Oxidative Stress, Cell Activation and Viral Infection
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Place for an antioxidant therapy in human immunodeficiency virus (HIV) infection

S. Baruchel[1,2] G. Bounous[2], P. Gold[2]

1 McGill University, Dept. of Pediatrics; McGill AIDS Centre, Montreal, Qc, H3H 1P3, Canada
2 McGill University, Dept of Medicine; McGill AIDS Centre, Montreal, Qc, H3G 1A4, Canada

Summary

Oxidative stress, a known activator of HIV replication in vitro, has a potential role as a cofactor of HIV disease progression. Arguments supporting the role of oxidative stress as a cofactor in HIV activation are summarized in this review. The role of intracellular antioxidants such as glutathione (GSH), and drugs and nutriceutical agents promoting GSH synthesis, are discussed. The review also includes the early results of nutritional interventions based on a diet enriched with IMMUNOCAL™, a whey protein concentrate prepared in a proprietary manner.

Introduction

In human immunodeficiency virus (HIV) mediated infection, after primary infection and viral dissemination, most patients have a period of "clinical latency" that may last for years (Pantaleo et al., 1993). Factors that stimulate HIV to replicate and to determine the period of latency are poorly understood in vivo. Results from close observations have shown HIV-infected individuals to have a decreased level of acid-soluble thiol; in particular, cysteine and GSH in plasma and leucocytes (Buhl et al., 1989; Eck et al., 1991) suggesting that oxidative stress may play an important role in the progression to full blown acquired immunodeficiency syndrome (AIDS) (Baruchel and Wainberg, 1992; Halliwell and Cross, 1991).

Rationale for an antioxidant therapy in HIV infection

Oxidative stress occurs when the balance between free radical generation and antioxidant defense is upset. In such cases, active oxygen species and free radicals are so reactive and short-lived that their levels are difficult to measure directly. For these reasons, most methods measure only the product of oxidative stress. GSH is a tripeptide and a major intracellular antioxidant which accounts for over 90% of the intracellular non-protein thiols. One mechanism of action of GSH is through removal of intracellular H_2O_2 by providing substrate for GSH peroxidase, the major H_2O_2 removing enzyme.

Indirect evidence indicates that HIV infection is associated with an increased consumption of antioxidants. The concentration of intracellular GSH in the peripheral blood mononuclear cells and lymphocytes of asymptomatic HIV-seropositive patients was found to be lower than in the healthy control group (Roederer et al., 1990; Smith et al., 1990; Staal et al., 1992). In addition, GSH levels were profoundly depressed in patients with AIDS and AIDS-related complex.

HIV-infected patients commonly excrete higher than average quantities of malondialdehyde into their urine, reflecting an increased level of lipid peroxidation. Rhesus monkeys that were acutely infected with the simian immunodeficiency virus had lower plasma thiol levels within two weeks of infection than uninfected animals. These early metabolic changes coincide with a rise in the level of urinary neopterin, a non-specific marker of macrophage activation (Eck et al., 1991).

A high plasma glutamate is also associated with a low level of thiol, leading to a reduction of intracellular GSH and impairment in T cell function (Eck et al., 1989). The consumption of intracellular non-protein thiol in these situations may play a role in increased oxidative stress. HIV-infected individuals show a 30-40% decrease in GSH in both CD4+ and CD8+ T cells. This decrease is due primarily to the specific removal from the circulation of a class of T cells with high GSH content (Roederer et al., 1990).

Oxidative stress and hydroxyradical formation can lead to increased lipid peroxidation and modification in both membrane fluidity and receptor conformation (Carson et al., 1986). In the absence of a proper antioxidant system, the DNA repair capacity of the cells may be altered and functional lymphocytes may be destroyed in situ (apoptosis) or suffer from impaired function (Ameisen and Capron, 1991; Arends and Wyllie, 1991). Although lower magnitude oxidative bursts are associated with lymphocyte activation and are regulated by intracellular GSH (Hamilos and Wedner, 1985; Hamilos et al., 1989), depletion of GSH is associated with immunosuppression and down regulation of IL-2 receptors (Suthantiran and May, 1990). Moreover, HIV-infected patients are also known to manifest increased radiation sensitivity when GSH is depleted (Hughes-Davies et al., 1991; Valis, 1991).

Oxidative stress stimulates HIV replication in vitro

HIV gene expression can be activated in vitro by oxidative stress. H_2O_2 can induce the expression of HIV in human T cell lines by activating transcription of nuclear factor kappa-B (NF-kappaB) (Duh, 1989). NF-kappaB is inactive in the cytosol when complexed to a second regulatory molecule termed IkappaB. Activation with a variety of oxidative stimuli results in the dissociation of the complex between NF-kappaB and IkappaB and subsequent translocation of NF-kappaB to the nucleus. NF-kappaB binding activity in the nucleus is modulated by

oxidoreduction in vitro. Activation of NF-kappaB nuclear binding activity by oxidative stress is specific and occurs at low concentration (Paeck et al., 1991; Toledano and Leonard, 1991). A variety of antioxidants, including GSH, GSH ester, pentoxyphylline, desferrioxamine and N-acetylcysteine can block activation of NF-kappaB (Baruchel et al., 1993; Fazely et al., 1991; Kalebic et al., 1991).

Mechanism of oxidative stress in HIV infection

Cytokines are important constituents in the regulation of the immune response. Tumor necrosis factor a (TNF-α) can up-regulate HIV expression in chronically infected T cells and monocytes (Griffin et al., 1990; Mellors et al., 1991; Poli et al., 1990). In this regard, TNF-α acts synergistically with either interleukin-6 or granulocyte-macrophage colony stimulating factor (GM-CSF) (Poli et al., 1990). TNF-α triggers virus expression by the induction of transcription of an activating factor which binds to NF-κB in the promoter region of the HIV long terminal repeat. This, in turn, results in increased transcription of HIV RNA and the eventual production of viral progeny (Paeck et al., 1991).

TNF-α is produced by activated macrophages and may contribute to disease progression by activation of HIV replication through the action of NF-κB. There are many controversies concerning the role of TNF-α in the activation of HIV infection in vivo. TNF-α levels have not been found to be constantly elevated in AIDS patients sera (Lahdevirta et al., 1988).

Intracellular thiol levels regulate lymphocyte functions

A number of studies have shown that depletion of GSH inhibits T cell proliferation. In fact, there is a critical need for adequate intracellular GSH levels for T cell proliferation (Fidelus and Tsan, 1986; Fidelus et al., 1987). Reduction of GSH by 10-40% in T cells completely inhibits T cell activation. While depletion of GSH leads to inhibition of some T-cell functions, supplementation can augment others functions, both in vitro and in vivo. GSH added exogenously augments lymphocyte proliferation in response to lectin. 2-L-Oxothiazolidine-4-carboxylate, a cysteine precursor which increases GSH levels, acts synergistically with concanavalin A to stimulate T cells. An increased of previously-lowered GSH levels in mice augments activation of cytolytic T cells, demonstrating the importance of GSH in vivo (Fidelus et al., 1987).

Oxidative stress and wasting syndrome

HIV-infected patients have a higher rate of resting energy expenditure associated with an increased fat oxidation rate and an increased concentration of IL-6. This cytokine is closely interrelated with TNF-α and IL-1 which appear to have metabolic effects related to tissue wasting probably through the generation of hydroxyradicals. In summary, oxidative stress may

be implicated in the pathophysiology of the wasting syndrome experienced by AIDS patients (Hommes et al., 1991).

Use of antioxidant in HIV infection ?

Several authors have found that various thiols alter HIV expression in vitro. Kalebic et al. (1991) have found that the thiols GSH, GSH monoester, and N-acetylcysteine block HIV expression in vitro. They have suggested that these thiol agents may have therapeutic value in HIV-infected patients. This group used the U1 cell line, chronically infected with the human immunodeficiency virus. Expression of HIV is minimal in these cells unless activated by stimuli such as PMA, TNF-α, or IL-6. Some of these cytokines may stimulate HIV activity, in part, by stimulating production of reactive oxygen species (ROS) (Yamauchi, 1990), as discussed above.

The HIV-infected U1 cells were pre-treated with various concentration of thiols, then the activators PMA, TNF-α or IL-6 were added and the cells incubated for various periods of time. HIV activity was estimated by measuring the activity of the viral enzyme reverse transcriptase, HIV messenger RNA and synthesis of the major HIV viral proteins. Reverse transcriptase activity in the cell culture supernatant without thiol treatment increased ten to thirty-fold when treated with either PMA or TNF-a and three to five fold when treated with IL-6. A single pre-treatment with GSH at 1, 5 or 15 mM suppressed reverse transcriptase activity markedly in a dose dependant manner regardless of whether PMA, TNF-α or IL-6 was used as the activator. The duration of exposure to the thiols was important. Pre-treatment with thiols for six hours was more effective than pre-treatment for three hours or the simultaneous addition of thiols with the viral inducers. At thiol concentration of 15 mM, all three thiols inhibited the induction of the total HIV protein synthesis induced by PMA, TNF-α or IL-6. Other reducing agents that are not directly used for GSH synthesis were also found to affect HIV replication in acute or chronic in vitro system.

Harakeh et al. (1990) studied the action of ascorbic acid on HIV activity in a chronically HIV-infected T-lymphocytic cell line and found that the presence of nontoxic concentrations of ascorbic acid. in the cell culture medium reduced the level of extracellular reverse transcriptase activity by 99% and the expression of p24 antigen by 90%. Moreover, the presence of ascorbic acid caused both a time and dose-dependent decrease in HIV activity in acutely infected CD4+ T-lymphocytes. The molecular mechanism by which ascorbic acid suppresses HIV activity is unknown, but the close interrelation between ascorbic acid and GSH metabolism is intriguing.

N-Acetylcysteine (NAC) at 10mM/L caused less than a two-fold inhibition of RT and conferred a synergistic effect (approximatively eight-fold inhibition) when added in conjunction with ascorbic acid. Long term experiments have shown continuous exposure to ascorbate was necessary for HIV suppression.

Pentoxyphylline, another antioxidant agent, has shown some anti-HIV activity in chronically and acutely-infected cell systems (Fazely et al., 1991). More re cently, a lipoic acid has shown some activity in the inhibition of NF-kappaB induced by TNF-α or by PMA. This inhibitory action of a lipoic acid was found to be very potent and only 4 mM was needed for a complete inhibition, whereas 20mM was required for N-acetyl cysteineSuzuki et al., 1992). Moreover, in vitro and in vivo applications of a lipoic acid have been associated with an increase in GSH concentration (Busse et al., 1992).

In our laboratory, we have tested 2-L-oxothiazolidine4 carboxylate (OTC) on the U1 cell line system chronically infected with HIV-1 and stimulated by PMA. Inhibition of HIV activity was measured as reflected by reverse transcriptase (RT) activity and P24 antigen expression. OTC, a GSH prodrug, provided a cysteine delivery system. At 2 mM concentration, OTC inhibits HIV expression with 80% inhibition of RT activity and partial inhibition of P24 antigen expression.

We also tested other types of antioxidants, specifically desferrioxamine on the acutely HIV-infected cell system MT4. We have shown that DFO at an in vitro concentration similar to the one achieved in clinical situations blocked HIV expression probably by inhibition of NF-kappaB (Baruchel et al., 1993; Paeck et al., 1991). Many antioxidants were found to be of potential use in this regard. Some compounds which do not replenish GSH may have beneficial effects by inhibiting ROS production and sparing GSH to some extent.

Antioxidant in vivo

Various agents such as diethyldithiocarbamate (DDTC), lipoic acid, 2-L-oxothiazolidine-4-carboxylate have shown some GSH-promoting activity in non-infected animals, however except for DDTC none of these compounds have been studied in HIV-infected animals. We have studied OTC in non-HIV infected animals, and found a GSH-promoting activity in various tissues including bone marrow.

IMMUNOCAL™

IMMUNOCAL™ is a whey protein concentrate prepared in a proprietary fashion so as to preserve the most thermosensitive molecule of whey, such as serum albumin, in their undenaturated form (serum albumin contains 6 glutamyl-cysteine groups per molecule). This group of milk proteins, when prepared appropriately, can produce a glutamyl-cysteine delivery system. IMMUNOCAL™ was kindly provided through courtesy of Immunotec Research Corporation Ltd., Montreal, Qc..

Bounous et al have shown that the humoral immune response of mice fed with 20g of IMMUNOCAL™/100g of diet was higher than mice fed formula diets of similar nutritional efficiency containing 20g/100g diet of any other types of commercially available semipurified food protein, such as casein. Bounous et al have further shown that the immunoenhancing activity of IMMUNOCAL™ concentrate is related to increased production of splenic GSH during the oxygen-requiring antigen driven clonal expansion of lymphocytes (Bounous et al., 1988; 1993).

Recent experiments in Japan have shown spleen cells of BALB/c male mice fed a 25g IMMUNOCAL™ concentrate/100g diet for 4 weeks had an increased immune response to SRBC in vitro and a higher content of L3T4+ cells than mice fed on isocaloric diet with 25g pure casein/100d diet. Similarly, the spleen L3T4+/Lyt-2+ ratio was 1,36 ± 0.07 in IMMUNOCAL™ fed mice and 0.55 ± 0.07 in the casein control group ($p<0.001$) (Hirai et al., 1990). This background represents the rationale for using IMMUNOCAL™ as a food supplementation in HIV-infected individuals.

Antioxidants in HIV infected individuals

Only a few reports are available on the use of antioxidants in HIV infected individuals.

Diethyldithiocarbamate

The only antioxidant drug widely studied in clinical trials has been diethyldithiocarbamate (DDTC). Despite encouraging early preliminary reports in two randomized placebo control studies and one randomized non-placebo study in AIDS patients, a larger study in asymptomatic individuals has failed to prove any benefit from the use of DDTC (Hersh et al., 1991; Lang et al., 1988; Picolet et al., 1993; Reisinger et al., 1990). The heterogenicity of the patients in the former study gives rise to the question of the benefits of antioxidant therapy in all HIV- infected patients. It also reinforces the argument for clear entry criteria into any study focusing on antioxidant metabolism and the need for careful monitoring of the oxidative stress status of patients before conducting any clinical trial using antioxidants.

Glutathione and Glutathione Pro-drugs

Recently published studies on the bio-availability of orally administrated GSH in human have been disappointing. These studies have shown no significant increase in plasma cysteine or GSH after oral administration of 3g of GSH in healthy volunteers. This may be due to hydrolysis of GSH by intestinal and hepatic gamma-glutamyl transferase (Yamauchi, 1990). However, oral administration of 25 mg OTC/kg in healthy volunteers and in HIV-infected individuals alone, or in association with intramuscular injection of 800 mg GSH per day, was successful in increasing the total GSH in the blood (Witschin et al., 1992).

N-acetylcysteine (NAC), another GSH-promoting agent, is currently under investigation. Four hours after a single dose of NAC given orally to HIV-infected patients, the concentration of cysteine in plasma and mononuclear cells increases and GSH concentration is moderately higher than before, or 2 hours after NAC administration. A sustained increased in intracellular cysteine may be necessary to normalize intracellular glutathione. This may be accomplished by repeat administration of NAC (Giorgi et al., 1992; Mihm et al., 1991; Ruffmann and Wendel, 1991).

Desferrioxamine (DFO)

A retrospective study looking at the median daily dose of desferrioxamine in a cohort of 69 HIV-1 infected thalassemic patients and the rate of progression to stage IV CDC classification over 6 years concluded that patients who received 40 mg/Kg daily of DFO as a chelating agent for iron overload had an 11% risk of progression as compared to 40% for those who received less than 40mg/kg/day ($p < 0.001$). When the dose was taken as a continuous variable it was found that the rate of progression was significantly slower in thalassemic patients receiving a higher dose ($p<0.003$). (Costagliola/Baruchel personal communication).

IMMUNOCAL™

Based on the animal experiments, a pilot study was undertaken at McGill University in order to evaluate the effect of a diet enriched with IMMUNOCAL™ in four HIV-infected asymptomatic individuals and one AIDS patient during a period of 3 to 5 months. IMMUNOCAL™ was dissolved and taken in a cold flavored drink in quantities progressively increased from 8.4 to 39 g per day. In 4 HIV asymptomatic patients who regularly took the product, no side effects were noted and the patient's body weight increased progressively during the study by an amount varying from 2 to 7 kg. As expected, the blood mononuclear cells' GSH content was below normal value in all patients at the onset of the study. In 3 of the asymptomatic HIV-infected patients, the GSH level increased over a 3 month period, and in one case returned to normal values (70% increased in concentration). Three comparable patients on their usual standard diet

over the same period showed some weight loss and no change in their blood GSH mononuclear cell content. After 5 months of treatment, the patient with AIDS showed weight stabilisation and had his intracellular GSH reach normal values. These preliminary data indicate that, whenever patients maintained their overall energy and protein intake, they either stabilized or increased their body weight as well increasing their GSH in lymphocytes. This confirms the potential for IMMUNOCAL™ as a glutamyl-cysteine delivery system (Bounous et al., 1989).

This new nutritional approach to HIV-positive individuals or patients suffering from full blown AIDS must be considered a nutriceutical form of therapy and is currently under investigation in a population of children suffering from AIDS and wasting syndrome in order to confirm and extend our preliminary data and to attempt to correlate the GSH modulating activity of IMMUNOCAL™ with both quantitative and qualitative immunological parameters.

Conclusion

There is certainly enough rationale and in vitro data to consider antioxidant therapy in association with antiretroviral therapy in HIV infection. Caution should probably be exercised before using any antioxidant in order to avoid any toxic side effects. An important prerequisite is good pharmacological monitoring of oxidative stress as an important part of any such trial. The parameters of such activity must be clearly defined if such therapy is to gain general utility. In addition, studies of viral burden will be necessary in the patients under investigation.

Furthermore, it must be noted that whereas lowering levels of reactive oxygen species may have beneficial biological effects, it is possible that excessive antioxidant protection could have some deleterious effects such as possible paradoxical immunosuppression. A good candidate for clinical monitoring of oxidative stress is GSH. Good biochemical techniques are available for measuring GSH in mononuclear cells. Flow-cytometric evaluation of GSH in T cell subsets is also available, but needs some standardisation and good correlation with the biochemical assay.

Pro-drugs of GSH such as N-acetyl-cysteine and 2-L-oxothiazolidine are currently in phase 1 investigations but results are not yet available. Nutritional interventions through a modified dairy product is an attractive approach to a cysteine delivery system and GSH elevation, and studies are currently ongoing in order to confirm our preliminary results.

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