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Biochemical Pharmacology 68 (2004) 1231–1238
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Administration of HDAC inhibitors to reactivate HIV-1 expression in latent cellular reservoirs: implications for the development of therapeutic strategies

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Received 23 April 2004; accepted 7 May 2004

Abstract

The discovery of powerful antiviral compounds in the 90's raised the hope that the human immunodeficiency virus type 1 (HIV-1) might be eradicated. However, if these drugs succeed in decreasing and controlling viral replication, complete eradication of the virus is nowadays impossible. The persistence of virus even after long periods of highly active antiretroviral therapy (HAART) mainly results from the presence of cellular reservoirs that contain transcriptionally competent latent viruses capable of producing infectious particles after cellular activation. These latently infected cells are a permanent source for virus reactivation and lead to a rebound of the viral load after interruption of HAART. Activation of HIV gene expression in these cells combined with an effective HAART has been proposed as an adjuvant therapy that could lead to the elimination of the latently infected cells and then to the eradication of the infection. In this context, we have previously demonstrated that deacetylase inhibitors (HDACi) synergize with TNF-induced NF-κB to activate the HIV-1 promoter. The physiological relevance of the TNF/HDACi synergism was shown on HIV-1 replication in both acutely and latently HIV-infected cell lines. Based on these results, we propose the administration of deacetylase inhibitor(s) together with continuous HAART as a new potential therapeutic perspective to decrease the pool of latent HIV reservoirs by forcing viral expression.

Keywords: HIV-1; Transcription; Latency; Reservoirs; HDCAi; HAART

1. Introduction

1.1. The HIV-1 reservoirs: a major obstacle to the eradication of the virus

The development of highly active antiretroviral therapies (HAART) has dramatically improved the survival and quality of life of HIV-1-infected individuals. Unfortunately, whereas these treatments significantly reduce the levels of viral RNA in plasma and lymphoid tissues, cessation of even prolonged highly suppressive HAART regimens results in viral load rebound to pre-therapy levels, indicating that antiretroviral therapy of this type is unable to completely eliminate HIV-1 [1–3].

This failure has been attributed in part to the presence of a long-lived, stable population of latently infected resting memory $CD4^+$ T cells that are not eliminated by the antiviral treatment [4–6]. Indeed, while many HIV-susceptible cells are fast-turnover cells, this small part of memory T cells are long-lived cells [7,8]. These infected cells can go dormant and stay in tissues for years despite effective HAART, thereby serving as the HIV-1 reservoirs in vivo [6]. These reservoirs have such a slow rate of decay during HAART that their eradication during a human lifespan is unlikely [9,10].

As with all retroviruses, HIV-1 integrates into the genome of the host cell. As a consequence, the activity of the integrated viral genome, or provirus, is greatly influenced by the metabolic and activation state of the host cell. The generation of latency is thought to occur after HIV infection of a transcriptionally active cell, which predominantly

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0006-2952/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2004.05.040

results in productive infection and cell death. However, if viral transcription decreases before either viral or immunologic cytopathic effects, the virus can become latent (reviewed in [3]). Viral genes expression in these latently infected cells can be reactivated by a wide variety of signals including cytokines such as interleukin-2, tumor necrosis factor alpha/SF2 (referred to as TNF hereafter), macrophage colony-stimulating factor (MCSF), antigens and other T-cell mitogens, glucocorticoid and thyroid hormones, bacterial infections, lipopolysaccharides, and small molecules. Consequently, if HAART is ceased, viremia rapidly re-emerges, regardless of the duration of drug therapy [1,11–13].

It has been proposed that one possible solution to the problem of HIV-1 latency is to purge these reservoirs by deliberately forcing HIV-1 gene expression in these latently infected cells in presence of HAART to prevent spreading of the infection by the newly synthesized viruses [11]. Such treatment could reduce the number of latently infected cells by causing them to be directly killed by the cytopathic action of the virus or to be destroyed by the immune system. The definition of such strategies is clearly dependent on the knowledge of the molecular mechanisms regulating HIV-1 latency and reactivation from latency.

2. Molecular aspects of HIV-1 latency

At the cellular level, two major forms of HIV-1 latency have been described: pre-integration latency and post-integration latency [14]. The first one cannot be taken into account for the formation of the long-term viral reservoirs and will not be further discussed in this review. Among the proposed mechanisms for HIV-1 post-integration latency are: (i) mutations in the Tat-TAR axis [15,16]; (ii) the lack of activation-dependent host transcription factors in resting cells [17–21]; (iii) transcriptional silencing based on chromatin structure at the site of integration [22–25]; (iv) epigenetic modifications (reviewed in [26]).

2.1. Mutations in the Tat-TAR axis

HIV transcription is characterized by two temporally distinct phases. The early phase relies on cellular transcription factors. However, because of a transcriptional elongation defect in the basal HIV-1 promoter, the 5′ long terminal repeat (LTR), most transcripts cannot elongate efficiently and terminate rapidly after initiation. However, a few transcripts elongate throughout the genome, resulting in transcription of the viral transactivator Tat. The late phase of transcription occurs when enough Tat protein has accumulated. Tat binds to TAR, an RNA hairpin loop formed at the 5′ end of all nascent HIV-1 transcripts (reviewed in [27]) (Fig. 1), recruits the pTEFb complex and causes the hyperphosphorylation of the RNA polymerase II, thereby dramatically increasing its ability to elongate. In several latently infected cell lines, which are used as models for studying HIV-1 post-integration latency, the proviruses harbor mutations in their Tat-TAR transcriptional axis [15,16]. Indeed, we have examined the sequence of the Tat protein encoded by proviruses integrated in the latently infected U1 and ACH2 cell lines and the sequence of their respective LTRs. The U1 cell line contains two distinct forms of Tat: one Tat cDNA lacks an ATG initiation codon, while the other contains a mutation at aa 13 (H13L). Both Tat cDNA are defective in terms of transcriptional activation of the HIV-1 LTR [16]. The Tat cDNA amplified from the ACH2 virus is fully functional in terms of transcriptional activation, but the ACH2 LTR contains a point mutation in the Tat responsive element TAR. This latter mutation impairs the Tat-responsiveness of the LTR [15].

Although the significance of such mutants in the generation of HIV-1 latency in infected individuals in vivo has not been established, the studies performed in these latently infected cell lines have led to the concept that transcription inhibition is critical for the establishment and maintenance of HIV-1 latency.

2.2. The NF-κB signaling pathway

The early phase of HIV-1 transcription depends on cellular transcription factors. Therefore, the simplest mechanism for latency involves the absence in resting $CD4^+$ T cells of transcription from the HIV-1 promoter. This could result from the absence in resting cells of host transcription factors that are required for the activation of gene expression from the HIV-1 5′ LTR [17,20,21]. Indeed, the U3 region of the HIV-1 LTR contains binding sites for cellular transcription factors that function as positive regulators of T-cell activation-specific gene expression in normal, uninfected T cells (Fig. 1). The notion that latent infection of T cells might involve the absence of the requisite host transcription factors has been postulated following the demonstration that, in transformed T-cell lines carrying an integrated copy of the HIV-1 genome, up-regulation of HIV-1 gene expression following exposure to TNFα is mediated through nuclear factor-kappa B (NF-κB) [20]. Indeed, the enhancer located in LTR U3 region contains two binding sites for NF-κB, which play a central role in the activation pathway of the HIV-1 provirus (Fig. 1). Various studies have reported that the κB-binding sites as well as the NF-κB proteins are critical for LTR promoter activity and important for optimal HIV-1 replication (reviewed in [28]). In addition, the antigenic stimulation of T cells induces NF-κB activation by stimulation of PKC. Different groups have shown that phorbol ester derivatives [29,30], which induce direct activation of the PKC, potently induce expression of latent viruses in peripheral blood mononuclear cells and in quiescent T cells of infected individuals [31,32]. Induction of other transcription factors, such as AP-1 by the mitogen-activated protein

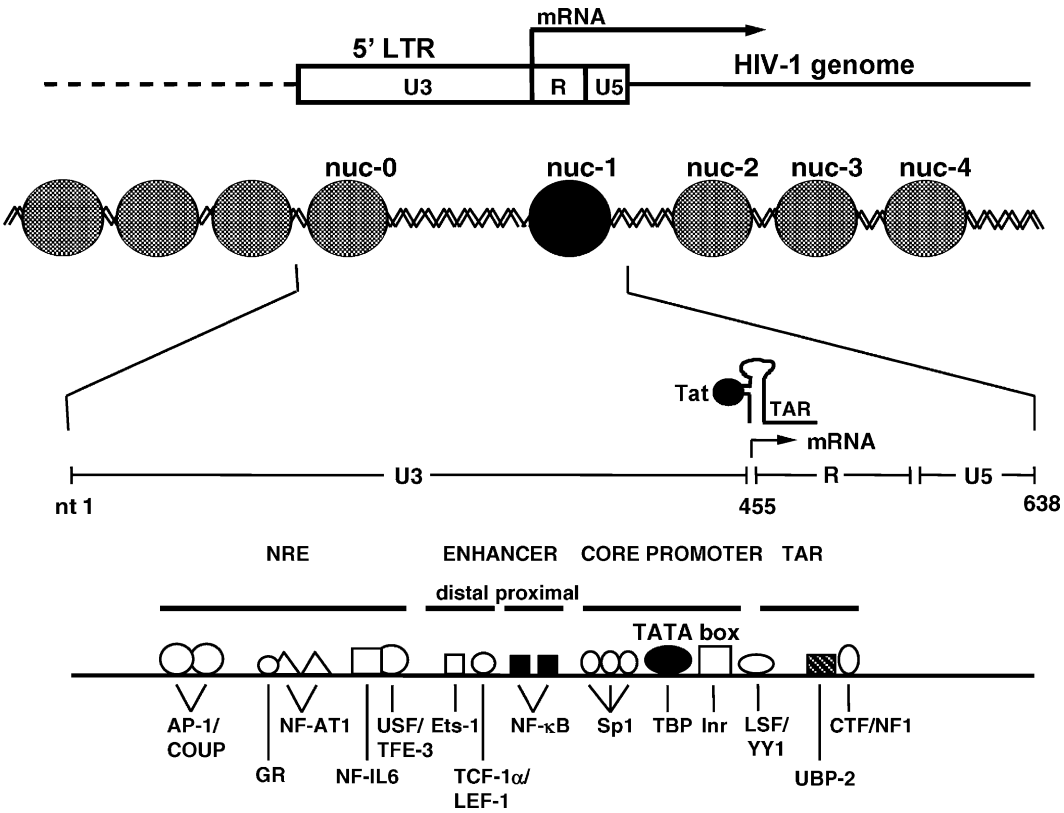

Fig. 1. Transcription elements in the 5′LTR of HIV-1. The U3, R and U5 regions of the LTR and binding sites for several transcription factors, as well as other sequence elements are indicated. Nucleotide (nt) + 1 is the start of U3 in the 5′LTR. The arrow at the U3/R junction denotes the start site of transcription. The region corresponding to the transactivating response (TAR) element is indicated. The mapping of nucleosome positioning in the 5′LTR and leader regions of HIV-1 is also represented. During transcriptional activation, a single nucleosome (nuc-1 in black) located immediately downstream of the transcription start, is specifically remodeled. This change is the only one observed in the complete genome of HIV-1 upon transcriptional activation.

kinase pathway and Sp1, also activate HIV LTR activity. However, their action is largely enhanced via interaction with NF-κB [33,34]. Like NF-κB, activation of the transcription factor nuclear factor of activated T cells (NF-AT) is induced early after T-cell stimulation and is critical to T-cell activation and proliferation [35].

2.3. The chromatin structure

Following infection, the HIV-1 proviral DNA is integrated into the host genome. Here, chromatin conformation seems to repress transcription from the integrated LTR promoter (reviewed in [36,37]). We have previously shown that, independently from the integration site, nucleosomes in the 5′ LTR are precisely positioned [25]. In the transcriptionally silent provirus, these nucleosomes define two large nucleosome-free regions. The first region corresponds to the enhancer/promoter area and the second region spans the primer-binding site region immediately downstream of the 5′LTR. These two open regions of chromatin are separated by a single nucleosome called nuc-1, that is specifically and rapidly disrupted during transcriptional activation (Fig. 1). The position of nuc-1 in the close proximity of the transcription start site and its disruption/remodeling during transcriptional activation led us to postulate that chromatin plays a crucial role in the repression of HIV-1 transcription during latency and that nuc-1 disruption is necessary for transcriptional activation [24,25]. In addition, studies from David Margolis group have demonstrated that the host factors YY1 and LSF cooperatively recruit histone deacetylase 1 (HDAC1) to the HIV-1 LTR and inhibit transcription by maintaining nuc-1 in an hypo-acetylated state [38,39]. In agreement with their data, pyrole-imidazole polyamides, which block the binding of the LSF–YY1 complex to the LTR and consequently the recruitment of HDAC-1 close to nuc-1, have been shown to induce reactivation of HIV-1 expression [38,39]. These experiments highlight the repressive role played by the nucleosome nuc-1 in the generation of post-integration latency. However, although the chromatin structure adopted by the integrated HIV-1 provirus is independent of the integration site, it has been demonstrated that the location of the provirus in hetero- or euchromatin locus could be an additional element to consider in the generation of post-integration latency [22,23].

2.4. Acetylation of histones and non-histone proteins

There is now strong evidence that both transcriptional activation and silencing are mediated at least in part

through the recruitment of enzymes that control protein acetylation. Acetylation of specific lysine residues within nucleosomal histones is generally linked to chromatin disruption and transcriptional activation of genes. Consistent with their role in altering chromatin structure, many transcriptional co-activators possess intrinsic histone acetyltransferase (HAT) activity that is critical for their function (reviewed in [40]). Similarly, co-repressor complexes include proteins that have histone deacetylase (HDAC) activity (reviewed in [41]). Importantly, reversible acetylation is also a critical post-translational modification of non-histone proteins, including general and specific transcription factors, co-activators, non-histone structural chromosomal proteins, and nuclear import factors. In the case of HIV-1, there is ample evidence that viral transcription is regulated by protein acetylation. Indeed, we and others have demonstrated the transcriptional activation of the HIV-1 promoter in response to deacetylase inhibitors (HDACi) (such as trichostatin A (TSA), trapoxin (TPX), valproic acid (VPA) and sodium butyrate (NaBut)). This occurs in ex vivo transiently or stably transfected HIV-1 LTR promoter reporter constructs [42–45], in latently HIV-1-infected cell lines [44–47], on in vitro chromatin-reconstituted HIV-1 templates [48,49], as well as in the context of a de novo infection [45]. HIV-1 transcriptional activation following treatment with HDACi is associated with nuc-1 remodeling (reviewed in [36]). Moreover, it has been demonstrated that acetylation of the HIV-1 transactivator Tat by p300, by P/CAF, and by human GCN5 is important for its transcriptional activity (review in [26,50–52]).

HDACi, such as TSA, strongly induce HIV-1 transcription, although only a small subset (<2%) of cellular genes have their expression modified by TSA [53]. The ability of the HIV-1 promoter to respond specifically to TSA might reflect a complex regulatory link between deacetylation and transcriptional repression of the LTR and highlights the importance of protein acetylation in the regulation of HIV-1 expression and especially in its reactivation from latency.

2.5. A regulatory link between NF-κB signaling pathway and protein acetylation

The transcription factor NF-κB and the regulation of protein acetylation both play a central role in the HIV-1 activation pathway. To better understand the molecular mechanisms regulating HIV-1 reactivation from latency, we have recently extended our studies on the TSA inducibility of the viral promoter (LTR) and focused on the functional role of the κB sites in this TSA response. We have demonstrated that HDACi (such as TSA and NaBut) synergize with TNF-induced NF-κB to activate transcription from the HIV-1 LTR [45]. This synergism requires intact κB sites and was observed with LTRs from subtypes A to G of the HIV-1 group M (major) [45]. Importantly, the physiological relevance of the TNF/TSA(NaBut) synergism was shown both on HIV-1 reactivation in a model cell line for post-integration latency and on HIV-1 replication in the context of a de novo viral infection [45].

Therefore, our results open new therapeutic strategies aimed at forcing viral expression and at contributing, in the presence of an efficient HAART, to a reduction of the pool of latently HIV-infected cellular reservoirs (see Section 3 here below).

We have unraveled the molecular mechanisms underlying the TNF/TSA synergism. In mammalian cells, there are five known members of the NF-κB/Rel family: p65 (RelA), c-Rel, RelB, p50, and p52. The most widely studied and most abundant form of NF-κB is a heterodimer of p50 and p65. In unstimulated cells, NF-κB is sequestered in the cytoplasm in an inactive form through interaction with its inhibitor IκBα, but NF-κB can be induced transiently by various stimuli (including inflammatory cytokines (TNFα, IL-1), bacterial lipopolysaccharides, viral proteins, mitogens, UV light) (Fig. 2).

Mechanistically, we have demonstrated that TSA and NaBut prolonge TNF-induced NF-κB binding to DNA and the intranuclear presence of p65 [54]. We have shown that the p65 subunit of NF-κB is acetylated in vivo. However, this acetylation is weak, suggesting that other mechanisms could be implicated in the potentiated binding and transactivation activities of NF-κB after TNF + TSA versus TNF treatment. Western blot and immunofluorescence confocal microscopy experiments have revealed a delay in the cytoplasmic reappearance of the IκBα inhibitor, which correlated temporally with the prolonged intranuclear binding and presence of NF-κB [54]. This delay was due neither to a defect in IκBα mRNA production, nor to a nuclear retention of IκBα, but rather to a persistent proteosome-mediated degradation of IκBα. We have demonstrated that TSA prolongs the activity of the IkB kinase (IKK) complex [54]. This prolongation of IKK activity could explain, at least partially, the delayed IκBα cytoplasmic reappearance observed in presence of TNF + TSA and the TNF/TSA synergism observed on the HIV-1 promoter [54] (Fig. 2).

3. HDACi: a new hope for HIV-1 eradication?

We have demonstrated a synergistic effect of TNF and HDACi on reactivation of HIV-1 expression in the latently infected U1 cell line (see Section 2.5). In addition, our preliminary experiments performed on CD8-depleted PBMCs isolated from aviremic patients suggest that HDACi are able to reactivate HIV-1 expression in latently infected cells (unpublished results). It is important to note that an array of cytokines, including the proinflammatory cytokines TNF and interleukin-1 (inducers of NF-κB), are already copiously expressed in the microenvironment of the lymphoid tissues, which harbor latent viral reservoirs [55]. Therefore, our results suggest that the use of

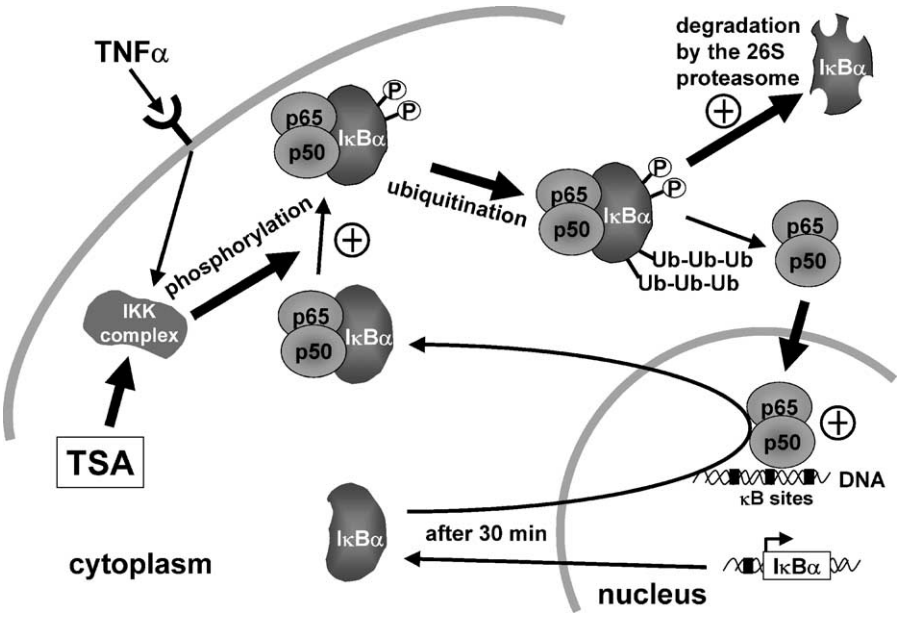

Fig. 2. The NF-kB signaling pathway and the effect of TSA. In unstimulated cells, NF-κB (represented by the protein dimer p50/p65) is sequestered in the cytoplasm in an inactive form through interaction with inhibitory IκB proteins including IκBα, IκBβ and IκBε. Upon activation of NF-κB by various stimuli (including inflammatory cytokines (TNFα, IL-1), bacterial lipopolysaccharides, viral proteins, mitogens, UV light), IκBs are rapidly phosphorylated by a macromolecular IκB kinase (IKK) complex, ubiquitinated and degraded by the 26S proteasome. The released NF-κB then translocates to the nucleus, where it can activate transcription from a wide variety of promoters, including that of its own inhibitor IκBα. The newly synthesized IκBα enters the nucleus, enhances NF-κB removal from DNA, and takes it back to the cytoplasm, thus restoring the inducible cytoplasmic pool of NF-κB. Thus, the de novo expression of IκBα proteins, which display nucleocytoplasmic shuttling properties, participates in a negative feedback system ensuring a transient NF-κB transcriptional response. Mechanistically, we have demonstrated that TSA prolongs the activity of the IKK complex (indicated by a + sign) and thus prolongs the phosphorylation of IκBα. The phosphorylation of IκBα by IKK is critical for its proteasome-mediated degradation. Therefore, the prolongation of IKK activity could explain the persistent degradation of neosynthesized IkBα observed in presence of TNF + TSA (indicated by a + sign) and the resulting prolonged presence of NF-κB in the nucleus (indicated by a + sign).

deacetylases inhibitors in the treatment of HIV infection may represent a valuable approach for purging the latently infected reservoirs in HAART-treated individuals. These HDACi would synergize with the cytokines already present at increased level in the serum of the HIV-infected individuals.

HDACi present several advantages in the context of an anti-HIV-1 adjuvant therapy. First, an ideal adjuvant agent would induce expression of HIV-1 without inducing global T-cell activation to prevent the generation of new target cells for the neo-synthesized virus. Indeed, HDACi do not induce proliferation or activation of T cells [56–58]. Recent data even suggest that HDACi inhibit CD4 T-cell proliferation in a dose-dependent manner [59]. Second, although infected resting $CD4^+$ T cells represent the major long-term HIV-1 reservoirs, other cell types (macrophages, dentritic cells and other non-T cells) clearly contribute to the persistence of HIV-1 during HAART [60,61]. As viral suppression in T cells becomes today increasingly effective, these alternative reservoirs may take on even greater relative importance as sites for viral persistence and as targets for purging. HDACi have been shown to act in a broad spectrum of cell lines and therefore, in contrast to agents that specifically induce T cells, they could target the different cellular HIV-1 reservoirs. Third, this class of agents is safely administrated for several years for other diseases, including beta-chain hemoglobinopathies (such as beta-thalassemia and sickle cell anemia) [62,63], epilepsy and bipolar disorders [64–66]. Moreover, HDACi are potent inducers of apoptosis and growth inhibition in transformed cells originating from lymphoid cells [56–58]. As HDACi are relatively non-toxic to normal cells, they are now considered as good candidates for novel cancer therapy. Among the different agents that have been identified as having HDACi activity (including TSA, TPX, oxamflatin, apicidin, phenylbutyrate, suberoylanilide hydroxamic acid (SAHA), pyroxamide, and FR901228), at least the latter four are currently used in clinical trials for evaluation of their anticancer efficacy [67].

The fourth element in favour of the use of HDACi in antiHIV treatment is the ability of these drugs to induce the transcriptional activation of several HIV-subtypes LTRs. Indeed, an increasing number of non-B HIV-1 subtype infections are currently diagnosed. We have shown that, in addition to the prototypical subtype B LTR, the LTRs from subtypes A to G of the HIV-1 major group M were also activated synergistically by TSA and TNF, and that the amplitude of the synergism correlates with the number of κB sites in the respective LTRs, which varies from one (subtype E) to three (subtype C) [45]. These data suggest that HDACi could be used to induce HIV-1 expression in a subtype non-specific manner.

A fifth important element is the fact that HDACi are already safely administrated to HIV-1 patients for years. Indeed, it is well documented that patients with HIV-1 are at an increased risk for the development of multiple neurologic manifestations including seizures [68]. Therefore, HIV-positive patients typically receive long-term anticonvulsant therapy following an initial episode of seizure activity [69]. Historically, these patients have been treated with many anticonvulsant agents including valproic acid which possess, in addition to its neurologic properties, HDACi activities. Supporting our hypothesis based on the ability of HDACi to induce HIV-1 replication in vivo, an increase in viral load of some HIV-positive patients receiving both HAART and valproic acid has been observed [70]. However, this phenomenon has not been observed in all patients receiving this combinatory therapy [69], suggesting that in some patients the increase in the viral load can be controlled by the antiretroviral treatment.

4. Discussion

HIV-1 latency represents a major problem in the eradication of HIV-1 in infected individuals treated with HAART. Indeed, even though the antiretroviral drugs protect uninfected cells from HIV infection, the HIV-infected cells are not affected by this therapy. In the absence of virus gene expression, latently infected cells differ from their uninfected counterparts by only the presence of 10 kb of viral DNA integrated into the host cell genome. These cells are not eliminated by the immune system or by viral-induced death. These reservoirs are thought to consist mainly of latently infected resting memory $CD4^+$ T cells and show striking stability, with a $t_{1/2}$ of 44 months. At this rate of decay, eradication of a reservoir consisting of only 10^6 memory $CD4^+$ T cells would take 73 years [10]. One possible solution to the problem of HIV-1 latency is to deliberately administrate agents that activate viral gene expression in the presence of HAART to prevent the spreading of the infection by the newly synthesized viruses [11]. The ideal HIV-1 inducing agent would be potent, orally available, non-toxic, active in a wide variety of latently infected cell types, and capable of penetrating anatomic sanctuaries such as the central nervous system. HDACi present such properties: (i) they are potent inducers of the HIV-1 expression; (ii) they can induce viral replication in different cell types and activate transcription of different HIV-1 subtype LTRs; (iii) some HDACi are already used in therapy against various diseases and several new molecules are under clinical trials; (iv) the HDACi valproic acid has already be given to HIV-positive patients for the treatment of neurological pathologies. These latter studies have demonstrated the ability of this molecule to cross the blood/brain barrier and the possibility to use HDACi as adjuvants to HAART.

Clinical efforts to reduce the latent HIV-1 reservoirs have so far been largely unsuccessful. Although treatment with interleukin-2 improves $CD4^+$ T-cell counts and reduces the HIV-1 reservoirs in some patients, systematic clinical studies have failed to demonstrate a consistent diminution of the pool of latently infected cells or of viral rebound following cessation of therapy [71,72]. Attempts to improve the ability of the endogenous immune system to combat HIV-1 by structured therapy interruptions (which are defined as a precise schedule according to which patients are put on and off therapy over a defined period of time) have also failed to reduce viral reservoirs and may actually promote the emergence of drug- and CTL-resistant viruses [73]. Cytoreductive therapy with cyclophosphamide in combination with HAART has also not reduced the cellular reservoirs of the virus [74]. Given these circumstances, together with the emerging problems of drug resistance and toxicity due to HAART, the possibility of treating persistent HIV-1 infection by inductive therapy with HDACi deserves further investigations.

Acknowledgments

We thank Arsène Burny for helpful comments on the manuscript. The work performed in C.V.L.'s laboratory is supported by grants from the ''Fonds National de la Recherche Scientifique'' (FNRS, Belgium), Télévie-Program of the FNRS, ''Action de Recherche concertée du Ministère de la Communauté française'' (ULB, ARC program no. 98/03-224), Internationale Brachet Stiftung, the CGRI-INSERM cooperation, ''Fortis Banque Assurance'', ''Fédération Belge contre le Cancer'', ''Région Wallonne'' (Program WALEO 021/5110) and Theyskens-Mineur Foundation. D.D. is supported by a post-doctoral fellowship from the Région Wallonne (grant 021/5110). V.Q. and C.V.L. are Chargé de Recherches and Maître de Recherches of the FNRS, respectively.

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