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Nucleoside and Nucleotide Reverse Transcriptase Inhibitors Induce Aging by Inhibiting Telomerase Function

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Abstract

The telomeres existing at the end of the eukaryotic chromosome, play an important role in localization, pairing of homologous chromosomes during cell division and synapsis formation, while telomerase is involved in maintenance of the telomere length. The application of antiHIV-1 molecules particularly NRTIs have been shown to interfere with telomerase function thereby inducing aging processes. Since the application of these molecules has already indicated production of oxidative stress and toxicity in AIDS patients, their adverse impact on telomerase function may further worsen the situation. In addition, the negative influence of antiHIV-1 regimens on certain host factors involved in telomerase function may enhance aging. HAART changes the landscape of the disease by progressively decreasing the progression of HIV-1, but exerts prolonged adverse effects on the telomerase function. Though there is no exact information available on this issue, intensive efforts are needed to explore regulation of telomerase expression in HIV infected individuals and particularly those receiving antiretrovirals.

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Introduction

Human Immunodeficiency Virus Type-1 (HIV-1) belongs to the retroviridae family and to the Lentivirus genus. It is the etiologic agent of the acquired immunodeficiency syndrome (AIDS) that targets the CD4 $^{+}$ T-lymphocyte cells of the human immune system[1]. The number of CD4 $^{+}$ T-lymphocyte cells in healthy males and females is reported to be 1200/ul and 1000/ul, respectively. The rapid destruction of these cells due to HIV-1 infection makes the infected individual immune-compromised and prone to several opportunistic infections. The number of CD4^{+} T-lymphocyte cells <200/µl or lower than this is considered According to an estimate of UNAIDS, there were approximately 33 million people worldwide living with HIV-1 and an emerging newly infected 2.7 million individuals[2]. India inhabits 2.1 million HIV-1 infected people equivalent to the third largest population in the world living with HIV-1. According to the National AIDS Control Organisation (NACO), there are two high risk groups of HIV-1 infected individuals, which include sex workers (SW) and those men who do sex with men (MSM).

The antiretrovirals approved by FDA-USA being prescribed to the doctors to treat the HIV infected individuals are over two dozen in number. They have been categorised in six groups as they act on different specific targets in different manners so as to block a specific stage in the life cycle of HIV-1. Depending on the condition of the disease, the physicians recommend the patients to take a combination of these drugs which is called as highly active antiretroviral therapy (HAART). In order to block the reverse transcription and synthesis of proviral DNA (cDNA), the drugs targeting the activity of HIV-1 reverse transcriptase (HIV-1RT) have been placed into two different groups: (1) competitive nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) which are substrate (dNTP) analogs and bind at the active site of the enzyme include abacabir (ABC), emtricitabine (FTC), lamivudine (3TC), tenofovir disoproxil fumarate (DF, TDF), and Zidovudine (AZT); and (2) the non-competitive reverse transcriptase inhibitors (NNRTIs), which bind at a site in the enzyme other than active site include doravirine (DDR), efavirenz (EFV), etravirine (ETR), nevirapine (NVP), and rilpivirine (RPV)[3].

The activities of viral protease and integrase have also been targeted by drugs to arrest viral progression. The viral protease catalyses the processing/ cleaving of a large polypeptide chain into small pieces for the proper organisation and packaging of virions. The antiHIV-1 protease drugs (PIs) include atazanavir (ATV), darunavir (DRV), fosamprenavir (FPV), ritonavir (RTV), sequinavir (SQV), and tipranavir (TPV). The chemical agents which interfere in the normal biochemical function of the viral integrase, which catalyses the integration of proviral DNA (cDNA) into human genome include dolutegravir (DTG) and raltegravir (RAL) [3]. The inhibitors of these two enzymes, however, have been found not to modulate or regulate the telomerase function.

Other targets in the HIV-1 life cycle have also been exploited to develop antiretroviral (ART) drugs for example the drugs which block docking and fusion of HIV-1 with the CD4 receptors of the T-lymphocytes include enfuvirtide (T-20). The CCR5, a co-receptor responsible for viral fusion at the surface of macrophages, dendrides and glial cells may be inactivated by maraviroc (MVC). In addition, a compound which may stop the post-attachment on the surface of certain immune cells containing CD4 receptors has been approved as ibalizumab-uiyk (IBA). In HARRT, a combination of either of the aforesaid drugs is prescribed to the patients to significantly reduce viremia or to eliminate the viruses from the body[4]. These drugs, however, do not adversely influence the activity of telomerase.

Telomerase, recognised as an anti-aging enzyme has been found to exhibit the potential to reverse the age-related attrition of telomere ends[5]. Telomerase has a catalytic subunit known as TERT telomerase reverse transcriptase (TERT-TRT). This enzyme has varied enzyme functions in different sub-cellular systems i.e. in the nucleus it acts as an RNA dependent DNA polymerase (TERT-TERC); and in mitochondria it acts as an RNA dependent RNA polymerase (RdRP or TERT-RMRP). The TERT alone has the ability to interact with key regulators to protect the cells and their sub-cellular organelles in order to promote their universal sustenance and rejuvenation

capacity. It also interacts with RNA elements, RMPR, tRNA, and TERC. A close observation of TERT structure indicates the presence of highly conserved amino acid sequences similar to those of other viral polymerases and hence it displays potential to catalyse synthesis of cDNA and also double stranded RNA. Hence, similar to HIV-1RT, inhibition of TERT activity takes place by antiHIV1-RT regimen, especially by NRTIs, and viral proteins[5].

It has been observed that one of the domains of TERT possesses a specific structure containing integral RNA (TER) which acts as a viable template for addition of nucleotides. The TERT exhibits the ability to support a specific catalytic function i.e. RNA dependent RNA polymerization (RdRP)[6]. In addition, it also displays template independent terminal transferase activity[7]. These two unique catalytic properties of this enzyme distinguish it from other RTs commonly contained in the retroviruses and other transposons. The RdRP activity associated to TERT is not required for synthesis of telomere and hence unrelated to its main function. Probably, it could be imparting extra-telomeric functions associated to carcinogenesis[6,8]. As a RdRP enzyme, TERT catalyses addition of rNTPs to the 3'OH terminus of the growing chain, which might be due to enough flexibility of the catalytic pocket or substrate binding region of this RT as compared to some other retrotransposon RTs in human genome. TERT's RdRP activity has also been found to be associated with its intracellular trafficking into mitochondria in order to mitigate oxidative stress[9-11] via offering protection of mtDNA against from free radicals mediated oxidative damage[12,13], thereby preventing the mitochondrial dysfunctions. However, the mechanism of interaction of NRTIs with the RdRP function of telomerase is not yet known.

It appears that telomerase modulation acts as a key player in the therapy of HIV-1 infection. The Induced expression of viral proteins and presence of some antiHIV-1 RT drugs promote TERT via its up regulation. The interactions between viral proteins and ARTs with the telomerase may induce immune deficiency in host and enhance level of oxidative stress in mitochondria which are associated with ageing. This mini review article is an endeavour to present the updated and comprehensive information on the modulation of host telomerase activity by NRTIs/NNRTIs and induction of ageing processes there off.

Accelerated Ageing in HIV Infected Patients

Patients with HIV infection are more prone to other deadly diseases than HIV-uninfected individuals. Life expectancy for many patients; particularly those with low CD4+cell counts is still shorter than that for the general population[14]. It has been apparent that people infected with this virus have an elevated risk for certain cancers, most notably Kaposi sarcoma (KS) and non-Hodgkin lymphoma[15]. There is no reason to expect that antiretroviral therapy provides protection from development of malignancies at ages than that of general population. A large part of the ageing phenotype is imbalance between inflammatory and anti-inflammatory networks, which results in the low grade chronic pro-inflammatory status of ageing, "inflamm-ageing"[16]. It is linked to immunosenescence, and on the whole they are the major contributory factors to the increased frequency of morbidity and mortality among elderly people. The study of Chang et al. (1994)[17] discovered gamma Herpesvirus, in AIDS associated Kaposi sarcoma. Kaposi sarcoma is the most common neoplasm occurring in persons with AIDS; approximately 15 to 20% of AIDS patients develop this neoplasm, which only occurs in immune-competent individual[6]. A number of epidemiologic studies have shown elevated risk of lung cancer among HIV-infected individuals. The study of Sansoni et al. (2008)[18] described that loss of immune related to remodelling where some functions were reduced and others remained unchanged. The immune system during ageing is a progressive event and an age-dependent decline of the virgin T-cells (CD95-)[19].

The use of nucleos(t)ide reverse transcriptase inhibitors (NRTI) remains the backbone of many initial highly active antiretroviral therapy (HAART) regimens for the treatment of HIV infection[20]. Standard antiretroviral therapy (ART) is the combination of at least three antiretroviral (ARV) drugs to suppress the virus and stop the progression of HIV-1 mediated disease i.e. AIDS. Since 1996, an effective HIV/AIDS treatment came into practice in the form of highly active antiretroviral therapy (HAART), that can progressively supress the

replication of HIV, partially restore immunity, reduce morbidity, and extend longevity[21]. A huge reduction in the rate of mortality of AIDS patients has been observed, particularly in early stages of the disease[22]. A combination of HIV/AIDS and HAART likely exhibits long -term effects on the mitochondrial genome and thus activity in many deleterious events result from, are triggered or are enhanced by oxidative stress and mitochondrial dysfunction[23]. Active combination of antiretroviral treatment leads to onset of age related diseases associated to the cardiovascular ailments and bone diseases in AIDS patients with the inhibition of telomerase activity, which finally leads to ageing[24,25]. The primary mechanism of HIV-1 infection mediated impact on the activity of telomerase function concerns senescence and ageing culminating into shortening of telomere. The study of von Zglinicki et al.[26] has indicated long-term chronic inflammation and/or oxidative stress which contributes to telomere shortening in monocytes. In addition telomere length has high correlates to ageing. The telomeres might be more vulnerable in old age and then might contribute to the development of Alzeimer's Disease (AD). Telomere's shortening in white blood cells (WBCs) have been shown to alter the immune function[27,28].

Telomerase Activity and NRTI

The adaptive immune response depends on the functions of T and B lymphocytes. T cells can be divided into CD4+ ("helper") and CD8+ (cytotoxic) T cells. The function of CD4+ T cells is to stimulate CD8+ T lymphocytes to kill target cells and B cells to produce antibodies. The function of CD8+ cells is to kill cells infected with intracellular pathogens such as viruses or cells transformed into cancer cells[29]. It has been estimated that subsequent cell division from a naïve cell to millions of effector cells results in telomere shorteneing. The shorter telomere has been observed in memory T cells than the naive CD4+T lymphocytes[30].

Telomeres are responsible for genome stability and protection of eukaryotic chromosomes from shortening or degradation. Telomeres constitute conjugate protein (nucleoprotein) structures located at the ends of chromosomes with short, conserved and highly repetitive sequences (TTAGGG), which prevent end-to-end fusions and other structural and functional cell abnormalities[31]. In humans, telomeres are bound by shelterin, a multimeric-protein complex, comprising TRF1, TRF2, RAP1, TIN2, TPP1 and POT1. It interacts with single stranded and double stranded telomeric DNA[32]. The pattern of shortening of telomeres has been clearly demonstrated. These findings indicate that the telomeric loss in normal hematopoietic cells is rapid within the first year of life (equating to 15 to 30 stem cell divisions) and then continues at a slow decline until 50 to 60 years of age. After which the decline again accelerates[33]. The active form of telomerase, a ribonucleoprotein complex, consists of two principal subunits called as telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC)[34,35]. It is responsible for maintaining the length of the telomere. The enzyme catalyses the addition of the hexanucleotide repeats to the telomeric ends[35].

Leeansyah *et. al.*[36] have reported that the application of NRTIs may inhibit telomerase activity *in vitro* in activated PBMCs and *ex vivo* in PBMCs from HIV-infected patients[37]. Liu et al.[37] have explored that 3′-azido-2′,3′-dideoxynucleosides inhibit telomerase activity thereby causing shortening of telomere length. HAART combinations that include zidovudine (ZDV) and lamivudine (3TC) have been shown to be highly effective in blocking the HIV-1 transmission and replication.[38] However, in foetuses the addition of ZDV may cause telomere shortening via inhibition of telomerase activity[38]. Further, abacavir as one of the most efficacious nucleoside analogues against HIV-1 RT used in treatment of AIDS patients has been shown to inhibit the human telomerase activity[39]. Similarly, other nucleoside triphosphate analogs such as AZT, 3'-deoxy- 2',3'-didehydrothymidine (d4T), and Ara-G have been demonstrated to efficiently inhibit telomerase activity *in vitro*, thereby causing consistent and rapid telomere shortening in growing Tetrahymena[40,41]. Most of the NRTIs (lamivudine, abacavir, zidovudine, emitricitabine and tenofovir) have been shown to exert inhibitory effect on the activity of telomerase. In addition to NRTIs, the heterocyclic isothiazolones have been proposed to significantly interfere with the telomerase activity by modifying a cysteine residue (s) in or near the reverse transcriptase active site of telomerase[42,43]. The NNRTIs (Lamivudine with zidovudine or stavudine and either of these molecules such as nevirapine or

efavirenz zidovudine (AZT), didanosine (ddI), and abacavir (ABC) have been shown to inhibit the catalytic activity of telomerase, which in turn leads to senescence and cell death thereby causing ageing. Application of NNRTIs has already been shown to induce oxidative stress and generate toxicity in AIDS patients[44-,46]. Therefore, a combination of NNRTIs associated oxidative stress and toxicity with that of inhibition in telomerase function may further enhance ageing processes. It is quite possible that NRTIs would also be adversely influencing certain host factors responsible for expression of genes encoding telomerase thereby reflecting into significant reduction in enzyme activity. Reverse transcriptase inhibitors (RTIs) inhibit transcription of viral RNA into proviral DNA (cDNA). Mitochondrial toxicity due to use of NRTIs in HIV-infected adults has been reported in most patients taking zidovudine, lamivudine, stavudine, and didanosine[47]. However, exact mechanism of action is not yet known to delineate the interaction of anti RT molecules with telomerase enzyme structure. It is therefore required to carry out extensive research to properly understand the regulation of telomerase expression in AIDS patients receiving NRTIs/NNRTIs treatment and induction of ageing processes. This information may help understand to develop strategies to combat NRTIs/NNRTIs mediated modulation of host telomerase function and enhanced ageing events.

Conclusion

The catalytic function of telomerase has been recognised as specialized enzyme activity similar to the reverse transcriptase, which catalyses the *de novo* replication of telomeric DNA repeats. This enzyme also exhibits the activities of terminal transferase, and RNA-dependent RNA polymerase, which differentiates the characteristics of telomerase from other RTs. It reflects on to the susceptibility of human cellular telomerase towards NRTs. However, the NNRTIs have been observed not to modulate the telomerase function unlike to HIV-1RT[48]. However, the exact mechanism of action of NRTIs on human telomerase function and consequent event of induction of ageing in HIV-1 infected individuals is not yet clearly known. Also, the inhibition of RdRP function of this RT by NRTIs is not well understood. These findings indicate that there is urgent need of extensive investigations towards evaluation of the impact of NRTIs on the off-targets in the patients receiving HAART for longer duration. The information would also be useful to develop strategies in order to overcome NRTIs mediated ageing in these patients.

List of Abbreviations

NRTIs: Nucleosidereverse - transcriptase inhibitors

NNRTIs: Non-nucleoside reverse-transcriptase inhibitors

HIV: Human Immunodeficiency Virus

AIDS: Acquired Immune Deficiency Syndrome

KS: Kaposi sarcoma

HAART: Highly Active Antiretroviral therapy

TERT: Telomerase Reverse Transcriptase

TERC: Telomerase RNA Component

RTIs: Reverse-transcriptase inhibitors

Ethics Approval and Consent to Participate

Not Applicable

Consent for Publication

All authors have consented for publication

Availability of Data and Materials

The data collected and anaysed by all the authors

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Competing Interests

Authors declare no competing interest

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