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CIRRHOSIS AND LIVER FAILURE

Randomized controlled trial assessing the effect of simvastatin in primary biliary cirrhosis

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Abstract

Background: This study evaluated the effect of statins in Primary biliary cirrhosis (PBC) on endothelial function, anti-oxidant status and vascular compliance. *Methods:* Primary biliary cirrhosis patients with hypercholesterolaemia were randomized to receive 20 mg simvastatin or placebo in a single blind, randomized controlled trial. Body mass index, blood pressure, glucose, liver function, lipid profile, immunoglobulin levels, serological markers of endothelial function and anti-oxidant status were measured as well as vascular compliance, calculated from pulse wave analysis and velocity, at recruitment and again at 3, 6, 9 and 12 months. *Results:* Twenty-one PBC patients ($F = 20$, mean age = 55) were randomized to simvastatin 20 mg ($n = 11$) or matched placebo ($n = 10$). At completion of the trial, serum cholesterol levels in the simvastatin group were significantly lower compared with the placebo group (4.91 mmol/L vs. 6.15 mmol/L, $P = 0.01$). Low-density lipoprotein (LDL) levels after 12 months were also significantly lower in the simvastatin group (2.33 mmol/L vs. 3.53 mmol/L, $P = 0.01$). After 12 months of treatment, lipid hydroperoxides were lower (0.49 μmol/L vs. 0.59 μmol/L, $P = 0.10$) while vitamin C levels were higher (80.54 μmol/L vs. 77.40 μmol/L, $P = 0.95$) in the simvastatin group. Pulse wave velocity remained similar between treatment groups at 12 months (8.45 m/s vs. 8.80 m/s, $P = 0.66$). Only one patient discontinued medication owing to side effects. No deterioration in liver transaminases was noted in the simvastatin group. *Conclusions:* Statin therapy in patients with PBC appears safe and effective towards overall reductions in total cholesterol and LDL levels. Our initial study suggests that simvastatin may also confer advantageous effects on endothelial function and antioxidant status.

Primary biliary cirrhosis (PBC) is a chronic autoimmune disease of the liver, primarily affecting middle-aged women, characterized by slow progressive destruction of the bile canaliculi leading to cholestasis, fibrosis and cirrhosis (1). PBC-associated hypercholesterolaemia, occurring in 75–95% of cases, directly affects bile acid synthesis. Elevated bile acid levels associated with PBC may then lead to deleterious effects through direct hepatocyte injury or possibly as a consequence of endothelial dysfunction. The role of elevated cholesterol and its effect on the biosynthetic pathway of bile acid synthesis may therefore afford a potential opportunity for therapeutic modulation in patients with PBC.

We have previously reported endothelial dysfunction, inflammation and anti-oxidant deficiency in a prospective study of 51 PBC patients compared with 34 healthy control subjects. High sensitivity C-reactive protein (hsCRP), intracellular and vascular cell adhesion molecules (sICAM and sVCAM) were all significantly higher (469.14 vs. 207.13 μg/dL, $P < 0.001$; 768.12 vs. 308.03 ng/ml, $P < 0.001$; 708.40 vs. 461.31 ng/ml, $P < 0.001$), while the anti-oxidant vitamins ascorbate, vitamin E and vitamin A were all significantly lower in PBC patients (39.91 vs. 72.68 μmol/L, $P < 0.02$; 1.08 vs. 1.81 μmol/L, $P = 0.001$; 2.63 vs. 3.14 μmol/L, $P < 0.001$) (2).

However, contrary to these adverse inflammatory responses, we identified a lower pulse wave velocity in PBC patients compared with control subjects (8.22 m/s vs. 8.78 m/s, $P = 0.02$) suggesting a possible reduction in vascular risk (2). Such a reduction in vascular risk is

consistent with a 7 year prospective evaluation by Crippin *et al.* of 312 PBC patients where there was no statistical difference in atherosclerotic death rates between PBC patients compared with age- and sex-matched controls (3). Longo *et al.* also reported that no definitive increase in vascular risk in 400 PBC patients followed up over 6.2 years as their incidence of cardiovascular events was similar to that of the general population (4).

HMG-CoA reductase inhibitors (statins) are used primarily for the prevention of cardiovascular disease through advantageous effects on lipid homeostasis. Brugts *et al.* reported a significant reduction in major coronary events (odds ratio 0.70, 95% CI 0.61 to 0.81) with statin therapy from their meta-analysis of 70 388 patients (5). A more recent meta-analysis by Reriani *et al.*, assessing 46 clinical trials and a total of 2706 patients, demonstrated that statin therapy significantly improved both central and peripheral endothelial function (6).

Through reductions in LDL cholesterol levels, statins have been postulated as a potential beneficial treatment for PBC patients (7–10). Initial reports by Kurihara *et al.* have described reductions in cholesterol and total bile acid levels in two PBC patients treated with pravastatin with additional histological improvements in one patient (9). Other small case series have described beneficial effects of simvastatin in PBC patients ranging from a 34% reduction in total cholesterol levels at 30 days to additional significant improvements between 12 and 37% in total cholesterol, LDL cholesterol, alkaline phosphatase, γ-glutamyltransferase and immunoglobulin M levels (7, 8, 10).

Combined with suboptimal nutritional reserves, patients with chronic liver disease often have a reduced metabolic clearance, which can lead to abnormally elevated serum levels of pharmaceutical agents such as statins where serum levels have been recorded up to 20 times greater than the desired therapeutic range (11). Although statins are generally safe, hepatotoxicity can occur, which, although infrequent, can be severe and in rare circumstances, may lead to liver failure (12, 13). However, there is little evidence to suggest that patients with chronic liver disease are more susceptible to hepatonecrosis than those with normal hepatic function (14). Unfortunately, longer term safety data as well the effects of statins on endothelial function, anti-oxidant status and vascular compliance in patients with PBC have not yet been reported (15).

The primary objectives of this study were to evaluate the effect of statins in PBC on endothelial function, anti-oxidant status and vascular compliance. The secondary objectives were to assess the safety profile and overall effect of statins on lipid homeostasis in PBC patients.

Methods

Patient recruitment

Male and female (non-pregnant) PBC patients, between the ages of 20 and 75, with serum cholesterol greater than 5.0 mmol/L were recruited from the Liver Clinic at the Royal Victoria Hospital. PBC was defined by the presence of (i) positive antimitochondrial antibodies to a titre of >1:40 on two occasions along with (ii) elevation of alkaline phosphatase (ALP) or (iii) compatible or diagnostic liver histology (16). Patients with known hypertension (blood pressure>160/90 mmHg), diabetes mellitus, a history of cardiovascular disease and those already prescribed lipid lowering agents or hormonal preparations were excluded from this study. The use of ursodeoxycholic acid was not considered a reason for inclusion or exclusion.

Following written informed consent, patients were subsequently recruited to a single-blind, randomized controlled trial to receive 20 mg simvastatin or matched placebo orally at night for 12 months. Patient treatment randomization and allocation was performed independently by the Department of Research Pharmacology in the Royal Victoria Hospital at the initial baseline visit. Simvastatin and matched placebo dosages were titrated to 40 mg if serum cholesterol levels remained elevated greater than 5.0 mmol/L after 3 months of treatment.

All pretrial medications remained unchanged. Patients were withdrawn from this study if plasma bilirubin, ALP, γ-glutamyl transpeptidase (GGT), alanine aminotransferase (ALT) or aspartate transaminase (AST) levels doubled compared with baseline levels, if a reduction in plasma albumin level (<35 g/L) was detected or if any adverse clinical concerns occurred during this study period. This study was approved by the Northern Ireland Research Ethics and Royal Victoria Hospital Clinical Governance Committees.

Patient assessment

Baseline clinical parameters

Following completion of initial baseline examinations and investigations at recruitment, patients were subsequently assessed again at 3, 6, 9 and 12 month post-treatment randomization. Following an overnight fast, each subject had a clinical assessment performed including blood pressure (mmHg) and body mass index [BMI = weight (kg) divided by height (metres) squared].

Biochemical assessment

Blood sampling

Fasting peripheral venous blood samples for plasma glucose, serum liver function tests, lipid profiles and immunoglobulins were performed routinely at each assessment time-point by the laboratories of the Royal Victoria Hospital. For safety purposes, liver enzymes were additionally checked at 6 weeks following recruitment.

Plasma samples for ascorbate were centrifuged immediately while serum collected for lipid hydroperoxides,

cell adhesion molecules and lipid soluble antioxidants was clotted for 15 min and then centrifuged. All samples were transferred to 2-ml tubes (Sarstedt Ltd, Drinagh, Wexford, Ireland) and stored at −80°C. All commercial assay analyses were performed according to the manufacturers' guidelines. Intra- and interassay coefficients of variation were within satisfactory limits according to the manufacturers' guidelines.

Plasma lipid hydroperoxides

These were measured spectrophotometrically using the Ferrous Oxidation-Xylenol Orange-version 1-assay (FOX 1), which was used to determine hydroperoxides (HPO) in the aqueous phase of serum. Hydroperoxides oxidize ferrous ions to ferric ions in dilute acids and the resultant ferric ions were then determined using ferric sensitive dyes as an indirect measure of hydroperoxide concentration.

Intracellular and vascular cell adhesion molecules

These were measured using commercially available ELISA kits from Eli-pair (Diaclone, Besançon, France).

Ascorbate

Concentrations were determined by the enzymatic oxidation of ascorbic acid and subsequent quinoxaline formation to generate a fluorescent derivative measured on the Cobas Fara centrifugal analyser as described by Vuillemier *et al.* (17).

Lipid soluble antioxidants

These levels were measured using a high performance liquid chromatography technique (HPLC) using diode array detection to assess retinol, γ-tocopherol, α-tocopherol, lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene and lycopene according to the method of Craft and Soares (1992) (18). The detection limits for retinol and the tocopherols were 0.05 nmol/L while 0.005 nmol/L was used for carotenoids.

High-sensitive C-reactive protein

This was measured with a latex-enhanced immunoturbidimetric assay (Randox Pharmaceuticals, Curmlin, Ireland) using an ILab 600 biochemical analyser and ILab 600 computer software (Instrumentation Laboratories, Warrington, UK).

Estimation of vascular compliance

The methodologies of pulse wave analysis (PWA) and pulse wave velocity (PWV) gated to the cardiac cycle have previously been described (19–21). After the patient had rested in the supine position in a temperature-controlled room for a minimum of 15 min, radial pulse wave analysis was recorded with a Millar tonometer and the Sphygmocor system model SCORPx, incorporating the pulse wave velocity system Model SCOR-Vx (SPC-301; Millar instruments and Atcor medical, Sydney, Australia). For pulse wave analysis, triplicate measurements were made with the patient in the supine position from the radial artery of the dominant arm and the average reading calculated. The Sphygmocor analysis software automatically processed the radial artery waveform data using a generalized transfer function. Generating measures of vascular compliance including augmentation index calibrated to 75 beats per min (AgIx75), time to reflectance (TR), Buckberg's subendocardial viability ratio (SEVR) and ejection duration percentage (ED%). Calculation of PWV was similar to PWA with the analysis gated to the cardiac cycle with separate readings from the dominant radial artery (distal site) and ipsilateral carotid artery (proximal site). Carotid-radial PWV was measured rather than carotid-femoral because of ease of reproducibility and acceptability to patients.

Statistical analysis

Analyses were performed using the Statistical Program for Social Sciences (SPSS 15.0 for windows; SPSS Inc., Chicago, IL, USA). Data were presented as mean and standard deviation. Comparisons between the interventional simvastatin and placebo groups were calculated using the Mann–Whitney *U*-test. Data on repeated measurements within groups over the time periods were analysed using the Wilcoxon signed-rank test. A P value less than 0.05 was deemed statistically significant.

Results

Patient demographics

Twenty-one PBC patients were successfully recruited (20 female, mean age=55.0 years) to receive simvastatin therapy ($n = 11$) or placebo ($n = 10$) (see Fig. 1 for recruitment algorithm). The mean age of participants in each group was similar (simvastatin: 55.64 ± 9.82 years vs. placebo: 54.40 ± 12.62 years, $P = 0.82$). The age range in the simvastatin group was 34–70 years and in the placebo group was 41–75 years. Body mass index was similar between treatment groups (simvastatin: 27.00 ± 5.39 vs. placebo: 27.84 ± 4.26, $P = 0.81$) as were other baseline clinical parameters (Table 1).

Assessment of routine haematological and biochemical indices

Baseline fasting glucose, lipid profile and immunoglobulins were comparable between the two groups at baseline. There was no significant difference in fasting glucose between the simvastatin and placebo treatment

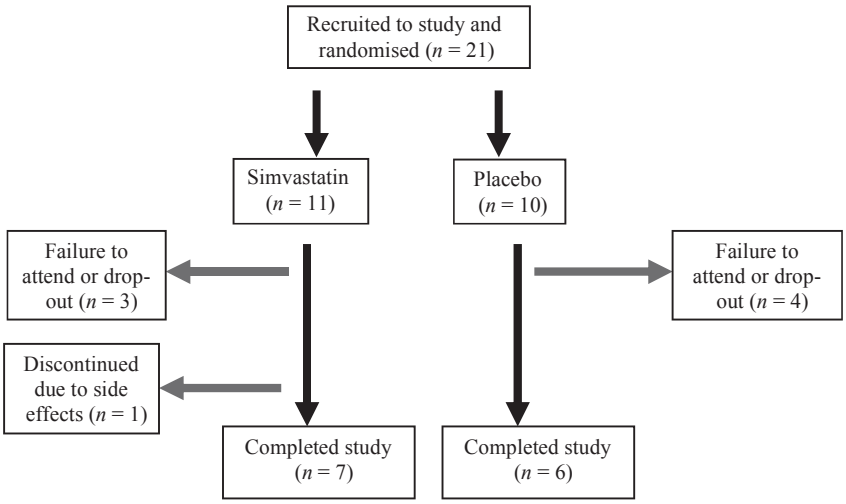

Fig. 1. Trial recruitment algorithm.

groups at each follow-up time-point. There was also no difference in fasting glucose when follow-up assessments were compared with baseline separately within the treatment groups (Table 1).

Baseline cholesterol (simvastatin: 6.23 ± 1.38 vs. placebo: 6.16 ± 0.98 $P = 0.56$) and LDL (simvastatin: 3.9 ± 1.26 vs. placebo: 3.88 ± 0.81, $P = 0.69$) levels were similar between the two groups. At completion of the trial, cholesterol levels in the simvastatin group were significantly lower compared with the placebo group (simvastatin: 4.91 ± 0.72 mmol/L vs. Placebo: 6.15 ± 0.75 mmol/L, $P = 0.01$). LDL levels in the simvastatin group were also significantly lower compared with the placebo group after 12 months of treatment (simvastatin: 2.33 ± 0.74 mmol/L vs. placebo: 3.53 ± 0.49 mmol/L, $P = 0.01$) (Table 1).

This reduction in cholesterol levels for the simvastatin group at 12 months was also significant when compared with baseline within groups separately (simvastatin: 6.23 ± 1.38–4.91 ± 0.72 mmol/L, $P = 0.016$ and placebo: 6.16 ± 0.98–6.15 ± 0.75 mmol/L, $P = 0.22$). A similar reduction was identified when LDL levels were compared with baseline within groups separately (simvastatin: 3.9 ± 1.26–2.33 ± 0.74 mmol/L, $P = 0.02$ and placebo: 3.88 ± 0.81–3.53 ± 0.49 mmol/L, $P = 0.69$).

Baseline immunoglobulin A was significantly lower in the statin group (simvastatin: 2.37 ± 0.94 g/L vs. placebo: 3.4 ± 0.91 g/L, $P = 0.03$), but was non-significantly lower by completion of the trial (simvastatin: 2.45 ± 0.75 g/L vs. placebo: 3.49 ± 1.18 g/L, $P = 0.14$).

Assessment of liver function

Liver function test indices were similar between the groups at baseline including ALT (simvastatin: 53.27 ± 43.78 U/L vs. placebo: 61.50 ± 48.63 U/L, $P = 0.82$) and AST (simvastatin: 51.27 ± 34.15 U/L vs. placebo: 59.10 ± 29.66 U/L, $P = 0.52$). At 12 months, there remained no significant difference between treatment groups in ALT (simvastatin: 43.57 ± 20.01 U/L vs. placebo: 74.50 ± 34.46 U/L, $P = 0.82$) or AST (simvastatin: 50.14 ± 25.39 U/L vs. placebo: 64.00 ± 23.74 U/L, $P = 0.14$) levels. Other liver function test indices also remained similar between treatment groups throughout this study (Table 1).

Endothelial function

Lipid hydroperoxides

Initial baseline levels were similar for both treatment groups (simvastatin: 0.53 ± 0.15 μmol/L vs. placebo 0.59 ± 0.20 μmol/L, $P = 0.82$). There was no significant difference between treatment groups at 12 months (simvastatin: 0.49 ± 0.10 μmol/L vs. placebo: 0.59 ± 0.11 μmol/L, $P = 0.10$). However, a trend for lower lipid hydroperoxide levels was identified in the simvastatin group at 12 months while no change was identified in the placebo group when lipid hydroperoxide levels were compared separately within groups with baseline (simvastatin – baseline: 0.53 ± 0.15 μmol/L vs. 12 months: 0.49 ± 0.1 μmol/L, $P = 0.81$ and placebo – baseline: 0.59 ± 0.2 μmol/L vs. 12 months: 0.59 ± 0.11 μmol/L, $P = 0.31$) (Table 2).

Cell adhesion molecules

There was no significant difference in sICAM and sVCAM levels between the simvastatin and placebo treatment groups at each time-point (Table 2). However, a trend for lower sICAM levels was identified in the simvastatin group at 12 months while no change was identified in the placebo group when sICAM levels were compared separately within groups with baseline (simvastatin – baseline 842 ng/ml vs. 12 months:

Table 1. Comparison of baseline and 12 month fasting glucose, liver function, lipid profile and immunoglobulin levels between treatment groups using the Mann–Whitney *U*-test

	Baseline			12 Months		
Variable	Statin Mean (SD)	Placebo Mean (SD)	*P* value	Statin Mean (SD)	Placebo Mean (SD)	*P* value
Systolic BP (mmHg)	130.82 (12.83)	135.90 (12.57)	0.74	126.10 (27.30)	129.50 (19.04)	0.84
Diastolic BP (mmHg)	74.91 (19.43)	75.50 (18.76)	0.96	75.00 (12.52)	76.50 (8.87)	0.81
Glucose (mmol/L)	5.21 (0.84)	5.33 (0.93)	0.90	5.01 (1.07)	5.42 (2.01)	0.86
Bilirubin (μmol/L)	22.00 (27)	16.30 (9.98)	0.52	16.14 (10.85)	15.83 (9)	0.86
ALT (U/L)	53.27 (43.78)	61.50 (48.63)	0.82	43.57 (20.01)	74.50 (34.46)	0.14
AST (U/L)	51.27 (34.15)	59.10 (29.66)	0.52	50.14 (25.39)	64.00 (23.74)	0.39
GGT (U/L)	225.36 (182.3)	155.80 (122.34)	0.56	278.00 (196.32)	270.33 (141.05)	1.00
ALP (U/L)	225.55 (143.15)	208.50 (154.2)	0.71	279.57 (241.81)	260.33 (165)	0.84
Albumin (g/L)	40.55 (2.42)	41.50 (4.38)	0.32	41.57 (3.36)	42.50 (2.07)	0.91
Cholesterol (mmol/L)	6.23 (1.38)	6.16 (0.98)	0.56	4.91 (0.72)	6.15 (0.75)	**0.01**
HDL (mmol/L)	1.81 (0.42)	1.61 (0.53)	0.54	2.03 (0.28)	1.99 (0.34)	1.00
LDL (mmol/L)	3.90 (1.26)	3.88 (0.81)	0.69	2.33 (0.74)	3.53 (0.49)	**0.01**
HDL/LDL Ratio	3.53 (0.75)	4.15 (1.29)	0.24	2.45 (0.50)	3.14 (0.51)	0.07
IgA (g/L)	2.37 (0.94)	3.40 (0.91)	**0.03**	2.45 (0.75)	3.49 (1.18)	0.14
IgG (g/L)	13.48 (3.33)	15.27 (4.28)	0.36	13.79 (1.67)	15.95 (4.32)	0.63
IgM (g/L)	3.57 (1.74)	3.44 (1.64)	0.91	3.95 (2.12)	4.47 (1.84)	0.63

ALP, alkaline phosphotase; ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; BP, blood pressure; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Ig, immunoglobulins. Bold values indicate $P < 0.05$.

Table 2. Comparison of baseline and 12 month serological endothelial function, anti-oxidant status and hsCRP between treatment groups using the Mann–Whitney *U*-test

	Baseline			12 Months		
Variable	Statin Mean (SD)	Placebo Mean (SD)	*P* value	Statin Mean (SD)	Placebo Mean (SD)	*P* value
HPO (μmol/L)	0.53 (0.15)	0.59 (0.20)	0.82	0.49 (0.10)	0.59 (0.11)	0.10
sICAM1 (ng/ml)	842.00 (405.52)	851.20 (492.01)	0.96	580.80 (325.51)	844.50 (551.90)	0.56
sVCAM1 (ng/ml)	676.00 (241.37)	714.33 (566.08)	0.60	967.86 (531.72)	694.50 (289.98)	0.37
Vitamin C (μmol/L)	73.84 (26.66)	82.51 (29.46)	0.41	80.54 (21.68)	77.40 (24.72)	0.95
α-carotene (μmol/L)	0.03 (0.02)	0.05 (0.06)	1.00	0.05 (0.03)	0.06 (0.07)	0.45
α-tocopherol (μmol/L)	26.94 (6.25)	23.29 (8.11)	0.11	23.90 (4.49)	25.76 (7.91)	0.53
β-carotene (μmol/L)	0.21 (0.16)	0.20 (0.22)	0.76	0.38 (0.41)	0.23 (0.21)	0.30
β-cryptoxanthin (μmol/L)	0.05 (0.03)	0.04 (0.03)	0.28	0.04 (0.02)	0.07 (0.04)	0.23
γ-tocopherol (μmol/L)	3.23 (1.29)	3.06 (1.61)	0.76	2.56 (1)	2.53 (0.99)	0.95
Lutein (μmol/L)	0.13 (0.06)	0.11 (0.06)	0.43	0.14 (0.08)	0.12 (0.04)	0.91
Lycopene (μmol/L)	0.17 (0.09)	0.30 (0.28)	0.43	0.30 (0.19)	0.60 (0.54)	0.23
Retinol (μmol/L)	1.48 (0.80)	1.33 (0.59)	0.86	1.29 (0.41)	1.62 (0.70)	0.53
Zeaxanthin (μmol/L)	0.03 (0.02)	0.03 (0.02)	0.88	0.03 (0.01)	0.03 (0.01)	0.42
hsCRP (μg/dL)	818.97 (1000.39)	494.33 (488.02)	0.23	574.93 (501.64)	300.54 (376.60)	0.07

HPO, hydroperoxides; sICAM1, soluble intracellular adhesion molecule-1; sVCAM1, soluble vascular cell adhesion molecule-1.

580.8 ng/ml, $P = 0.13$ and placebo – baseline: 851.2 ng/ml vs. 12 months 844.5 ng/ml, $P = 0.63$).

Antioxidant status

Initial baseline levels were similar for both treatment groups. After 12 months, serum anti-oxidant levels including vitamin C, vitamin E (α-tocopherol, γ-tocopherol), vitamin A, lutein, zeaxanthin, α-carotene and lycopene did not change significantly from baseline when the treatment groups were directly compared or when assessed within groups separately over time (Table 2).

However, despite the lack of significance, a trend was identified for an advantageous effect of simvastatin on

vitamin C levels over time (simvastatin – baseline: 73.84 μmol/L vs. 12 months: 80.54 μmol/L, $P = 0.94$ and placebo – baseline: 82.51 μmol/L vs. 12 months: 77.40 μmol/L, $P = 0.16$).

Furthermore, although the mean β-carotene levels were not significantly different between the treatment groups at baseline (simvastatin: 0.21 ± 0/16 μmol/L vs. placebo: 0.2 ± 0.22 μmol/L, $P = 0.76$) or 12 months (simvastatin: 0.38 ± 0.41 μmol/L vs. placebo: 0.23 ± 0.21 μmol/L, $P = 0.30$), a significant trend for increased β-carotene levels was identified in the simvastatin group at 12 months while no change was identified in the placebo group when β-carotene levels were compared separately within groups with baseline (simvastatin – baseline: 0.21 ± 0.16 μmol/L vs. 12 months: 0.38 ± 0.41 μmol/L, $P = 0.03$ and Placebo – baseline: 0.20 ± 0.22 μmol/L vs. 12 months: 0.23 ± 0.21 μmol/L, $P = 0.30$) (Table 2).

High sensitive C-reactive protein

Baseline hsCRP levels were similar between treatment groups. At 12 months, there was no significant difference in hsCRP levels between treatment groups when compared with each other over time or when compared within groups separately (Table 2).

Vascular compliance

Baseline PWA assessed by AgIx75, TR, SEVR and ED% indices was similar between the treatment groups (Table 3). Mean PWV was also similar between the treatment groups at baseline (simvastatin: 8.50 ± 0.72 m/s vs. placebo: 8.46 ± 1.18 m/s, $P = 0.70$). By completion of the trial, there was no difference in PWA indices between the treatment groups when compared both between and within groups. PWV analysis also remained similar between the treatment groups at trial completion (simvastatin: 8.45 ± 1.1 m/s vs. placebo: 8.8 ± 0.99 m/s, $P = 0.66$) (Table 3).

Trial completion

Seven of 11 (63.6%) patients from the interventional group and six of 10 (60%) from the placebo group completed this study. Three patients (two from the placebo group) were successfully enrolled and issued medication, but did not take any medication and failed to attend for follow-up analysis. One patient prescribed simvastatin 40 mg developed mild myalgia at 9 months and discontinued medication. This patient remained in this study on an intention to treat basis. No patients discontinued therapy owing to clinical concern or deterioration of liver enzymes. In the simvastatin group, four of the 11 (36.4%) patients required a dose increase to 40 mg after 3 months as a result of total cholesterol remaining greater than 5.0 mmol/L, whereas six of 10 (60%) patients in the placebo group received an increase.

Discussion

Although PBC is strongly associated with hypercholesterolaemia, it is still uncertain whether PBC itself confers any additional increase in cardiovascular risk (4). Endothelial dysfunction and anti-oxidant depletion associated with PBC may represent either a predisposition to cardiovascular disease or may simply relate to a secondary phenomenon of disease activity (2, 22–24).

Previous researchers have identified well-established links between PBC and hyperlipidaemia and between raised lipids and endothelial dysfunction (4). Longo *et al.* found that more than 75% of patients with PBC had serum total cholesterol levels >5.2 mmol/L, whereas Creager *et al.* found that in patients with hypercholesterolaemia, there was a markedly decreased effect of nitrovasodilators (e.g. endothelium-derived relaxing factor) on the vascular smooth muscle of resistance vessels in the forearm (25). This reduced response to endothelium-dependent vasodilators reflects endothelial dysfunction and is thought to be caused by defects in the bioactivity of nitric oxide in hypercholesterolaemic patients (26).

The beneficial effects of statins are thought to extend beyond cholesterol reduction and there is increasing recognition of cholesterol-independent or "pleiotropic" effects of statins, including improvement of endothelial function and reduction in oxidative stress and vascular inflammation (27). The role of statin therapy in PBC patients remains yet unclear where therapeutic benefits

Table 3. Comparison of baseline and 12 month vascular compliance variables between treatment groups using Mann–Whitney *U*-test

	Baseline			12 Months		
Variable	Statin Mean (SD)	Placebo Mean (SD)	*P* value	Statin Mean (SD)	Placebo Mean (SD)	*P* value
HR (bpm)	68.21 (8.31)	68.20 (6.31)	0.80	68.24 (6.98)	72.67 (2.83)	0.36
AgIx75 (%)	30.66 (9.45)	27.20 (11.19)	0.62	28.24 (8.74)	22.78 (13.91)	0.56
TR (msec)	135.12 (10.53)	136.33 (10.39)	0.92	136.19 (10.81)	135.72 (10.72)	0.95
ED (%)	37.33 (4.11)	37.30 (3.01)	0.67	37.00 (2.74)	37.50 (2.22)	0.76
SEVR (%)	140.42 (29.62)	136.13 (15.89)	0.79	142.00 (19.91)	139.22 (15.16)	0.92
PWV (m/s)	8.50 (0.72)	8.46 (1.18)	0.70	8.45 (1.1)	8.80 (0.99)	0.66

AgIx75, augmentation index calibrated to 75 beats per minute; ED, ejection duration; HR, heart rate; PWV, pulse wave velocity; SEVR, subendocardial viability ratio; TR, time to reflectance.

may relate to beneficial effects on lipid homeostasis as well as potential advantageous anti-inflammatory effects (28). Currently, there is also a paucity of prospective data assessing the effects of statin therapy in PBC patients and an absence of randomized controlled trials.

Stojakovic *et al.* have recently produced a prospective report of the beneficial effects of 10 mg atorvastatin therapy in 19 early-stage PBC patients with hypercholesterolaemia and additional cardiovascular risk factors. Similar to our study where we identified a 21% reduction in total cholesterol and 40% reduction in LDL, they also observed a similar reduction of 28% in total cholesterol and 31% in oxidized LDL levels after 12 months of treatment. However, no appreciable beneficial effects were identified in HDL or triglycerides (15). These findings are also similar to Stojakovic *et al.*'s initial 12 week pilot study of atorvastatin prescribed at doses of 10 mg/day, 20 mg/day and 40 mg/day for 4 weeks, respectively, in 15 patients with early PBC, which showed 35% reductions in cholesterol and a 41% decrease in LDL (29). No other prospective studies of statin therapy in PBC patients have been conducted, but in further retrospective studies, Abu Rajab *et al.* identified a 30% mean reduction in cholesterol in 58 PBC patients on statin therapy for a mean duration of 41 months, whereas Stanca *et al.* (2008) identified a 31% reduction in total cholesterol and 33% reduction in LDL from their retrospective assessment of 15 PBC patients with hypercholesterolaemia treated with 10–20 mg of atorvastatin for 1 year (30, 31).

In terms of serological markers of endothelial dysfunction, we did not identify significant improvements with statin therapy, but did note trends towards improvement. In contrast, Stojakovic *et al.* identified significant reductions in endothelial inflammation as shown by lower sVCAM-1 (baseline: 114 ng/ml vs. 48 weeks: 85 ng/ml, $P = 0.028$) and oxidized LDL (baseline: 13 mU/L vs. 12 months: 9 mU/L, $P = 0.004$) levels. Cangemi *et al.* has also shown a 58.9% reduction in oxidized LDL by 30 days in hypercholesterolaemic patients without liver disease randomized to receive atorvastatin 10 mg/day instead of placebo (32). Although the sVCAM-1 findings of Stojakovic *et al.* were not replicated in our own study, we did identify a non-significant 31% reduction in sICAM-1 in the statin treatment group.

Aboutwerat *et al.* has shown that lipid peroxidation markers are significantly elevated in PBC compared with age- and sex-matched controls (plasma and urinary 8-isoprostane, $P < 0.001$; plasma malondialdehyde, $P = 0.007$) (24). Furthermore, Cangemi *et al.* has demonstrated significant attenuation of oxidative stress as determined by reduced urinary isoprostanes after only 3 days (-18.8%, $P < 0.01$) of statin therapy and after 30 days, these authors identified an even stronger reduction in isoprostanes (-37.1%, $P < 0.01$) (32). Treatment with atorvastatin has also been reported to significantly limit serum lipid peroxidation through reductions in LDL thiobarbituric acid reactive substances in large animal hypercholesterolaemia models (33). Our study identified a possible trend towards the prevention of serum lipid peroxidation as determined by the FOX-1 assay in participants randomized to statin therapy where lipid hydroperoxides were reduced by 8%. However, these effects were not statistically significant.

Our own group has previously identified antioxidant depletion in PBC, which is congruent with the finding of significantly reduced levels of glutathione (30% less in PBC) in this study by Aboutwerat *et al.* (2, 24). The results of our study have demonstrated a trend towards increased Vitamin C levels in PBC patients with hypercholesterolaemia treated with statins, but again, this was a non-significant improvement. Lui *et al.* has previously investigated the effects of atorvastatin (10 mg/day) administration for 5 months on antioxidant status in 19 hypercholesterolaemic patients and found that statin therapy had no effect on Vitamin C (baseline: 35.23 μmol/L vs. 5 months: 35.70 μmol/L, $P = 0.429$) (34). Therefore, if an advantageous effect of statin therapy on Vitamin C exists in PBC, it may be unique to the PBC population. However, this question may only be addressed by a further larger study.

In our study, vascular compliance was unaffected by statin therapy with PWV and other indices remaining static in this study population. Flow-mediated vasodilatation is another frequently used technique for non-invasively assessing endothelial function. However, within our centre, a long-standing and established expertise existed in the measurement of pulse wave velocity (PWV) and pulse wave analysis (PWA) (2, 35, 36). Therefore, strict adherence to our established process using this investigative tool was decided upon to ensure that the most robust clinical data were obtained.

Stojakovic *et al.*, contrary to our study, identified significant improvements in vascular function as assessed by flow-mediated dilation of the brachial artery (baseline: 7.16% vs. 48 weeks: 8.36%, $P < 0.05$). However, the findings of Stojakovic *et al.* were limited by the absence of a placebo-controlled patient group. Ott *et al.* more recently randomized 29 hypercholesterolaemic patients without liver disease to rosuvastatin or placebo for 42 days where PWA was assessed at rest and after infusion of NG-monomethyl-L-arginine (L-NMMA). These authors identified beneficial effects of rosuvastatin therapy on markers of endothelial function including a significantly lower resting central augmentation index over this study period (18.3% vs. 21.9%, $P = 0.027$) as well as a greater central augmentation index response to L-NMMA in the rosuvastatin group compared with placebo (rosuvastatin: 20.5% vs. 25.7%, $P = 0.001$ and placebo: 24% vs. 24.7%, $P = 0.632$) (37).

In contrast to the general population, PBC patients also have marked increases in plasma LDL, including the LDL subfraction lipoprotein-X (38). Despite the advantageous effect of simvastatin on lipid homeostasis,

particularly total cholesterol and LDLs, it remains unclear whether lipid modulation in PBC patients confers any improvements in cardiovascular risk or mortality in patients with PBC and hyperlipidemia (39). Although previous research from our own group and elsewhere has alluded to no increase in the risk of cardiovascular disease in the PBC population, there may be a subgroup of approximately 10% of PBC patients who have additional disorders of lipid metabolism, like PBC patients with additional cardiovascular risk factors, may be at increased risk of cardiovascular complications (2, 31, 39). Statin therapy may be beneficial in this subpopulation of PBC patients, but there remains significant concern regarding the safety profile of statins in PBC and as such, there has been a reluctance to commence this therapy, particularly as previous safety data pertains to retrospective cohort studies (40, 41). Therefore, the final aim of this study was to assess the safety of statins in PBC, especially in terms hepatotoxicity and transaminase derangement.

Our study suggests that simvastatin is an effective and safe therapy when administered to PBC patients where no evidence of hepatotoxicity or derangement of transaminases was identified. Although we have only reported a small series, our randomized placebo-controlled clinical trial demonstrates that simvastatin therapy is well tolerated in compliant patients with only one patient developing mild myalgia throughout the 12 month follow-up period. This safety profile is consistent with previous reports in the literature. Stanca *et al.* documented the discontinuation of statin therapy owing to myalgia in only one of 16 PBC patients, whereas Stojakovic *et al.* described two other PBC patients who discontinued statins as a result of significant increases in ALP and creatinine kinase levels (15, 30). Finally, Abu Rajab *et al.* reported no adverse affects of statins in PBC (31). Our prospective RCT provides additional safety data regarding the use of simvastatin in PBC.

A potential limitation of this study is the wide age range of participants included. In addition, although all patients had well-compensated disease, there was no uniformity in the length of time the disease was present. The exclusion criterion based on blood pressure was also set high (160/90 mmHg) in our methodology. The current definition of hypertension is 140/90 mmHg and by these standards, three participants from the placebo group and four participants from the simvastatin group would be considered hypertensive.

This study was the first placebo-controlled RCT comparing the effect of simvastatin in PBC patients with hypercholesterolaemia. In addition, by including up to 12 months of follow-up, it is also one of the longest reported prospective studies of statins in PBC patients (15). However, the authors acknowledge the low patient recruitment volume and suggest that larger studies over extended follow-up periods may be required to fully elicit the effects of statin therapy on longer term cardiovascular outcomes.

Conclusion

Statin therapy in patients with PBC appears safe and effective towards overall reductions in total cholesterol and LDL levels. Our initial study suggests that simvastatin may also confer advantageous effects on endothelial function and antioxidant status through reductions in lipid hydroperoxides coupled with an improved vitamin C status. However, as these trends were not statistically significant, further large-scale studies are warranted.

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