Atorvastatin reduces serum cholesterol and triglycerides with limited improvement in vascular function in adults with sickle cell anemia

Although red cell rigidity is clearly the principal pathophysiological component of sickle cell anemia (SCA), there is substantial evidence also implicating disordered blood vessel wall function. Many of these abnormalities, such as vascular dysfunction and associated biomarkers, are also seen in atherosclerosis and other vasculopathies, and improve with statin therapy. Besides lowering serum cholesterol, statins indirectly act as Rho kinase inhibitors which activate endothelial nitric oxide synthase (NOS). NOS-related blood flow increases within one month in hypercholesterolemic patients treated with a low dose of atorvastatin (10 mg daily),[1] and within 24 h in healthy subjects treated with high-dose atorvastatin (80 mg daily).[2] Statins have shown promise for SCA in the sickle cell mouse,[3] in human neutrophils *ex vivo*,[4] and in children with sickle cell disease.[5]

SCA subjects were selected from the NIH sickle cell cohort to enrich for vascular dysfunction evidenced by any of the following characteristics: a higher than median plasma level of soluble vascular cell adhesion molecule (sVCAM-1) (>795 ng/mL; 14 subjects),[6] or a tricuspid regurgitant velocity (TRV) ≥2.5 m/s by Doppler echocardiography (11 subjects; 2 of the high sVCAM-1 group also had TRV ≥2.5 m/s).[7] Other inclusion and exclusion criteria were similar to a previous study.[8] African-American adults without sickle cell trait served as controls only for characterization of baseline blood flow, and did not participate in the treatment study. All subjects signed informed consent forms for a research protocol approved by the institutional review board of the National Heart, Lung and Blood Institute (*ClinicalTrials.gov identifier NCT00072826*). Data were analyzed with the Prism 4.0 statistical package (GraphPad Software, La Jolla, CA, USA), with Spearman's correlations, paired t-test, and one-way or two-way analysis of variance of the mean (ANOVA) with repeated measures as appropriate. $P<0.05$ was considered significant.

Twenty-five subjects with SCA (11 male, 14 female; median age 36 years) and 10 healthy subjects (6 male, 4 female; median age 37 years) were enrolled. Detailed laboratory characteristics are provided in the *Online Supplementary Table S1*.

Forearm blood flow (FBF) measurements were performed by means of strain gauge venous-occlusion plethysmography. Briefly, a mercury-filled silastic strain gauge was placed around the widest portion of the forearm, and connected to a plethysmograph calibrated to measure the percent change in volume. The plethysmograph is connected to a computer for FBF measurements following inflation of a wrist cuff to suprasystolic pressure to exclude the hand circulation. A blood pressure cuff on the upper arm was inflated to 50 mmHg for 7 s with a rapid cuff inflator in order to occlude venous outflow, but not arterial inflow, into the forearm. This causes indiscernible distention of the forearm at a rate proportionate to arterial inflow. A series of 7 sequential blood flow measurements are averaged for each blood flow value. Blood pressure was recorded directly from the intra-arterial catheter immediately after each series of flow measurements.

Subjects with sickle cell disease underwent catheterization of the brachial artery with baseline blood sampling. FBF was measured following 20 min of normal saline infused at 1 mL/min into the brachial artery. The endothelium-independent vasodilator sodium nitroprusside was infused at 0.8, 1.6, and 3.2 μg/min, respectively, in order to test the vascular responsiveness to an NO donor. After 3

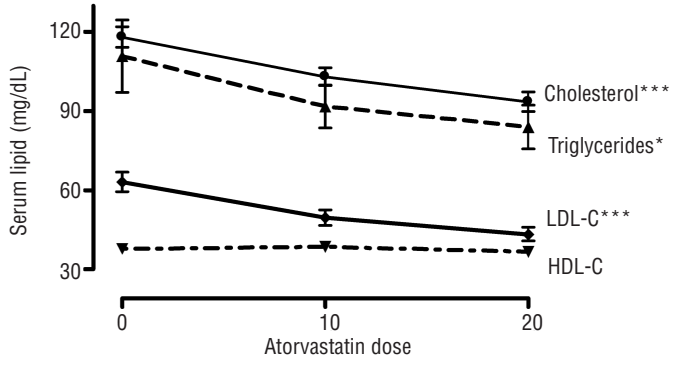

Figure 1. Effect of atorvastatin on serum lipid levels. Administration of atorvastatin to 25 subjects with sickle cell disease at 10 or 20 mg daily yielded a dose-dependent significant reduction of total serum cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C).

Table 1. Specific and percentage changes in lipid level. Data points represent mean values and error bars indicate the standard error of the mean. Significance was tested by analysis of the variance of the mean with repeated measures, and post-test for linear trend.

Lipid		Baseline	Atorvastatin 10 mg	Atorvastatin 20 mg	P
Cholesterol	Mean ± SEM	118±4	103±3	94±2	***
	% change from baseline		-13%	-21%	
Triglycerides	Mean ± SEM	111±14	92±8	84±8	*
	% change from baseline		-17%	-24%	
LDL-C	Mean ± SEM	63±4	50±3	43±3	***
	% change from baseline		-21%	-31%	
HDL-C	Mean ± SEM	38±2	38±2	37±2	n.s.
	% change from baseline		+2%	-3%	

***P<0.0001, one-way ANOVA with repeated measures; P<0.0001, post-test for linear trend; *P<0.05, one-way ANOVA with repeated measures; P<0.01, post-test for linear trend.

min of each infusion, forearm flows were measured. After a 20-min rest period, another baseline measurement was obtained and the endothelium-dependent vasodilator acetylcholine was infused at 7.5, 15, and 30 μg/min, respectively, to test for endothelial release of relaxant factors including NO. After 3 min of each infusion, forearm flows were measured. Following a 30-min rest period, the NOS inhibitor L-NMMA was infused at 4 and 8 μmol/min, to test basal production of NO. After 3 min of each infusion, FBF was measured. At the conclusion of the measurements on L-NMMA at 8 μmol/min, combined infusion of L-NMMA 8 μmol/min with acetylcholine at 30 μg/min for 3 min was administered, and then FBF was measured. The extent to which L-NMMA reduced acetylcholine-induced vasodilation indicate the degree of agonist-stimulated NOS-dependent FBF.

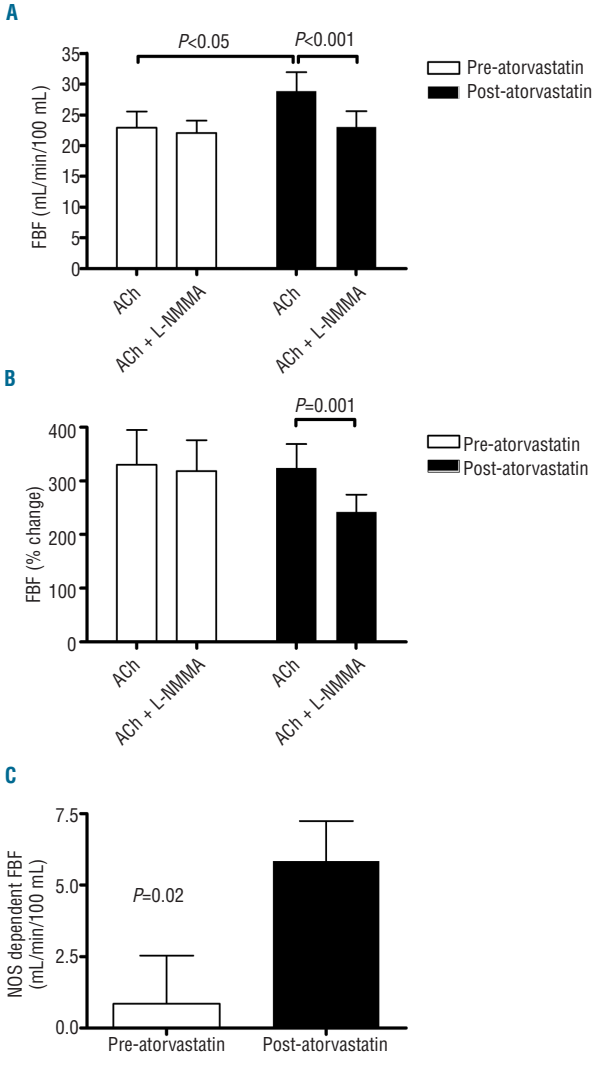

Figure 2. Atorvastatin use is associated with an increase in agonist-induced, NOS-dependent forearm blood flow. Vasodilatory responses to brachial artery infusions of acetylcholine (ACh, 30 μg/min) and the NOS inhibitor L-NG-monomethylarginine (L-NMMA, 8 μmoL/min) were measured by venous occlusion strain gauge plethysmography before and after four weeks of atorvastatin administration. Prior to atorvastatin administration (white bars), the L-NMMA-sensitive fraction of ACh response was negligible, but after atorvastatin treatment response was statistically significant, expressed in absolute forearm blood flow (A), or as percentage change in blood flow (B). The amount of acetylcholine-induced, NOS-dependent blood flow calculated for each subject is significantly increased after atorvastatin treatment (C). Bars indicate means, and error bars indicate standard error of the mean. Significance was tested by paired t-test.

The subjects with SCA demonstrated baseline FBF characteristics that confirmed the previously reported defect in nitric oxide-dependent blood flow (*Online Supplementary Figure S1*).[8,9] Our recruitment strategy successfully enriched for SCA subjects with vascular dysfunction. All enrolled SCA subjects had a blunted vasodilatory response to a nitric oxide (NO) donor compared to the healthy subjects that recapitulates that previously reported in SCA mice,[10,11] and in approximately three-quarters of sickle cell anemia patients.[8,9,12]

Atorvastatin significantly reduced serum cholesterol, low density lipoprotein cholesterol (LDL-C), and triglyceride levels in a dose-dependent manner (Figure 1). These changes were equivalent in both male and female subgroups. There was no change in serum high density lipoprotein cholesterol (HDL-C). These observations indicate successful inhibition of HMG CoA reductase, the intended target of statin drugs, and provide functional evidence of subjects' adherence to study treatment. Despite the statistically significant favorable effect of atorvastatin upon lipid profiles, vasodilatory responses to an NO donor and NOS inhibitor were not affected (*Online Supplementary Figure S2*). Because change in NOS inhibitor response was the pre-specified primary outcome variable, this is, therefore, a negative study. However, some of the important pre-specified secondary outcome variables showed statistically significant changes in vascular function induced by atorvastatin therapy. Following the combined total of four weeks of atorvastatin treatment, a small, but statistically significant increase in ACh responsiveness was observed, suggesting increased function of endothelium (P=0.002, *Online Supplementary Figure 2C*). The vasodilatory response to the highest dose of ACh was examined with and without the higher dose of the NOS inhibitor L-NMMA. Not only was the ACh peak vasodilatory response to ACh greater on atorvastatin (mean±standard error of the mean 23.0±2.6 *vs.* 28.9±3.1 mL/min/100mL, P<0.05, paired t-test), the decrease in ACh response elicited by NOS blockade was statistically significant on atorvastatin (28.9±3.1 *vs.* 23.0 ±2.6 mL/min/100mL, P<0.001), but not prior to atorvastatin (Figure 2A). This observation was also supported by the relative change in FBF under those conditions (323±45 *vs.* 241± 34% FBF increase from baseline, P=0.001; Figure 2B). There was a marked change in the amount of NOS-dependent, ACh-stimulated blood flow following atorvastatin treatment (0.9±1.7 *vs.* 5.8±1.4 mL/min/100mL, P=0.02; Figure 2C), suggesting improved endothelial NOS function. In gender subgroup analyses, females consistently demonstrated significantly higher vasodilatory responses to ACh than males, but males and females responded equivalently to atorvastatin. The blood flow results are negative for the primary hypothesis, but the secondary outcome blood flow variables suggest some degree of favorable response to atorvastatin, which must be interpreted with caution.

Several markers of endothelial activation, inflammation and clinical outcome did not change during the four weeks of atorvastatin treatment, including hemoglobin levels, fetal hemoglobin, lactate dehydrogenase, bilirubin, C-reactive protein, plasma levels of sVCAM-1, monocyte chemokines RANTES and MIP-1b (*Online Supplementary Figure S3*), or tricuspid regurgitant velocity. These results also point to the overall lack of effectiveness of atorvastatin 20 mg in improving relevant vascular biomarkers in adults with SCA.

No evidence of toxicity was observed during the four

weeks of treatment with atorvastatin. Serum alanine aminotransferase and creatine kinase levels remained unchanged from baseline. Adverse events during the study included 4 acute pain episodes, 3 of which required hospitalization, equivalent to 1.59 hospitalizations per patient-year during enrollment on the study, this is the same as in SCA population statistics. One of these episodes involved transient mental status changes, a small pulmonary infiltrate and mild transient renal insufficiency, all of which resolved after transfusion of two units of packed red blood cells. This patient was removed from the study due to an exclusion criterion of transfusion within two weeks of entry or end point.

Much has been written about the pleiotropic effects of statins in the general population to improve clinical outcomes related to vasculopathies such as atherosclerosis. Part of this activity derives from statin activation of NOS activity. It has been speculated that these vasculoprotective effects might be useful in SCA.[3-5] Our SCA clinical trial of atorvastatin at moderate doses with physiological outcome measures is a negative study for its primary purpose. However, there are still several findings of scientific value that emerge from this project: 1) reproducing previous baseline findings of nitric oxide resistance in SCA; 2) developing an enrichment strategy for recruitment that resulted in 100% of the SCA subjects having characteristics of nitric oxide resistance; 3) secondary outcome variables indicating some evidence that endothelial function is being impacted, albeit to a smaller degree than expected. This last finding raises the question as to whether higher doses of atorvastatin or earlier intervention for longer duration should be considered for future investigation to delay vascular dysfunction in SCA patients.

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The online version of this article has a Supplementary Appendix.

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References

1. Perticone F, Ceravolo R, Maio R, Cloro C, Candigliota M, Scozzafava A, et al. Effects of atorvastatin and vitamin C on endothelial function of hypercholesterolemic patients. Atherosclerosis. 2000;152(2):511-8.
2. Laufs U, Wassmann S, Hilgers S, Ribaudo N, Bohm M, Nickenig G. Rapid effects on vascular function after initiation and withdrawal of atorvastatin in healthy, normocholesterolemic men. Am J Cardiol. 2001;88(11):1306-7.
3. Solovey A, Kollander R, Shet A, Milbauer LC, Choong S, Panoskaltsis-Mortari A, et al. Endothelial cell expression of tissue factor in sickle mice is augmented by hypoxia/reoxygenation and inhibited by lovastatin. Blood. 2004;104(3):840-6.
4. Canalli AA, Proença RF, Franco-Penteado CF, Traina F, Sakamoto TM, Saad ST, et al. Participation of Mac-1, LFA-1 and VLA-4 integrins in the in vitro adhesion of sickle cell disease neutrophils to endothelial layers, and reversal of adhesion by simvastatin. Haematologica. 2011;964:526-33.
5. Hoppe C, Kuypers F, Larkin S, Hagar W, Vichinsky E, Styles L. A pilot study of the short-term use of simvastatin in sickle cell disease: effects on markers of vascular dysfunction. Br J Haematol. 2011;153(5):655-63.
6. Kato GJ, Martyr S, Blackwelder WC, Nichols JS, Coles WA, Hunter LA, et al. Levels of soluble endothelium-derived adhesion molecules in patients with sickle cell disease are associated with pulmonary hypertension, organ dysfunction, and mortality. Br J Haematol. 2005;130(6):943-53.
7. Gladwin MT, Barst RJ, Castro OL, Gordeuk VR, Hillery CA, Kato GJ, et al. Pulmonary hypertension and NO in sickle cell. Blood. 2010;116(5):852-4.
8. Gladwin MT, Schechter AN, Ognibene FP, Coles WA, Reiter CD, Schenke WH, et al. Divergent nitric oxide bioavailability in men and women with sickle cell disease. Circulation. 2003;107(2):271-8.
9. Belhassen L, Pelle G, Sediame S, Bachir D, Carville C, Bucherer C, et al. Endothelial dysfunction in patients with sickle cell disease is related to selective impairment of shear stress-mediated vasodilation. Blood. 2001;97(6):1584-9.
10. Kaul DK, Liu XD, Fabry ME, Nagel RL. Impaired nitric oxide-mediated vasodilation in transgenic sickle mouse. Am J Physiol Heart Circ Physiol. 2000;278(6):H1799-806.
11. Nath KA, Shah V, Haggard JJ, Croatt AJ, Smith LA, Hebbel RP, et al. Mechanisms of vascular instability in a transgenic mouse model of sickle cell disease. Am J Physiol Regul Integr Comp Physiol. 2000;279(6):R1949-55.
12. Eberhardt RT, McMahon L, Duffy SJ, Steinberg MH, Perrine SP, Loscalzo J, et al. Sickle cell anemia is associated with reduced nitric oxide bioactivity in peripheral conduit and resistance vessels. Am J Hematol. 2003;74(2):104-11.

Predictors and short-term outcomes of hyperleukocytosis in children with acute myeloid leukemia: a report from the Children's Oncology Group

Adults and children with acute myeloid leukemia (AML) who present with a high initial white blood cell count (WBC) have poor outcomes, and a consistent association with induction death has been observed when the initial WBC is 100×10^9/L or over.[1] However, rates of induction death have varied between trials.[2,3] Furthermore, major advances in supportive care have been incorporated into routine clinical care such as prompt transfusion of platelets, leukapheresis, hydroxyurea, urate oxidase and hemodialysis. With these advances in supportive care, it is unclear whether poor outcomes with hyperleukocytosis in pediatric AML continue to be problematic.

The Children's Oncology Group (COG) adopted a modified AML Medical Research Council backbone and