Published in final edited form as:

Br J Haematol. 2011 June ; 153(5): 655–663. doi:10.1111/j.1365-2141.2010.08480.x.

A pilot study of the short-term use of simvastatin in sickle cell disease: effects on markers of vascular dysfunction

Carolyn Hoppe¹, Frans Kuypers², Sandra Larkin², Ward Hagar³, Elliott Vichinsky¹, and Lori Styles¹

¹Department of Haematology/Oncology, Children's Hospital & Research Center Oakland, Oakland, CA, USA

²Red Cell Laboratory, Children's Hospital Oakland Research Institute, Oakland, CA, USA

³Adult Sickle Cell Center, Children's Hospital Oakland Research Institute, Oakland, CA, USA

Summary

Sickle cell disease (SCD) is characterized by progressive vascular injury and its pathophysiology is strikingly similar to that of atherosclerosis. Statins decrease inflammation and improve endothelial function in cardiovascular disease, but their effect in SCD is not known. In this pilot study, we examined the safety and effect of short-term simvastatin on biomarkers of vascular dysfunction in SCD. We treated 26 SCD patients with simvastatin, 20 or 40 mg/d, for 21 d. Plasma nitric oxide metabolites (NOx), C-reactive protein (CRP), interleukin-6 (IL-6), vascular cell adhesion molecule-1 (VCAM-1), tissue factor (TF) and vascular endothelial growth factor (VEGF) were analyzed and responses to simvastatin were compared between the two treatment groups. Simvastatin increased NOx levels by 23% in the low-dose ($P = 0.01$) and 106% in the moderate-dose ($P = 0.01$) groups, and by 52% overall ($P = 0.0008$). CRP decreased similarly in both dose groups and by 68% overall ($P = 0.02$). Levels of IL-6 decreased by 50% ($P = 0.04$) and 71% ($P < 0.05$) in the low- and moderate-dose groups, respectively. Simvastatin had no effect on VEGF, VCAM1 or TF. Simvastatin was well-tolerated and safe. Our preliminary findings showing a dose-related effect of simvastatin on levels of NOx, CRP and IL-6 suggest a potential therapeutic role for simvastatin in SCD.

Keywords

sickle cell disease; statin; nitric oxide; inflammation

Sickle cell disease (SCD) is now recognized as a chronic, progressive vasculopathy that can lead to irreversible organ damage. This chronic organ damage is the most frequent cause of death beyond childhood (Powars *et al*, 2005). Although red blood cell sickling and rheologic abnormalities are central to the pathophysiology of SCD, the endothelium and its response to injury cells plays a major role in the vaso-occlusive manifestations of this disease. Reperfusion injury resulting from transient occlusive events, oxidative stress and chronic inflammation lead to a state of sustained endothelial activation (Hebbel *et al*, 2004). Systemic inflammation has been demonstrated experimentally and clinically by increased leukocyte production and elevations in pro-inflammatory cytokines (Belcher *et al*, 2003, 2000; Blann *et al*, 2003; Bourantas *et al*, 1998; Schnog *et al*, 2004a). Nearly every

manifestation of SCD correlates with leukocytosis and, even at baseline, patients exhibit an acute-phase response marked by elevated levels of tumour necrosis factor (TNF), interleukins and high sensitivity C-reactive protein (hsCRP) (Bourantas *et al*, 1998; Platt, 2000; Singhal *et al*, 1993). Increased expression of leukocyte adhesion molecules has also been associated with more severe disease and experimental studies support a direct role for leukocytes in microvascular occlusion (Okpala *et al*, 2002; Turhan *et al*, 2002).

In addition to inflammation, pathophysiologic responses to endothelial injury in SCD also involve endothelial adhesion and activation of coagulation. Cellular interactions with the endothelium trigger increased expression of tissue factor (TF) and vascular cell adhesion molecule (VCAM-1), causing further adherence of sickle red cells and leukocytes, as well as platelet activation (Kaul *et al*, 1995; Solovey *et al*, 1998, 2001). At the same time, production of cytoprotective mediators, such as nitric oxide (NO), are consumed by the oxidative by-products of ischemia/reperfusion injury (Chiang & Frenette, 2005; Kato & Gladwin, 2008; Makis *et al*, 2000).

Decreased NO bioavailability has been observed at 'steady state', and in association with vaso-occlusive pain and acute chest syndrome (ACS) (Gladwin *et al*, 1999; Morris *et al*, 2000; Sullivan *et al*, 2008). Related studies have shown that plasma NO consumption and the uninhibited production of reactive oxygen species exacerbate vascular injury through activation of inflammatory responses (Klings & Farber, 2001; Osarogiagbon *et al*, 2000). Restoring normal endothelial function with therapeutic agents that target inflammation and NO dysregulation may thus have a favourable impact on the vasculopathy of SCD.

Emerging data indicate that many of the clinical benefits of statins are independent of their cholesterol-lowering effects and are largely conferred through modulation of NO, via inhibition of Ras/Rho proteins and direct upregulation of endothelial nitric oxide synthase (eNOS) (Hernandez-Perera *et al*, 1998; Kano *et al*, 1999; Laufs *et al*, 1998). The hypothesis that statins afford protection from endothelial injury through their direct action on NO production has been confirmed by studies in eNOS deficient mice subjected to stroke and myocardial ischemia (AminHanjani *et al*, 2001; Asahi *et al*, 2005; Endres & Laufs, 2004; Endres *et al*, 1998; Sironi *et al*, 2003). In subsequent studies, statins have been shown to down-regulate inflammation, adhesion and thrombosis through NO-mediated pathways (Diomedea *et al*, 2001; Egashira *et al*, 2000; Kimura *et al*, 1997; Lefer *et al*, 1999; Niwa *et al*, 1996; Pruefer *et al*, 1999; Sparrow *et al*, 2001). The clinical benefit derived from statins has been demonstrated by improved endothelial function in several disease populations, including diabetes, stroke and metabolic syndrome as well as cardiovascular disease (Cucchiara & Kasner, 2001; Danesh & Kanwar, 2004; Endres *et al*, 1998; Feske *et al*, 2009; Hernandez-Perera *et al*, 1998; Kureishi *et al*, 2000; Laufs *et al*, 1998; Rikitake *et al*, 2005; Tan *et al*, 2002; van de Ree *et al*, 2003; Yamada *et al*, 2000).

The mechanistic potential of statins to improve vascular function may be extended to the pathophysiology of SCD. Data from transgenic mouse models indicate that statins attenuate endothelial activation in SCD. Solovey *et al*, (2004) found that in sickle mice subjected to hypoxia-reperfusion injury, lovastatin inhibited an expected increase in TF expression. In a more recent study, pretreatment with statins improved survival in pneumococcus-infected sickle mice with direct demonstration of an anti-inflammatory effect. Preliminary data from a limited study in SCD patients with pulmonary hypertension showed improvement in measures of NO-dependent vascular dysfunction after treatment with atorvastatin (Kato *et al*, 2004).

Although simvastatin has been extensively studied and marketed for use in other patient populations, its clinical safety and potential efficacy has not been studied in SCD. The aim

of this pilot study was to examine the effect of treatment with short-term simvastatin (20 and 40 mg/d) on plasma NO and selected biomarkers of endothelial dysfunction (IL-6, hs-CRP, VCAM-1, TF, and VEGF) in subjects with SCD.

Methods

Study subjects

Patients at least 13 years of age, with all genotypes of SCD were eligible. Patients were recruited at their baseline status without acute pain or SCD-related complications requiring hospitalization. Patients treated with hydroxyurea (HU) at a stable dose (>3 months) were eligible.

Exclusion criteria consisted of a pretreatment serum total cholesterol <100 mg/dl, triglycerides (TG) below lower normal limits (LNL) (<40 mg/dl), creatine kinase (CK) greater than upper normal limits (UNL) (>232 U/l), serum creatinine >1.5-fold UNL (1.8 mg/dl), alanine transaminase (ALT) >2-fold UNL (>130 U/l). Other exclusion criteria were pregnancy/lactation, red cell transfusion or hospitalization within the 30 d prior to enrollment, current treatment with statins, amiodarone or other drugs with known metabolic interactions with statins (e.g. cytochrome P450 3A4 metabolism), an underlying musculoskeletal disorder, a positive urine toxicology screen or known history of cocaine or amphetamine use. This study was approved by the Institutional Review Board at Children's Hospital & Research Center Oakland (CHRCO). All subjects or their parents provided written informed consent.

Study design

The study was a phase I/II safety and dose escalation trial in which participants were treated with one of two doses (20, 40 mg/d) of oral simvastatin daily for 21 d, followed by a taper over 4 d. A dose-escalating approach was used, whereby demonstration of clinical safety in all subjects enrolled in the low dose (20 mg/d) group was required prior to enrollment of subjects in the moderate dose (40 mg/d) group. Serial non-fasting blood samples were collected at baseline, days 7, 14 and 21 during treatment, and days 25 and 39 after treatment, for laboratory evaluation of clinical safety and measurement of plasma biomarker levels.

Measurement of plasma biomarkers

Lipid profiles, complete blood counts and routine chemistries were conducted using standard laboratory techniques in the clinical laboratory at CHRCO. Plasma samples were assayed for the concentration of NO metabolites, nitrite/nitrate (NO_x), using an NO chemiluminescence analyzer (Sievers Model 280 NOA; Sievers Instruments, Boulder, Colorado, USA), as previously described (Braman & Hendrix, 1989). Hs-CRP was measured by a high-sensitivity (0.01 mg/dl) Plasma hs-CRP was determined by a latex particle enhanced immunochemistry assay (Vitros Model 5,1 FS Chemistry Systems, Johnson & Johnson Clinical Diagnostics Inc., Rochester, NY, USA). Plasma IL-6 and VCAM-1 were measured by ultrasensitive ELISA according to the manufacturer's instructions (Quantikine HS Human IL-6 Immunoassay, Parameter Human sVCAM-1 Immunoassay; R&D Systems, Minneapolis, MN, USA). Plasma TF and VEGF were similarly measured with a commercial ELISA kit (R&D Systems).

Statistical analysis

Descriptive and experimental measures were expressed as mean $\pm$ SD. Baseline characteristics were compared between the two treatment groups using unpaired *t*-tests for continuous variables and Fisher's exact test for discrete variables. Changes in biomarker levels from baseline in response to simvastatin were assessed using matched paired *t*-tests.

Spearman correlations were performed where appropriate. A two-tailed $P < 0.05$ was considered significant. Statistical analysis was performed with STATA (version 9.0; STATA Corporation, College Station, TX, USA) and GraphPad Prism statistical software (version 5.01; GraphPad, San Diego, CA, USA).

Results

Baseline characteristics

A total of 26 subjects participated in the study, with 14 subjects in the low dose (20 mg/d) group and 12 subjects in the moderate dose (40 mg/d) group.

A greater number of subjects with a HbSS genotype were enrolled in the low dose group ($n = 12$; 86%) compared to the moderate dose group ($n = 5$; 42%) ($P = 0.04$). Of the 17 subjects with HbSS, nine were on stable hydroxyurea (HU) therapy. With the exception of SCD genotype, there were no clinically significant differences between the two dose groups at baseline.

Simvastatin Effects on Lipid Profiles and Clinical Safety Measurements

Transient, asymptomatic elevations in CK (2–3X UNL) were observed in two subjects on day 21 of treatment, with a rapid return to baseline within 1 week after discontinuation of simvastatin. Five subjects experienced a mild sickle cell pain episode during the study, but did not require hospitalization. All five subjects were managed in the outpatient setting with oral pain medications. Consistent with previous studies, plasma NOx levels rapidly declined in these five subjects during the vaso-occlusive episode (VOE) (Fig 1) (Morris *et al*, 2000). Levels of hs-CRP and IL-6 simultaneously increased, returning to baseline after resolution of the VOE. As this study was intended to determine the effects of simvastatin treatment at 'steady-state', data points corresponding to these vaso-occlusive pain events were not included in the analysis.

Simvastatin reduced total cholesterol and low-density lipoprotein (LDL) cholesterol levels, indicating general compliance with the study drug. This effect did not appear to be dose-related, as the total cholesterol and LDL cholesterol levels decreased by the same extent in both treatment groups. Serum high-density lipoprotein (HDL) cholesterol and TG levels were unaffected by simvastatin (Table I). Renal, hepatic and hematologic studies, as measured by serum complete metabolic panel and complete blood count (CBC), were also unaffected by simvastatin treatment. Simvastatin had no effect in either treatment group on laboratory markers of hemolysis, including haemoglobin (Hb), reticulocyte count, lactate dehydrogenase (LDH), and total bilirubin.

Simvastatin effects on plasma NO and markers of inflammation, coagulation and adhesion

Changes in biomarker levels for the two treatment groups are shown in Table II. Plasma NOx levels increased by 23% in the low dose group, and by 106% in the moderate dose group (Fig 2). Plasma IL-6 levels decreased by 50% ($P = 0.04$) and 71% ($P < 0.05$) in the low and moderate dose groups, respectively. The change in hs-CRP levels did not show the same dose-effect, decreasing by 73% and 60% in the low and moderate dose groups, respectively. After discontinuation of simvastatin on day 21, levels of NOx, IL-6 and hs-CRP returned to baseline within 2 weeks. Analysis of the two treatment groups combined showed a significant impact of simvastatin on the levels of NOx ($P = 0.0008$), hs-CRP ($P = 0.02$) and IL-6 ($P = 0.01$) (Table II). Although VEGF levels decreased overall ($P = 0.04$), this effect did not reach significance in the analysis of individual treatment groups. Similarly, simvastatin had no effect on plasma levels of sVCAM-1 or TF levels.

Plasma NOx levels varied with respect to HU use in subjects with HbSS. Baseline NOx levels were higher in subjects on HU therapy ($40 \pm 26 \mu\text{mol/l}$; $n = 9$) compared to those who were not taking HU ($15.4 \pm 12.4 \mu\text{mol/l}$; $n = 8$) ($P = 0.02$). However, the observed increase in NOx levels after simvastatin treatment was much greater in subjects who were not taking HU, rising by 98% from baseline in this group ($P = 0.002$) compared to only 16% in HU-treated subjects ($P = 0.13$) (Fig 3). Excluding the subjects who were on HU therapy from the overall analysis further increased the magnitude of the simvastatin treatment effect on NOx levels, with NOx increasing by 50% ($P = 0.02$) in the low dose group and by 120% ($P = 0.01$) in the moderate dose group. Hydroxyurea therapy had no significant effect on hs-CRP and IL-6 levels at baseline, or after simvastatin treatment.

Levels of hs-CRP correlated with IL-6 (Spearman $r = 0.5$; $P = 0.03$) and with NOx (Spearman $r = 0.5$; $P < 0.05$). No correlations were found between the increase in NOx level and levels of the other biomarkers measured (hs-CRP, IL-6, VEGF, VCAM, TF). There were no significant correlations between the increase in NOx with simvastatin and hematologic or lipid parameters.

Discussion

Sickle cell disease is characterized by chronic inflammation and sustained endothelial activation (Hebbel, 2004). With the exception of lipid deposition, the vasculopathy of SCD is remarkably similar to that of atherosclerosis. Markers of endothelial dysfunction, including CRP and IL-6, sVCAM-1, selectins, TF and VEGF are similarly elevated in both diseases (Cleator & Vaughan, 2008; Devaraj *et al*, 2006b; Fontaine *et al*, 2003; Grundy, 2002; Hebbel, 2004; Jialal *et al*, 2006; Li & Fang, 2004).

Numerous experimental studies have shown that statins modulate inflammation, both *in vitro* and *in vivo*. Decreased levels of inflammatory mediators have been observed after treatment with statins in clinical trials of atherosclerotic heart disease, diabetes, stroke and metabolic syndrome (Albert *et al*, 2001; Blake & Ridker, 2000; Kinlay *et al*, 2008; Koh, 2000; Sacks *et al*, 1996; Shah & Newby, 2003). However, there have been no studies to date examining the effect of statins on biomarkers of inflammation and endothelial damage in patients with SCD. In this pilot efficacy study, short-term treatment of SCD subjects with simvastatin (20 and 40 mg/d) had a favourable impact on biomarkers of endothelial injury and inflammation, as indicated by a dose-related increase in NOx, and concomitant reductions in hs-CRP and IL-6 levels.

Patients with SCD exhibit elevated levels of circulating CRP and other inflammatory mediators, even at 'steady state' (Akinola *et al*, 1992; Krishnan *et al*, 2010; Makis *et al*, 2006; Pathare *et al*, 2004). We observed similarly elevated levels of hs-CRP and IL-6 in subjects at baseline, prior to treatment with simvastatin. These results indicate a state of sustained low-grade inflammation in SCD during asymptomatic periods. The correlation between baseline hs-CRP and IL-6 found in our study population is not surprising, as IL-6 produced in response to subclinical vaso-occlusion stimulates production of acute phase proteins such as CRP (Yudkin *et al*, 2000). Moreover, in the five subjects who developed a mild VOE during the study, we observed a sharp decline in NOx and simultaneous increase in hs-CRP and IL-6 levels at the onset of reported symptoms. Decreased NOx levels have previously been reported in association with vaso-occlusive pain events and acute chest syndrome (ACS) (Morris *et al*, 2000; Reiter *et al*, 2002; Schnog *et al*, 2005). These findings also agree with previous studies showing positive correlations between hs-CRP levels and the frequency of hospitalizations for VOE, as well as the severity of SCD (Krishnan *et al*, 2010; Makis *et al*, 2006). We cannot rule out the possibility that the increase in NO (via upregulation of neuronal NOS) with simvastatin treatment may have contributed to the VOE

observed in this study. With larger numbers and longer duration, a clinical trial is necessary to definitively answer this question. Although it was not possible to isolate the influence of SCD genotype on biomarker levels in this limited study, subjects with an HbSS genotype tended to have lower baseline NOx levels and higher baseline hs-CRP and IL-6 levels. Further studies are needed to address the influence of genotype on these biomarkers of endothelial dysfunction in SCD.

Plasma NOx levels increased with simvastatin in both treatment groups and more than doubled in the moderate dose group, consistent with numerous studies in other vascular disease populations showing statin-associated increases in NO production (Cucchiara & Kasner, 2001; Danesh & Kanwar, 2004; Laufs, 2003; Liao & Laufs, 2004; Romano *et al*, 2000; Seljeflot *et al*, 2002). We cannot exclude the possible influence of dietary NO sources on the measured changes in NOx levels, as subjects were not required to fast or restrict their diet prior to collection of blood samples. However, the consistent pattern of NOx changes in response to simvastatin suggests that dietary NO had a nominal effect on our results.

Hydroxyurea has been shown to increase intravascular generation of NO in SCD (Gladwin *et al*, 2002; Glover *et al*, 1999) and patients treated with HU demonstrate elevated plasma NOx levels (Cokic *et al*, 2003; Gladwin *et al*, 2002; Huang *et al*, 2002; Sato *et al*, 1997). In our sub-analysis examining the effect of HU on biomarker responses to simvastatin, the observed increase in NOx levels was restricted almost exclusively to subjects who were not on HU therapy. Despite the small number of subjects included in this analysis, it is conceivable that subjects who were regularly taking HU had achieved a ceiling effect in NOx levels, beyond which simvastatin had little further effect.

Our results showing a dose-related increase in NOx and a simultaneous decrease in IL-6 levels imply that simvastatin may be more effective at higher doses. The lack of a similar dose-effect of simvastatin on hs-CRP levels agrees with findings from a recent meta-analysis of clinical cardiovascular disease trials showing the absence of correlation between statin dose and hs-CRP level (Balk *et al*, 2003; Genser *et al*, 2008; Liem *et al*, 2008; van de Ree *et al*, 2003).

Nitric oxide deficiency, resulting from oxidant-induced consumption or loss of biological function, drives the inflammatory, pro-adhesive and thrombotic processes that cause endothelial injury. The vasculoprotective effects of statins beyond cholesterol lowering have been shown in several experimental studies to be modulated by NO through direct upregulation of eNOS (Endres *et al*, 1998; Hernandez-Perera *et al*, 1998; Kureishi *et al*, 2000; Laufs *et al*, 1998; Yamada *et al*, 2000). In animal models, pretreatment with simvastatin has been shown to upregulate eNOS, decrease adhesion molecule expression and attenuate leukocyte rolling (Gertz *et al*, 2003; Hernandez-Perera *et al*, 1998; Laufs *et al*, 2000, 1998; Pruefer *et al*, 2002; Santizo *et al*, 2002). Similar anti-inflammatory effects were recently demonstrated in statin-treated sickle cell mice showing prolonged survival following pneumococcal infection (Rosch *et al*, 2010).

In a recent study of patients with metabolic syndrome, simvastatin was found to decrease hs-CRP and IL-6 levels through regulation of nuclear transcription factors, NFkB and Akt (Devaraj *et al*, 2006a). In vitro studies of human monocytes incubated with lovastatin have documented similar changes in IL-6 levels and NFkB activity (Bustos *et al*, 1998; Grip *et al*, 2002; Ortego *et al*, 1999).

Solovey *et al*, previously demonstrated that hypoxia/reoxygenation-induced endothelial TF expression could be inhibited by lovastatin in sickle mice and more recently documented that endothelial downregulation of TF is modulated by eNOS (Solovey *et al*, 2010, 2004). Similar results have been reported in other vascular disease populations showing that statins,

through NO-mediated upregulation, decrease TF expression and thereby oppose coagulation activation (Undas *et al*, 2005). In our study, simvastatin only modestly decreased plasma TF levels without reaching statistical significance. The lack of an effect on TF levels in our study may be explained by the involvement of other transcription factors or mechanistic pathways that are independent of statins. It is also possible that the optimal dose required to have an effect on TF had not been reached in this pilot study. Alternatively, as TF is differentially expressed in various tissues, circulating plasma levels may not reflect tissue levels. Solovey *et al*, (2004) found that TF expression was localized to the pulmonary vasculature in sickle mice. Moreover, treatment with lovastatin had no effect on TF expression in monocytes, and only modestly decreased pulmonary endothelial VCAM expression after hypoxic challenge. Plasma VCAM levels were similarly unaffected by simvastatin in our study. Additional studies, particularly in humans, are needed to examine tissue-specific biomarker levels and the influence of statins on TF, VCAM and other mediators of endothelial dysfunction.

Despite initial concerns over the cholesterol-lowering effects of simvastatin on red cell membrane biology and the potential for increased hemolysis, we found no relationship between the reduction in cholesterol and laboratory measures of hemolysis, such as Hb, reticulocyte count, LDH and total bilirubin levels in our study population. Moreover, the observed increase in NOx levels after simvastatin did not correlate with Hb, reticulocyte count, LDH or total bilirubin, suggesting that the effect of simvastatin on NOx may be independent of hemolytic activity.

Except for transient, asymptomatic increases in CK levels, simvastatin was well-tolerated with minimal toxicity in our study population. Five subjects reported mild SCD-related pain that did not differ from their clinical history in terms of frequency, intensity and duration. Four subjects were being treated with HU for a history of recurrent vaso-occlusive episodes and two subjects reported weekly episodes that were controlled with oral opioids.

Although confirmation in larger studies is needed, the results of this pilot study show overall improvements in the levels of NOx and downstream inflammatory markers associated with endothelial dysfunction in SCD. In addition, the positive safety profile of statins observed in the general population is substantiated by the tolerability and lack of serious adverse events in our study population of SCD patients. The present pilot study should lead to larger, randomized, placebo-controlled trials to assess both clinical and mechanistic effects of simvastatin treatment on endothelial function and vascular inflammation in patients with SCD.

Acknowledgments

This work was supported by the FDA Office of Orphan Products Development Grant # 1R01FD-03080-01-A1 (Clin-icalTrials.gov ID NCT00508027) and Grant #UL1 RR024131-01 from the National Center for Research Resources (NCRR).

References

- Akinola NO, Stevens SM, Franklin IM, Nash GB, Stuart J. Subclinical ischaemic episodes during the steady state of sickle cell anaemia. *Journal of Clinical Pathology*. 1992; 45:902–906. [PubMed: 1385483]
- Albert MA, Staggers J, Chew P, Ridker PM. The pravastatin inflammation CRP evaluation (PRINCE): rationale and design. *American Heart Journal*. 2001; 141:893–898. [PubMed: 11376301]
- Amin-Hanjani S, Stagliano NE, Yamada M, Huang PL, Liao JK, Moskowitz MA. Mevastatin, an HMG-CoA reductase inhibitor, reduces stroke damage and upregulates endothelial nitric oxide synthase in mice. *Stroke*. 2001; 32:980–986. [PubMed: 11283400]

- Asahi M, Huang Z, Thomas S, Yoshimura S, Sumii T, Mori T, Qiu J, Amin-Hanjani S, Huang PL, Liao JK, Lo EH, Moskowitz MA. Protective effects of statins involving both eNOS and tPA in focal cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism*. 2005; 25:722–729. [PubMed: 15716855]
- Balk EM, Lau J, Goudas LC, Jordan HS, Kupelnick B, Kim LU, Karas RH. Effects of statins on nonlipid serum markers associated with cardiovascular disease: a systematic review. *Annals of Internal Medicine*. 2003; 139:670–682. [PubMed: 14568856]
- Belcher JD, Marker PH, Weber JP, Hebbel RP, Vercellotti GM. Activated monocytes in sickle cell disease: potential role in the activation of vascular endothelium and vaso-occlusion. *Blood*. 2000; 96:2451–2459. [PubMed: 11001897]
- Belcher JD, Bryant CJ, Nguyen J, Bowlin PR, Kielbik MC, Bischof JC, Hebbel RP, Vercellotti GM. Transgenic sickle mice have vascular inflammation. *Blood*. 2003; 101:3953–3959. [PubMed: 12543857]
- Blake GJ, Ridker PM. Are statins anti-inflammatory? Current Controlled Trials in Cardiovascular Medicine. 2000; 1:161–165. [PubMed: 11714433]
- Blann AD, Marwah S, Serjeant G, Bareford D, Wright J. Platelet activation and endothelial cell dysfunction in sickle cell disease is unrelated to reduced antioxidant capacity. *Blood Coagulation and Fibrinolysis*. 2003; 14:255–259. [PubMed: 12695748]
- Bourantas KL, Dalekos GN, Makis A, Chaidos A, Tsiara S, Mavridis A. Acute phase proteins and interleukins in steady state sickle cell disease. *European Journal of Haematology*. 1998; 61:49–54. [PubMed: 9688292]
- Braman RS, Hendrix SA. Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium (III) reduction with chemiluminescence detection. *Analytical Chemistry*. 1989; 61:2715–2718. [PubMed: 2619057]
- Bustos C, Hernandez-Presa MA, Ortego M, Tunon J, Ortega L, Perez F, Diaz C, Hernandez G, Egido J. HMG-CoA reductase inhibition by atorvastatin reduces neointimal inflammation in a rabbit model of atherosclerosis. *Journal of the American College of Cardiology*. 1998; 32:2057–2064. [PubMed: 9857893]
- Chiang EY, Frenette PS. Sickle cell vaso-occlusion. *Hematology/oncology Clinics of North America*. 2005; 19:771–784. v. [PubMed: 16214643]
- Cleator JH, Vaughan DE. Clinical implications of the contrasting effects of in vivo thrombin receptor activation (protease-activated receptor type 1) on the human vasculature. *Journal of the American College of Cardiology*. 2008; 51:1757–1759. [PubMed: 18452781]
- Cokic VP, Smith RD, Beleslin-Cokic BB, Njoroge JM, Miller JL, Gladwin MT, Schechter AN. Hydroxyurea induces fetal hemoglobin by the nitric oxide-dependent activation of soluble guanylyl cyclase. *Journal of Clinical Investigation*. 2003; 111:231–239. [PubMed: 12531879]
- Cucchiara B, Kasner SE. Use of statins in CNS disorders. *Journal of the Neurological Sciences*. 2001; 187:81–89. [PubMed: 11440749]
- Danesh FR, Kanwar YS. Modulatory effects of HMG-CoA reductase inhibitors in diabetic microangiopathy. *FASEB Journal*. 2004; 18:805–815. [PubMed: 15117885]
- Devaraj S, Chan E, Jialal I. Direct demonstration of an antiinflammatory effect of simvastatin in subjects with the metabolic syndrome. *Journal of Clinical Endocrinology and Metabolism*. 2006a; 91:4489–4496. [PubMed: 16968805]
- Devaraj S, Glaser N, Griffen S, Wang-Polagruto J, Miguelino E, Jialal I. Increased monocytic activity and biomarkers of inflammation in patients with type 1 diabetes. *Diabetes*. 2006b; 55:774–779. [PubMed: 16505242]
- Diomedea L, Albani D, Sottocorno M, Donati MB, Bianchi M, Fruscella P, Salmona M. In vivo anti-inflammatory effect of statins is mediated by nonsterol mevalonate products. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2001; 21:1327–1332.
- Egashira K, Ni W, Inoue S, Kataoka C, Ki-tamoto S, Koyanagi M, Takeshita A. Pravastatin attenuates cardiovascular inflammatory and proliferative changes in a rat model of chronic inhibition of nitric oxide synthesis by its cholesterol-lowering independent actions. *Hypertension Research*. 2000; 23:353–358. [PubMed: 10912772]

- Endres M, Laufs U. Effects of statins on endothelium and signaling mechanisms. *Stroke*. 2004; 35:2708–2711. [PubMed: 15375300]
- Endres M, Laufs U, Huang Z, Nakamura T, Huang P, Moskowitz MA, Liao JK. Stroke protection by 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase. *Proceedings of the National Academy of Sciences of the United States of America*. 1998; 95:8880–8885. [PubMed: 9671773]
- Feske SK, Sorond FA, Henderson GV, Seto M, Hitomi A, Kawasaki K, Sasaki Y, Asano T, Liao JK. Increased leukocyte ROCK activity in patients after acute ischemic stroke. *Brain Research*. 2009; 1257:89–93. [PubMed: 19133241]
- Fontaine C, Dubois G, Duguay Y, Helledie T, Vu-Dac N, Gervois P, Soncin F, Mandrup S, Fruchart JC, Fruchart-Najib J, Staels B. The orphan nuclear receptor Rev-Erbalpha is a peroxisome proliferator-activated receptor (PPAR) gamma target gene and promotes PPARgamma-induced adipocyte differentiation. *The Journal of Biological Chemistry*. 2003; 278:37672–37680. [PubMed: 12821652]
- Genser B, Grammer TB, Stojakovic T, Siekmeier R, Marz W. Effect of HMG CoA reductase inhibitors on low-density lipoprotein cholesterol and C-reactive protein: systematic review and meta-analysis. *International Journal of Clinical Pharmacology and Therapeutics*. 2008; 46:497–510. [PubMed: 18826864]
- Gertz K, Laufs U, Lindauer U, Nickenig G, Bohm M, Dirnagl U, Endres M. Withdrawal of statin treatment abrogates stroke protection in mice. *Stroke*. 2003; 34:551–557. [PubMed: 12574574]
- Gladwin MT, Schechter AN, Shelhamer JH, Ognibene FP. The acute chest syndrome in sickle cell disease. Possible role of nitric oxide in its pathophysiology and treatment. *American Journal of Respiratory and Critical Care Medicine*. 1999; 159:1368–1376. [PubMed: 10228097]
- Gladwin MT, Shelhamer JH, Ognibene FP, Pease-Fye ME, Nichols JS, Link B, Patel DB, Jankowski MA, Pannell LK, Schechter AN, Rodgers GP. Nitric oxide donor properties of hydroxyurea in patients with sickle cell disease. *British Journal of Haematology*. 2002; 116:436–444. [PubMed: 11841449]
- Glover RE, Ivy ED, Orringer EP, Maeda H, Mason RP. Detection of nitrosyl hemoglobin in venous blood in the treatment of sickle cell anemia with hydroxyurea. *Molecular Pharmacology*. 1999; 55:1006–1010. [PubMed: 10347241]
- Grip O, Janciauskiene S, Lindgren S. Atorvastatin activates PPAR-gamma and attenuates the inflammatory response in human monocytes. *Inflammation Research*. 2002; 51:58–62. [PubMed: 11926313]
- Grundey SM. Obesity, metabolic syndrome, and coronary atherosclerosis. *Circulation*. 2002; 105:2696–2698. [PubMed: 12057978]
- Hebbel RP. Special issue of microcirculation: examination of the vascular pathobiology of sickle cell anemia. *Microcirculation*. 2004; 11:99–100. [PubMed: 15280085]
- Hebbel RP, Osarogiagbon R, Kaul D. The endothelial biology of sickle cell disease: inflammation and a chronic vasculopathy. *Microcirculation*. 2004; 11:129–151. [PubMed: 15280088]
- Hernandez-Perera O, Perez-Sala D, Navarro-Antolin J, Sanchez-Pascuala R, Hernandez G, Diaz C, Lamas S. Effects of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, atorvastatin and simvastatin, on the expression of endothelin-1 and endothelial nitric oxide synthase in vascular endothelial cells. *Journal of Clinical Investigation*. 1998; 101:2711–2719. [PubMed: 9637705]
- Huang J, Hadimani SB, Rupon JW, Ballas SK, Kim-Shapiro DB, King SB. Iron nitrosyl hemoglobin formation from the reactions of hemoglobin and hydroxyurea. *Biochemistry*. 2002; 41:2466–2474. [PubMed: 11841242]
- Jialal I, Devaraj S, Singh U. C-reactive protein and the vascular endothelium: implications for plaque instability. *Journal of the American College of Cardiology*. 2006; 47:1379–1381. [PubMed: 16580525]
- Kano H, Hayashi T, Sumi D, Esaki T, Asai Y, Thakur NK, Jayachandran M, Iguchi A. A HMG-CoA reductase inhibitor improved regression of atherosclerosis in the rabbit aorta without affecting serum lipid levels: possible relevance of up-regulation of endothelial NO synthase mRNA. *Biochemical and Biophysical Research Communications*. 1999; 259:414–419. [PubMed: 10362523]

- Kato GJ, Gladwin MT. Evolution of novel small-molecule therapeutics targeting sickle cell vasculopathy. *JAMA*. 2008; 300:2638–2646. [PubMed: 19066384]
- Kato GJ, Hunter C, Hunter L, Dejam A, Machado R, Mack K, Villagra J, Nichols J, Coles W, Sachdev V, Cannon R, Gladwin MT. Atorvastatin therapy restores nitric oxide-dependent vascular responsiveness in patients with sickle cell disease. *Blood*. 2004; 104:72a.
- Kaul DK, Fabry ME, Costantini F, Rubin EM, Nagel RL. In vivo demonstration of red cell-endothelial interaction, sickling and altered microvascular response to oxygen in the sickle transgenic mouse. *Journal of Clinical Investigation*. 1995; 96:2845–2853. [PubMed: 8675655]
- Kimura M, Kurose I, Russell J, Granger DN. Effects of fluvastatin on leukocyte-endothelial cell adhesion in hypercholesterolemic rats. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 1997; 17:1521–1526.
- Kinlay S, Schwartz GG, Olsson AG, Rifai N, Szarek M, Waters DD, Libby P, Ganz P. Inflammation, statin therapy, and risk of stroke after an acute coronary syndrome in the MIRACL study. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2008; 28:142–147.
- Klings ES, Farber HW. Role of free radicals in the pathogenesis of acute chest syndrome in sickle cell disease. *Respiratory Research*. 2001; 2:280–285. [PubMed: 11686897]
- Koh KK. Effects of statins on vascular wall: vasomotor function, inflammation, and plaque stability. *Cardiovascular Research*. 2000; 47:648–657. [PubMed: 10974215]
- Krishnan S, Setty Y, Betal SG, Vijender V, Rao K, Dampier C, Stuart M. Increased levels of the inflammatory biomarker C-reactive protein at baseline are associated with childhood sickle cell vasocclusive crises. *British Journal of Haematology*. 2010; 148:797–804. [PubMed: 19995398]
- Kureishi Y, Luo Z, Shiojima I, Bialik A, Fulton D, Lefer DJ, Sessa WC, Walsh K. The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. *Nature Medicine*. 2000; 6:1004–1010.
- Laufs U. Beyond lipid-lowering: effects of statins on endothelial nitric oxide. *European Journal of Clinical Pharmacology*. 2003; 58:719–731. [PubMed: 12634978]
- Laufs U, La Fata V, Plutzky J, Liao JK. Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation*. 1998; 97:1129–1135. [PubMed: 9537338]
- Laufs U, Endres M, Custodis F, Gertz K, Nickenig G, Liao JK, Bohm M. Suppression of endothelial nitric oxide production after withdrawal of statin treatment is mediated by negative feedback regulation of the rho GTPase gene transcription. *Circulation*. 2000; 102:3104–3110. [PubMed: 11120702]
- Lefer DJ, Jones SP, Girod WG, Baines A, Grisham MB, Cockrell AS, Huang PL, Scalia R. Leukocyte-endothelial cell interactions in nitric oxide synthase-deficient mice. *American Journal of Physiology*. 1999; 276:H1943–H1950. [PubMed: 10362674]
- Li JJ, Fang CH. C-reactive protein is not only an inflammatory marker but also a direct cause of cardiovascular diseases. *Medical Hypotheses*. 2004; 62:499–506. [PubMed: 15050096]
- Liao JK, Laufs U. Pleiotropic Effects of Statins. *Annual Review of Pharmacology and Toxicology*. 2005; 45:89–118.
- Liem AH, van de Woestijne AP, Zwinderman AH, Visseren FL, Jukema JW. Determinants of CRP level in statin-treated patients. *Current Medical Research and Opinion*. 2008; 24:1065–1068. [PubMed: 18312742]
- Makis AC, Hatzimichael EC, Bourantas KL. The role of cytokines in sickle cell disease. *Annals of Hematology*. 2000; 79:407–413. [PubMed: 10985359]
- Makis AC, Hatzimichael EC, Stebbing J, Bourantas KL. C-reactive protein and vascular cell adhesion molecule-1 as markers of severity in sickle cell disease. *Archives of Internal Medicine*. 2006; 166:366–368. [PubMed: 16476879]
- Morris CR, Kuypers FA, Larkin S, Vichinsky EP, Styles LA. Patterns of arginine and nitric oxide in patients with sickle cell disease with vaso-occlusive crisis and acute chest syndrome. *Journal of Pediatric Hematology/oncology*. 2000; 22:515–520. [PubMed: 11132219]
- Niwa S, Totsuka T, Hayashi S. Inhibitory effect of fluvastatin, an HMG-CoA reductase inhibitor, on the expression of adhesion molecules on human monocyte cell line. *International Journal of Immunopharmacology*. 1996; 18:669–675. [PubMed: 9089010]

- Okpala I, Daniel Y, Haynes R, Odoemene D, Goldman J. Relationship between the clinical manifestations of sickle cell disease and the expression of adhesion molecules on white blood cells. *European Journal of Haematology*. 2002; 69:135–144. [PubMed: 12406006]
- Ortego M, Bustos C, Hernandez-Presa MA, Tunon J, Diaz C, Hernandez G, Egidio J. Atorvastatin reduces NF-kappaB activation and chemokine expression in vascular smooth muscle cells and mononuclear cells. *Atherosclerosis*. 1999; 147:253–261. [PubMed: 10559511]
- Osarogiagbon UR, Choong S, Belcher JD, Vercellotti GM, Paller MS, Hebbel RP. Reperfusion injury pathophysiology in sickle transgenic mice. *Blood*. 2000; 96:314–320. [PubMed: 10891467]
- Pathare A, Al Kindi S, Alnaqdy AA, Daar S, Knox-Macaulay H, Dennison D. Cytokine profile of sickle cell disease in Oman. *American Journal of Hematology*. 2004; 77:323–328. [PubMed: 15551290]
- Platt OS. Sickle cell anemia as an inflammatory disease. *Journal of Clinical Investigation*. 2000; 106:337–338. [PubMed: 10930436]
- Powars DR, Chan LS, Hiti A, Ramicone E, Johnson C. Outcome of sickle cell anemia: a 4-decade observational study of 1056 patients. *Medicine (Baltimore)*. 2005; 84:363–376. [PubMed: 16267411]
- Pruefer D, Scalia R, Lefer AM. Simvastatin inhibits leukocyte-endothelial cell interactions and protects against inflammatory processes in normocholesterolemic rats. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 1999; 19:2894–2900.
- Pruefer D, Makowski J, Schnell M, Buerke U, Dahm M, Oelert H, Sibelius U, Grandel U, Grimminger F, Seeger W, Meyer J, Darius H, Buerke M. Simvastatin inhibits inflammatory properties of *Staphylococcus aureus* alpha-toxin. *Circulation*. 2002; 106:2104–2110. [PubMed: 12379581]
- van de Ree MA, Huisman MV, Princen HM, Meinders AE, Kluft C. Strong decrease of high sensitivity C-reactive protein with highdose atorvastatin in patients with type 2 diabetes mellitus. *Atherosclerosis*. 2003; 166:129–135. [PubMed: 12482559]
- Reiter CD, Wang X, Tanus-Santos JE, Hogg N, Cannon RO III, Schechter AN, Gladwin MT. Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease. *Nature Medicine*. 2002; 8:1383–1389.
- Rikitake Y, Kim HH, Huang Z, Seto M, Yano K, Asano T, Moskowitz MA, Liao JK. Inhibition of Rho kinase (ROCK) leads to increased cerebral blood flow and stroke protection. *Stroke*. 2005; 36:2251–2257. [PubMed: 16141422]
- Romano M, Mezzetti A, Marulli C, Ciabattini G, Febo F, Di Ienno S, Roccaforte S, Vigneri S, Nubile G, Milani M, Davi G. Fluvastatin reduces soluble P-selectin and ICAM-1 levels in hypercholesterolemic patients: role of nitric oxide. *Journal of Investigative Medicine*. 2000; 48:183–189. [PubMed: 10822898]
- Rosch JW, Boyd AR, Hinojosa E, Pestina T, Hu Y, Persons DA, Orihuela CJ, Tuoma-nen EI. Statins protect against fulminant pneumococcal infection and cytolysin toxicity in a mouse model of sickle cell disease. *Journal of Clinical Investigation*. 2010; 120:627–635. [PubMed: 20093777]
- Sacks FM, Pfeffer MA, Moya LA, Rouleau JL, Rutherford JD, Cole TG, Brown L, Warnica JW, Arnold JM, Wun CC, Davis BR, Braunwald E. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. *New England Journal of Medicine*. 1996; 335:1001–1009. [PubMed: 8801446]
- Santizo RA, Xu HL, Galea E, Muyskens S, Baughman VL, Pelligrino DA. Combined endothelial nitric oxide synthase upregulation and caveolin-1 downregulation decrease leukocyte adhesion in pial venules of ovariectomized female rats. *Stroke*. 2002; 33:613–616. [PubMed: 11823678]
- Sato K, Akaike T, Sawa T, Miyamoto Y, Suga M, Ando M, Maeda H. Nitric oxide generation from hydroxyurea via copper-catalyzed peroxidation and implications for pharmacological actions of hydroxyurea. *Japanese Journal of Cancer Research*. 1997; 88:1199–1204. [PubMed: 9473738]
- Schnog JB, Mac Gillavry MR, van Zanten AP, Meijers JC, Rojer RA, Duits AJ, ten Cate H, Brandjes DP. Protein C and S and inflammation in sickle cell disease. *American Journal of Hematology*. 2004a; 76:26–32. [PubMed: 15114593]
- Schnog JB, Teerlink T, van der Dijs FP, Duits AJ, Muskiet FA, CURAMA Study Group. Plasma levels of asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor,

- are elevated in sickle cell disease. *Annals of Hematology*. 2005; 84:282–286. [PubMed: 15599544]
- Seljeflot I, Tonstad S, Hjermann I, Arnesen H. Reduced expression of endothelial cell markers after 1 year treatment with simvastatin and atorvastatin in patients with coronary heart disease. *Atherosclerosis*. 2002; 162:179–185. [PubMed: 11947912]
- Shah SH, Newby LK. C-reactive protein: a novel marker of cardiovascular risk. *Cardiology in Review*. 2003; 11:169–179. [PubMed: 12852794]
- Singhal A, Doherty JF, Raynes JG, McAdam KP, Thomas PW, Serjeant BE, Serjeant GR. Is there an acute-phase response in steady-state sickle cell disease? *Lancet*. 1993; 341:651–653. [PubMed: 7680738]
- Sironi L, Cimino M, Guerrini U, Calvio AM, Lodetti B, Asdente M, Balduino W, Paoletti R, Tremoli E. Treatment with statins after induction of focal ischemia in rats reduces the extent of brain damage. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2003; 23:322–327.
- Solovey A, Gui L, Key NS, Hebbel RP. Tissue factor expression by endothelial cells in sickle cell anemia. *Journal of Clinical Investigation*. 1998; 101:1899–1904. [PubMed: 9576754]
- Solovey AA, Solovey AN, Harkness J, Hebbel RP. Modulation of endothelial cell activation in sickle cell disease: a pilot study. *Blood*. 2001; 97:1937–1941. [PubMed: 11264155]
- Solovey A, Kollander R, Shet A, Milbauer LC, Choong S, Panoskaltis-Mortari A, Blazar BR, Kelm RJ Jr, Hebbel RP. Endothelial cell expression of tissue factor in sickle mice is augmented by hypoxia/reoxygenation and inhibited by lovastatin. *Blood*. 2004; 104:840–846. [PubMed: 15073034]
- Solovey A, Kollander R, Milbauer LC, Abdulla F, Chen Y, Kelm RJ Jr, Hebbel RP. Endothelial nitric oxide synthase and nitric oxide regulate endothelial tissue factor expression in vivo in the sickle transgenic mouse. *American Journal of Hematology*. 2010; 85:41–45. [PubMed: 20029945]
- Sparrow CP, Burton CA, Hernandez M, Mundt S, Hassing H, Patel S, Rosa R, Hermanowski-Vosatka A, Wang PR, Zhang D, Peterson L, Detmers PA, Chao YS, Wright SD. Simvastatin has anti-inflammatory and antiatherosclerotic activities independent of plasma cholesterol lowering. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2001; 21:115–121.
- Sullivan KJ, Kissoon N, Gauger C. Nitric oxide metabolism and the acute chest syndrome of sickle cell anemia. *Pediatric Critical Care Medicine*. 2008; 9:159–168. [PubMed: 18477929]
- Tan KC, Chow WS, Tam SC, Ai VH, Lam CH, Lam KS. Atorvastatin lowers C-reactive protein and improves endothelium-dependent vasodilation in type 2 diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism*. 2002; 87:563–568. [PubMed: 11836286]
- Turhan A, Weiss LA, Mohandas N, Collier BS, Frenette PS. Primary role for adherent leukocytes in sickle cell vascular occlusion: a new paradigm. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99:3047–3051. [PubMed: 11880644]
- Undas A, Brummel-Ziedins KE, Mann KG. Statins and blood coagulation. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2005; 25:287–294.
- Yamada M, Huang Z, Dalkara T, Endres M, Laufs U, Waeber C, Huang PL, Liao JK, Moskowitz MA. Endothelial nitric oxide synthase-dependent cerebral blood flow augmentation by L-arginine after chronic statin treatment. *Journal of Cerebral Blood Flow and Metabolism*. 2000; 20:709–717. [PubMed: 10779015]
- Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis*. 2000; 148:209–214. [PubMed: 10657556]

Fig 1.

Change in plasma NOx levels during a vaso-occlusive episode (VOE) in five subjects. Plasma NOx levels initially increased after initiation of simvastatin treatment (day 0). Plasma NOx levels subsequently decreased coincident with, or shortly before, the reported vaso-occlusive episode (arrows). NOx levels returned to baseline after resolution of the vaso-occlusive episode.

Fig 2. Effect of simvastatin treatment on plasma levels of (A) NO, (B) IL-6, and (C) hs-CRP in subjects with SCD. Plasma biomarker levels in 26 subjects with SCD treated with simvastatin, 20 mg/d ($n = 14$) or 40 mg/d ($n = 12$). (A) Plasma levels of nitric oxide metabolites (NOx) before, during and after treatment with simvastatin. (B) Plasma IL-6 levels before, during and after treatment with simvastatin. (C) Plasma hs-CRP levels before, during and after treatment with simvastatin. Day 0 = start of treatment, day 21 = discontinuation of treatment, day 39 = follow-up off treatment.

Fig 3.

Effect of hydroxyurea therapy on plasma NOx responses to simvastatin. Plasma NOx levels (SD) at baseline (day 0), and after simvastatin treatment (day 21) in HbSS subjects on hydroxyurea (HU) therapy ($n = 9$) and not on HU therapy ($n = 8$). The increase in NOx levels in response to simvastatin was much greater in the group that was not on HU therapy compared to the group on HU therapy.

Table 1
Effect of simvastatin treatment (20, 40 mg/d) on clinical safety parameters and lipid profiles in subjects with SCD.

	Simvastatin, 20 mg/d (n = 14)		Simvastatin, 40 mg/d (n = 12)	
	Baseline	Day 21	Baseline	Day 21
Lipid panel				
Total cholesterol, mg/dl	124 (20)	108 (20)	134 (34)	116 (24)
LDL cholesterol, mg/dl	67 (18)	53 (18)	67 (30)	52 (22)
Triglycerides, mg/dl	93 (36)	95 (49)	81 (57)	80 (47)
HDL cholesterol, mg/dl	41 (8)	39 (8)	41 (9)	40 (8)
Haemoglobin, g/dl	9.4 (1.8)	9.2 (1.7)	10.3 (1.5)	10.4 (1.3)
Reticulocytes, %	9.4 (4.2)	10 (5.8)	6.2 (4.6)	6.3 (4.9)
LDH, U/l	987 (259)	1219 (210)	890 (140)	890 (170)
AST, U/l	40 (14)	46 (20)	41 (8)	46 (10)
ALT, U/l	33 (15)	37 (12)	16 (8)	19 (12)
Total bilirubin, mg/dl	3.4 (2.2)	3.2 (2.0)	2.3 (2.0)	2.6 (2.4)
BUN, mg/dl	8 (3.6)	8.6 (3.3)	7.8 (2.9)	8.1 (2.9)
Creatinine, mg/dl	0.79 (0.25)	0.82 (0.29)	0.72 (0.13)	0.76 (0.19)
Creatine kinase, U/l	72 (40)	129 (126)	76 (38)	96 (72)

Data are presented as mean (SD).

Table II

Biomarker levels at baseline and after treatment with simvastatin (day 21) in low-dose (20 mg/d), moderate-dose (40 mg/d) and combined groups.

	Low-dose, 20 mg/d (n = 14)			Moderate dose, 40 mg/d (n = 12)			Combined group (n = 26)		
	Baseline	Day 21	P-value	Baseline	Day 21	P-value	Baseline	Day 21	P-value
NOx (μmol/l)	31 (25)	38 (25)	0.01	18.5 (13)	38.2 (25)	0.01	25.4 (21)	38.1 (24)	0.0008
hs-CRP (mg/l)	9.9 (16)	2.7 (1.8)	0.1	6.0 (6.5)	2.4 (1.7)	0.02	8.0 (12.2)	2.5 (1.7)	0.02
IL-6 (pg/ml)	1.2 (1.3)	0.6 (0.4)	0.04	0.45 (0.42)	0.13 (0.12)	0.05	0.98 (1.2)	0.41 (0.42)	0.01
VEGF (pg/ml)	451 (345)	287 (305)	0.05	260 (140)	230 (160)	0.4	364 (283)	262 (246)	0.04
VICAM1 (ng/ml)	1335 (718)	1291 (660)	0.6	609 (255)	523 (152)	0.4	1098 (703)	1087 (647)	0.9
TF (pg/ml)	165 (78)	156 (110)	0.7	307 (73)	271 (128)	0.3	195 (96)	180 (121)	0.4

Data are presented as mean (SD).