



NIH PUBLIC ACCESS

Author Manuscript

AIDS. Author manuscript; available in PMC 2013 December 16.

Published in final edited form as:

AIDS. 2008 June 19; 22(10): . doi:10.1097/QAD.0b013e3282fd6df4.

Valproic acid without intensified antiviral therapy has limited impact on persistent HIV infection of resting CD4+ T cells

Nancy M. Archin^a, Joseph J. Eron^a, Sarah Palmer^b, Anne Hartmann-Duff^a, Jeffery A. Martinson^c, Ann Wiegand^b, Nicholas Bandarenko^a, John L. Schmitz^a, Ronald J. Bosch^d, Alan L. Landay^c, John M. Coffin^b, and David M. Margolis^a

^aUniversity of North Carolina at Chapel Hill, Chapel Hill, North Carolina ^bHIV Drug Resistance Program, NCI, NIH, Frederick, Maryland ^cRush-Presbyterian-St Luke's Medical Center, Chicago, Illinois ^dHarvard School of Public Health, Boston, Massachusetts, USA

Abstract

Objectives—Valproic acid and intensified antiretroviral therapy may deplete resting CD4+ T-cell HIV infection. We tested the ability of valproic acid to deplete resting CD4+ T-cell infection in patients receiving standard antiretroviral therapy.

Methods—Resting CD4+ T-cell infection was measured in 11 stably aviremic volunteers twice prior to, and twice after Depakote ER 1000 mg was added to standard antiretroviral therapy. Resting CD4+ T-cell infection frequency was measured by outgrowth assay. Low-level viremia was quantitated by single copy plasma HIV RNA assay.

Results—A decrease in resting CD4+ T-cell infection was observed in only four of the 11 patients. Levels of immune activation and HIV-specific T-cell response were low and stable. Valproic acid levels ranged from 26 to 96 µg/ml when measured near trough. Single copy assay was performed in nine patients. In three patients with depletion of resting CD4+ T-cell infection following valproic acid, single copy assay ranged from less than 1–5 copies/ml. Continuous low-level viremia was observed in three patients with stable resting CD4+ T-cell infection (24–87, 8–87, and 1–7 copies/ml respectively) in whom multiple samples were analyzed.

Conclusion—The prospective addition of valproic acid to stable antiretroviral therapy reduced the frequency of resting CD4+ T-cell infection in a minority of volunteers. In patients in whom resting CD4+ T-cell infection depletion was observed, viremia was rarely detectable by single copy assay.

Keywords

antiretroviral therapy; HIV; latency; resting CD4+ T cells; valproic acid

Introduction

Despite its benefits, antiretroviral therapy (ART) cannot eliminate HIV infection [1–3]. Low levels of HIV RNA are detected in the plasma of many patients despite ART [4–6], and can be quantitated by a sensitive assay to limit of one copy of HIV RNA per milliliter [7].

Another barrier to the eradication of HIV infection is the early establishment of a latently infected pool of resting memory CD4⁺ cells [8–10].

Histone deacetylase (HDAC) is a critical regulator of HIV latency. The action of HDAC at the HIV promoter maintains quiescence of HIV gene expression in infected resting CD4⁺ lymphocytes [11–18]. HDAC inhibition leads to HIV outgrowth from resting CD4⁺ T cells of aviremic patients [19,20]. Resting CD4⁺ T-cell infection (RCI) was depleted when intensified ART was given in combination with valproic acid (VPA), an anticonvulsant and nonselective HDAC inhibitor [21]. We sought to test the ability of VPA to deplete RCI when combined with standard ART.

Methods

HIV-infected volunteers receiving stable ART with plasma HIV-1 RNA less than 50 copies/ml and a CD4 cell count of more than 300 cells/ μ l for at least 6 months were enrolled. Leukapheresis was performed on two occasions prior to the addition of VPA (on day –49 to –27 day 0), and at 12 and 16 weeks after the addition of VPA (Depakote ER 1000 once a day). Other enrollment criteria have been described [21]; because of its interaction with VPA, volunteers receiving zidovudine were excluded. Informed consent was obtained from all patients.

Lymphocytes were obtained by continuous-flow leukapheresis. Isolation of resting CD4⁺ T cells, recovery and quantification of replication competent virus was performed as described [21]. Briefly, following purification resting CD4⁺ T cells were incubated for 2 days with the HIV integrase inhibitor L-870812 (1 μ mol/l; gift of D. Hazuda, Merck Research Laboratory, West Point, Pennsylvania, USA) and efavirenz (15 nmol/l) or abacavir (4 μ mol/l) as determined by treatment history to insure the decay of any preintegrated HIV DNA. A total of 93.6 million resting CD4⁺ T cells were plated in replicate dilutions of 2.5 million (36 cultures), 0.5 million (six cultures), and 0.1 million (six cultures) cells per well and stimulated with 1 μ g/ml PHA-L (Remel, Lanexa, Kansas, USA), a five-fold excess of allogeneic irradiated peripheral blood mononuclear cells (PBMCs) from a seronegative donor, and 20 U/ml interleukin (IL-2) for 24 h. Cells were then washed and fed with CD8-depleted PBMCs collected from a selected HIV seronegative donor. These donor cells were screened for adequate CCR5 expression, collected by leukapheresis and stored in aliquots. Assays were performed with matched patient–donor pairs. Infected units per billion (IUPB) were estimated as described [21]. An observed depletion of greater than 50% is significant as this is greater than the 0.3 log₁₀ standard deviation of our assay.

Flow cytometry analysis was performed on lymphocytes isolated at leukapheresis as described in refs [21–24]. Cell surface phenotypes were characterized as follows: naive T cells, CD45RA⁺ CD62L⁺; memory T cells, CD45RO⁺ CD45RA[–]; activated T cells, HLADR⁺ CD38⁺ or HLADR⁺ CD69⁺ CD25⁺. CD8⁺ and CD4⁺ T cells were analyzed.

Additional studies were performed in batch on leukocytes from patients 1 to 6 preserved by rate-controlled freezing, after thawing and overnight incubation. HIV-specific responses were analyzed as described in ref. [24] using individual 15-mer peptides from HIV-1 consensus subtype B Nef (49 peptides), HIV-1 consensus subtype B Gag (123 peptides), and HIV-1 consensus subtype B Pol (221 peptides) obtained from the National Institute of Health (NIH) AIDS Research and Reference Reagent program. Baseline T-cell activation was obtained on the media control samples. Cytokine production (IFN- γ , IL-2) was also evaluated in the CD4⁺ and CD8⁺ T-cell populations.

Plasma HIV-1 RNA concentrations were measured by Roche Amplicor (Roche Molecular Systems, Branchburg, New Jersey, USA) with an assay detection limit of 50, and an ultra-

sensitive, quantitative real-time, reverse transcriptase (RT)-PCR single copy assay (SCA) capable of detecting and quantifying plasma HIV-1 RNA to a limit of detection of 1 copy/ml [25]. Seminal HIV-1 RNA was measured by NucliSens QT (bioMérieux, Durham, North Carolina, USA) with an assay detection limit of 400 copies/ml. Seminal fluid was collected at the same time points as leukapheresis. SCA was performed on serum samples collected at the screening visit, at 27–49 days before the start of VPA therapy, on days 0, 3, and weeks 1, 2, 4, 8, 12, and 16 after the start of VPA therapy.

Results

Twelve HIV-infected men, stably suppressed on ART, entered this study. ART included protease inhibitors (five patients), nonnucleoside reverse transcriptase inhibitors (six patients), or both (one patient). CD4 cell counts at entry ranged from 581 to 1121 cells/ μ l (27–52%).

After determination of the frequency of resting cell infection circulating at two time points, 1000 mg VPA (Depakote ER) was administered once a day for 16 weeks. One patient was removed from the study prior to the evaluation of VPA effect for protocol violations.

Total VPA levels were measured near trough, prior to daily dosing, and ranged from 26 to 96 μ g/ml. Dose was increased if trough levels were consistently below 40 μ g/ml; adjustment to 1250 mg was required in one patient. VPA therapy was generally well tolerated. Four patients reported minor adverse effects that resolved without specific intervention. Patient 8 reported depressive symptoms not clearly related to study drug.

CD4+ and CD8+ cells showed no changes in CD38+ HLADR+ (data not shown). In the first six patients, studies of HIV-specific immune responses were undertaken. VPA did not alter the proportions of naive, effector, or central memory CD4+ or CD8+ T cells (data not shown). HIV-specific T-cell responses to viral peptide antigen were low and unaltered following VPA therapy (data not shown).

VPA therapy combined with standard ART depleted resting cell infection in only four of the 11 patients studied (Table 1). Median VPA level at trough was not significantly different in patients with RCI depletion (56 μ g/ml) compared with those without (49 μ g/ml).

Given the observation of intermittent or low-level viremia in some patients, additional assays were performed to insure that outgrowth represented integrated provirus. After negative selection over antibody columns, when sufficient cells were available additional cell sorting was performed to obtain resting CD4+ populations of more than 99.9% purity in five patients. As fewer cells (34–49 million resting CD4+ T cells) were available after sorting, the point estimate of IUPB obtained in this assay is less precise. However, the frequency of RCI in these highly purified cell preparations did not differ significantly from a parallel assay performed without additional sorting (Table 2) or from the pooled results of the standard assay (Table 1).

To measure more accurately the extent of viremia to a limit of detection of 1 copy/ml, SCA was performed. SCA measurements correlated with Amplicor results (Table 1). In patient 9, intermittent viremia of more than 50 copies/ml by Amplicor assay was observed. In this patient SCA was routinely positive at 24–87 copies/ml. Therefore in this patient, results appeared consistent with variation in PCR-based detection of stable low-level viremia, as described in a longitudinal study [26]. In contrast, in patient 8 lack of viremia detected by Amplicor assay early in the study was associated with viremia of less than 30 copies/ml by SCA, whereas after week 8 clinical suspicions of nonadherence and intermittent Amplicor assays of more than 50 copies/ml were associated with SCA of at least 68 copies/ml.

We did not observe an association between duration of aviremia and RCI depletion. Four patients with depletion of RCI had HIV RNA less than 50 copies/ml for a mean of 15 months (median 13, range 10–23), compared with a mean of 19 months (median 14, range 7–45) in seven patients without depletion of RCI.

An association was observed between residual or low-level viremia and the stable RCI following VPA therapy. Depletion was not observed in patients 8 and 9, in whom intermittent viremia was observed, nor in patient 7 who had residual viremia by SCA (Table 1). Residual viremia was rarely detectable in patients 6, 11, and 12, although only in patient 6 were we able to perform the SCA repeatedly. In contrast, viral load was repeatedly less than 5 copies/ml in all three patients with a significant decline in RCI in whom the SCA was performed (patients 1, 2, and 3).

In some patients, viremia may be detected in genital fluids when it is not detectable in plasma [27]. Seminal fluid was provided by patients 1, 3, 5, 6, and 8, but HIV RNA was undetectable (limit of detection 400 copies/ml) at all available time points.

Discussion

Current antiretroviral therapy does not eliminate persistent resting cell infection. Therapies that selectively target the quiescent provirus and induce its expression could open new avenues for combating persistent HIV infection.

We studied the effect of the addition of VPA to standard ART on RCI. There was a decline in RCI of more than 50% in four of 11 patients receiving VPA therapy combined with standard ART. We did not observe large fluctuations or an apparent increase in resting CD4+ T-cell infection after the addition of VPA to ART, as reported [28].

Our observations suggest that in the majority of patients on standard ART, VPA alone is not sufficient to reduce RCI. Notably, there appeared to be an association of a decline in RCI with the absence of low-level viremia. Jones and Perelson [29] have recently suggested that intermittent low-level viremia to levels above approximately 7 copies/ml may prevent the decay of chronically infected cell populations despite continuous ART. Plasma viremia was observed to be uniformly less than 5 copies/ml in all three patients with a significant decline of RCI in whom SCA was performed. However, SCA was less than 5 copies/ml in three patients without a decline in RCI, although a limited number of assays were available in these patients.

One potential result of the induction of expression of latent provirus might be the appearance of low-level viremia in the peripheral blood, if viral expression was robust enough to escape the tissue reservoir and appear in the peripheral circulation. We did not observe evidence of a change in low-level viremia as measured by SCA throughout VPA therapy, similar to the stability of low-level viremia in patients treated with ART, VPA, and enfuvirtide [21].

Cells with an activated phenotype or HIV-specific immune cells might appear in the peripheral blood following induction of latent provirus. However, we found the number of these cells to be low, as is common in patients stably suppressed on ART, and without correlation to the level of RCI after VPA therapy.

Further depletion of RCI by VPA or response to VPA in a larger subset of patients might be observed if higher doses of VPA were administered. However, these modest findings suggest that alternate approaches may be needed to substantially deplete RCI in the majority of HIV-infected patients with stable suppression of viremia. If therapies that induce

remission or eradication of HIV infection are ever achieved, they will have to be effective over periods of treatment measured in months rather than years. Alternate approaches to intensify ART or utilize more potent HDAC inhibitors [30,31] are in development.

Acknowledgments

We are grateful to D. Parker, M. Cheema, and G. Lehrman for technical assistance, L. Ngo for study coordination, S. Fiscus, M. Kerkau, F. Ashton and the UNC CFAR Virology, Immunology, and Clinical Core facilities, J. Whitesides and the Duke BSL3 flow cytometry facility, L. Manor and the staff of the UNC GCRC, and to the dedicated staff of the UNC Blood Bank. We also thank Drs D. Wohl, C. Hicks, and M. Hudgens for study safety reviews. Depakoate ER was kindly provided by Abbott Laboratories. Funding for the study was provided by National Institutes of Health grants AI45297 and AI064074 to D.M.M., R00046 to the UNC GCRC, AI50410 to the UNC CFAR, and the Yudenfriend family. Most importantly, this effort would not have been possible without the selfless contribution of the study volunteers.

References

1. Gulick RM, Mellors JW, Havlir D, Eron JJ, Gonzalez C, McMahon D, et al. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. *N Engl J Med.* 1997; 337:734–739. [PubMed: 9287228]
2. Hammer SM, Saag MS, Schechter M, Montaner JS, Schooley RT, Jacobsen DM, et al. International AIDS Society-USA Panel. Treatment for adult HIV infection: 2006 recommendations of the International AIDS Society-USA panel. *JAMA.* 2006; 296:827–843. [PubMed: 16905788]
3. May MT, Sterne JA, Costagliola D, Sabin CA, Phillips AN, Justice AC, et al. Antiretroviral Therapy (ART) Cohort Collaboration. HIV treatment response and prognosis in Europe and North America in the first decade of highly active antiretroviral therapy: a collaborative analysis. *Lancet.* 2006; 368:451–458. [PubMed: 16890831]
4. Dornadula G, Zhang H, VanUitert B, Stern J, Livornese L Jr, Ingerman MJ, et al. Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy. *JAMA.* 1999; 282:1627–1632. [PubMed: 10553788]
5. Di Mascio M, Dornadula G, Zhang H, Sullivan J, Xu Y, Kulkosky J, et al. In a subset of subjects on highly active antiretroviral therapy, human immunodeficiency virus type 1 RNA in plasma decays from 50 to <5 copies per milliliter, with a half-life of 6 months. *J Virol.* 2003; 77:2271–2275. [PubMed: 12525664]
6. Zhang L, Ramratnam B, Tenner-Racz K, He Y, Vesanen M, Lewin S, et al. Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy. *N Engl J Med.* 1999; 340:1605–1613. [PubMed: 10341272]
7. Maldarelli F, Palmer S, King MS, Wiegand A, Polis MA, Mican J, et al. ART suppresses plasma HIV-1 RNA to a stable set point predicted by pretherapy viremia. *PLoS Pathog.* 2007; 3:e46. [PubMed: 17411338]
8. Wong JK, Hezareh M, Gunthard HF, Havlir DV, Ignacio CC, Spina CA, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science.* 1997; 278:1291–1295. [PubMed: 9360926]
9. Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science.* 1997; 278:1295–1300. [PubMed: 9360927]
10. Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, Baseler M, et al. Presence of an inducible HIV latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci USA.* 1997; 94:13193–13197. [PubMed: 9371822]
11. Margolis DM, Somasundaran M, Green MR. Human transcription factor YY1 represses human immunodeficiency virus type 1 transcription and virion production. *J Virol.* 1994; 68:905–910. [PubMed: 8289393]
12. Romero F, Gabriel M, Margolis DM. Repression of HIV-1 through the novel cooperation of the human factors YY1 and LSF. *J Virol.* 1997; 71:9375–9382. [PubMed: 9371597]

13. Coull J, Romero F, Sun JM, Volker JM, Galvin KM, Davie JR, et al. The human factors YY1 and LSF repress the human immunodeficiency virus type-1 long terminal repeat via recruitment of histone deacetylase 1. *J Virol*. 2000; 74:6790–6799. [PubMed: 10888618]
14. Imai K, Okamoto T. Transcriptional repression of human immunodeficiency virus type 1 by AP-4. *J Biol Chem*. 2006; 281:12495–12505. [PubMed: 16540471]
15. Marban C, Suzanne S, Dequiedt F, de Walque S, Redel L, Van Lint C, et al. Recruitment of chromatin-modifying enzymes by CTIP2 promotes HIV-1 transcriptional silencing. *EMBO J*. 2007; 26:412–423. [PubMed: 17245431]
16. Williams SA, Chen LF, Kwon H, Ruiz-Jarabo CM, Verdin E, Greene WC. NF-kappaB p50 promotes HIV latency through HDAC recruitment and repression of transcriptional initiation. *EMBO J*. 2006; 25:139–149. [PubMed: 16319923]
17. He G, Margolis DM. Counter-regulation of chromatin acetylation and histone deacetylase occupancy at the integrated promoter of human immunodeficiency virus type 1 by the HIV-1 activator tat and the HIV-1 repressor YY1. *Mol Cell Biol*. 2002; 22:2965–2973. [PubMed: 11940654]
18. Ylisastigui L, Coull JJ, Rucker V, Bosch RJ, Brodie SJ, Corey L, et al. Polyamides reveal a role for repression in viral latency within HIV-infected donors' resting CD4+ T cells. *J Infect Dis*. 2004; 190:1429–1437. [PubMed: 15378435]
19. Ylisastigui L, Archin N, Lehrman G, Bosch RJ, Margolis DM. Coaxing human immunodeficiency virus type 1 from resting CD4+ T cells: can the reservoir of HIV be purged? *AIDS*. 2004; 18:1101–1108. [PubMed: 15166525]
20. Quivy V, Adam E, Collette Y, Demonte D, Chariot A, Vanhulle C, et al. Synergistic activation of human immunodeficiency virus type 1 promoter activity by NF-kappaB and inhibitors of deacetylases: potential perspectives for the development of therapeutic strategies. *J Virol*. 2002; 76:11091–11103. [PubMed: 12368351]
21. Lehrman G, Hogue IB, Palmer S, Jennings C, Spina CA, Wiegand A, et al. Depletion of latent HIV infection in vivo. *Lancet*. 2005; 36:549–555. [PubMed: 16099290]
22. Al-Harthi L, Siegel J, Spritzler J, Pottage J, Agnoli M, Landay A. Maximum suppression of HIV replication leads to the restoration of HIV-specific responses in early HIV disease. *AIDS*. 2000; 14:761–770. [PubMed: 10839583]
23. Betts MR, Nason MC, West SM, De Rosa SC, Migueles SA, Abraham J, et al. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. *Blood*. 2006; 107:4781–4789. [PubMed: 16467198]
24. Nomura LE, Emu B, Hoh R, Haaland P, Deeks SG, Martin JN, et al. IL-2 production correlates with effector cell differentiation in HIV-specific CD8+ T cells. *AIDS Res Ther*. 2006; 3:18. [PubMed: 16859558]
25. Palmer S, Wiegand AP, Maldarelli F, Bazmi H, Mican JM, Polis M, et al. New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. *J Clin Microbiol*. 2003; 41:4531–4536. [PubMed: 14532178]
26. Nettles RE, Kieffer TL, Kwon P, Monie D, Han Y, Parsons T, et al. Intermittent HIV-1 viremia (Blips) and drug resistance in patients receiving HAART. *JAMA*. 2005; 293:817–829. [PubMed: 15713771]
27. Gunthard HF, Havlir DV, Fiscus S, Zhang ZQ, Eron J, Mellors J, et al. Residual human immunodeficiency virus (HIV) Type 1 RNA and DNA in lymph nodes and HIV RNA in genital secretions and in cerebrospinal fluid after suppression of viremia for 2 years. *J Infect Dis*. 2001; 183:1318–1327. [PubMed: 11294662]
28. Siliciano JD, Lai J, Callender M, Pitt E, Zhang H, Margolick JB, et al. Stability of the latent reservoir for HIV-1 in patients receiving valproic acid. *J Infect Dis*. 2007; 195:833–836. [PubMed: 17299713]
29. Jones LE, Perelson AS. Transient viremia, plasma viral load, and reservoir replenishment in HIV-infected patients on antiretroviral therapy. *J Acquir Immune Defic Syndr*. 2007; 45:483–493. [PubMed: 17496565]
30. Marks PA, Breslow R. Dimethyl sulfoxide to vorinostat: development of this histone deacetylase inhibitor as an anticancer drug. *Nat Biotechnol*. 2007; 25:84–90. [PubMed: 17211407]

31. Archin, N.; Espeseth, A.; Duff, A.; Cheema, M.; Parker, D.; Hazuda, D., et al. Expression of Latent HIV induced by a selective class I HDAC inhibitor. Abstract of the 14th Annual Conference on Retroviruses and Opportunistic Infections; Los Angeles, California. 2007; [abstract 501]

Table 1

Frequency of infection of resting CD4+ T cells from patients on standard ART alone and ART with VPA.

Patient	ART IUPB ^c	ART and VPA IUPB ^c	Less than 50% depletion IUPB on VPA	One single copy assay ^b	
				Range	Median
1	187	90	Yes	<1-1	<1
2	52	22	Yes	<1-3	1
3	70	22	Yes	<1-5	2
4	183	3	Yes	ND	ND
5	112	120	No	ND	-
6	54	75	No	<1-1	<1
7	531	625	No	1-7	3
8	17 951	9438	No ^a	8-77	25
9	4890	3300	No ^a	24-87	37
10	696	1253	No	<1	-
11	57	83	No	<1-1	-

ART, antiretroviral therapy; IUPB, International Union of Photobiology; ND, not defined; SCA, single copy assay; VPA, valproic acid.

^aNonconsecutive episodes of viremia of more than 50 copies during study.

^bSCA data (RNA copies/ml) represents a range from six to 10 determinations from screening visit to week 24 end-of-study visit, but for patient 10 (two assays) and 11 (three assays).

^cIUPB represents pooled data from two assays pre-VPA and two assays after addition of VPA.

Table 2

Frequency of infection of resting CD4+ T cells.

Patient	IUPB standard assay	IUPB sorted assay
1	45	66
6	57	101
7	625	320
8	8000	17 950
9	2835	2551

Variance of the sorted assay is greater than the standard assay, as fewer cells are evaluated. Frequency was calculated after column purification (IUPB standard assay) and column purification followed by sorting (IUPB sorted assay) at one time point while receiving ART and VPA. IUPB, International Union of Photobiology; VPA, valproic acid.