelica sinensis* have been approved in in vitro and in vivo studies and clinical trials; then, agents with the promising effects can be used in the HIV treatment protocol. However, the concern is the potential prooxidant properties of GSH which may lead to the formation of ROS and free radicals through participation in metal ion-mediated reactions (25).

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