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Simvastatin Reduces Vaso-occlusive Pain in Sickle Cell Anaemia: A Pilot Efficacy Trial

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Summary

Sickle cell anaemia (SCA) is a progressive vascular disease characterized by episodic vaso-occlusive pain. Despite the broad impact of inflammation on acute and chronic clinical manifestations of SCA, no directed anti-inflammatory therapies currently exist. Statins are cholesterol-lowering agents shown to confer protection from vascular injury by suppressing inflammation. We previously documented a reduction in soluble biomarkers of inflammation in patients with sickle cell disease treated with simvastatin. To determine the potential clinical efficacy of simvastatin, we treated 19 SCA patients with single daily dose simvastatin for 3 months and assessed changes from baseline in the frequency and intensity of diary-reported pain and levels of circulating nitric oxide metabolites (NOx), high sensitivity C-reactive protein (hs-CRP), vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), ICAM-3, E-selectin, and vascular endothelial growth factor (VEGF). Treatment with simvastatin resulted in a significant reduction in the frequency of pain (p=0.0003), oral analgesic use (p=0.003) and circulating hs-CRP (p=0.003), soluble (s)E-selectin (p=0.01), sICAM-1 (p=0.02), sICAM-3 (p=0.02) and sVEGF (p=0.01). Simvastatin had no effect on pain intensity or levels of NOx, sP-selectin and sVCAM-1. The observed reductions in pain rate and markers of inflammation were greatest in subjects receiving hydroxycarbamide (HC), suggesting a synergistic effect of simvastatin. These results provide preliminary clinical data to support a larger trial of simvastatin in SCA.

Keywords

sickle cell anaemia; simvastatin; vaso-occlusive pain; inflammation; clinical effect

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Disclosure of Interests

The authors have no competing interests.

Introduction

Vaso-occlusive pain crises (VOC) are the clinical hallmark and the leading cause of morbidity in sickle cell anaemia (SCA), accounting for the vast majority of sickle cell-related hospital admissions. Up to 30% of adults and 10% of children with SCA experience sickle cell pain on a daily basis, suggesting that the pathology of vaso-occlusion is ongoing and unremitting, even during so-called “steady-state” periods (Aisiku, *et al* 2009).

The pathophysiology of vaso-occlusion involves multiple interrelated processes that have been increasingly linked to inflammation (Owusu-Ansah, *et al* 2016). Erythrocyte sickling and haemolysis trigger acute inflammation marked by monocyte activation, elaboration of inflammatory cytokines and abnormal expression of endothelial adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), E-selectin and P-selectin (Brown, *et al* 2001, Hebbel and Vercellotti 1997, Shiu, *et al* 2000). These circulating mediators of inflammation and adhesion have been shown to exacerbate sickling in experimental models and correlate with clinical disease severity (Dworkis, *et al* 2010, Kaul, *et al* 2004, Krishnan, *et al* 2010, Makis, *et al* 2006, Qari, *et al* 2012). Repeated cycles of ischaemia reperfusion promote a chronic inflammatory state that results in progressive vascular injury and multi-organ dysfunction (Hebbel 2004, Kato, *et al* 2009, Okpala, *et al* 2002, Osarogiagbon, *et al* 2000).

Treatment with hydroxycarbamide (HC, also termed hydroxyurea [HU]) is recommended in children and adults to reduce the frequency of acute VOC, but has not been shown to prevent disease progression and the chronic morbid complications associated with SCA (Yawn, *et al* 2014). Given the complex pathobiology of vaso-occlusion, a multimodal approach to treatment that includes agents with complementary mechanisms of action is warranted.

Statins (3-Hydroxy-3-methyl-glutaryl CoA (HMG-CoA) reductase inhibitors) have been shown to improve endothelial function independent of their lipid-lowering effects, by suppressing inflammation and restoring nitric oxide (NO) production (De Caterina, *et al* 1995, Laufs 2003, Nohria, *et al* 2006, O’Driscoll, *et al* 1997, Takemoto, *et al* 2002). The anti-inflammatory properties of statins exert their effects via several mechanisms involved in the pathology of sickle cell vaso-occlusion (Antonopoulos, *et al* 2012, Holschermann, *et al* 2006, Jain and Ridker 2005, Piechota-Polanczyk and Jozkowicz 2016, Tousoulis, *et al* 2014, van der Meij, *et al* 2013, Wang, *et al* 2005). In sickle cell mice, statins inhibited pulmonary endothelial tissue factor (TF) and VCAM-1 expression after hypoxic challenge and resulted in prolonged survival following pneumococcal infection (Rosch, *et al* 2010). Simvastatin inhibited ICAM-1 expression and sickle neutrophil adhesion to stimulated endothelial cells in vitro (Canalli, *et al* 2008, Canalli, *et al* 2011, Silveira, *et al* 2013, Solovey, *et al* 2004). The mechanistic role of statins in modulating endothelial nitric oxide synthase (eNOS) expression is supported by pilot studies showing improvement in NOS-dependent blood flow after atorvastatin treatment in adult SCA patients with underlying endothelial dysfunction (Bereal-Williams, *et al* 2012). We previously demonstrated clinical safety and improvement in soluble markers of inflammation in sickle cell disease (SCD) patients treated with short-term simvastatin (Hoppe, *et al* 2011). The primary objective of this study

was to determine the potential clinical efficacy of simvastatin in reducing the frequency and intensity of vaso-occlusive pain in SCA.

Methods

Study Subjects

The study included patients with SCA (Hb SS or Hb S/β^0 thalassaemia genotype) who were at least 10 years of age and had a history of ≥ 3 vaso-occlusive pain episodes requiring treatment with a prescribed oral or parenteral analgesic in the preceding year. Patients were recruited at their baseline status without acute pain or other SCA-related symptoms. Patients receiving treatment with HC at a stable dose for ≥ 3 months were eligible.

Laboratory exclusion criteria were defined as a pre-treatment serum total cholesterol < 2.3 mmo/l or triglycerides (TG) 0.34 mmol/l, creatine kinase (CK) greater than upper normal limit (UNL) (>232 u/l), serum creatinine greater than 1.5-fold UNL, alanine transaminase (ALT) greater than 2-fold UNL. Clinical exclusion criteria were pregnancy/lactation, red cell transfusion or hospitalization within the 30 days prior to enrolment, 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. The study was approved by the Institutional Review Board at University of California, San Francisco (UCSF) Benioff Children's Hospital Oakland (BCHO). All subjects or their parents provided written informed consent.

Study Design

The study was a single centre, open label, non-randomized trial assessing the efficacy of simvastatin (ClinicalTrials.gov Identifier: NCT00508027) in reducing daily vaso-occlusive pain events in paediatric and adult patients with SCA. All subjects received simvastatin in a single oral dose 40 mg (weight >60 kg), 30 mg (weight 45–60 kg), 25 mg (weight 35–44 kg) daily for 3 months. The primary outcome measure was the change from baseline in the frequency and intensity of diary-reported vaso-occlusive pain after 3 months of treatment with simvastatin. Baseline pain was assessed using diary data collected over a period of one month prior to initiation of simvastatin. A web-based electronic diary (eDiary) accessed by smartphone was used to report daily sickle cell-related pain, pain intensity, use of oral or parenteral analgesics and visits to a medical facility. The eDiary was previously developed for use in SCD and included well-validated metrics to assess pain specifically attributed to SCA-related vaso-occlusion, including mild daily pain as well as more severe pain associated with an acute VOC (Jacob, *et al* 2013a, Jacob, *et al* 2014, Jacob, *et al* 2013b, Jacob, *et al* 2012). The eDiary also included a section for comments using free text to describe pain that was not considered to be sickle cell-related pain.

Vaso-occlusive pain was defined as the occurrence of sickle cell-related pain within the preceding 24 h. Study participants were asked to report the intensity of their sickle cell pain, on a scale from 0 (none) to 9 (worst) in the preceding 24 h, specific pain medications used, and visits to a medical provider for sickle cell-related pain. The smartphone was pre-

programmed with automated reminders prompting subjects to complete the daily diary. Subjects took an average of 3 minutes to complete the eDiary and submitted daily diaries for one month prior to treatment and for 3 months during treatment with simvastatin.

Pain rate was defined as the sum of days a subject reported sickle cell-related pain divided by the total number of daily diaries completed, expressed as a percentage. Days of hospitalization for an acute VOC were excluded from the analysis. Pain intensity was evaluated by a visual analogue scale (VAS) comprised of a horizontal line anchored by the words “none” and “worst pain” at each end that was automatically quantified on a 9-unit metric to generate a score. Pain intensity was measured as the sum of a subject’s highest daily pain score divided by the total number of pain days reported. While all medications used were recorded in the e-diary, only oral or parenteral opioids and non-steroidal anti-inflammatory drugs (NSAIDS) were included in the analysis. The frequency of analgesic use was defined as the sum of days a subject reported use of either opioid or NSAID to manage sickle cell-related pain divided by the total number of daily diaries completed, expressed as a percentage.

Clinical and laboratory assessments were performed at baseline, at monthly visits during treatment and at follow-up one month after discontinuation of simvastatin. Serial non-fasting blood samples for clinical safety studies and for plasma biomarkers were collected at each visit. Lipid profiles, complete blood counts and routine chemistries were conducted using standard laboratory techniques in the clinical laboratory at UCSF BCHO. Plasma high sensitivity C-reactive protein (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). Blood samples for the remaining biomarker studies were centrifuged at 3000g at room temperature and the plasma aliquots were frozen and stored at −80 °C until analysis. Plasma samples were assayed for the concentration of NO metabolites, nitrite/nitrate (NOx), using an NO chemiluminescence analyser (Sievers Model 280 NOA; Sievers Instruments, Boulder, Colorado, USA), as previously described (Braman and Hendrix 1989); VCAM-1 and vascular endothelial growth factor (VEGF) were measured by enzyme-linked immunosorbent assay (ELISA; Invitrogen, Camarillo, CA) according to the manufacturer’s instructions; ICAM-1, ICAM3, P-selectin and E-selectin were analysed on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) using a bead-based immunoassay (eBioscience, San Diego, CA), as previously described (Elshal & McCoy 2006). The minimum detection limit of the ELISA assay for VCAM-1 was 0.5 ng/mL. The sensitivity of the immunoassay for E-selectin was1.2 ng/ml; ICAM-1, 5.3 ng/ml; ICAM-3, 4.8 ng/ml and for P-selectin it was 5.7 ng/ml.

Monitoring for adverse events, including muscle pain, weakness and gastrointestinal symptoms, as well as adherence to simvastatin was performed by review of the daily e-diary record and by direct questioning at each visit. A pill count was performed monthly and at the final study visit. The total study duration with follow-up was 5 months.

Statistical Analysis

Descriptive statistics were used to summarize baseline patient characteristics, expressed as mean ± SD or median with interquartile range. The effects of simvastatin treatment were

assessed by comparing the frequency and intensity of pain within subjects before and after 3 months of treatment. Changes from baseline in pain measures, opioid analgesic use and biomarker levels were assessed using Wilcoxon matched-pairs signed rank test for non-parametric data. A two-tailed $P<0.05$ was considered significant. Statistical analysis was performed with GraphPad Prism statistical software (version 5.01; GraphPad software Inc., San Diego, CA, USA).

Results

Subject characteristics

Twenty-four subjects met the eligibility criteria for the study. Of these, 2 subjects were lost to follow-up prior to study initiation, 3 subjects were unable to adhere to the protocol and 19 subjects completed the study. All study participants had SCA (17 HbSS, 2 Hb S/β^0 thalassaemia) with a mean age of 18.5 years (range 10–34 years), 13 (68%) were female and 10 (53%) were receiving stable treatment with hydroxycarbamide.

Overall adherence to treatment with simvastatin, as assessed by verbal report and monthly pill counts, was 82% (range, 70%–100%). A total of 5 serious adverse events (SAE) were reported in 3 subjects who were hospitalized for VOC requiring hospitalization. In one subject, the hospitalization was complicated by severe hyperhaemolysis following transfusion. This subject was withdrawn from the study during the treatment period, but was included and contributed a total of 74 study days to the analysis. There was one AE, possibly related to simvastatin, in a subject who developed a facial rash after one month of treatment. None of the study subjects developed clinical symptoms of myopathy or hepatitis during the treatment period.

Simvastatin effects on lipid profiles and clinical laboratory measurements

Routine laboratory values (complete blood count, serum chemistry panel and hepatic transaminase) and creatine kinase levels remained stable on serial follow-up with no observed differences from baseline (Table I).

Simvastatin led to a significant decrease from baseline in serum lipid levels, with a decrease in total cholesterol by 9% and in low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) by 26% and 38%, respectively. There was a modest 5% decrease in non-fasting triglyceride levels. As expected, high-density lipoprotein (HDL) levels did not change following treatment with simvastatin.

Simvastatin effects on vaso-occlusive pain

The median diary completion rate (number of diaries completed/total number of study days) was 74% (range, 53%–97%). Diary adherence was similar during the baseline (median 76%, range 40%–94%) and treatment (median 73%, range 47%–99%) periods. Pain related to SCD was reported on 20% of total diary days, the majority (91%) of which were rated as mild (>0–4, 61%) to moderate (>4–7, 31%). Severe pain (>7) was reported by 7 subjects on 8% of days and led to an emergency department visit or hospitalization in 4 of these

subjects. Subject enrolment was evenly distributed across the months of the year. There was no apparent seasonal effect on the frequency or intensity of pain.

Treatment with simvastatin led to an 85% reduction in the overall rate of pain, from a baseline of 20% to 3% (p=0.0003) (Figure 1A). Oral analgesic use also decreased by 67%, from 9% to 3% of pain days after treatment (p=0.003). However, pain intensity did not change in response to simvastatin (Figure 1B). In a separate analysis examining the effect of simvastatin by HC use, the reduction in both pain rate and oral analgesic use remained significant in both subgroups of patients treated with either simvastatin alone or in combination with HC (Table II). A numerical decrease in pain intensity was observed in the subgroup on HC therapy, but did not reach significance.

Simvastatin effects on soluble biomarkers of inflammation

Plasma levels of hs-CRP, NOx and VEGF were evaluated in all subjects. Soluble (s)ICAM-1, ICAM-3, VCAM-1, E-selectin and P-selectin levels were measured in only 14 of the 19 subjects, due to a change in availability of the adhesion marker assay kit. Baseline levels of these biomarkers were consistent with previous studies showing elevated levels in SCA patients compared to healthy, age- and race-matched control subjects (Al Najjar, *et al* 2016, Bhagat, *et al* 2012, Blann, *et al* 2008, Conran, *et al* 2004, Duits, *et al* 1996, Gurkan, *et al* 2005, Hatzipantelis, *et al* 2013, Kanavaki, *et al* 2012, Kato, *et al* 2005, Krishnan, *et al* 2010, Makis, *et al* 2006, Mohan, *et al* 2005, Qari, *et al* 2012, Rees, *et al* 1995, Saleh, *et al* 1999).

Simvastatin-associated changes in biomarker levels are shown in Table II. Treatment with simvastatin led to a significant reduction in plasma levels of hs-CRP, sE-selectin, sICAM-1, sICAM-3 and sVEGF. The greatest change was in hs-CRP with a 37% decrease from baseline (p=0.003). More modest changes were found in the levels of sVEGF, sE-selectin, sICAM-1 and sICAM-3, which decreased by 10–20% from baseline following simvastatin. Neither plasma nitric oxide (NOx) metabolites nor sP-selectin levels changed significantly after treatment.

In a limited sub-analysis comparing biomarker changes in subjects on HC therapy with those who were not on HC, the decreases in hs-CRP, sE-selectin, sICAM-1, sICAM-3 and sVEGF observed in response to simvastatin were localized exclusively to the subgroup on HC therapy. The treatment effect of simvastatin alone was no longer significant in subjects who were not on HC. The persistent and more dramatic reduction in biomarker levels found in the combined treatment group suggests that simvastatin and HC may act synergistically (Table III, Table IV). Notably, serum biomarker levels were similar in the HC and non-HC subgroups at baseline.

Discussion

In this single centre pilot study of simvastatin in adolescents and adults with SCA, treatment for up to 3 months led to a dramatic reduction in the rate of sickle cell related pain and oral analgesic use, improvement in soluble biomarkers of inflammation and an acceptable safety profile.

Study participants reported a baseline frequency of sickle cell pain on 20% of days and used an analgesic medication on 50% of these days, consistent with mounting evidence that pain in SCA is more common than previously believed (Dampier, et al 2004, Smith, et al 2011, Smith, et al 2008).

Simvastatin was associated with an 85% reduction in the frequency of self-reported pain events and a parallel reduction in analgesic use. Despite the marked decline in pain rate, pain intensity did not change with simvastatin. Although several well-validated measures of sickle cell-related pain were included in the eDiary, analysis of pain intensity was limited to VAS scores, which may not have fully captured the multi-dimensional nature of pain intensity in this disease. Not surprisingly, pain intensity scores were highest prior to an acute VOC requiring management in the emergency department or hospital. However, there were too few VOC requiring a visit to a medical facility in this relatively short study to assess an effect of simvastatin on health care utilization.

In addition to a decline in the rate of pain, treatment with simvastatin led to a decrease in several circulating mediators of inflammation including hs-CRP, sICAM-1, sICAM-3, sE-selectin and sVEGF. These markers of inflammation and endothelial adhesion are elevated in SCD and other vascular disorders characterized by endothelial dysfunction and indicate a state of sustained low-grade inflammation (Hatzipantelis, *et al* 2013, Mohan, *et al* 2005). The reduction in hs-CRP and other inflammatory mediators following treatment with simvastatin are consistent with our prior study in SCD patients and several multi-centre studies in non-SCD populations (Blake and Ridker 2000, Hoppe, *et al* 2011, Kinlay, *et al* 2008, Koh 2000, Sacks, *et al* 1996, Shah and Newby 2003). Notably, in individuals with low or normal cholesterol, but high hs-CRP levels, statins significantly reduced the risk of stroke, disease progression and mortality (Albert, *et al* 2001).

Mechanistic studies have corroborated an independent anti-inflammatory effect of statins through NO-mediated regulation of nuclear transcription factors NFkB and Akt (Bustos, *et al* 1998, Devaraj, *et al* 2006, Grip, *et al* 2002, Ortego, *et al* 1999). In experimental models of ischaemia/reperfusioninjury including sickle mice, statins have been shown to prevent vaso-occlusion and improve microvascular blood flow through inhibition of NFκB-dependent adhesion molecules, VCAM, ICAM and selectins (Bao, *et al* 2004, Belcher, *et al* 2003, De Caterina, *et al* 1995, Kollander, *et al* 2010, Liuni, *et al* 2010, Palmer, *et al* 1988, Selvaraj, *et al* 2003, Solovey, *et al* 2001). Collectively, these studies provide a plausible explanation for the reduction in soluble markers of inflammation (hs-CRP) and endothelial activation (sICAM-1, sICAM-3, sE-selectin, VEGF) observed following simvastatin treatment in this study.

Remarkably, the greatest changes in biomarker levels were found in the subgroup of patients who were also receiving HC therapy. The observed decrease in hs-CRP, sICAM-1, sICAM-3, sE-selectin and VEGF levels remained significant in patients treated with simvastatin alone, but was even more dramatic in patients receiving concomitant HC therapy. Hydroxycarbamide has proven efficacy in reducing the frequency of VOC and has been shown to inhibit expression of several adhesion molecules including E- and P-selectin, ICAM and VCAM-1 (Almeida, *et al* 2015, Conran, *et al* 2004, Kato, *et al* 2005, Saleh, *et al*

1999, Verger, *et al* 2014). Localization of the treatment effect to the HC subgroup suggests that simvastatin may augment the established effect of HC in reducing sICAM-1, sICAM-3 and sE-selectin levels in subjects receiving combined treatment. Despite the small number of subjects in this analysis, it is conceivable that SCA patients treated with HC may derive greater benefit from co-treatment with simvastatin. These results must be considered in light of the potential bias introduced as a result of participation in the study, including improvement in HC compliance. If confirmed, these results have promising therapeutic implications, in that simvastatin may potentially benefit patients who are already on HC, as well as those who are intolerant or have a suboptimal response to HC.

As demonstrated in our previous study, sVCAM-1 and sP-selectin levels did not change in response to treatment with simvastatin in this study. These results are not altogether surprising, as circulating levels may not reflect the differential temporal and tissue-specific expression of these markers during vaso-occlusion. In sickle mice, treatment with statins inhibited TF and VCAM-1 expression in pulmonary endothelium, but not monocytes (Chi, *et al* 2003, Hebbel and Mohandas 1994, Solovey, *et al* 2004, Solovey, *et al* 2001). Kato et al (2005) previously reported a reduction in sVCAM-1 levels in SCA patients following treatment with HC. It is thus conceivable that simvastatin had no additional effect in lowering sVCAM-1 levels beyond that achieved by HC in this study.

In contrast to our previous study showing an increase in NOx following simvastatin, simvastatin had no direct effect on levels of plasma NOx. However, this effect was eliminated in patients who developed acute VOC (Hoppe, *et al* 2011). The lack of a treatment effect on plasma NOx levels in this study may have reflected the greater disease severity in subjects with SCA enrolled in this study. An alternative and perhaps more likely explanation is that measurement of plasma NOx may not accurately estimate endogenous NO bioavailability in SCA patients, as suggested by other studies showing conflicting results (Akinsheye and Klings 2010, Lamarre, *et al* 2014, Lopez, *et al* 1996, Lopez, *et al* 2000). Aside from hs-CRP, there are no clinically approved assays for the biomarkers in this study and measurements may have been affected by several factors, including circadian variation, short half-life, post-prandial effects and assay stability (Moshage 1997).

Safety

There were no clinical safety concerns or simvastatin-related adverse events in this study. As in our prior study, simvastatin resulted in a relatively modest and stable decrease in total cholesterol and LDL-C levels. Moreover, simvastatin had no effect on haematological parameters, arguing against the hypothetical risk of red blood cell underproduction due to excessive cholesterol lowering. These results extend the safety profile found in our previous study and are supported by data from large clinical trials documenting the overall safety of simvastatin in diverse populations (Florentin and Elisaf 2012, Giorgi, *et al* 2011, Jiang, *et al* 2014).

Limitations

Despite the significant effects of simvastatin observed in this study, a larger, controlled trial is necessary to confirm these results. Given the variability in clinical symptoms within and

among patients with SCA, a period of one month may not have adequately reflected subjects' baseline status. Environmental factors may also contribute to the clinical variability of SCD, including the frequency of acute pain. This single institution study was conducted in a geographic area with less seasonal variation and fluctuations in climate than other regions, thus limiting the generalizability of the treatment effect observed. Variable compliance with daily diary submissions ranging between 47% and 97% may have influenced the results of this study. Missing data limited the analysis to pre- and post-measurements rather than multiple measurements over time. However, this pilot study was intended to collect preliminary efficacy data needed for future studies.

Conclusions

The results of this study, showing a reduction in the frequency of vaso-occlusive pain and a corresponding decrease in serum markers of inflammation, suggest that simvastatin alone or in combination with HC may show promise as a core preventative therapy for SCA. These findings provide preliminary clinical data to support a larger trial evaluating the long-term safety and efficacy of simvastatin in SCA.

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CH performed the research; CH and LS designed the research study; EJ contributed essential tools; FK and SL contributed essential reagents and performed the laboratory analyses; CH, LS and EJ analysed the data; CH and EV wrote the paper.

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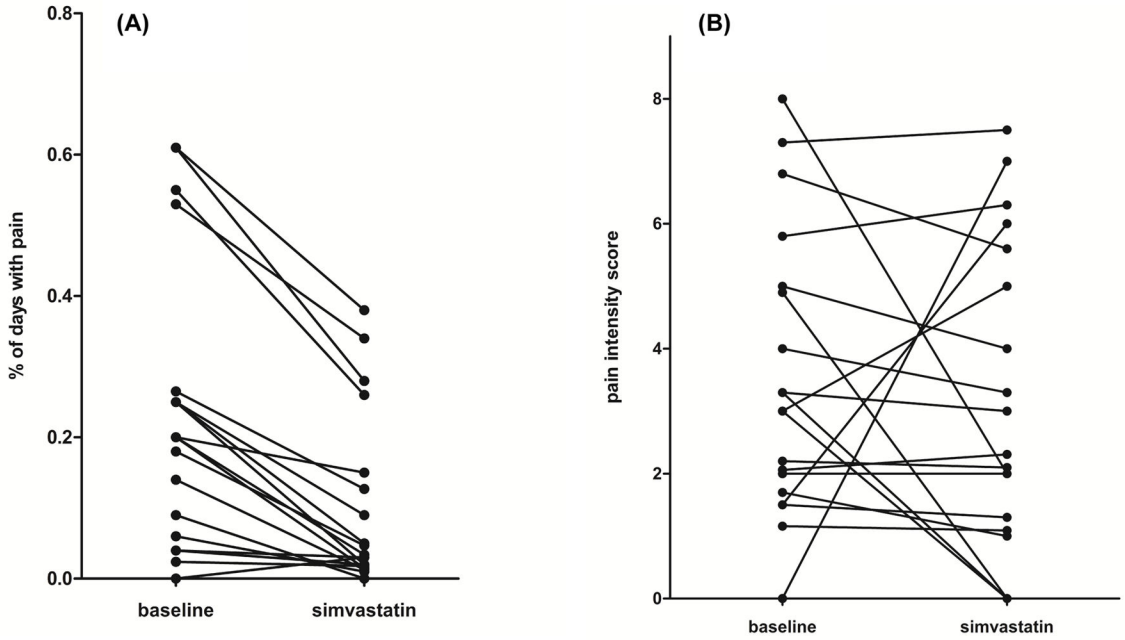

Figure 1.

Effect of simvastatin on patient reported days with pain and pain intensity. (A) Within-subject changes in the proportion of total study days with reported pain. (B) Within-subject changes in the intensity of reported sickle cell pain. Pain intensity was evaluated using the numerical rating score from the visual analogue scale. Results are expressed as median (25, 75th percentile).

Table I

Effect of simvastatin treatment on lipid profiles and clinical laboratory parameters

Laboratory	Baseline	Treatment	% change	p-value
Total cholesterol (mmo/l)	2.97 (2.69, 3.36)	2.71 (2.28, 2.90)	−9	0.0002
LDL-C (mmo/l)	1.37 (1.16, 1.71)	1.01 (0.83, 1.45)	−26	0.0003
VLDL-C (mmo/l)	0.62 (0.38, 0.69)	0.39 (0.31, 0.52)	−38	0.0021
HDL-C (mmo/l)	0.98 (0.88, 1.09)	0.93 (0.88, 1.09)	−5	0.6864
Triglycerides (mmo/l)	1.06 (0.82, 1.41)	1.01 (0.78, 1.11)	−5	0.0405
WBC count (x 10^9/l)	8.5 (6.4, 11.3)	7.1 (5.9, 9.8)	−16	0.0584
Hb (g/l)	94 (85, 111)	93 (87, 108)	−1	0.7944
ARC (x 10^9/l)	0.29 (0.18, 0.37)	0.21 (0.14, 0.32)	−28	0.0732
Platelet count (x 10^9/l)	331 (284, 445)	301 (269, 385)	−10	0.3838
Total bilirubin (μmol/l)	35.9 (23.9, 56.4)	34.2 (17.1, 46.2)	−5	0.1270
ALT (u/l)	25.0 (13.0, 30.0)	22.0 (18.0, 31.0)	−12	0.9133
AST (u/l)	54.0 (41.0, 81.0)	53.0 (37.0, 80.0)	−2	0.7601
Creatinine (μmol/l)	44.2 (35.4, 61.9)	44.2 (35.4, 53.0)	0	0.7339
Creatine kinase (U/L)	43.0 (34.0, 52.0)	44.0 (37.0, 55.0)	2	0.3437

Values are expressed as median (25^{th}, 75^{th} percentile)

ALT, alanine transaminase; ARC, absolute reticulocyte count; AST, aspartate transaminase; Hb haemoglobin; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; VLDL-C, very low-density lipoprotein-cholesterol; WBC, white blood cell.

Table II

Changes in pain frequency, intensity and analgesic use after 3 months of simvastatin treatment in total study group and by concomitant hydroxycarbamide use

	Total group				On HC (n=10)				Not on HC (n=9)			
	Baseline	**Simvastatin**	**% change**	**p-value**	**Baseline**	**Simvastatin**	**% change**	**p-value**	**Baseline**	**Simvastatin**	**% change**	**p-value**
Pain rate	0.20 (0.06, 0.27)	0.03 (0.02, 0.15)	−85	0.0003	0.15 (0.04, 0.32)	0.03	−80	0.006	0.25 (0.16, 0.41)	0.047	−81	0.013
Analgesic use	0.09 (0, 0.22)	0.03 (0, 0.09)	−67	0.003	0.08 (0, 0.2)	0.03 (0, 0.09)	−63	0.04	0.09 (0.03,0.23)	0.03 (0.01,0.09)	−67	0.04
Pain intensity	3.0 (1.7, 5.0)	2.3 (1.1, 5.6)	−23	0.2762	2.7 (1.5, 5.2)	2.2 (1.3,4.5)	−19	0.43	3.0 (2.0,5.9)	3.0 (0.5,6.3)	0	0.57

Values are expressed as median and 25–75% interquartile range

Analgesic medications included oral or parenteral opioids and non-steroidal anti-inflammatory drugs.

HC, hydroxycarbamide

Table III

Change in soluble biomarker levels after 3 months of simvastatin treatment

Biomarker	Baseline	Simvastastin treatment	% change	P-value
NOx (μM)	27.5 (15.4, 34.6)	28.2 (22.1, 36.0)	+2.5	0.40
hs-CRP (mg/l)	1.90 (1.70, 9.90)	1.20 (0.90, 2.30)	−37	0.003
sE-selectin (ng/ml)	302.3 (132.3, 542.5)	273.2 (114.5, 417.4)	−10	0.01
sP-selectin (ng/ml)	474.5 (304.9, 705.9)	360.5 (269.8, 635.1)	−24	0.32
sVEGF (ng/ml)	122.3 (83.0, 195.0)	98.3 (81.8, 163.8)	−20	0.01
sICAM-1 (ng/ml)	511.6 (381.3, 631.9)	428.0 (361.6, 629.2)	−16	0.02
sICAM-3 (ng/ml)	154.2 (86.7, 176.8)	128.8 (76, 156)	−16	0.02
sVCAM-1 (ng/ml)	992.4 (796.8, 1778)	903.6 (706.8, 1664)	−9	0.12

Values are expressed as median and 25–75% interquartile range

hs-CRP, high sensitivity C-reactive protein; NOx, nitrite/nitrate; sE-selectin, soluble E-selectinsICAM-1, -3, soluble intercellular adhesion molecule 1, 3; sP-selectin, soluble P-selectin; sVCAM-1, vascular cell adhesion molecule 1; sVEGF, soluble vascular endothelial growth factor.

Table IV

Comparison of changes in soluble (s) biomarker levels after simvastatin by hydroxycarbamide use.

Biomarker	HC (n=10*)				No HC (9*)			
	baseline	simvastatin	%change	p-value	baseline	simvastatin	%change	p-value
NOx (μM)	21.4	28	+31	0.39	30	28.2	−6	0.82
hs-CRP (mg/l)	1.8 (1.2,9.9)	1.05 (.58,1.7)	**−42**	**0.008**	3	2.2	−27	0.15
sE-selectin (ng/ml)	232.4	177.6	**−24**	**0.03**	370.3	355.3	−4	0.44
sP-selectin (ng/ml)	405.8	352.2	−13	.94	487.3	586	20	0.25
sVEGF (ng/ml)	148.9	96.0	**−36**	**.03**	98.6	116.9	19	0.13
sICAM-1 (ng/ml)	462.9	377.4	**−86**	**.008**	537.6	573.3	7	0.56
sICAM-3 (ng/ml)	160.4	128.8	**−20**	**.008**	151.8	124.2	−18	0.85
sVCAM-1 (ng/ml)	822.1	749.6	−9	0.58	1110	1129	2	0.20

*Analyses of sE-selectin, sP-selectin, ICAM-1, ICAM-3 and VCAM-1 was limited to 14 subjects (8 on HC, 6 not on HC)

HC, hydroxycarbamide; hs-CRP, high sensitivity C-reactive protein; NOx, nitrite/nitrate; sE-selectin, soluble E-selectinsICAM-1, -3, soluble intercellular adhesion molecule 1, 3; sP-selectin, soluble P-selectin; sVCAM-1, vascular cell adhesion molecule 1; sVEGF, soluble vascular endothelial growth factor.