Supplemental Digital Content

Use of rosuvastatin in HIV-associated chronic obstructive pulmonary disease: A randomized pilot study

Running head: Rosuvastatin in HIV pulmonary disease

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Methods:

Choice of rosuvastatin: Rosuvastatin was selected because of its safety in HIV and postulated effects on COPD and inflammation [25-28]. A relatively low dose of rosuvastatin was chosen in order to avoid interactions with antiretroviral medications.

Exclusion criteria: Subjects were excluded if they had an absolute or relative contraindication to statins (abnormal renal or liver function, history of diabetes mellitus requiring medication or hemoglobin A1C greater than 6.5%), were acutely ill (hospitalized for a non-psychiatric or non-traumatic cause within 4 weeks of randomization, increasing respiratory symptoms or fevers in the prior 4 weeks), currently using another anti-inflammatory or immunosuppressive medication (excluding aspirin), or had a contra-indication to statin use (current use of other medications with known interactions with rosuvastatin, allergy or adverse reaction to statins). We also excluded individuals who had a contraindication to performing pulmonary function testing.

Randomization procedure: An adaptive randomization was chosen to maximize enrollment by allowing participants to get a potentially more effective therapy. The randomization procedure adapts the randomization probabilities as the trial progresses to increase the number of participants on the more successful therapy. To adapt the randomization, hsCRP 30 days post-randomization was chosen because it is an acute phase reactant that is rapidly altered on statin therapy, allowing us to adapt treatment to the response within the study time-frame [30, 31]. For purposes of randomization, treatment was considered a success if the 30-day hsCRP declined by 10% or more from baseline.

Pulmonary function: Pre- and post-bronchodilator (480µg albuterol) spirometry and measurement of DLco were performed per American Thoracic Society standards [27, 28]. Hankinson and Neas reference equations for predicted values were used for spirometry and DLco, respectively. DLco was corrected for hemoglobin and carboxyhemoglobin [29, 30].

Chest CT scans: Non-contrasted CT scans of the chest were acquired during an inspiratory breath hold at 100 mAs and reconstructed using the GE "standard" kernel at 0.625 mm thickness at end-inspiration. CT image data were analyzed by a single reader using a standardized approach at the University of Pittsburgh. The lung was segmented from the CT images [37], and the density mask technique [38] used to quantify the percentage of lung voxels below a threshold of -950 Hounsfield units (HU) as indicative of emphysema [39, 40].

Biomarkers and PBMC gene expression: Levels of the inflammatory biomarkers IL-6, IL-8, sCD14, and sCD163 were measured in serum via ELISA (R&D, Minneapolis, MN) at baseline and 24 weeks. Based on our previous work linking endothelin-1 (ET-1), a marker of vascular dysfunction also produced by inflammatory cells, to lung function in HIV [41], we also measured serum levels of ET-1 (R&D, Minneapolis, MN). Serum aliquots were stored at -80˚C and assayed after a single thaw. Technical replicates were performed in triplicate, and replicates with coefficients of variation greater than 20% were rejected and the assay repeated. Expression levels of IL-6, IL-8, sCD14, sCD163, and ET-1 mRNA in PBMCs were measured by real-time RT-PCR at baseline and 24 weeks (n=8 samples available for rosuvastatin group, n=11 for placebo). Total RNA from PBMCs was isolated and purified with RNeasy columns (Qiagen, Hilden, Germany). Two hundred ng of RNA from each sample was reverse transcribed with reverse transcriptase. Real-time RT-PCR amplification was performed as described [42]. The Ct values for each assayed gene were normalized to the endogenous control mRNA glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and then to a baseline calibrator sample.

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Power calculations and selection of outcomes: The trial sample size was decided on based on the R34 funding mechanism with the goal of supporting pilot studies for infrastructure. As UCLA did not find eligible participants, we reduced the goal to two sites and 22 participants. Thirty-day change in hsCRP was the primary outcome in terms of the adaptive randomization design as other markers were not expected to change sufficiently in a time frame to influence randomization. The primary clinical outcome of interest was change in FEV1% predicted compared between the rosuvastatin and placebo groups.

Statistical analyses: The response-adaptive design induces dependencies between a participant's treatment assignment and the outcomes of the previous participants. Thus, simulations were used to determine the statistical significance when comparing the treatment and control groups on the baseline and the outcome measures. For each of the 1000 simulations, the null distribution was estimated by re-assigning a treatment group to the participants using the response-adaptive design algorithm. The observed values from our study were compared to the simulated null distribution and the percentage of simulated values that were equal to or more extreme than the observed value was computed to obtain the p-value.

Study flow: Medical record review was then performed to determine eligibility. The first participant was randomized on May 8, 2013; recruitment ended August 27, 2014 and the last follow-up was February 13, 2015. Forty-four subjects were eligible, and 36 consented to participate (Supplemental Figure 1). Twenty-two were randomized, with 21 completing the

study. One subject in the placebo group was terminated prior to week 4 due to an elevated creatinine kinase and chose not complete the remainder of the study.

Results: Radiographic emphysema was minimal at baseline in both groups and did not change significantly in either group at 24 weeks. There were no significant changes in the St. George's Respiratory Questionnaire scores.

Biomarkers and PBMC gene expression: Serum IL-6 and IL-8 did not change significantly in either group. Serum sCD14 levels increased in the placebo group (217,929 ng/ml median change, p=0.0029) and did not change in the rosuvastatin group (-30,525 ng/ml median change, p=0.56). There was also a significant difference in comparison of the change between the two groups (p=0.032). Serum sCD163 levels also increased in the placebo group (64 pg/ml median change, p=0.003), but did not change in the rosuvastatin group (12.5, p=0.72) without a significant change between the groups. ET-1 levels decreased in the rosuvastatin group (median change = -0.32 pg/ml, p=0.005) and were unchanged in the placebo group (median change =-0.164 pg/ml, p=0.28), with a significant difference in the comparison of change in the groups (p=0.003). Expression of IL-6 mRNA in PBMCs decreased in the rosuvastatin group (median fold-change = -0.12, p=0.023) and did not change in the placebo group (median fold-change = -0.005, p=0.52). IL-8 mRNA expression tended to have a bigger decrease in the rosuvastatin group, but did not reach statistical significance (-7.64 versus -0.35, p=0.083). There were no significant changes in gene expression of sCD14 or sCD163 in either group. ET-1 mRNA expression decreased significantly in the rosuvastatin group (median fold-change = -0.32, p=0.005), but did not change in the placebo group (median fold-change = -0.16, p=0.62). There were no differences in mRNA expression of biomarkers between groups.

Adverse events: Similar numbers of participants reported myopathy in the rosuvastatin (n=3) and placebo groups (n=4). One participant in each group reported abdominal pain or nausea.

Each group had one participant with elevated creatine kinase levels and one participant in the placebo group had an elevated creatinine.

Table 1: Baseline pulmonary characteristics for the cohort and by treatment group.

	All (N=22) **Median (Q1, Q3)** **n (%)**	**Placebo (N=11)** **Median (Q1, Q3)** **n (%)**	**Rosuvastatin (N=11)** **Median (Q1, Q3)** **n (%)**	**Simulation** **p-value**
FEV_1 % predicted	82.7 (78.0, 90.0)	82.9 (80.1, 85.2)	80.9 (75.3, 91.7)	0.42
FVC % predicted	85.4 (77.6, 94.1)	81.0 (77.1, 88.3)	91.7 (81.3, 98.6)	0.10
FEV_1/FVC	0.78 (0.71, 0.81)	0.79 (0.77, 0.82)	0.73 (0.67, 0.8)	0.02
GOLD COPD, n (%)	4 (18.2)	1 (9.1)	3 (27.3)	0.22
DLco % predicted	64.1 (56.7, 67.5)	65.7 (60.9, 67.8)	61.7 (54.9, 66.8)	0.55
Fraction of lung voxels below -950 HU	1.4 (0.6, 3.1)	0.6 (0.5, 1.2)	1.5 (1.1, 3.8)	0.24
St. George's Total Score	2.8 (0.8, 8.4)	2.5 (0.8, 7.4)	2.9 (1.0, 15.1)	0.65
St. George's Activity Score	6.1 (0.0, 17.1)	6.2 (0.0, 14.7)	6.0 (0.0, 26.3)	0.84
St. George's Symptom Score	5.0 (0.0, 13)	4.9 (2.5, 7.3)	5.1 (0.0, 16.3)	0.17

Abbreviations: GOLD COPD, Chronic obstructive pulmonary disease by Global Initiative on Chronic Lung disease criteria; DLco, diffusing capacity for carbon monoxide adjusted for hemoglobin and carboxyhemoglobin; FEV_1, forced expiratory volume in one second after bronchodilator; FVC, forced vital capacity after bronchodilator; HU, Hounsfield units; SD, standard deviation; Q, quartile

Supplementary Figure 1: CONSORT diagram

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