

HAART BLOCKS THE ACCELERATED EROSION OF TELOMERES INDUCED BY HIV INFECTION

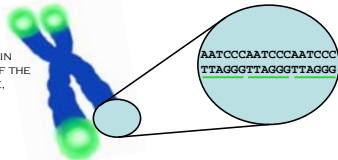
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ABSTRACT

Background: HIV infection induces rapid lymphocyte turnover and the accelerated loss of telomere sequence that is believed to contribute to AIDS associated immunosenescence. In this study we asked if HAART, by reducing HIV viral load and restoring T cell counts, also arrested accelerated telomere erosion and if telomere length was restored in T cells repopulating the circulation. **Design:** Blood samples from 16 HIV patients that were previously characterized for telomere length in 1995 and subsequently treated with HAART, were collected and telomere lengths of peripheral blood mononuclear cells (PBMCs) were determined and compared in parallel, pre- and post-HAART. **Methods:** Archived DNA samples from our previous study and PBMCs of the same individuals ten years after beginning HAART were purified, and telomere length was measured in the same gels by terminal restriction fragment (TRF) analysis. **Results:** Treatment with HAART blocked the accelerated telomere erosion normally seen in PBMCs during HIV infection ($p < 0.0001$). Patients on HAART showed an average loss of 65 bp/year compared to the ~52 bp/year loss seen in age matched populations and the >180 bp/yr seen upon HIV infection in the absence of HAART. **Conclusions:** HAART blocks the accelerated telomere erosion seen in the circulating component of the immune system during HIV infection. However, it does not restore telomere sequences previously lost, indicating that repopulation of PBMCs from progenitor cells or activation of telomerase does not restore replication potential. Preservation of telomere length should be considered as a potential advantage favoring earlier HAART use.

INTRODUCTION

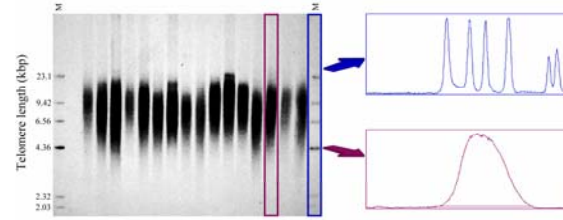
- Telomeres are structures located at eukaryotic chromosome ends that are critical to chromosome integrity and protection.
- As telomere length gradually shortens, so does the apparent residual replicative potential of the cell.
- The strong relationship between subject age and telomere length allows estimates derived from measurements of telomere length to be used as a cellular "mitotic clock".¹
- Recent correlative evidence has linked telomere length to the probabilities of mortality from infectious and cardiovascular disease and as a consequence to lifespan.³
- A variety of disease states and disease treatment regimens increase telomere erosion both *in vivo* and *in vitro*, which may lead to the eventual compromise of immune function.



- Human immunodeficiency virus (HIV) type 1 infection results in a sustained high level of viral replication matched by a strong immune response characterized by the accelerated turnover of T lymphocytes.
- Analyses of HIV-infected individuals have consistently shown accelerated telomere shortening in PBMCs compared to uninfected age-matched controls (5-10 fold acceleration)²
- In HIV-infected individuals, differences in attrition rates were present between subpopulations of cells, with CD8+ cells shortening at a rate three times that of CD4+ cells, although both populations showed an accelerated loss.²
- HAART suppresses HIV replication, lowers plasma HIV RNA to undetectable levels and leads to a significant recovery of CD4+ cell counts.
- The majority of work in the area is cross-sectional in nature. Using a longitudinal study design, we followed telomere length in PBMCs from 16 HIV patients receiving HAART over 10+ years

METHODS

Genomic DNA isolated from PBMCs is digested with restriction enzymes, electrophoresed and hybridized to radiolabelled telomere specific probe. Analysis of the resulting autoradiogram allows for the mean terminal restriction fragment (mTRF) length to be determined by comparing the weighted center of mass for each sample to a known molecular weight marker.



RESULTS

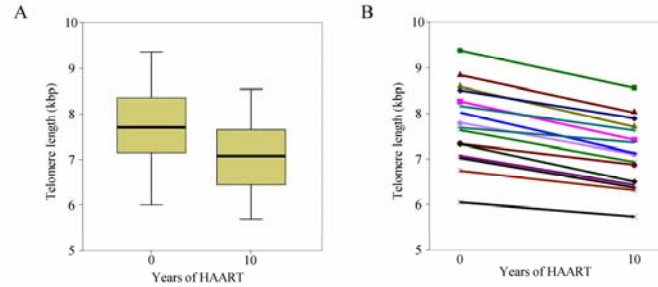


Figure 1: Telomere length changes in HAART-treated HIV patients. A) Box-and-whisker plots for pre- and post-HAART individuals. Plots represent median TRF (center line), upper and lower quartiles (top and bottom of box), and extreme values in data set (whiskers). B) Each individual line represents the rate of mTRF loss of a single patient in the study.

ID #	Age (yrs) & Gender	Baseline		10 years of HAART		Δ mTRF (kbp)
		CD4+ count (cells/μl)	Pre-HAART mTRF (kbp)	CD4+ count (cells/μl)	Post-HAART mTRF (kbp)	
1	28 F	225	8.86	400	8.05	0.81
2	29 M	4	7.03	640	6.37	0.66
3	51 F	378	7.33	400	6.87	0.46
4	36 M	343	8.16	160	7.62	0.54
5	35 M	98	6.75	430	6.31	0.44
6	30 F	328	7.32	445	6.5	0.82
7	42 M	379	7.06	275	6.46	0.6
8	50 M	574	7.64	640	6.93	0.71
9	32 M	342	8.01	350	7.12	0.89
10	35 M	634	8.62	1425	7.73	0.89
11	28 M	239	9.38	720	8.56	0.82
12	27 M	721	8.51	510	7.89	0.62
13	32 M	59	7.78	620	7.1	0.68
14	42 M	692	8.26	830	7.41	0.85
15	43 M	168	6.05	175	5.74	0.31
16	37 F	338	7.69	570	7.37	0.32
Mean	36.1	345.1	7.78	536.9	7.13	0.65

Table 1: Patient demographics and telomere length measurements in HIV-infected individuals. Baseline pre-HAART information from 1995 includes age, gender, CD4+ count and pre-mTRF. Ten year HAART information includes CD4+ count & post-mTRF.

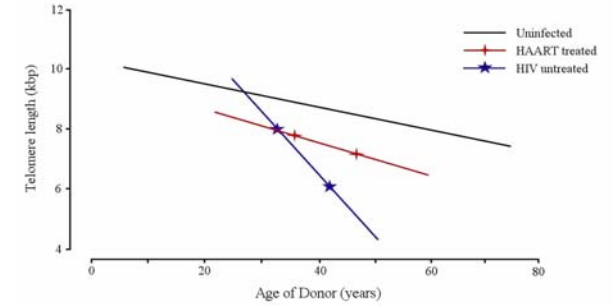


Figure 2: Differential rate of telomere loss in untreated HIV individuals and HAART treated patients. Plots were generated from the linear regression of mTRF vs age in uninfected controls, untreated HIV patients and HIV patients undergoing HAART treatment.

CONCLUSIONS

- HAART nearly completely blocked the HIV-associated accelerated telomere loss in PBMCs.
- Factors such as patient age, gender and initial viral load did not correlate to mTRF loss.
- Consistent changes in telomere length during HAART was seen regardless of patient starting telomere length or the stage of disease when treatment was initiated.
- While it might be argued that the decreased losses seen simply represent an increased level of longer telomere containing CD4+ cells in the samples, our data does not support this. Some patients showed a loss of CD4+ counts after treatment, while others showed large increases. If the decrease were simply due to the increases proportion of CD4+ cells in the sample, we would expect the group with large increases to have the smaller Δ mTRF. This correlation is not seen.
- Our long term longitudinal study indicates that the shorter-term responses to HAART seen in other studies, are unable to stably restore telomere sequences once lost, indicating that repopulation of PBMCs from progenitor cells or activation of telomerase does not restore replication potential. Despite the fact that clinical correlations at present remain unclear, our results argue the case for earlier intervention with antiretroviral therapy in managing HIV infection.

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