

Failure of atorvastatin to modulate CSF HIV-1 infection

Results of a pilot study

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ABSTRACT

Background: HIV-1 infection of the CSF space is nearly universal in untreated systemic infection, and correlates strongly with intrathecal and systemic immunoactivation and CSF pleocytosis. Based on the potential immunomodulatory and antiviral properties of HMG-CoA reductase inhibitors (statins), we examined the effect of atorvastatin on CSF HIV-1 infection and associated CSF abnormalities in a small pilot study.

Methods: Seven male HIV-1-infected, antiretroviral-naïve subjects with a mean blood CD4+ T cell count of 473 cells/ μ L were studied in an open-label, single-arm pilot study to assess the effects of 80 mg atorvastatin daily for 8 weeks. The primary endpoint was the change in CSF HIV-1 RNA levels, both absolutely and relative to plasma HIV-1 RNA, at 4 and 8 weeks of treatment. Other outcome measures included CSF white blood cell counts and neopterin concentrations as indices of intrathecal immunoactivation, and blood HIV-1 RNA levels, neopterin concentrations, and T lymphocyte counts. Effects on blood lipids were used to monitor the established biologic effects of atorvastatin and treatment adherence.

Results: No significant changes in CSF virologic and inflammatory indices or in systemic HIV-1 infection were observed during atorvastatin treatment despite potent reduction of blood lipids.

Conclusion: Atorvastatin showed no appreciable effect on CSF HIV-1 infection or intrathecal immunoactivation in this small uncontrolled study and thus appears to have little promise as an immunomodulatory adjuvant therapy for CNS HIV-1 infection, at least in neuroasymptomatic subjects with preserved CD4+ T cell counts. *Neurology*® 2008;71:521-524

GLOSSARY

ADC = AIDS dementia complex; **ART** = antiretroviral therapy; **HIVE** = HIV encephalitis; **HMG-CoA** = 3-hydroxy-3-methylglutaryl coenzyme A; **LP** = lumbar puncture; **WBC** = white blood cell.

HIV-1 infection of the CSF space is a nearly universal aspect of untreated HIV-1 infection, from primary exposure through advanced AIDS.¹ Though usually clinically silent and seemingly benign, in some individuals infection evolves to more invasive HIV encephalitis (HIVE) accompanied by the cognitive and motor function abnormalities of the AIDS dementia complex (ADC).² While combination antiretroviral therapy (ART) is effective in suppressing CSF infection in most patients, even in the setting of drug resistance,³ the effect of HIV-1 on the CNS remains a clinical problem. Patients not treated with ART still present with ADC. Moreover, in concert with the increased survival related to therapy, the prevalence of milder neurocognitive impairment may have actually increased,⁴ perhaps in part related to early sub-clinical brain injury or ongoing injury despite treatment. This has led to the search for adjuvant modes of treatment to mitigate brain injury by interfering with virus-driven immunopathologic pathways downstream from infection.⁵

We undertook this pilot study to test whether atorvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, might be a candidate adjuvant treatment

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Table 1	Study entry criteria
	>18 years of age
	Informed consent
	Off ART >6 weeks
	Plasma and CSF HIV-1 RNA >1,000 copies/mL
	Blood CD4+ T cells >150 cells/ μ L
	ADC stage <1
	Statin treatment
	None in >6 months
	No prior reaction to statin
	No interacting medications
	No enhanced risk of LP (CNS mass, bleeding diathesis)
	No active CNS diseases
	No active infections
	Screening blood levels*
	Hemoglobin >10 g/dL
	AST and ALT <2.5 \times upper limit normal
	CK <3 \times upper limit of normal

*Also used to monitor toxicity and determine drug discontinuation.

ART = antiretroviral therapy; ADC = AIDS dementia complex; LP = lumbar puncture.

through modulation of CSF HIV-1 infection and intrathecal immunoactivation. In addition to impairing cholesterol synthesis, HMG-CoA reductase inhibitors reduce biosynthesis of isoprenoid pyrophosphatases involved in the post-translational prenylation of key regulatory proteins involved in immune responses.⁶ On this basis statins have been recommended for clinical trials in a number of immunologic diseases. Conflicting evidence suggests statins may also more directly inhibit HIV-1 replication.⁷

METHODS This open-label, uncontrolled pilot study examined the effects of atorvastatin at the high end of standard dosage for 8 weeks. Subject entry criteria are summarized in table 1. Primary outcomes were the changes from baseline in CSF HIV-1 RNA levels and in the difference between plasma and CSF HIV-1 \log_{10} RNA levels. Secondary outcomes included changes in CSF white blood cell (WBC) counts and neopterin concentrations, and in blood HIV-1 RNA and neopterin levels. We also measured effects on blood CD4⁺ and CD8⁺ T cells and on the CSF: blood albumin ratio as an index of blood–brain barrier integrity.⁸ Serum cholesterol, LDL cholesterol, and triglycerides were used to monitor the known biologic effects of atorvastatin and treatment adherence. This study was approved by the University of California San Francisco Committee on Human Research, and informed consent was obtained from all subjects.

After meeting eligibility criteria, subjects began atorvastatin 80 mg/day for the next 8 weeks. At baseline, weeks 4 and 8, and after a 6-week drug washout period, study visits included a lum-

Table 2	Baseline subject characteristics
Characteristics	Mean (SD)
Age, y	43.6 (4.8)
Time since HIV diagnosis, y	11.3 (7.4)
Blood T cells (cells/μL)	
CD4+	473 (180)
CD8+	1,527 (572)
HIV-1 RNA (\log_{10} copies/mL)	
Plasma	4.30 (0.34)
CSF	3.85 (0.65)
Plasma:CSF difference	0.47 (0.55)
CSF WBCs (per μL)	7 (4–8)*
Neopterin (nMol/L)	
Plasma	15.3 (4.3)
CSF	17.1 (5.8)
CSF: blood albumin ratio	5.13 (2.21)
Serum lipids (mg/dL)	
Cholesterol	177.6 (43.3)
LDL	111.9 (33.3)
Triglycerides	100.7 (47.8)

*Median (IQR).

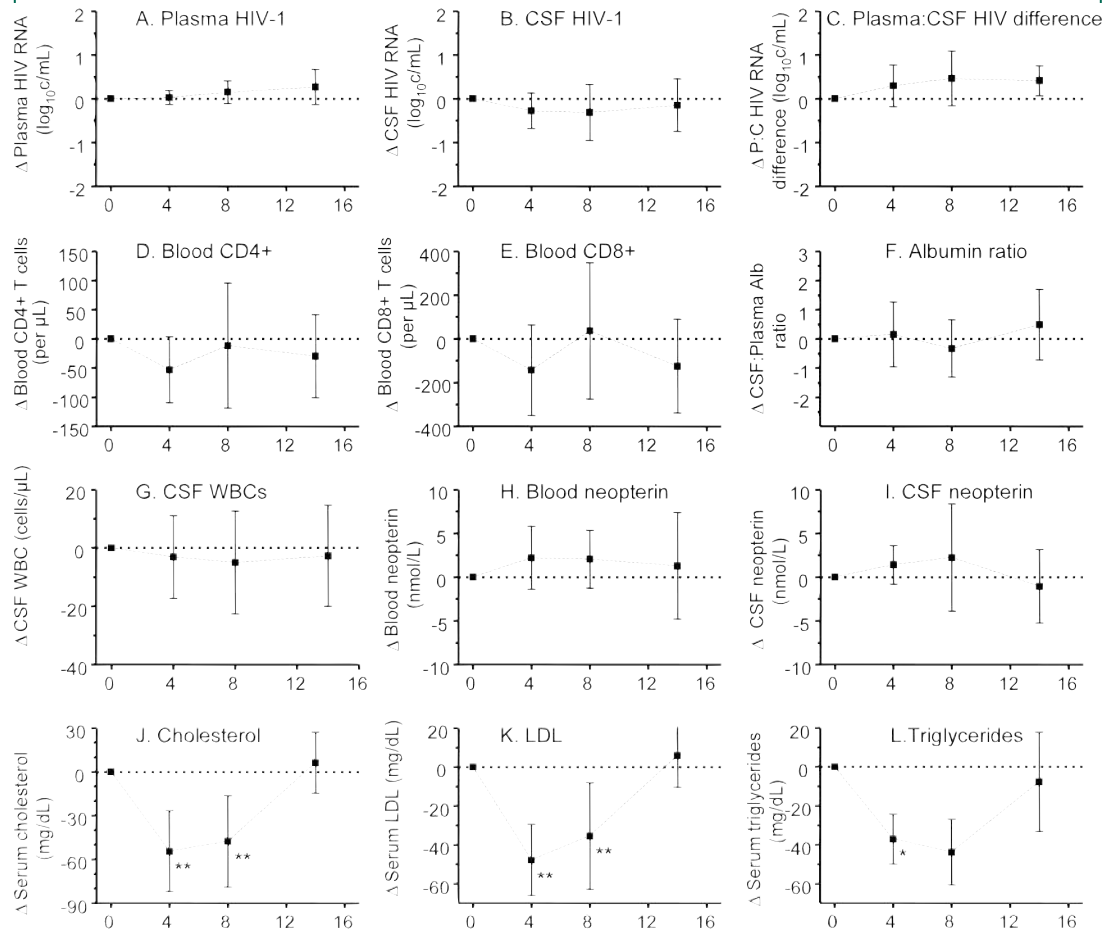
bar puncture (LP) and concurrent blood sampling using previously described methods.^{3,5} HIV-1 RNA and neopterin concentrations were measured in cell-free CSF and plasma as previously described,⁵ while other measurements were performed in the San Francisco General Hospital Clinical Laboratories using standard clinical methods.

Changes from baseline to subsequent study visits were analyzed by simple paired *t* tests and repeated measures analysis of variance with Dunnett post hoc comparison. All *p* values were two-sided with values <0.05 considered significant in this initial comparison. Statistical analyses used Prism 5 (GraphPad Software Inc, San Diego, CA) while power calculations used GraphPad StatMate 2.00.

RESULTS Of 16 subjects screened, 8 were excluded because of low CSF HIV-1 RNA (*n* = 5), elevated serum transaminase (*n* = 3), or elevated serum CK (*n* = 1). The remaining 8 subjects entered the study and were prescribed atorvastatin. One electively discontinued participation at week 4; his blood lipid profiles showed no drug effect, and he was excluded from analysis for therapy nonadherence.

Table 2 summarizes the baseline characteristics of the remaining seven subjects. Six completed the study without adverse event. One subject discontinued atorvastatin from week 4 (included in analysis) through week 8 (excluded from analysis) of the study due to elevations in serum transaminases, but continued study participation through the washout period and week 14 visit. For repeated measures analysis of variance analysis, his 4-week results were carried forward and included in the 8-week data.

Figure Changes in viral, immune, and lipid measurements during atorvastatin treatment



Panels show the mean changes from baseline and 95% CIs for (A) plasma HIV-1 RNA, (B) CSF HIV-1 RNA, and (C) plasma:CSF \log_{10} difference in HIV-1 RNA concentrations; (D) blood CD4+ T cells, (E) blood CD8+ T cells, and (F) CSF:plasma albumin ratio; (G) CSF WBC counts; (H) blood neopterin, and (I) CSF neopterin concentrations; and serum (J) cholesterol, (K) LDL cholesterol, and (L) triglyceride levels. The designations of significance (* $p < 0.05$; ** $p < 0.01$) derive from paired t test using observed values. Analysis of variance analysis with post hoc testing showed similar results with significant changes confined to the lipid measurements.

Changes from baseline in the outcome measures are shown in the figure. There were no significant changes in the virological measures: plasma HIV-1 RNA remained steady (A), and a minor but insignificant reduction in the mean CSF HIV-1 RNA (B) and increase in the plasma:CSF \log_{10} difference (C) were noted. There were no significant changes in blood CD4+ and CD8+ T cells or in blood-brain barrier permeability to albumin (D–F) or in CSF WBC count (G) and neopterin (H) or in blood neopterin (I). The absence of virologic and anti-inflammatory effects contrasted with substantial reductions in serum cholesterol, LDL, and triglycerides (J–L) that returned to baseline values during the washout.

DISCUSSION This pilot study was undertaken as an initial step in exploring statins as an adjuvant treatment of CNS HIV-1 infection, and more particularly in assessing whether atorvastatin might reduce

CSF HIV-1 infection and intrathecal immunoreactivation through its immunomodulatory properties. Our underlying mechanistic hypotheses involved the following reasoning: 1) if CSF levels of HIV-1 RNA reflect local viral production by trafficking short-lived CD4+ T cells,⁹ and 2) if both traffic of these cells into the CSF and their viral output are enhanced by immune activation,⁵ and 3) if atorvastatin is capable of down-modulating these processes, then 4) atorvastatin should reduce CSF HIV-1 RNA concentrations absolutely and in relation to the plasma viral load. We noted neither of these latter effects: CSF and plasma HIV-1 RNA (and their ratio) were not significantly altered by treatment, despite potent effects of atorvastatin on blood lipids.

We also hypothesized that atorvastatin would mitigate the characteristic CNS immune activation of chronic HIV-1 infection, and would reduce the increased CSF WBC counts and elevations in CSF

neopterin, a soluble biomarker of intrathecal immune activation.^{5,10} However, these markers also remained unchanged through the course of treatment. Our group also recently completed a randomized, placebo-controlled pilot study focused on the effect of atorvastatin on T cell activation in blood (n = 10); this also failed to show an effect (unpublished data). Thus, while atorvastatin can exert immunomodulatory activity in vivo, this either does not counteract the mechanisms operating in HIV-1 infection or is too weak in relation to this potent HIV-1 effect.

This pilot study was subject to the inherent limitations of its size, duration, and absence of an untreated control group for comparison, raising concern for Type II error. A small though insignificant decrease in CSF HIV-1 and increase in the plasma:CSF difference (figure, B and C) were noted, and we cannot fully dismiss the possibility that the study was underpowered to detect a mild effect of the drug or that CSF HIV-1 would decline further with longer exposure. However, if the observed CSF virologic changes were indeed real, based on these pilot results a controlled study would require 50–70 subjects in each of two arms to have an 80% power to detect this difference between groups. Moreover, even if they had proved significant, these effects were too weak to justify a study of this size, especially in the absence of an effect on CSF WBC or neopterin levels or on systemic HIV-1 replication—particularly when viewed in comparison to the potent effects of combination ART on these variables.³

If CNS immune activation is an important component in the pathogenesis of ADC/HIVE, our study sug-

gests that statin therapy has little promise as an immunomodulatory adjuvant therapy in this setting.

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