Antioxidant Therapy- A Relevant Therapeutic Adjunct For Antiretroviral Therapy In PwHAs In Ghana

Author(s): Dr. Christian Obirikorang, Dr. Francis Agyemang Yeboah, Mr. Lawrence Quaye

Corresponding Author: 
Dr. Christian Obirikorang, 
Biomedical Scientist, Dept of Molecular Medicine, School of Medical Sciences, KNUST, Dept of Molecular Medicine, School of Medical Sciences, KNUST, Kumasi - Ghana

Submitting Author: 
Dr. Christian Obirikorang, 
Biomedical Scientist, Dept of Molecular Medicine, School of Medical Sciences, KNUST, Dept of Molecular Medicine, School of Medical Sciences, KNUST, Kumasi - Ghana

Article ID: WMC001039
Article Type: Research articles
Submitted on: 19-Oct-2010, 06:41:46 PM GMT Published on: 19-Oct-2010, 09:39:15 PM GMT
Article URL: http://www.webmedcentral.com//article_view/1039
Subject Categories: INFECTIOUS DISEASES
Keywords: Reactive oxygen species, Antioxidant, Oxidative stress, CD4 count, HIV, Malondialdehyde

How to cite the article: Obirikorang C, Yeboah F, Quaye L. Antioxidant Therapy- A Relevant Therapeutic Adjunct For Antiretroviral Therapy In PwHas In Ghana. WebmedCentral INFECTIOUS DISEASES 2010;1(10):WMC001039

Source(s) of Funding: 
No source of funding

Competing Interests: 
We declare that we have no competing interests.
Antioxidant Therapy- A Relevant Therapeutic Adjunct For Antiretroviral Therapy In Plwhas In Ghana

Abstract

Background: Reactive Oxygen Species (ROS) has been implicated in the stimulation of replication and progression of HIV infection. This study was aimed at investigating the levels of oxidative stress and the relevance of antioxidant therapy as an adjunct for highly active antiretroviral therapy (HAART) in people living with HIV/AIDS (PLWHA) in Ghana.

Methods: Two hundred and twenty-eight (228) people living with HIV/AIDS (PLWHAs) were recruited from an established HIV/ART centre in Ghana. The subjects were placed in three groups according to their CD4 counts: CD4+ counts Group 1: ≥ 500 cell mm⁻³; Group 2: 200-499 cells mm⁻³; and Group 3: <200 cells mm⁻³. One hundred (100) sex, age-matched and healthy HIV-seronegative individuals were used as control. Venous blood samples was taken and analyzed for CD4+ count and markers of oxidative stress which included Malondialdehyde (MDA), Ferric Reducing Ability of Plasma (FRAP), Vitamin C and E, Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx).

Results: The mean MDA concentrations in Group 1 (2.68±0.09 mmol L⁻¹), Group 2 (2.77±0.10 mmol L⁻¹) and Group 3 (3.17±0.13 mmol L⁻¹) were significantly elevated when compared to the control group (1.54±0.04 mmol L⁻¹) (p<0.0001). The mean FRAP concentrations in Group 1 (563.4±17.70 µmol L⁻¹), Group 2 (463.3±13.28 µmol L⁻¹) and Group 3 (342.0±9.93 µmol L⁻¹) were significantly decreased when compared to the control group (923.3±36.91 µmol L⁻¹) (p<0.0001). Significant decreases in the levels of SOD (p<0.0001), GPx (p<0.0001), Vitamin C and E (p<0.0001) was observed in the patient group compared to the control group.

Conclusions: Results from this study clearly show that, severe oxidative stress occurs in the HIV seropositive patients in comparison with healthy controls and increases significantly disease progression. Adverse effects of ROS might therefore be alleviated with antioxidant therapy as a combination therapy with antiretroviral drugs at cellular level and this might lead to a concomitant improvement in the general health status of PLWHAs.

Introduction

Humans infected with human immunodeficiency virus (HIV) have been shown to be under oxidative stress. Perturbations of the antioxidant defense system in HIV-infected humans include changes in ascorbic acid, tocopherol, carotenoids, selenium, superoxide dismutase and glutathione. In addition, elevated levels of hydroperoxides and malondialdehyde are found in plasma of HIV-infected individuals. Enhanced oxidative stress increases the replication of HIV, possibly via activation of nuclear factor kappa B (NF-κB), a transcription factor that stimulates replication of HIV and certain cytokines e.g. tumor necrosis factor-α (TNF-α) [1]. HIV responds to transcriptional stimuli similar to those leading to the induction of a series of cellular genes during T cell activation. [2]. HIV induced oxidative damage is influenced by the extent of oxidative stress and the activity of the body’s antioxidant defences which include dietary and non-dietary antioxidants and antioxidant enzymes. Management of PLWHAs in Ghana is focused on the administration of antiretroviral drugs and as such assessing oxidative stress levels, its role in HIV/AIDS progression and augmenting therapy with antioxidants might greatly improve the care and management of PLWHAs.

Methods

This prospective case control comparative study was conducted in an established anti retroviral therapy (ART) centre located at Cape Coast, the capital of the Central region of Ghana. The study was conducted between August 2007 and May 2008 and was approved by the Committee on Human Research, Publications and Ethics (CHRPE), School of Medical
Sciences, Kwame Nkrumah University of Science & Technology (KNUST), Kumasi, Ghana. All patients enrolled in the study completed a written informed consent in accordance with the Helsinki Declaration. Demographic details of the study participants were completed and venous samples collected from the patients before the initiation of ART into vacutainer® plain and EDTA tubes after an overnight fast (12-16 h) from the patients. Anticoagulated blood was used for the analysis of CD4 count, superoxide dismutase and glutathione peroxidase and the coagulated blood was allowed to clot and spun at 3000rpm for 10 minutes. The serum was collected and used for the assay of markers of oxidative stress. In all two hundred and twenty-eight (228) confirmed People Living with HIV/AIDS (PLWHAs) were included in the study. PLWHAs were staged in three (3) groups according to the Centres for Disease Control and Prevention Criteria (CDC) classification system that emphasizes the importance of CD4 T-lymphocyte testing in clinical management of HIV-infected persons. CD4+ counts Group 1: ≥ 500 cells mm⁻³; Group 2: 200-499 cells mm⁻³; and Group 3: < 200 mm⁻³. One hundred (100) sex and age-matched healthy HIV seronegative individuals served as controls. Inclusion criteria for the study include HIV positivity and not taking multivitamins/minerals supplements for at least 3 months prior to the taking of the blood samples and be clinically stable with no observable opportunistic infections. Exclusion criteria were as follows: smoking, initiation of antioxidant vitamin therapy before study, diabetes, kidney or liver dysfunction.

The CD4+ T-lymphocytes count was determined using the Becton Dickinson (BD) FASCount system (Becton, Dickinson and Company, California, USA). The BD FASCount system uses flow cytometry for the quantification of the CD4 T-Lymphocytes. CD4 testing is the recognized gold standard for the staging of HIV/AIDS, guiding treatment decisions and evaluating effectiveness of therapy.

MDA levels were determined by the MDA-Thiobarbituric acid (TBA) test which employs the reaction of MDA and TBA in acid solution. The method used for this assay was based on that of Kamal et al. (1989) [3] where serum was treated with trichloroacetic acid (TCA) and % TBA. Absorbances were measured at 535 nm with results being expressed as µmol L⁻¹ using the extinction coefficient of 1.56 x 10⁴L mmol cm⁻¹.

Total antioxidant capacity was measured by FRAP assay according to the method of Benzie & Strain, (1996) [4]. Serum vitamin C was determined by micro technique using Dinitrophenyhydrazine (DNPH) as described by Natelson (1961) [5]. Serum Vitamin E was measured by the method described by Baker and Frank, (1968) [6]. Glutathione peroxidase (GPx) was determined using the RANDOX RANSEL® reagent and superoxide dismutase (SOD) was determined using the RANDOX RANSOND® reagent.

Statistical Analysis

The OUTLIERS preliminary test for detection of error values was applied for statistical analysis. The results were given as mean ± (SEM). Correlations were evaluated using the Pearson’s correlation test. For all statistical comparisons, the level of significance was set at p < 0.05. Data analysis was done using GraphPad Prism for Windows version 4.02 (GraphPad Software, San Diego, CA, USA).

Results

Demographic characteristics of patients and controls are shown in Table 1. There was no significant difference in the age when the test subjects were compared to the control group (p=0.43). However, significant difference in mean CD4 counts were observed when the control group was compared to the test subjects (p<0.0001) and CD4 groups 2 and 3 (p <0.0001) respectively. The FRAP test showed a significant difference (F₃, 228=119.9; p<0.0001) when the test subjects were compared to the control group. Using the Bonferroni’s multiple comparison test for FRAP, there were significant differences between the control group and the three CD4 groups (p<0.0001) and within the three CD4 groups when they were compared against each other (p<0.0001) as shown in Fig. 1B. For serum MDA concentrations, the study found significant differences between the control group and the three CD4 groups (p<0.0001). In Fig. 3A, a Pearson’s correlation between the serum MDA levels of the test subjects and CD4 counts showed significant negative correlation (R= -0.292; p<0.0001). Serum Vitamin C and E concentrations were significantly and considerably reduced in the three CD4 groups compared to the control subjects (p<0.0001). Likewise, a strong positive correlation between the serum Vitamin C (R= 0.472; p< 0.001) and Vitamin E (R= 0.766; p<0.0001) levels were observed when the test subjects were compared to the controls (Figures 3C and 4A respectively). The mean blood SOD and GPx levels of the test subjects correlated linearly with CD4 counts and the difference were statistically significant (R= 0.812; p<0.001; R=0.652; p< 0.001) as shown in figure 4B and 4C respectively. One-way ANOVA revealed significant differences between blood SOD (F₃, 228
Initial infection with human immunodeficiency virus (HIV) is followed by an asymptomatic period of variable duration characterized by low or absent virus replication, stable or slowly decreasing numbers of CD4 T-helper cells and qualitative defects in T-cell function [7]. The pathogenesis of HIV infection involves dynamic interactions between the virus and the host immune system which result in immune activation throughout the course of infection. The degree of activation of the immune system can be monitored by measuring the serum levels of a variety of cellular markers that correlate with clinical progression of HIV disease [8,9,10]. The likelihood and timing of development of clinical AIDS following seroconversion, for any particular individual, are not readily predictable, thus the use of nonclinical disease markers has become critically important for patient management [11].

Formation of highly reactive oxygen-containing molecules is a normal consequence of a variety of essential biochemical reactions. Oxidants such as superoxide radicals (O$_2^-$), hydrogen peroxides (H$_2$O$_2$), hydroxyl radicals (HO$^*$) and lipid peroxides (LOOH) have been shown to play important roles in human disease [12]. Oxidants that are produced by stimulated neutrophils and/or macrophages can induce cellular injury and lysis of bystander cells both in vivo and in vitro [13]. As a means of dealing with this, most cells have enzymes and other molecules with antioxidant capabilities that can protect against the adverse effects of free radical reaction. Oxidative stress occurs when the balance between pro-oxidants and anti-oxidants is disturbed in favour of the former. According to a model of disease pathogenesis proposed by Fuchs et al. (1991) [14], HIV and opportunistic infections directly or indirectly leads to an oxidative stress. The pro-oxidative conditions cause activation of free radical-producing immune cells, enhancement of viral replication and weakening of the antioxidant defence system. This cycle becomes autocatalytic and facilitates disease progression [15].

The results of this study demonstrated that the total antioxidant capacity of human serum as measured by FRAP decreases in HIV positive patients compared to the HIV negative control and correlated positively with CD4 of the HIV positive subjects. The mechanism behind the significant reduction in total FRAP could likely be due to increased oxidative stress induced by an increase in free radicals production. Therefore initiation of antioxidant therapy in HIV infection could effectively inhibit the severity of infection and further improve survival.

The significantly greater release of MDA in severe cases as compared to mild progressive disease observed in the study suggests that oxidative stress leads to MDA formation which is a reflection of severities in HIV infection and anti-oxidant deprivation. Based on CD4 counts, the mild and severe groups had progressive diminishing levels of anti-oxidant (i.e. SOD, GPx, FRAP, ascorbic acid and alpha-tocopherol) in comparison to the control group. Levels of MDA were significantly increased in HIV seropositive patients in this study as confirmed by the findings of Fuchs et al. (1991) [14]. The observed increase of oxidative stress processes in these patients may modify proteins and DNA by addition reactions [16]. The main reason for high MDA levels in HIV seropositive patients in this study could be due to decreased activity of the defense system protecting tissues from free radical damage. This study demonstrates decrease in SOD activity at the early stages of HIV infection when the CD4 count is greater than 500 cells mm$^{-3}$. Flores et al., (1993) [17] found that the HIV Tat protein down-regulates the synthesis and overrides the induction of the MnSOD, which is induced by oxidative stress. Total SOD activity was measured in this study and includes activities of CuZnSOD and MnSOD. It may therefore be presumed that the decreases in total SOD activity could have been a result of inhibition of MnSOD by HIV Tat protein. Though the effect of Tat protein on EC-SOD has not been properly described, EC-SOD activity is strongly influenced by inflammatory cytokines such as TNF-alpha [18, 19, 20].

Blood glutathione peroxidase-levels were initially higher in HIV positive individuals compared to the HIV-negative control group and then fell significantly below that of the control group as the CD4 count declined. The initial increase in GPx observed in this study is in agreement with previous work [21, 22, 23]. It is therefore likely that GPx activity first increases under lipid peroxidation via an adaptative response, and then decreases as a result of its consumption, as observed with CD4 count decrease. These activities correspond to an aggravation of the disease with development of opportunistic infections and thus an increased production of ROS. HIV infection also causes increased oxidative stress during erythropoiesis, which, in turn, increases the level of...
GPX-1 expression, the predominant form of the enzyme found in erythrocytes [24]. The positive correlation between the CD4 counts and glutathione peroxidase level observed in this study is likely due to increased consumption of GSH by the GSH-Px reaction and/or decreased release into the circulation from the liver, the site of GSH production. Considering the role of GSH in immune function, the loss of thiol compounds, especially of GSH, represents a critical feature of HIV-disease.

Plasma ascorbate was lower in subjects with HIV infection. This means that such subjects have higher vitamin C requirements than do persons without HIV infection. Although oxidized ascorbate can be recycled, increased oxidative stress is assumed to increase ascorbate depletion [25]. Thus, plasma ascorbate may be decreased by the chronic immune activation of HIV infection even if dietary intake is at a level judged to be adequate for healthy persons [26]. The lower plasma ascorbate in HIV-positive patients found in this study is in agreement with previous observations [27, 28, 29]. This suggests that vitamin C utilization is increased by HIV infection and higher intakes during HIV infection should help prevent oxidative damage and maintain normal immune function. Vitamin C has been shown to inhibit HIV replication in acutely and chronically infected T cells and to inhibit HIV reactivation in T cells stimulated by TNF-alpha [30, 31]. The depressed plasma ascorbate levels found in HIV-infected patients in addition to the positive correlation with the CD4 counts suggest that they are less protected against viral replication. Ascorbic acid reduces the levels of extracellular reverse transcriptase activity and the expression of p24 antigen in an HIV-infected T-lymphocyte cell line [30]. It may therefore be suggested from this study that, observed decreases in ascorbic acid would lead to an increase in the nuclear transcription factor NF-kB which would result in increase viral replication. Vitamin C and E supplements have been shown to decrease oxidative damage and tend to decrease disease severity in HIV-positive Canadian adults [27]. High intakes of vitamin C have also been associated with a lower risk of progression to AIDS in US [32]. Studies in Tanzania [33] and Kenya [34] also indicate lower risk of death and increase in CD4 T lymphocyte respectively due to HIV-infection following multi-nutrient supplements containing vitamins C and E.

Vitamin E which is the major lipid soluble antioxidant is present in cellular membranes and protects against lipid peroxidation [35]. As confirmed by this study, previous works had indicated lower plasma alpha-tocopherol concentrations in subjects with HIV infection or AIDS than in healthy controls [36, 37, 38]. The significant positive correlation between CD4 count and alpha-tocopherol is also consistent with the report that plasma alpha-tocopherol concentrations decrease with time during HIV infection as CD4 count declines [39]. Earlier works suggest that vitamin E may increase immune response to antigens, improve host resistance against challenge with microorganisms, and enhance B and T cell lymphocyte functions, as well as phagocytic function [40, 41]. The effects of vitamin E on immune function and the potential benefits of vitamin E therapy in HIV-infected patients have been documented [42,43, 44]. The majority of evidence put forth by these studies suggests that vitamin E is not only necessary for proper functioning of the immune system, but also has important immuno-stimulatory properties. Studies in both humans and animal models have shown that vitamin E supplementation, in excess of the recommendation significantly increases humoral and cell-mediated immune responses to antigens and enhance phagocytic functions [43]. The progressive diminished vitamin E level in this study could lead to viral replication and faster progression from HIV to AIDS. The prevalence of overtly or marginally low serum vitamin E levels has been documented to range between 8% and 20% in studies of HIV positive individuals [45, 46, 47, 48].

Conclusion(s)

We thus conclude that HIV progression to AIDS is not a simple cellular event but a combination of other previously less observed factors like ROS as observed from this study. The overall effect is the selective and progressive destruction of cells through apoptosis and finally cell death. It is likely from this study that adverse effect of ROS can be alleviated by administration of antioxidants as a combination therapy with antiretroviral drugs which at cellular level will alleviate the effect of ROS and lead to the proper management of People Living with HIV/AIDS in Ghana.

Acknowledgement(s)

We wish to express our profound gratitude to all PLWHAs who availed themselves voluntarily to participate in this research.

Authors Contribution(s)
CO and LQ carried out the oxidative stress analysis, CD4+ counts, performed the statistical analysis and drafted the manuscript. FAY designed the study and its coordination and participated in the drafting of the manuscript. All authors read and approved the final manuscript.

References

24. Lacey, CJ, Murphy, ME, Sanderson, MJ, Monteiro,
Illustrations

Illustration 1

Table 1. Demographic Characteristics Subjects and Control. The values are expressed as mean ± SEM

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Total Subjects</th>
<th>CD4=500</th>
<th>CD4 230-500</th>
<th>CD4&lt;200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, (years)</td>
<td>38.76±1.73</td>
<td>37.19±0.84</td>
<td>38.72±2.58</td>
<td>37.43±1.57</td>
<td>36.60±1.68</td>
</tr>
<tr>
<td>Male, (n)</td>
<td>72</td>
<td>157</td>
<td>22</td>
<td>47</td>
<td>88</td>
</tr>
<tr>
<td>Female, (n)</td>
<td>28</td>
<td>71</td>
<td>21</td>
<td>15</td>
<td>34</td>
</tr>
<tr>
<td>CD4 count, (mm$^3$)</td>
<td>1045±457±177.53</td>
<td>776±171.17***</td>
<td>824±90±76.75</td>
<td>373±70±44.44***</td>
<td>84±30±77.53***</td>
</tr>
<tr>
<td>HIV serotype 1, (n)</td>
<td>-</td>
<td>138</td>
<td>22</td>
<td>34</td>
<td>82</td>
</tr>
<tr>
<td>HIV serotype 2, (n)</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>HIV serotype 1 and 2, (n)</td>
<td>-</td>
<td>89</td>
<td>20</td>
<td>27</td>
<td>42</td>
</tr>
</tbody>
</table>

Illustration 2

Figure 1. Effects of CD4 counts on (A) MDA, (B) FRAP and (C) GPx in Test subjects and Control group
Illustration 3

Fig 2. Effects of CD4 counts on (A) SOD, (B) Vit C and (C) Vit E in Test subjects and Control group

Illustration 4

Figure 3. Linear correlation between CD4 count and (A) MDA (B) FRAP and (C) Vitamin C.
Illustration 5

Figure 4. Linear correlation between CD4 count and (A) Vitamin E (B) SOD (C) GPx
Disclaimer

This article has been downloaded from WebmedCentral. With our unique author driven post publication peer review, contents posted on this web portal do not undergo any prepublication peer or editorial review. It is completely the responsibility of the authors to ensure not only scientific and ethical standards of the manuscript but also its grammatical accuracy. Authors must ensure that they obtain all the necessary permissions before submitting any information that requires obtaining a consent or approval from a third party. Authors should also ensure not to submit any information which they do not have the copyright of or of which they have transferred the copyrights to a third party.

Contents on WebmedCentral are purely for biomedical researchers and scientists. They are not meant to cater to the needs of an individual patient. The web portal or any content(s) therein is neither designed to support, nor replace, the relationship that exists between a patient/site visitor and his/her physician. Your use of the WebmedCentral site and its contents is entirely at your own risk. We do not take any responsibility for any harm that you may suffer or inflict on a third person by following the contents of this website.