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Negative Effect of Ascorbic Acid in Gene Regulation of Provirus Hiv-1

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ABSTRACT

To elucidate the inhibition mechanism of human immunodeficiency virus type 1 (HIV-1) replication by ascorbic acid, 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 HIV-1-long terminal repeat (LTR) chloramphenicol acetyl transferase (CAT) plasmid, we examined the action of ascorbic acid on tat dependent transcriptional activation of HIV-1 gene through enhancer/promoter of HIV-1-LTR. In tat expressing cells, ascorbic acid strongly reduced the levels of intracellular CAT activity in a dose dependent manner (5 to 100 ❍g/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.

KEYWORDS : HIV-1, Tat, Ascorbic acid, RNA elongation

Introduction

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 1,2,3. 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, glutatione (GSH), N-acetyl L-cysteine (NAC), butylated hydroxyanisole (BHA) or α-tocopherol/vitamin E to human diploid-cell culture 4,5. 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-κB), AP1 or USF, by redox regulation system 6,7,8. 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 mg of ascorbic acid per ml (0.57 mM), which is more low concentration than NAC as 30 mM (4.9 mg/ml) 5,9. Furthermore, it was not established whether ascorbic acid exerted a virus-specific effect or interacted directly with the activating substances.

We have investigated the action of ascorbic acid on HIV-1 life cycle under the controlled conditions *in vivo* and *in vitro*. Here, we report the effects of ascorbic acid on 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 trans-activator of transcription factor (tat) dependent HIV-1 genome RNA elongation system in HIV-1 infected cells.

It was reported by several research groups that continuous exposure of HIV-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 NF-κB, AP1 and USF, which be down-regulated by the redox system of antioxidants such as NAC, GSH, and BHA 4,9. When ascorbic acid was added together with NAC into culture medium, extra cellular 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 transcription 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. 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 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 mg/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 11,12,13,14,15. 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 68 kDa or 185 kDa 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 36 kDa or 140 kDa which directly interacts tat protein 12,13, then the activation of transcription and RNA elongation is activated by these proteins-tat/TAR complexes 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, 36 kDa, 86 kDa or 185 kDa 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. 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 mg/ml was attained in plasma in patients consuming oral ascorbic acid to achieve urinary levels about 1 mg/ml. These findings are consistent with a high bowel tolerance reported for AIDS patients.

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