Journal of Nutritional Medicine (1994) *4*, 393–401

ORIGINAL RESEARCH

Comparative Analysis of Ascorbate and AZT Effects on HIV Production in Persistently Infected Cell Lines

STEVE HARAKEH PhD AND RAXIT J. JARIWALLA PhD

Virology and Immunodeficiency Research Program, Linus Pauling Institute of Science and Medicine, 440 Page Mill Road, Palo Alto, CA 94306, USA

The effects of ascorbate (vitamin C) and azidothymidine (AZT) were examined on HIV expression in permanently infected and reporter cell lines. In T-lymphocytic HXB cells, constitutively producing moderate to high levels of virus, ascorbate suppressed HIV production and reduced the yield of infectious virus released into the culture supernatant. AZT, which has been reported to block de novo *infection of freshly infected cells, did not inhibit constitutive virus production in HXB cells. In latently infected ACH-2 T-cells, producing low basal level of virus, exposure to phorbol ester (PMA) caused about 10-fold increase in virus production. Pre-treatment of ACH-2 cells with ascorbate followed by PMA stimulation resulted in a dose-dependent reduction in the extracellular level of HIV reverse transcriptase activity. AZT treatment did not suppress HIV activation in PMA-stimulated ACH-2 cells. In mixed cultures of uninfected HLCD4-CAT and infected HL2/3 cells, ascorbate did not affect virus-induced* (tat-*mediated) transcriptional activation of the CAT reporter gene linked to the HIV long terminal repeat. These results reveal anti-HIV effects of ascorbate that offer potential for development of combined therapy with other agents.*

Keywords: vitamin C (ascorbate), zidovudine (AZT), reverse transcriptase, cytokine stimulation, latently infected ACH-2 line, T-lymphocytic cells.

INTRODUCTION

A characteristic of HIV, the human retrovirus linked to AIDS, is its ability to establish latent and chronic infection in primary lymphoid and peripheral blood cells of the infected host [1]. Latent HIV resides within host chromosomes as integrated provirus that is subject to activation by a variety of stimuli including exposure to viral/opportunistic pathogens, tumor-promoting/mitogenic agents, drugs and other xenobiotics. The action of such stimuli is associated with oxidative stress and the formation of inflammatory cytokines, both of which have been shown to induce the expression of latent HIV [2, 3].

Virus induction by cytokines and reactive oxygen intermediates is mediated through the activation of the cellular transcription factor NFkB that interacts with the kB binding site within the HIV long terminal repeat (LTR) [2]. Virus production is also facilitated through the action of viral regulatory proteins that transactivate HIV by binding to other sites within the LTR of viral RNA [4]. Chronically infected cells that produce HIV in the absence of

This publication is dedicated in the memory of Professor Linus Pauling.

exogenous stimulation contain constitutively active basal cellular factors that drive the continuous production of HIV.

Since integrated HIV represents a large pool of dormant virus, the development of strategies to suppress its expression can be considerably effective in slowing or stopping the progression of HIV. Studies in our laboratory have been focused on unraveling the role of micro-nutrients and antioxidant thiols in HIV infection. Towards this end, we have demonstrated that ascorbic acid or ascorbate (vitamin C) and thiols such as *N*-acetyl-cysteine (NAC) can suppress constitutive HIV production in chronically infected cells and block *de novo* (new) virus replication in cells acutely infected with HIV [5, 6]. Although NAC has been shown to inhibit HIV expression following cytokine stimulation [7, 8], the action of ascorbate on latent HIV has remained largely unknown. Furthermore, the effects of conventional AIDS treatment drugs such as the nucleoside analogue zidovudine (azidothymidine or AZT) on constitutive virus production in chronically infected cells are not known. In this paper, we compare the anti-HIV activity of ascorbate with that of AZT in constitutive virus-producing T-cells and in a latently infected T-cell line exposed to an inducing agent.

MATERIALS AND METHODS

Cell Lines

HXB (H9/HTLV-III$_B$). Source, growth conditions and properties of this chronically HIV-infected T-lymphocytic cell line have been previously described [5, 6].

ACH-2. (From M. Roederer, Herzenberg laboratory, Stanford University.) This latently HIV-infected T-cell line was grown and maintained in the same growth medium as that used for HXB cells (RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM-*L*-glutamine, 1 mM-pyruvate and gentamicin at 50 μg ml^{-1}).

HL 2/3. (Developed by B. Felber and G. Pavlakis.) This cell line was obtained from the NIAID Reference Reagent Repository. It was derived from co-transfection of HeLa cells with plasmids pSV2neo and HXB2/3gpt, a molecular clone of HIV-1, followed by selection of transformed cells in medium containing the antibiotic, geniticin [4].

HLCD4-CAT. (Developed by B. Felber and G. Pavlakis.) This cell line was also provided by the NIAID Reagent Repository. It is a derivative of HeLa cells that expresses the CD_4^+ surface receptor and contains an integrated copy of the CAT gene linked to HIV LTR. Upon viral infection, the expression of the CAT gene is activated by HIV *tat* protein [4].

Assay of Infectious Virus Yield

Infectivity of virus in culture supernatant (from HXB cells) was assayed using the HLCD4-CAT cell line as described by Ciminale *et al.* [4]. Briefly, HLCD4-CAT cells were seeded in six-well clusters at a density of 1×10^6 cells per well. After overnight incubation at 37°C, medium was aspirated and cells exposed to supernatants containing virus (100–200 μl) which was allowed to adsorb at 37°C. After 90 min, virus was aspirated, cultures washed once with calcium–magnesium free phosphate-buffered saline (PBS), refed growth medium and incubated for 48 h. At this time, cells were harvested, lyzed and extracts assayed for CAT activity as described later (see co-cultivation assays).

Assay of HIV Production in HXB Cells

This was assessed by measuring reverse transcriptase (RT) activity or p24 antigen level in the culture supernatant as previously described [5]. RT activity was expressed as the amount of tritiated dTMP incorporated (cpm per 0·75 ml of culture supernatant). The p24 value was expressed as ng per 0·75 ml of culture supernatant.

Virus Activation Protocol in ACH-2 Cells

ACH-2 cells were grown overnight at 8×10^5 cells ml^{-1} with or without vitamin/drug to obtain cells in the logarithmic phase. Cell density was adjusted to 1×10^6 cells ml^{-1}, phorbol 12-myristate 13-acetate (PMA) (~ 61 ng ml^{-1}) was added and incubation continued for 24 h in the presence or absence of fresh dose of vitamin/drug. An aliquot of cell suspension was used to determine cell viability by the Trypan blue dye exclusion test [5, 6]. For RT analysis, 1·5 ml of cell suspension (in duplicate) was spun in a refrigerated microcentrifuge (Tomy) at 12 000 rpm for 5 min. Cell pellet was set aside on ice for processing of intracellular RT. Supernatant was spun in the Tomy microcentrifuge at 12 000 rpm for 2 h, medium was aspirated and the pelleted virus was resuspended in 22·5 μl of RT-Detect lysis buffer (DuPont, Wilmington, Delaware) and frozen until assay of extracellular RT activity. For intracellular RT, stored cell pellet (duplicate samples) was resuspended in 50 μl of RT-Detect lysis buffer, subjected to five cycles of freeze/thaw, spun briefly in the microcentrifuge and the clarified extract was collected and frozen at -40°C until assay. For RT assays, frozen samples were thawed and 10 μl used to quantify enzymatic activity using the non-radioactive RT-Detect method of DuPont.

Measurement of CAT Induction in Co-cultivation Assays

In the simultaneous co-cultivation protocol, HL2/3 and HL CD4-CAT cell lines were washed with PBS, and 1×10^6 cells ml^{-1} of each line were mixed thoroughly and incubated in the presence of growth medium supplemented with or without ascorbate at 37°C in 5% CO_2/95% air.

For quantitation of CAT activity, cells were harvested at 16 or 48 h and lyzed in 150 μl of 0·25 M-TrisHCL, pH 7·8, followed by three cycles of freezing and thawing. CAT assays were performed as described by Gorman *et al.* [9]. The percentage of acetylated chloramphenicol was quantitated in a scintillation counter.

For the pre-treatment experiment, HLCD4-CAT cells were treated with various ascorbate concentration for 16 h, mixed with an equal number of HL2/3 cells and co-cultivated for various periods with daily addition of fresh ascorbate solution. Cultures were analyzed for induced CAT activity as previously described.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA). The differences between the means of treated and control groups were tested for significance using Fisher's least significant differences at $p = < 0{\cdot}05$ (Fisher PLSD). An effect was considered significant when the value ($+$ or $-$) of mean difference between groups exceeded Fisher PLSD in the one-factor ANOVA test.

RESULTS

Ascorbate Effect on Yield of Infectious Virus Released from Chronically Infected HXB Cells

Previous studies from our laboratory [5, 6] demonstrated that growth of chronically

TABLE 1. Effect of ascorbate on yield of infectious virus produced in HXB cells

Ascorbate conc. (μg ml^{-1})	CAT activity[a] (cpm)	CAT activity (% of control)
0	1829	100
100	1553	84.9
150	587	32.1

[a]expressed as cpm per 200 λ cell extract; data are mean of three replicate samples

HIV-infected HXB cells in the presence of non-toxic ascorbate concentrations resulted in significant reduction of extracellular HIV RT activity and p24 antigen level in the culture supernatant. To determine the influence of ascorbate on the infectivity of HIV particles released from chronically infected cells, we used the HLCD4-CAT indicator cell line to assay the level of infectious virus. This line contains an integrated copy of a reporter gene (CAT) linked to HIV promoter–enhancer sequences contained in its LTR. Reporter gene becomes activated upon expression of the viral transactivating protein, *tat* [4]. Quantitative induction of the CAT reporter protein is used as a measure of infectious virus [4]. For infectivity analysis, HXB cells were grown for 4 days in the presence and absence of supplemented ascorbate, the culture supernatants were collected and assayed for virus yield on HLCD4-CAT. The data, presented in Table 1, show that the yield of infectious HIV released from cells treated with 150 μg ml^{-1} ascorbate was reduced by 68% relative to untreated control, indicating that the inhibitory effect of ascorbate in chronically infected cells is associated with a decline in viral infectivity.

AZT Effects on Virus Production in Chronically Infected Cells

To determine the influence of AZT on constitutive HIV production in HXB cells, cultures were grown continuously in the presence of non-cytotoxic AZT concentrations (0·5–2 μg ml^{-1}) for a period of 6 days. Results for extracellular RT activity detected on days 4 and 6 following AZT treatment are summarized in Fig. 1 and those for extracellular p24 antigen level observed on days 2, 4 and 6 post-treatment are presented in Fig. 2.

Statistical analysis using the ANOVA test showed that the pair-wise comparison for RT activity is not significant for 0·5, 1·0 and 2·0 μg ml^{-1} AZT versus respective control in Fig. 1. The mean difference between treated and control pairs on day 4 ranged from $-0{\cdot}005$ to 0·148 (Fisher PLSD = 0·344) and that on day 6 ranged from 0·271 to $-0{\cdot}438$ (Fisher PLSD = 0·496). Pair-wise comparison for p24 antigen in Fig. 2 is also not significant for each AZT concentration tested versus respective control on days 2 and 4 and for 0·5 and 1·0 μg ml^{-1} AZT versus control on day 6 (mean difference range: $-1{\cdot}159$E-5 to $-8{\cdot}887$E-6 on day 2, 2·593E-5 to $-1{\cdot}894$E-5 on day 4 and $-3{\cdot}950$E-5 to 3·003E-5 on day 6; Fisher PLSD = 1·639E-5 on day 2, 4·481E-5 on day 4 and 3·760E-5 on day 6). The pair-wise comparison between 2·0 μg ml^{-1} AZT and control for p24 antigen in Fig. 2 was found to be significant on day 6. However, unlike the inhibitory effect on p24 antigen production seen with ascorbate [5], AZT at 2·0 μg ml^{-1} on day 6 exerted a stimulatory effect on HIV production.

Ascorbate and AZT Effects on Virus Activation in Latently Infected Cells

Unlike HXB cells that produce virus constitutively, ACH-2 cells express no or low levels of virus. However, ACH-2 can be induced by phorbol ester (PMA) or cytokines (e.g. TNF alpha or beta) to make substantial levels of virus. To evaluate ascorbate influence on virus

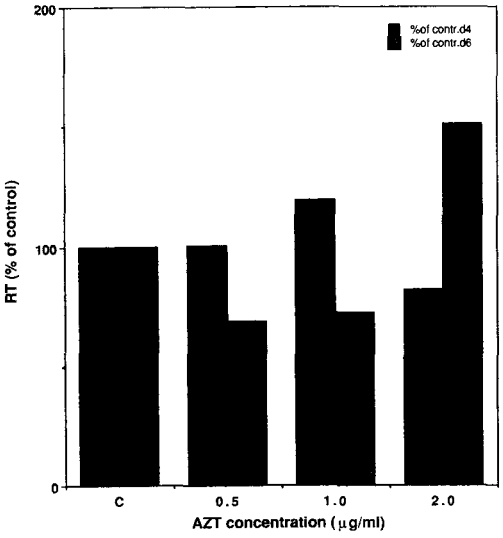

FIG. 1. AZT effects on RT production in chronically infected HXB cells. Shown is extracellular RT activity in the supernatant of AZT-treated cultures plotted as a percentage of that in respective untreated control on day 4 (contr. d4) and day 6 (contr. d6) following initiation of treatment.

activation, ACH-2 cells were pre-treated overnight with non-toxic concentrations of ascorbate (100–300 μg ml^{-1}) or AZT (1–5 μg ml^{-1}), exposed to PMA, and grown continuously in the presence of vitamin or drug for another 24 h. Extracellular HIV RT activity was determined and compared to that in control cultures. Data are presented in Fig. 3. Treatment of ACH-2 with PMA resulted in ~ 10-fold induction of virus. Pre-treatment with

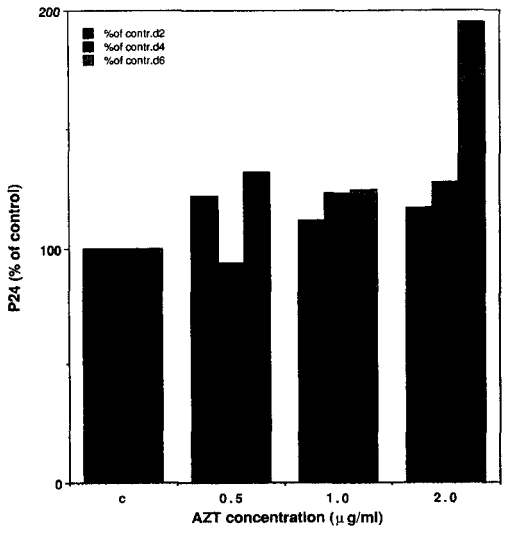

FIG. 2. AZT effects on P24 antigen production in HXB cells. Shown is the extracellular p24 antigen level in supernatant of AZT-treated cultures, plotted as a percentage of that in respective untreated control on day 2 (contr. d2), day 4 (contr. d4) and day 6 (contr. d6) following initiation of treatment.

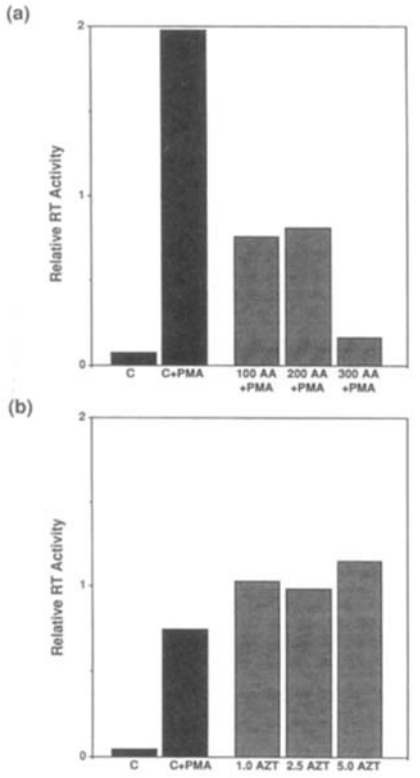

FIG. 3. Ascorbate and AZT effects on extracellular RT released from PMA-stimulated ACH-2 cells. (a) Relative RT activity in untreated control (C), PMA-stimulated control (C + PMA) and ascorbate-treated PMA-stimulated cultures (AA + PMA) at 24 h following PMA treatment. (b) Relative RT activity in untreated control (C), PMA-stimulated control (C + PMA) and AZT-treated PMA-stimulated cultures (AZT + PMA) at 24 h following PMA treatment. Ascorbate and AZT concentrations in (a) and (b) are in $\mu g\ ml^{-1}$.

ascorbate caused a dose-dependent decline in the level of RT released into the culture supernatant (Fig 3(a), significant at 95% by ANOVA for all ascorbate concentrations; mean difference range, 1·216 to 1·81; Fisher PLSD = 0·405). Pre-treatment with 1·0, 2·5 and 5·0 $\mu g\ ml^{-1}$ AZT had no significant inhibitory effect on virus activation (Fig. 3(b), mean difference range, $-0{\cdot}2865$ to $-0{\cdot}3412$; Fisher PLSD = 0·6417).

Effects of Ascorbate on HIV-induced Transcriptional Activation

To obtain insight into the mechanism of ascorbate inhibition of HIV production, we tested whether ascorbate affected transcriptional activation mediated by viral transactivating factors. Towards this end, we utilized mixed cultures of two cell lines: (i) HL2/3, which expresses the viral envelope gp120 glycoprotein on the cell surface and the viral transactivator protein *tat* in the nucleus and (ii) HLCD4-CAT, which expresses the CD4 receptor and contains an inducible CAT reporter gene linked to the HIV LTR. The viral LTR contains promoter/regulatory sequences for directing expression of linked genes. Co-cultivation of the two cell lines results in cell fusion due to CD4-gp120 interaction and leads to the induction of the CAT reporter gene caused by the HIV *tat* gene product (transferred from HL2/3 cells) acting upon the viral LTR (present in HLCD4-CAT cells). In this

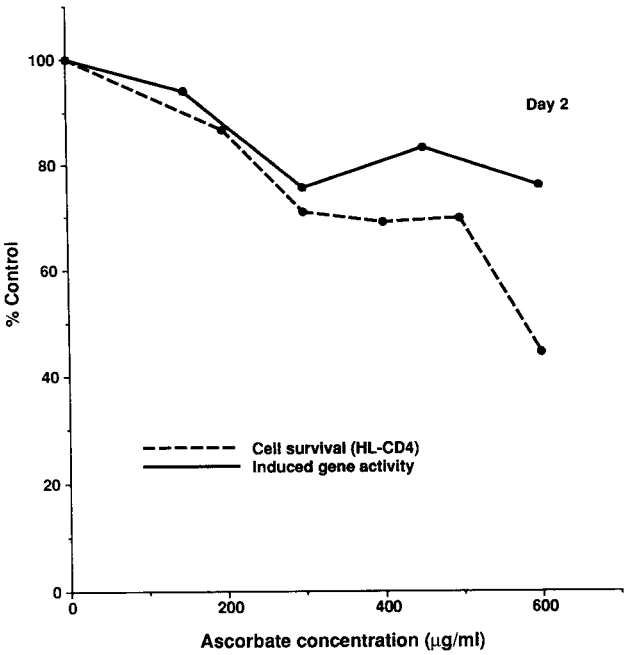

FIG. 4. Ascorbate effect on transcriptional activation of CAT reporter gene in mixed cultures of HL2/3 and HLCD4-CAT cell lines. Shown are cell survival in ascorbate-treated HLCD4-CAT culture and CAT enzymatic activity in ascorbate-treated co-cultures as a percentage of corresponding values in untreated controls prior to normalization of values to a fixed number of viable cells.

co-cultivation assay, the induction of the CAT gene is used as a measure of the effect of an anti-viral substance on transcriptional events in the HIV life cycle involving the action of the *tat* gene [4].

We examined the effect of a wide range of ascorbate concentrations on the expression of CAT activity following co-cultivation of the above cell lines in the presence of appropriate controls. Two types of experiments were done. First, HLCD4-CAT cells were pre-treated with various doses of ascorbate for 6 h and then co-cultivated with HL2/3 cells for 24–48 h, followed by assay of CAT activity. In the second experiment, ascorbate was added to cultures simultaneously at the time of co-cultivation and again at intervals of 24 h, followed by measurements of CAT activity at 24–48 h after cell mixing. Data from the simultaneous addition experiment are shown in Fig. 4. Analysis of survival of HLCD4-CAT cells by ANOVA showed that ascorbate concentrations at 200 $\mu g\ ml^{-1}$ and above produced dose-dependent reduction of cell growth (mean difference range, $4{\cdot}43 \times 10^4$ to $1{\cdot}81 \times 10^5$; Fisher PLSD = $3{\cdot}26 \times 10^4$). In contrast, when normalized to 1×10^4 viable cells, the ascorbate concentrations tested did not exert an inhibitory effect on induction of CAT activity (mean difference between control and 300 $\mu g\ ml^{-1}$, $-112{\cdot}66$; Fisher PLSD = 159·64), showing some stimulation of CAT relative to control at 600 $\mu g\ ml^{-1}$ (mean difference from control, -1162; Fisher PLSD = 159·64). Results from the ascorbate pre-treatment experiment also showed no significant inhibition of induced CAT activity (data not shown), indicating that ascorbate did not compete with the viral envelope glycoprotein for binding to the cell surface receptor. Both experiments rule out the inhibitory effect of ascorbate on *tat*-induced transcriptional activation of HIV LTR.

DISCUSSION

Experimental evidence presented in this paper has shown that ascorbate can reduce the yield of infectious virus constitutively produced in chronically HIV-infected cells as well as suppress the extracellular level of newly induced virus in a PMA-stimulated latently infected cell line. In contrast, AZT which primarily blocks *de novo* infection of freshly infected cells did not inhibit constitutive virus production nor suppress activation of latent virus in chronically infected cells. Because integrated HIV provirus in chronic or latent infection represents a large pool of viral burden in HIV-seropositive persons, results reported here have implications for therapy.

Preliminary reports from several clinical practitioners who have monitored AIDS patients taking large doses of ascorbate have consistently indicated observations of improved clinical benefit including amelioration of symptoms associated with opportunistic infections and Kaposi's sarcoma [10–12]. Furthermore, a recent prospective study in HIV-seropositive men showed that the daily use of a multi-vitamin supplement at baseline significantly correlated with delayed onset of AIDS compared to non-supplemented controls [13]. In that study, the reduction in hazard of developing AIDS approached significance for vitamin C. The ability of vitamin C to inhibit HIV replication in acutely, chronically and latently infected cells is consistent with these observations.

Currently prescribed drugs such as AZT confer only limited benefit in treatment of advanced disease [1]. Furthermore, a large clinical trial of early intervention in asymptomatic HIV-infected persons showed that AZT conferred some benefit in the first year but did not alter rate of disease progression or survival time over a longer monitoring period (M. Seligmann *et al.*, Ninth International AIDS Conference, Berlin, 6–11 June 1993). The small benefit from AZT treatment could be related to its limited effects on HIV expression in infected cells. Thus, whereas AZT can effectively inhibit viral replication in acutely infected cells, it was not found to affect HIV production in cytokine-stimulated latently infected cells [14]. Our data on the lack of AZT effect on PMA-induced HIV activation in the latently infected ACH-2 cell line (Fig. 3(b)) are in agreement with the independent findings of Poli *et al.* [14]. Other data presented in this study (Figs 1 and 2) have additionally shown that AZT is ineffective in suppressing constitutive production of virus in unstimulated chronically infected cells.

In marked contrast to AZT, ascorbate suppressed HIV production in both unstimulated chronically infected cells and in a PMA-stimulated latently infected cell line. The precise mechanism of ascorbate inhibition of HIV in infected cells has not been elucidated. Previous observations with unstimulated HXB cells showed that the level and size of viral RNA and proteins synthesized in ascorbate-treated cultures were not significantly different from corresponding patterns in untreated cultures [15, 16]. In the same investigation, enzyme assays on cell extracts from an unstimulated reporter cell line revealed a dose-dependent reduction by ascorbate in the activity of the reporter protein product expressed from HIV LTR [16]. These results indicated an inhibitory effect of ascorbate on HIV-specific protein activity in unstimulated cells.

Results from unstimulated cell lines tested in the present study are consistent with our previous findings. Thus, in experiments utilizing mixed cultures of unstimulated HL2/3 and HLCD4-CAT reporter cell lines (Fig. 4), ascorbate did not block the interaction of HIV gp120 envelope protein with CD4 cell surface receptor nor did it block transactivation of the HIV LTR by the viral transactivator protein *tat*. In a more recent experiment, unstimulated ACH-2 cells showed approximately five-fold lower basal levels of intracellular HIV RT activity following ascorbate treatment (data not shown). Taken together, these data support an inhibitory effect targeted at viral protein activity in unstimulated cells.

It is not presently known whether the inhibitory effect of ascorbate in stimulated cells is targeted at the same HIV components as those in unstimulated cells. Analysis of the intracellular factors that drive HIV expression following PMA or cytokine stimulation will

be necessary to demonstrate whether differences exist in components targeted by ascorbate in stimulated versus unstimulated cells.

It is worthy of note that other agents, such as NAC, shown to suppress HIV expression in PMA-stimulated cells [2], were found to inhibit HIV production only mildly in unstimulated HXB cells [3, 6]. A synergistic inhibitory effect of ascorbate and thiols was demonstrated in cultures of chronically infected cells [6]. The demonstration that ascorbate, unlike AZT and NAC, can efficiently suppress HIV replication in both unstimulated and stimulated cells provides a rationale for developing combined therapy protocols for testing it in conjunction with other agents with different mechanisms of action.

ACKNOWLEDGEMENTS

We are grateful to Dan McWeeney and John Shou for assistance with cell cultures/biochemical assays and Dr Shrirang Netke for help with statistical analysis. We also thank the AIDS Research and Reference Reagent Program and Drs Pavlakis and Felber, Division of AIDS, NIAID-NIH for providing the HeLa-derived reporter cell lines utilized in this study.

REFERENCES

[1] Fauci AS. Multifactorial nature of human immunodeficiency virus disease: implications for therapy. Science 1993; 2: 1011–18.
[2] Staal FJT, Ela S, Roederer M, Anderson MT, Herzenberg LA, Herzenberg LA. Glutathione deficiency and human immunodeficiency virus infection. Lancet 1992; 339: 909–12.
[3] Jariwalla RJ, Harakeh S. Ascorbic acid and AIDS: strategic functions and therapeutic possibilities. In: Watson, R, ed. Nutrition and AIDS. Boca Raton, Florida: CRC Press, 1994, Chapter 9, 117–39.
[4] Ciminale V, Felber B, Campbell M, Pavlakis G. A bioassay for HIV-1 based on Env-CD4 interaction. AIDS Res and Human Retroviruses 1990; 6: 1281–7.
[5] Harakeh S, Jariwalla RJ, Pauling L. Suppression of human immunodeficiency virus by ascorbate in chronically and acutely infected cells. Proc Natl Acad Sci USA 1990; 87: 7245–9.
[6] Harakeh S, Jariwalla RJ. Comparative study of the anti-HIV activities of ascorbate and thiol-containing reducing agents in chronically HIV-infected cells. Am J Clin Nutr 1991; 54: 1231–5S.
[7] Roederer M, Staal FJ, Raju PA, Ela SW, Herzenberg LA, Herzenber LA. Cytokine-stimulated human immunodeficiency virus replication is inhibited by N-acetyl-L-cysteine. Proc Natl Acad Natl USA 1990; 87: 4884–8.
[8] Kalebic T, Kinter A, Guide P, Anderson ME, Fauci AS. Suppression of HIV expression in chronically infected monocytic cells by glutathione ester, and N-acetyl cysteine. Proc Natl Acad Sci USA 1991; 88: 986–90.
[9] Gorman CM, Moffat LF, Howard BH. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol Cell Biol 1992; 2: 1044–51.
[10] Cathcart RF. Vitamin C in the treatment of AIDS. Medical Hypotheses 1984; 14: 423–33. (Reprinted in O'Connor T. Living With AIDS: Reaching Out. Corwin Publishers, 1987.)
[11] Brighthope IE. In: AIDS: remissions using nutrient therapies and megadose intravenous ascorbate. International Clinical Nutrition Review, April 1977, pp. 53–75; referred to in his book, The AIDS Fighters. Keats Publishing, Inc., 1988.
[12] Priestley J. Nutrient replacement therapy enhances survival and laboratory parameters of HIV positive patients. Abstract 2: B209, VIII Int. Conference on AIDS, July 1992, Amsterdam.
[13] Abrams B, Duncan D, Hertz-Picciotto I. A prospective study of dietary intake and acquired immune deficiency syndrome in HIV-seropositive homosexual men. J AIDS 1993; 6: 949–58.
[14] Poli G, Orenstein JM, Kinter A, Folks TM, Fauci AS. Interferon-alpha but not AZT suppresses HIV expression in chronically infected cell lines. Science 1989; 244: 575–7.
[15] Jariwalla RJ, Harakeh S. HIV suppression by ascorbate and its enhancement by a glutathione precursor. Abstract 2B207, Eighth Int. Conf. on AIDS, July 1992, Amsterdam.
[16] Harakeh S, Jariwalla RJ. Mechanistic analysis of ascorbate inhibition of human immunodeficiency virus. Chemico-Biological Interactions 1994; 91: 207–15.