TITLE OF STUDY

Statin modulation of monocyte activation: a pilot study for potential use in NeuroAIDS

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Study Protocol

Abstract

Neurological complications of HIV infection are common and persist even in treated people. Monocytes play a major role in the initiation and progression of neurocognitive dysfunction. Monocytes get activated and migrate to the brain where they produce soluble factors that cause inflammation. They also carry virus to the brain. During the course of the disease several factors contribute to immune activation (including monocyte activation), many of which play a role in progression of neurocognitive disease. We hypothesize that if immune activation is controlled in these chronic HIV-infected individuals, progression to neurological injury can be stopped. Therefore, we propose to study the effects of a statin drug with anti-inflammatory functions, on the monocyte activation status of these individuals. Eligibility: Subjects enrolled in the study will be on anti-HIV therapy, viral load less than 200 copies/ml for more than 6 months. Intervention: 1) Subjects on PI-based HAART will be administered 10 mg/day X 2weeks and then 20 mg/day; subjects on non-PI-based HAART will receive 20 mg/day X 2 weeks and then 40 mg/day Atorvastatin. 2) At specific time points during the study period blood will be drawn to study various immune and virological parameters.

Objectives

Our objectives are based on the hypothesis that statin treatment will reduce the inflammatory monocyte phenotype and negatively regulate the inflammatory cytokines that have been linked to neuropathogenesis and may have potential a adjunctive therapy in HIV-associated neurological disease. In this pilot project we propose to 1) Determine the effects of Atorvastatin on peripheral blood monocyte populations in a 12-week plot study in chronically HIV-infected people. 2) Determine the relationship between changes in monocyte phenotype following Atorvastatin treatment, and soluble markers of activation/ inflammation linked to neuropathogenesis, as well as activation status of T cells.

Primary outcome variable(s)

1) Peripheral blood monocyte population and surface markers: CD16, CD163, and CCR2. 2) Plasma levels of monocyte-associated inflammatory cytokines and chemokines: MCP-1, soluble CD163, and soluble CD14

Background

Two principal populations of human peripheral blood monocytes are recognized. The CD14+CD16- population comprises ~90% of the total monocytes in a healthy person and a CD14+CD16+ population the remaining 10% of the monocytes. The latter are also called the pro-inflammatory / non-classical subset, based on expression of inflammatory cytokines such as TNF-, IL-1 and IL-6, and enhanced antigen presentation capacity.

Several lines of evidence suggest that this inflammatory monocyte population, characterized by CD16 expression, plays a particularly important role in the pathogenesis of HIV-associated neurological disease including HIV encephalopathy (HIVE). This population is increased in chronic HIV-1 disease, and in particular is expanded in people with HIV encephalopathy or dementia. This monocyte population has a greater propensity for invasion into tissues. Furthermore several reports indicate that even though monocytes are not generally considered major reservoirs of infection in blood, there is preferential infection of this particular subset by the virus, and these cells may help virus traffic into tissues including brain. Finally HIVE is associated pathologically with the accumulation in brain of monocyte/macrophage cells that express CD16, CD163 and several other activation markers, and it is believed that release of both inflammatory mediators from these macrophage lineage cells, as well as viral products from infected cells within this population, are mainly responsible for the neuronal injury that occurs . Since CD163 is not normally expressed by resident microglia in normal brain tissue, the numerous CD163+ cells observed in SIVE / HIVE suggest emigration of monocytes into the CNS from the peripheral blood. Increased CD163+/CD16+ monocyte/ macrophages in the CNS have been associated with increased frequency of CD163+/CD16+ monocytes in the periphery and correlates significantly with increased viral load and CD4+ T-cell decline. Monocyte neuroinvasion is preceded by a dramatic increase in the number of peripheral blood monocytes as well as peak levels of viral load and proinflammatory mediators in plasma. These observations highlight the importance of this monocyte subset as a link between HIV / SIV CNS disease and peripheral immune pathogenesis and suggest that events in the periphery are central to the development of HIV-associated neuropathogenesis. Recent findings suggest a role for elevated LPS levels, a result of translocation of bacterial products from a leaky gut, in triggering monocyte activation during HIV infection, thereby contributing to HIV-dementia pathogenesis via trafficking of activated monocytes into brain (6, 70). Furthermore, elevated LPS levels compromise the integrity of the blood brain barrier which enhances monocyte transmigration into the CNS . LPS triggers the release of many inflammatory cytokines and other effectors including IL-1, IFN-, TNF-, IL-8, along with platelet activating factor and arachidonic acid metabolites and is a likely cause of immune activation in HIV infection. Of note, LPS up-regulates the expression of MCP-1, which is essential for the recruitment of monocytes into sites of inflammation. This is especially relevant to HIV-neuropathogenesis since elevated MCP-1 is associated with increased risk of progressing to HIV-dementia. Interestingly CD14 +CD16+ monocytes are a major source of MCP-1 and other proinflammatory proteins that recruit T cells and additional monocytes into the CNS. In addition, CD14+CD16+ monocytes promote highly efficient viral replication on differentiation into macrophages and also activate resting T cells for HIV-1 infection. It has been proposed that T-cell activation in HIV disease is an indirect consequence of monocyte activation and is of significance since it suggests that altered interactions between monocytes and T cells may contribute to dysregulated responses, which is a feature of HIV infection. Since HIV- dementia is partly a result of an inflammatory environment in the periphery over an extended period of time, it is important to identify

potential treatment methods of reducing systemic immune activation / inflammation in the setting of chronic HIV disease. In recent years since the advent of HAART, though the incidence of HAD has decreased, its prevalence has increased, leading to efforts at identifying adjunctive treatment options. Several studies report the neuroprotective effects of statins in neurodegenerative diseases like Alzheimer's and Parkinson's disease, and further trials are currently underway to test its effects in multiple sclerosis treatment. Since statins are well tolerated and have relatively few side effects, they may be considered for treatment of neurological complications in HIV infection. Statins (HMG-CoA reductase inhibitors), are cholesterol lowering drugs with pleiotropic immunomodulatory / anti-inflammatory properties which are independent of their cholesterol lowering ability. These effects include inhibition of cytokine / chemokine expression, inhibition of T- cell activation, inhibition of leukocyte adhesion and down-regulation of MHC II, to name a few. Preliminary animal studies have demonstrated the ability of statins to enhance bacterial clearance and attenuate pro-inflammatory responses to LPS . Other studies suggest that statins can attenuate the pro- inflammatory effects of endotoxin in astrocytes, microglia and endothelial cells. Based on in vitro data, another proposed effect of statins might be to lower viral load -an important factor in monocyte activation, although the limited studies that have evaluated that in vivo have reached different conclusions. Interestingly, activated CD16+ monocytes are also associated with an increased risk of coronary artery disease (CAD) and macrophages (in the vascular wall) are believed to play an important role in vascular injury, but whether statins alter the CD16+ population of monocytes as part of their well documented beneficial effects in CAD, has received little attention. Evidence of the immunomodulatory / anti-inflammatory effects of statins on the monocyte function is limited. No in vivo studies have yet been carried out to assess the effects of statins on the pro-inflammatory monocyte sub-population in chronic HIV disease. Moreover, it is unclear how modulation of the monocyte function by statins correlates with T cell responses. Recent evidence of a positive correlation between elevated plasma LPS levels, immune activation, disease progression, monocyte activation and CD4+ T cell depletion, suggests that all these parameters are inter-dependent. The inflammatory phenotype of monocytes makes them a potential therapeutic target for reducing disease burden. We hypothesize that treatment of chronic HIV-1 positive individuals with statins, would modulate monocytes from a pro- inflammatory towards an anti-inflammatory phenotype functionally by decreasing the number of CD14 +CD16+CD163+ monocytes. Statin treatment would also strongly reduce plasma MCP-1 levels (along with other anti-inflammatory effects), as demonstrated in our preliminary data and by others and would reduce systemic immune activation, which has direct implications for neuroAIDS pathogenesis. We also hypothesize that statin treatment would result in decreased viral load which would also result in decreased immune activation. In the long term, decreased immune activation as a result of statin treatment, would slow down the progression to AIDS and decrease the prevalence of HAD.

Design

This is an open label pilot to study the monocyte-related anti-inflammatory effects of Atorvastatin drug in chronic HIV-1 infected individuals who are on HAART. On-treatment data was compared with pre-treatment data.

Study duration

The duration of the study is one year. The time duration of treatment is 12 weeks, with a total duration of subject participation of 18 weeks.

Characteristics of the Study Population

Target population

We will enroll chronic HIV infected individuals who are on anti-retroviral HAART therapy (without changes in HAART drug combination for at least 3 months). Additionally, they will not be on any prior statin therapy. These individuals will have plasma viral loads less than 200copies/ml for 6 months. The effects of Atorvastatin treatment will be determined by comparing the various parameters during the treatment period to baseline levels.

Subjects at UPenn

15

Subjects at Sites Other than UPenn

0

Accrual

We will have access to the study subjects through the CFAR Clinical core. The initial proposed sample size was 20 subjects based on having sufficient power to detect a change in each of the outcome variables from baseline to week 12. Because of the modifications in the protocol involving: a) the use of Atorvastatin rather than Simvastatin, and b) the inclusion of hs-CRP as a screening test, both factors leading to increased costs, we have decided to decrease the total enrollment to 15 subjects. We believe this will not negatively impact the value of the study as this pilot is meant to generate data for future definitive studies.

Key inclusion criteria

1. Chronic HIV-1 infected individuals on HAART (no change in drug combination for at least 3 months prior to enrollment in the study), and be willing to continue on therapy for

the duration of the study.

2. HIV viral load less than 200 copies/ml for more than 6 months.

3. hs-CRP levels above the upper limit of normal (3 mg/L).

3. Willingness to use a method of contraception during the study period.

4. Willingness to have blood drawn.

5. If female, willingness to undergo pregnancy testing on a monthly basis and are not breastfeeding.

6. Ability to understand and willingness to sign the informed consent.

Key exclusion criteria

1. Concomitant use of fibric acid derivatives or other lipid lowering agents including patients on statins and Ezetimibe.

2. Use of any anti-inflammatory drugs (OTC or prescription) on a daily basis.

3. Pregnancy or breastfeeding.

4. Active drug use or alcohol abuse/dependence, which in the opinion of the investigators will interfere with the patient's ability to participate in the study.

5. Allergy or hypersensitivity to Atorvastatin or any of its components.

6. History of myositis or rhabdomyolysis with use of any statins.

7. Patients who are on concurrent immunomodulatory agents, including systemic corticosteroids (including nasal or inhaled steroids) will be ineligible for 3 months after completion of therapy with the immunomodulating agents.

8. History of inflammatory muscle disease such as poly or dermatomyositis.

9. Serious intercurrent illness requiring systemic treatment and/or hospitalization within 30 days of entry.

10. Evidence of active opportunistic infections requiring treatment or neoplasms that require chemotherapy during the study period.

11. Creatinine phosphokinase elevations (CPK) greater than 3 times the upper limit of normal.

12. Known active liver disease or AST/ALT greater than 2x the upper limit of normal.

13. Renal insufficiency indicated by serum creatinine 2 mg/dl.

14. Absolute neutrophil count (ANC) 1000/mm3, hemoglobin less than 10.0 g/dL for males or 9.0 g/dL for females, platelet count 100,000/mm.

15. HCV co-infection.

16. Ischemic heart disease.

17. NYHA Class III or IV congestive heart failure.

Subject recruitment

The CFAR Clinical Core will assist us in the identification and recruitment of subjects required for the study from the Clinical Core Cohort.

Subject compensation

Yes, subjects will be compensated a total of $300 by the end of the study. They will be paid $5 at the screening visit. Following enrollment in the study, they will be paid $20 at each of the subsequent study visits in cash and the balance of $195 will be paid at the end of the study by check.

Procedures

SAMPLE COLLECTION: Informed consent will be obtained from all individuals enrolled in the study. As shown in the 'study schedule', subjects will undergo screening tests for enrollment into the study at week -2. The treatment period will be for a total of three months, blood samples will be collected before starting statin treatment on day 0, and at 2 weeks, 6 weeks and 12 weeks through the duration of the treatment. Finally, blood samples will be collected 4 weeks after the end of treatment (washout period).

Study Schedule

		T=-2wk	T=0	T=2 wk	T=6 wk	T=12 wk	T=16 wk
	Atorvastatin treatment:		begin	+	+	end	
	Sample type:	**screen**	**baseline**	**treatment**	**treatment**	**treatment**	**Wash-out**
Safety labs	CBC	X		X	X	X	
	Metabolic panel (Cr, AST/ALT) & CPK	X		X	X	X	
Treatment monitoring labs	Lipid panel (non-fasting)	X		X		X	
Clinical outcome labs (plasma)	D-dimer		X	X		X	
	hsCRP	X		X		X	
Lymphocyte subset panel	CD3/CD4/CD8	X	X			X	
HIV VL	HIV viral load		X			X	
Urine Pregnancy		X		X	X	X	
PBMC FACS	CD14/16/163; CD3/4/8; CCR5/CXCR4/CX3CR1/CCR2; CD25/38/DR;TF		X		X	X	X
Monocyte transcriptiome analysis	Purified monocyte RNA (store, run if funds permit)		X		X	X	X
Plasma luminex	Soluble mediators (store)*	X	X	X	X	X	X
Plasma ELISA	sCD14/sCD163 /Tissue Factor (store)*	X	X	X	X	X	X
Total vol bld /time point		21.5	100	26	96.5	111.5	85

Analysis Plan

STATISTICAL ANALYSIS: Because this is a pilot study with a small sample size, any statistical test applied to the data will only be able to detect large changes or trends over time. Instead, our main goal will be to look for trends occurring in the data and to estimate means needed for accurate sample size calculations for a full study. The data will be analyzed as follows.

DESCRIPTIVE STATISTICS: Standard descriptive statistics will be used to summarize the sample characteristics at baseline. For continuous variables means, medians and ranges will be calculated, while frequencies and percentages will be calculated for categorical variables. The outcome variables (marker levels, viral loads, and cell counts) will also be summarized at baseline and each follow-up time point. In addition, the change in outcomes from baseline to week 12 will be calculated for each subject and statistically described. Further, graphical tests, such as stem-and-leaf plots, will be used to test for normality in the continuous variables, and guide in the choice of transformation where warranted.

CHANGES IN OUTCOME: The change in the outcomes from baseline to week 12 will be compared using paired t-tests (normally distributed) or Wilcoxon rank-sum tests (non-normal). Bivariate comparisons will be used to determine if the changes in the outcomes are associated with any of the baseline demographic or clinical characteristics. For categorical variables, t-tests, ANOVA, or non-parametric equivalents will be used to compare changes in outcomes between groups, while correlation coefficients will be calculated with continuous characteristic variables. Also, since outcome variables are taken at multiple times, they will be graphed to visually inspect for trends across times via Spaghetti plots. Longitudinal data analysis methods will be applied to test for changes over time, and if those changes are affected by any of the subject characteristic variables.