Fitting analysis provides further evidence for eradication of HIV/AIDS infection under combined liposome drug delivery treatment

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It is now evident that the commonly accepted strategy for treatment of HIV/AIDS by highly active antiretroviral therapy (HAART) will not lead to eradication of HIV in a reasonable time. This can be seen from the typical exponential viral load decay upon treatment, which reveals initial considerable but incomplete reduction of plasma HIV RNA with subsequent low level HIV persistence even in patients on effective anti-retroviral therapy. Here we show that the viral load follows a simple zero trend linear regression line under different treatment approaches recently proposed by us. This indicates a whole body HIV eradication might be achieved in a reasonable time.

Introduction

We recently proposed and evaluated a new approach for treatment of HIV/AIDS[1]. In contrast to HAART, it is based on simultaneous destroying the virus (both in the blood stream and its reservoirs) and rendering the non-infected target cells refractory to HIV attack. The latter was accomplished by blocking the cell's polyphosphoinositide transmembrane signaling system and, in turn, the generated second messengers, calcium release and the triggering of protein kinase C (PKC). The crucial role of the polyphosphoinositide pathway in the HIV life cycle is well documented in the literature[2–7]. We implemented this approach by using our liposome anti-HIV/AIDS preparation, FTL/AZT/PEBA, containing lithium as a specific non-competitive blocker of the polyphosphoinositide pathway[8, 9] and 3'-azido-3'deoxythymidine (AZT, azidothymidine) as an anti-retroviral agent, both encapsulated in liposomes. The results obtained by extended evaluation of our preparation on HIV-infected cell cultures, experimental animals and AIDS-suffering subjects showed that it is non toxic and such an approach may contribute considerably to successful AIDS therapy[1].

Results

The primary goal of the present work is to evaluate and interpret the best fit line for our experimental data of viral load measured in AIDS patients under treatment with FTL/AZT/PEBA, with the aim of providing additional evidence for its therapeutic abilities, advantages and mode of action as well as to use the graph as a tool for making prognosis of therapy outcome and to estimate the time necessary to reach the end point of the medication period. **Figure 1** illustrates that upon treatment with FTL/AZT PEBA the plasma HIV-1 RNA follows a simple downward (negative slope) linear regression line instead of the exponential one which is typical when non-liposome encapsulated (free) antiretrovirals, e.g. HAART are used and in contrast to ours does not lead to zero viral load[10–13]. Taking into account our previous findings[1] and the continual downward linear slope (**Figure 1**), we came to a conclusion that whole body eradication of HIV could be obtained in a reasonable time.

Discussion

These results could be explained by the advantageous features of the liposome drug delivery system[14–16], the dual action of lithium on both HIV-to-host cells signalling[2–7] and cytoplasmic HIV RT DNA conformational state[1]. Briefly, some of the liposomes carrying the active substances (lithium and AZT in this particular case) are phagocytised by the macrophages, both in the blood circulation and in the HIV sanctuary organs, with subsequent lysis and sustained release of the above components. Others simply adhere to the cell outer membrane surface, whereupon the drug molecules will diffuse through the liposome lipid bilayer and into the cell. A similar role is played by the liposome-to-cell fusion[16]. The above mechanisms of liposome drug delivery are of particular importance for creating constant optimal therapeutic intracellular concentrations of AZT and lithium, both into the phagocytic cells and the non-phagocytic HIV target cells, e.g. CD4+ T lymphocytes. Upon contact with HIV, these intrinsically quiescent cells become activated and are forced to proliferate via a specific receptor signaling mechanism, which is promoted by the polyphosphoinositide pathway[3, 4, 6]. As a result, productive and/or latent HIV infection develops. Liposome-delivered

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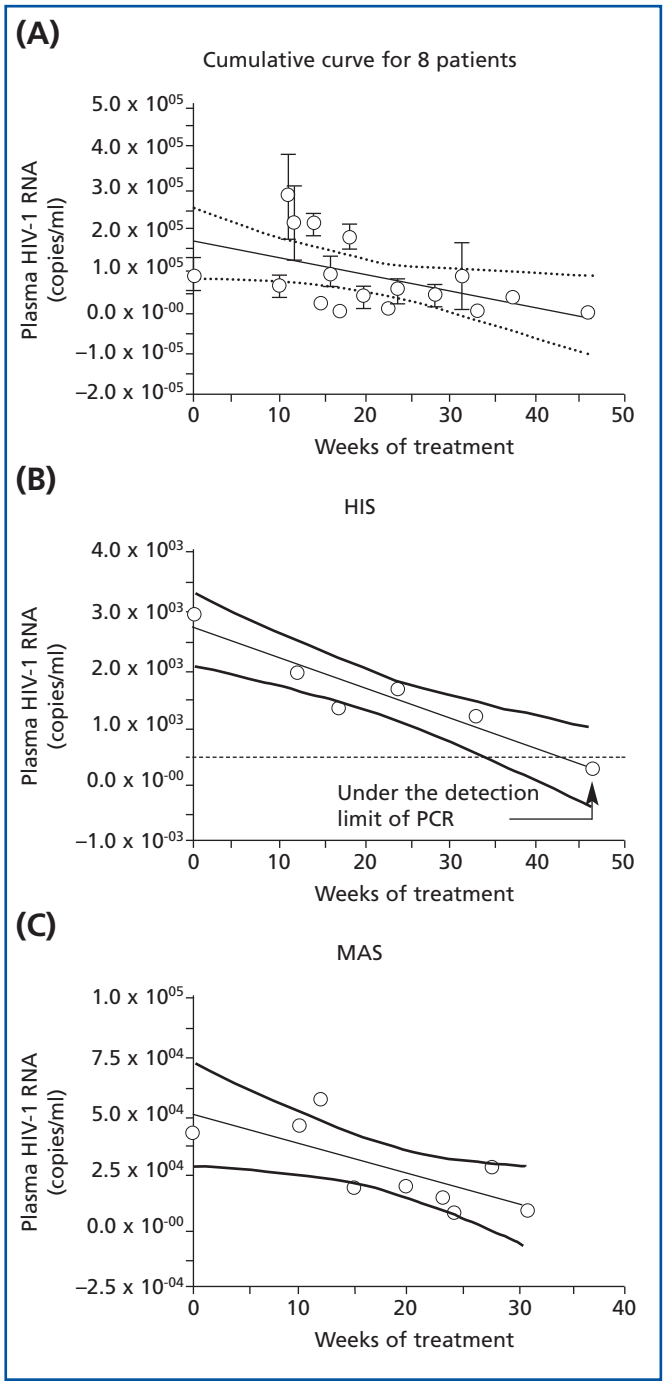

Figure 1. The estimated best fit line (linear regression) of the viral load (VL) decay obtained under treatment with FTL/AZT/PEBA. (A) Cumulative curve for 8 AIDS patients P = 0.041, r^2 = 0.25. The calculated time for obtaining zero VL is 51 weeks and 53 and 83 weeks for four and five orders of magnitude bellow zero respectively. (B) Representative example for patient (HIS) whose VL reached the detection limit of our PCR (500 copies/ml HIV-1 RNA, indicated by horizontal dashed line) within 46 weeks of treatment (P = 0.0041, r^2 = 0.8973). We calculated that 52 weeks are necessary for the patient's VL to reach the mathematical zero and respectively 71 and 244 weeks for 3 and 4 orders of magnitude bellow zero. (C) Representative example for patient (MAS) whose VL sharply decreased but did not reach the detection limit of our assay within 31 weeks of treatment. We calculated that 38 weeks are necessary to obtain zero VL and respectively 46 and 116 weeks for VL of four and five orders below the mathematical zero. We used Curve Expert version 1.36 (Hyams Development, USA) for preliminary automatic fit with 36 models and interpolation of the viral load with time. We used Prism version 2.01 (20) for further detailed analysis of the best fit linear regression line and estimation of the best fit parameters and calculation of the theoretical end point time necessary to obtain different viral loads. We also used Prism to construct the graphs. Amplicor HIV-1 Monitor test (Roche Diagnostic Systems, USA) was used to measure of HIV-1 RNA copies.

lithium stops such an activation process thus rendering the target non-infected CD4+ T cells refractory to HIV attack. This is achieved by inhibition of the key enzymes of the phosphoinositide pathway, the inositol monophosphatase (IMP) and inositol polyphosphate 1-phosphomonoesterase[7,9]. Besides, the liposome-delivered intracellular lithium ions may add a positive charge to the negative phosphate groups of the cytoplasmic HIV RT DNA with consequent conformational changes and inactivation of the molecule[1]. There are both experimental and computer simulation evidence that such interactions may take place[17]. Apparently, further integration of the cytoplasmic HIV DNA into the cell genomic one will thus be avoided. The above mechanism provides hope that one of the most stubborn HIV reservoirs, the latently infected CD4+ T cells[10], might be inactivated, thus contributing to whole body HIV eradication. At the same time, HIV DNA production is successfully blocked by AZT delivered by liposomes into the target cells. Recently, a subset of human natural killer (NK) cells that express CD4 and HIV co-receptors CCR5 and CXCR4 have been identified and proved susceptible to HIV-1 infection in a CD4-dependent manner[18]. The results presented provide strong evidence that such NK cells remain persistently infected with HIV-1 even in patients receiving HAART for 1–2 years. Apparently, the above described mechanism of action of FTL/AZT/PEBA may also contribute to the elimination of such non-T cell HIV reservoirs.

As illustrated (**Figure 1**), the prognosis of the therapy outcome might be considered optimistic in terms of reaching a complete cure as long as the viral load progression follows a downward linear slope with zero trend. Stable deviations from this regression model may indicate, for instance, either possible development of drug resistance or HIV rebound due to replenishment from ineradicable reservoir(s) with respective failure of the treatment. We investigated the theoretical time for zero viral load and obtained a reasonable treatment period of about 50 weeks (**Figure 1, A–C**). Since there is no assay with a detection limit below 25–50 plasma HIV-1 RNA copies per millilitre and our PCR limit is 500 copies/ml, we also calculated the time necessary to reach a viral load that is four and five orders of magnitude below the mathematical zero. Nevertheless, we obtained a reasonable result on treatment periods, depending on the viral load (**Figure 1**), which is far below the estimations based on HAART (over 60 years on average)[10].

Conclusion

In conclusion, we show that the linear zero trend viral load decay obtained for the first time under our HIV/AIDS treatment approach[1] suggests that a whole body eradication of the virus is achievable in a reasonable time. We consider

the following main points in support of this. First, the ability of FTL/AZT/PEBA to simultaneously and completely knock out HIV from both reproductively-infected and reservoir cells regardless of their type and organ localisation, based on the liposome drug delivery mechanisms described above. Second, rendering of the non-infected target cells refractory to HIV attack by blocking their polyphosphoinositide transmembrane signalling system, thus preventing infection expansion and improving the immune system status. And last but not least, creating conformational changes in *de novo* synthesised cytoplasmic HIV RT DNA with consequent inactivation of the molecule, thus stopping both further reproductive and/or latent HIV infection. The graph may also serve as a useful tool for early prediction of drug resistance and management of the therapy.

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