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BIOLOGICAL SIGNIFICANCE OF ASCORBIC ACID ON HIV-1 LIFE CYCLE

Takuma Hayashi[1,2] and Richard A. Young[3]

[1]Associate Professor, Dept. of Immunology and Infectious Disease, Shinshu University Graduate School of Medicine, Matsumoto, Nagano, Japan, [2]Business using Advanced Technology, Japan Science and Technology Agency (JST), Chiyoda, Tokyo, Japan, [3]Whitehead Institute for Biomedical Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA.

ABSTRACT

To elucidate the inhibition mechanism of human immunodeficiency virus type 1 (HIV-1) life cycle by ascorbic acid/vitamin c, we have investigated and compared the effect of noncytoxic concentrations of ascorbic acid on provirus HIV-1 replication. Using trans-activator of transcription factor (tat) expressing cells or non-expressing cells transfected reporter plasmid, which is constructed with HIV-1-long terminal repeat (LTR) and chloramphenicol acetyl transferase (CAT), we examined the action of ascorbic acid on tat dependent transcriptional activation of *HIV-1* gene through enhancer/promoter of HIV-1-LTR. In that expressing cells, ascorbic acid strongly reduced the levels of intracellular CAT activity in a dose dependent manner (5 to 100 μg/ml). Alternatively in non tat-expressing cells, CAT activity was reduced somewhat. Using other *in vivo* and *in vitro* experiments, ascorbic acid inhibited the activity of tat dependent *HIV-1* RNA elongation, but did not inhibit activity of basal transcriptional activation of *HIV-1* gene. The intracellular HIV-1 genome RNA patterns in ascorbic acid treated cells infected with *HIV-1* showed significant differences in the synthesis and the processing of individual *HIV-1* viral genome RNAs compared to the patterns of untreated controls. Tat dependent *HIV-1* transcription was specifically reduced, because in contrast to *HIV-1* transcription, transcriptional activities through adenovirus major late promoter, Rous sarcoma virus promoter or SV40 promoter were not reduced by treatment of ascorbic acid. Furthermore, the activation of transcription factors was not affected by treatment of ascorbic acid. These results show that ascorbic acid specifically inhibits the replication of HIV-1 on down-regulation of tat dependent *HIV-1* genome RNA elongation.

Key words: Human Immunodeficiency Virus Type 1 (HIV-1), RNA and DNA viruses.

INTRODUCTION

Ascorbic acid/Vitamin C is defined as the generic term for all compounds exhibiting the biological activity of L-Ascorbic Acid. Ascorbic acid is the most important vitamin in fruits and vegetables. It is the major water soluble anti oxidant within the body. Ascorbic Acid is the principal biologically active form but L-dehydroascorbic acid, an oxidation product also shows biological activity. Ascorbic acid is required for the prevention of scurvy and maintenance of healthy skin, gums and blood vessels. It functions in collagen formation, absorption of inorganic iron, reduction of plasma cholesterol level, enhancement of the immune system and reaction with singlet oxygen and other free radicals. As an antioxidant, it also reduces the risk of atherosclerosis and some forms of cancer.

Previous reports demonstrated the anti-viral activity of ascorbic acid against a broad spectrum of RNA and DNA viruses including polio virus, herpes virus, human immunodeficiency virus type 1 (HIV-1) *in vivo* and *in vitro* [1-3]. Already it has reported that the suppression of virus production and cell fusion in HIV-1 infected T-lymphocyte cell lines grown in the presence of non toxic concentration of ascorbic acid [4, 5]. Among the earliest studies on viral replication, it is reported that the growth of HIV-1, after the first replication cycle, was suppressed by the addition of ascorbic acid, glutathione-SH (GSH), N-acetyl L-cysteine (NAC), butylated hydroxyanisole (BHA) or α-tocopherol/vitamin E to human diploid-cell culture [4,5].

Corresponding Author :- **Takuma Hayashi** Email:- yoyoyo224@hotmail.com

Asian J. Pharm. Res. Vol 5, Issue 3, 195-198, 2015.

There is increasing evidence that reactive oxygen intermediates (ROIs) play an important role in cellular processes such as signal transduction and the controlling gene expression. As actions of GSH and NAC such as thiol-containing antioxidants on the replication of HIV-1 is previously reported, GSH and NAC reduce the target DNA binding activities of nuclear factor κ-B (NF- κ B), activator protein 1 **(**AP1), specificity protein 1 (SP1) or upstream stimulatory factor (USF), by redox regulation system [6-8]. These antioxidants such as GSH, NAC, BHA, and vitamin E reportedly play such as radical scavenger in the cytsole of cells stimulated by TNF-α or H2O2, and then the induction of NF- κB activity by these stimuli is blocked [5]. The suppression of the HIV-1 replication by GSH or NAC is caused by the inactivation of these transcriptional factors by redox regulation system [7, 8]. Ascorbic acid may be considered to play as antioxidant free radical scavenger such like GSH or NAC, thus it is possible to regulate the NF- κB DNA binding activity by ascorbic acid [9, 10]. However, the previous report shows that the life cycle of HIV-1 is suppressed by treatment of 100 μg of ascorbic acid per ml (0.57 mM), which is more low concentration than NAC as 30 mM (4.9 μg/ml) [5, 9]. Viability of control and cultures with ascorbic acid were determined using the trypan blue exclusion test. No toxicity of ascorbic acid was observed in result, when cultures were grown in presence of ascorbic acid at 20-100 μg/ml (Fig. 1A). Already it is reported that cytotoxic concentration of ascorbic acid (TCID50) is 1.06 mg/ml (6mM). Furthermore, it was not established whether ascorbic acid exerted a virus-specific effect or interacted directly with the activating substances.

We have investigated the biological function of ascorbic acid on HIV-1 life cycle under the controlled conditions *in vivo* and *in vitro*. Here, we report the biological significance of ascorbic acid on trans-activator of transcription factor (tat) dependent transcription activity through enhancer/promoter of HIV-1-long terminal repeat (LTR) using *in vitro* and *in vivo* experiments. In this report, we demonstrate that ascorbic acid specifically inhibits tat dependent HIV-1 genome RNA elongation system in HIV-1 infected cells.

Resistance to antiretroviral drug and HIV-1 mutants

The HIV is a lentivirus (a subgroup of retrovirus) that causes HIV infection and acquired immunodeficiency syndrome (AIDS). AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV subtype. Treatment consists of highly active antiretroviral therapy (HAART) which slows progression of the disease. As of 2010 more than 6.6 million people were taking them in low and middle income countries. Treatment also includes preventive and active treatment of opportunistic infections. Isolates of HIV-1 and HIV-2 with resistance to antiretroviral drugs arise through genetic mutations, which have been tracked and analyzed. There is currently no cure or effective HIV vaccine. Significant intra- and interindividual variability has been observed in response to use of pharmacological agents in treatment of HIV infection. Treatment of HIV infection is limited by high rates of adverse drug reactions and development of resistance in a significant proportion of patients as a result of suboptimal drug concentrations. The efficacy of antiretroviral therapy is challenged by the emergence of resistant HIV-1 mutants with reduced susceptibility to antiretroviral drugs, development of new antiretroviral drugs is therefore required for preventing the activation and replication of HIV in patients.

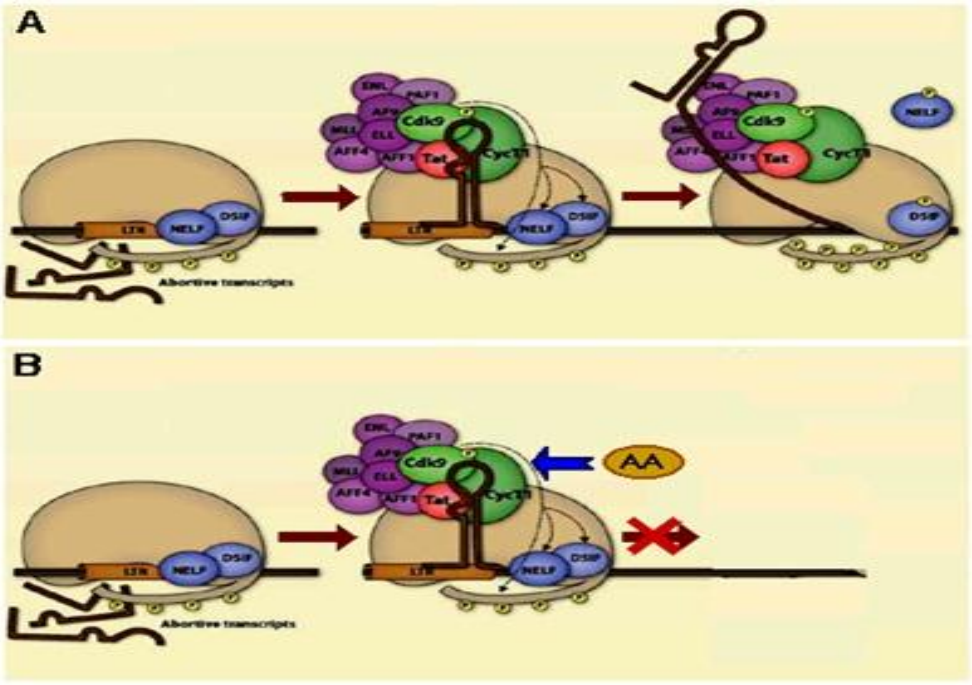
Antiviral effect of ascorbic acid

It was reported by several research groups that continuous exposure of HIV-1-infected cells to non-cytotoxic ascorbic acid concentrations resulted in significant inhibition of both virus replication in chronically HIV-1 infected cells and multinucleated giant-cell formation in acutely HIV-1 infected $CD4^+$cells [4, 5, 10]. However, the molecular mechanism by which ascorbic acid suppresses HIV-1 replication was not fully understood yet. There is increasing evidence that reactive oxygen intermediates (ROIs) play an important role in cellular processes such as signal transduction and the control of gene expression [6, 7]. The suppression of HIV-1 replication is caused by transcriptional factors, NF- κB, AP1 and USF, which be down-regulated by the redox system of antioxidants such as NAC, GSH, and BHA [4, 6, 9]. When ascorbic acid was added together with NAC into culture medium, HIV-1 viral reverse transcriptase (RT) was reduced to 20.0% of the control, compared with values of 30.0% and 50.0% seen, respectively, with ascorbic acid alone and NAC alone, suggesting that there are the different target point between ascorbic acid and NAC [4]. HIV-1 suppression by ascorbic acid was not due to secondary effects resulting from inhibition of cellular growth or metabolic activity. This paper supported that activities of transcriptional factors are not reduced by ascorbic acid treatment [10]. The experimental evidence in this paper has demonstrated that ascorbic acid could inhibit the HIV-1 replication by blocking the regulation on the step of tat dependent HIV-1-RNA elongation system. Ascorbic acid dose not inhibit activities of basal transcriptional factors containing RNA polymerase II and transcriptional factors, NF- κB, SP1, USF for HIV-1, however as shown in *in vitro* and *in vivo* exprements, tat dependent transcriptional activation are strongly reduced by ascorbic acid treatment. Further, an earliest report shows that HIV-LTR-directed β-galactosidase expression in transiently transfected Jurkat cells is not inhibited by ascorbic acid [10]. The *in vivo* experimental evidence presented in this paper has revealed that the inhibition of HIV-1 replication by treatment with ascorbic acid is caused by inhibition of tat dependent RNA elongation, but the basal transcriptional activation through HIV-LTR is not

Asian J. Pharm. Res. Vol 5, Issue 3, 195-198, 2015.

affected by treatment with ascorbic acid. Furthermore, comparison of intracellular HIV-1-RNA patterns in ascorbic acid treated cells with corresponding patterns of untreated controls showed significant differences in the synthesis of viral RNAs. Importantly, the smallest RNAs 2.0 kb were detected in cells treated by 20~100 μg/ml of ascorbic acid, tat protein translated from smallest RNAs possibly was exists in cells, but other length RNAs were not detected by RT-PCR. Thus, the results indicated in *in vitro* expreriments show that tat dependent RNA elongation system was strongly inhibited by ascorbic acid. It is demonstrated in several reports that tat could activate transcriptional activation and RNA elongation after forming initiation complex with cellular cofactors [11-15]. Furthermore the known species tropism of tat protein appears to arise from the fact that not only tat but also the cellular cofactor can markedly influence the RNA sequence specificity of the resultant protein complex [11, 12]. In earlier studies, molecular weight NELF or DSIF proteins expressing in $CD4^{+}$T-cell recognizes the loop structure in trans-activation response (TAR) and forms proteins-TAR complex [11, 12], other cellular proteins CDK9 and Cyclin T which directly interacts tat protein [12, 13], then the activation of transcription and RNA elongation is activated by these proteins-tat/TAR complexes including positive-transcription elongation factor b (P-TEFb) [2]. In other result, Mss1, which strongly expresses in T-lymphocytes, activates with tat the transcription through the promoter/enhancer of HIV-1-LTR, but activation mechanism by Mss1 is not revealed [14]. It was demonstrated by RT-PCR that expression of Mss1 mRNA gene was not suppressed in cells treated by ascorbic acid, expression of other cellular cofactors, AFF4, ELL2 or ENL have not been examined yet. Tat is demonstrated to recognize directory TFIID, TFIIB and transcription factor SP1 and binds and then activates the transcription as mediator between TAR and basal transcriptional factors [16, 17]. There are possible two reasons why HIV-1 gene expression is down regulated by ascorbic acid treatment. First, the expression of these cellular cofactors may be down regulated in cells treated by ascorbic acid. The second, the stereomatic conformation of tat protein may be changed by the treatment of ascorbic acid and be not able to play as the trans-activating mediator (Figure 1.). It is necessary to examine whether the tat activity is down regulated in the cells treated by ascorbic acid or not. Already, ascorbic acid is used for the treatment of AIDS and ascorbic acid at 90 μg/ml was attained in plasma in patients consuming oral ascorbic acid to achieve urinary levels about 1 μg/ml. These findings are consistent with a high bowel tolerance reported for AIDS patients.

Figure 1. Prior to positive-transcription elongation factor b (P-TEFb) recruitment, proviral transcription proceeds inefficiently, resulting in the production of abortive transcripts. (A) RNA Polymerase II processivity is highly increased following Tat-mediated recruitment of P-TEFb to the TAR RNA, where it also associates with the SEC. Cdk9 phosphorylates the negative elongation factors NELF and DSIF, resulting in the dissociation of NELF and the conversion of DSIF into a positive elongation factor, and the Ser2 residues of the C-terminal domain (CTD) of RNA Polymerase II, inducing efficient transcriptional elongation. (B) We are afraid that ascorbic acid (AA) modifies stereomatic conformation of protein-RNA complex with tat and cellular co-factors, resulting in the inability of tat dependent HIV-1 RNA elongation to upregulate the genome HIV-1 RNA-processing system.



Asian J. Pharm. Res. Vol 5, Issue 3, 195-198, 2015.

CONCLUSION

In the test tube, very high concentrations of vitamin C can prevent HIV from infecting new cells and prevent the activation and replication of HIV in dormant infected cells. Vitamin C also dramatically reduced the formation of syncytia, clumps of dysfunctional T-cells which form around an HIV-infected T-cell in the test tube. Syncytia tend to appear more frequently when CD4 cell counts are falling rapidly, and it has been theorised that their appearance may indicate an increased chance that AIDS-related illnesses will develop.

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