Biological effects of acute pravastatin treatment in patients after aneurysmal subarachnoid hemorrhage: a double-blind, placebo-controlled trial


Department of Neurosurgery, Addenbrooke’s Hospital, University of Cambridge, United Kingdom

Object. The authors previously demonstrated that acute pravastatin therapy in patients after aneurysmal subarachnoid hemorrhage (SAH) ameliorates vasospasm-related delayed ischemic neurological deficits. The object of this study was to continue to examine potential mechanisms of these beneficial effects.

Methods. Eighty patients with aneurysmal SAH (age range 18–84 years; time to onset 1.8 ± 1.3 days) were enrolled in a double-blind study and randomized to receive 40 mg of oral pravastatin or placebo daily for as long as 14 days. Daily transcranial Doppler ultrasonography and blood tests every 3 days (including full blood cell counts, coagulation profiles, fasting glucose and lipid profiles, and serum biochemistry) were performed during the trial period.

Results. No significant differences were found in baseline laboratory data between the trial groups. Subsequent measurements during the 14-day trial showed reduced low-density lipoprotein (LDL) cholesterol levels and total/high-density lipoprotein cholesterol ratios between Days 3 and 15 (p < 0.05), and increased D-fumer levels (p < 0.05) on Day 6, in the pravastatin group. Patients who received pravastatin but developed vasospasm had significantly lower baseline LDL cholesterol levels or a less extensive reduction in LDL cholesterol levels (p < 0.05), and greater increases in plasma fibrinogen (p = 0.009) and serum C-reactive protein on Day 3 (p = 0.007), compared with those patients without vasospasm. The reduction in LDL cholesterol levels on Day 3 in the placebo group correlated with the duration of normal cerebral autoregulation on the ipsilateral side of the ruptured aneurysm (p = 0.002).

Conclusions. In addition to functioning through a cholesterol-independent pathway, cerebrovascular protection from acute statin therapy following aneurysmal SAH may also function through cholesterol-dependent mechanisms.

(Key Words: cholesterol • inflammation • neuroprotection • statin therapy • subarachnoid hemorrhage)

Although aneurysmal SAH accounts for only 5% of all strokes, it is a devastating subtype of hemorrhagic stroke and disproportionately affects the relatively young (median age 52 years).12 Despite considerable advances in aneurysmal SAH diagnosis and intervention, high early mortality and disability rates still occur and are particularly prevalent when cerebral vasospasm results in DIND. The overall unfavorable outcome rate of aneurysmal SAH is as high as 38%, and combined early mortality and morbidity rates are 50% in the United Kingdom, which equates to 2500 patients either dying or sustaining severe disability in the United Kingdom every year.12

Extensive experimental and clinical research has been conducted in an effort to prevent or treat vasospasm, but current therapies are limited to induced “triple-H” (hypervolemic, hemodilution, and hypertensive) therapy or control of arterial carbon dioxide levels.56 In cases of refractory vasospasm, balloon angioplasty and superselective intraarterial injection of vasodilators (such as papaverine) have been used.12 Long-term outcomes from these local therapies, however, are disappointing.12 Although the deficiency in reliable treatment for preventing or reversing vasospasm, potential neuroprotective agents have been studied as alternatives.12,27,36

Statins, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase, have been discovered to produce neuroprotection by improving cerebral vasomotor reactivity and reducing cytokine responses to cerebral ischemia.22,52 Mechanisms for this neuroprotection have been thought to be independent of cholesterol reduction and exclusively

Abbreviations used in this paper: ALT = alanine aminotransferase; ANOVA = analysis of variance; CI = confidence interval; CPK = creatine phosphokinase; CRP = C-reactive protein; CT = computed tomography; DIND = delayed ischemic neurological deficit; eNOS = endothelial nitric oxide synthase; FEU = fibrinogen equivalent unit; HDL = high-density lipoprotein; IVH = intraventricular hemorrhage; LDL = low-density lipoprotein; mRS = modified Rankin Scale; NO = nitric oxide; OR = odds ratio; SAH = subarachnoid hemorrhage; SD = standard deviation; TCD = transcranial Doppler; WFNS = World Federation of Neurological Surgeons.
In this study, we sought to continue to assess the presence of hydrocephalus and/or IVH, and aneurysm location on cerebral angiography. Exclusion criteria were nonaneurysmal SAH, pregnancy, preictal statin therapy, and contraindications to statin use (such as a history of liver or renal dysfunction, or ALT level > 50 U/L).

After admission, clinical management of the aneurysmal SAH was conducted in the standard manner for the department, including administration of 60 mg oral nimodipine every 4 hours and moderate intravenous fluid supplementation (3 L/day). Daily TCD ultrasonography was used to measure vasospasm and assess cerebral autoregulation. Vasospasm was defined as mean flow velocity in the middle cerebral artery greater than 1.2 m/second with a Lindegaard ratio greater than 3. Severe vasospasm was defined as mean flow velocity greater than 2.0 m/second and a Lindegaard ratio greater than 3.33,34 Cerebral autoregulation was assessed using the transient hyperemic response test.34 Patients with symptomatic vasospasm were admitted to the Neurosciences Critical Care Unit for triple-H therapy. None of these patients underwent endovascular angioplasty. Factors that had the potential to affect patient outcome were documented, including ventriculitis, sepsis, modes of aneurysm treatment (endovascular versus clipping), operating neurosurgeons, and immediate postoperative deficits.

Vasospasm-related DIND was defined as the development of focal neurological deficits and/or a drop in the Glasgow Coma Scale score by two points or more27 and associated with vasospasm on TCD ultrasonography. Other possible conditions causing neurological deterioration (such as hydrocephalus, intracerebral hemorrhage, surgical complications, metabolic abnormalities, or infection) were excluded after repeated imaging (CT, xenon CT, and/or cerebral angiography) and metabolic screening. Patient outcome at discharge and at 6 months was defined according to the mRS as favorable (mRS Score 1 and 2) or unfavorable (mRS Score 3–6).2

Laboratory Tests

Detailed full blood cell counts (including differentiation), coagulation profiles (including fibrinogen and D-dimer), fasting glucose and lipid profiles, electrolytes, CRP levels, and muscle CPK levels were obtained at admission and repeated every 3 days. The serum lipid profile included total cholesterol level, LDL cholesterol level, HDL cholesterol level, total/HDL cholesterol ratio, and triglycerides. Suspected adverse effects were reported to the safety committee and the data were reviewed on an individual patient basis.

Statistical Analysis

All analyses were performed on an intent-to-treat basis, and probability values were two-sided. Data are presented as means ± SDs with 95% CIs. Statistical analysis was performed using Intercooled STATA statistical software (version 8.0 for Windows). The pre- and postrandomization characteristics were compared using the chi-square test; the Fisher exact test was used when any number of the cell was less than 5. For the characteristic of age, the t-test was used. All laboratory data were compared with the baseline data using repeated-measures ANOVA. When the ANOVA showed significant differences within subjects, tables of Dunnett correction were applied to identify significant changes over time. A probability value of less than 0.05 was considered statistically significant.

Results

Patient Recruitment

During 2004, 80 patients with aneurysmal SAH were recruited. Trial medications were begun within 1.8 ± 1.3 days (95% CI 0–4.3 days) from the initial bleed, and no trial-related complications were observed. Thirty-eight patients (47.5%) completed the 14-day trial. Of the remaining patients, 30 (37.5%) were well enough to be discharged early, 10 died (12.5%), and two patients (2.5%) prematurely withdrew. All 80 patients were included in the final analysis.

Prerandomization characteristics, including age, sex, WFNS grade, Fisher grade, hydrocephalus, IVH, and location of aneurysm, were well balanced between the trial groups. Factors that may have affected patient outcome after randomization, including insertion of external ventricular drains, ventriculitis, medical interventions, immediate postoperative deficits, operating neurosurgeons, and sepsis, were also well balanced between the trial groups.33

Development of Vasospasm

During the 14-day observation period, 42 patients (52.5%) developed vasospasm on TCD ultrasonography, including 20 (25.0%) on the ipsilateral side, six (7.5%) on the contralateral side, and 16 (20.0%) on both sides. The average number of days between the initial bleed and the onset of vasospasm was 5.2 ± 2.6 days (95% CI 4.3–6.0 days), and the average duration of vasospasm was 2.7 ± 3.5 days (95% CI 1.9–3.5 days).

Nineteen patients (23.8%) developed severe vasospasm, including 11 (13.8%) on the ipsilateral side, two (2.5%) on the contralateral side, and six (7.5%) on both sides. The
average number of days between the ictus and the onset of severe vasospasm was 6.8 ± 3.4 days (95% CI 4.9–8.6 days), and the average duration of severe vasospasm was 0.8 ± 1.8 days (95% CI 0.4–1.2 days).

Results on comparing vasospasm, DIND, cerebral auto-regulation, and outcome between the trial groups have been previously published.\(^\text{35,36}\) Briefly, the incidence rates of vasospasm and severe vasospasm on TCD ultrasonography were reduced in the pravastatin group by 32 (p = 0.006, log-rank test) and 42 (p = 0.044, log-rank test), respectively, with a shortened duration of severe vasospasm on TCD ultrasonography by 0.8 days (p = 0.068, t-test).

Logistic regression analysis results showed that an age less than 50 years (OR 4.09, 95% CI 1.36–12.26, p = 0.001), female gender (OR 5.06, 95% CI 1.14–21.85, p = 0.035), increased the risk (OR 3.98, 95% CI 1.07–14.76, p = 0.039) increased the risk of vasospasm, whereas pravastatin therapy reduced the risk (OR 0.39, 95% CI 0.13–1.14, p = 0.085). Similarly, female gender (OR 5.06, 95% CI 1.24–20.55, p = 0.023), poor WFNS grade (OR 4.05, 95% CI 1.07–15.34, p = 0.040), immediate postoperative deficits (OR 8.55, 95% CI 1.65–44.41, p = 0.011), and IVH (OR 3.98, 95% CI 1.07–14.76, p = 0.039) increased the risk of severe vasospasm, whereas pravastatin therapy reduced the risk (OR 0.16, 95% CI 0.04–0.74, p = 0.018).

Delayed Ischemic Deficits

Vasospasm-related DINDs occurred in 14 patients (17.5%; 12 in the placebo group and two in the pravastatin group) and were associated with new cerebral infarcts as noted on CT scans. The average number of days between the vasospasm and the onset of vasospasm-related DIND was 3.6 ± 1.7 days (95% CI 2.6–4.6 days).

The incidence of vasospasm-related DIND was significantly reduced by 83% in the pravastatin group (p < 0.001, log-rank test).\(^\text{35}\) The average number of days between the initial bleed and the onset of vasospasm-related DIND was 7.9 ± 2.9 days (95% CI 6.2–9.6 days), and no significant difference was noted between the trial groups. Logistic regression analysis identified pravastatin therapy as the only factor that influenced and reduced the incidence of DIND (OR 0.12, 95% CI 0.03–0.59, p = 0.009).

Laboratory Studies

Fibrinogen and D-dimer. In the entire study population, plasma fibrinogen levels increased acutely from Day 0 (3.74 ± 1.05 g/L, 95% CI 3.50–3.98 g/L) to Day 3 (5.06 ± 1.60 g/L, 95% CI 4.69–5.42 g/L, p < 0.001). This higher level was sustained throughout the entire 14-day trial period (p < 0.001). In contrast, there were no significant changes in plasma D-dimer levels. A higher level or a larger increase of fibrinogen on Day 3 was associated with the occurrence of vasospasm (p < 0.05; Table 1), whereas smaller increases in D-dimer on Day 3 or 6 correlated with an aggravating effect toward severe vasospasm (Table 2).

There were significant correlations between DIND and increases in plasma fibrinogen or D-dimer. Patients who developed DIND had larger increases in plasma fibrinogen or larger decrements in D-dimer, regardless of the allocation (Table 2).

Hemoglobin and Hematocrit. Similar progressive hematocrit dilution was noted in all patients regardless of the trial allocation (maximum values on Day 15: hemoglobin −1.65 g/dL, 95% CI −2.41 to −0.90 g/dL, p < 0.001; hematocrit −4.82%, 95% CI −6.89 to −2.74%, p < 0.001), particularly between Days 3 and 12 in those with an unfavorable outcome (maximum difference on Day 9: −4.28 g/dL, 95% CI −1.87 to 6.69 g/dL, p < 0.001; Fig. 1). There were negative correlations between levels of hemoglobin and CRP from Days 6 to 15 (r = −0.06 to −0.37, p < 0.05).

Leukocytes and Platelets. Within the entire study population, changes in the number of leukocytes, neutrophils, lymphocytes, and monocytes followed a biphasic pattern. Compared with baseline levels, the neutrophil count decreased significantly on Day 6 (−1.58 × 10⁹/L, 95% CI

### Table 1

<table>
<thead>
<tr>
<th>Laboratory Results</th>
<th>No Vasospasm (95% CI)</th>
<th>Vasospasm (95% CI)</th>
<th>Difference (95% CI)</th>
<th>p Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean Day 3 fibrinogen (g/L)</td>
<td>4.48 ± 1.47 (3.99–4.97)</td>
<td>5.59 ± 1.54 (5.10–6.09)</td>
<td>1.11 (0.43–1.80)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>mean Day 3 increases in fibrinogen (g/L)</td>
<td>0.63 ± 1.53 (0.12–1.14)</td>
<td>1.96 ± 1.70 (1.41–2.50)</td>
<td>1.33 (0.59–2.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>mean Day 3 increases in CRP (mg/L)</td>
<td>16.51 ± 61.26 (3.35 to 36.37)</td>
<td>62.31 ± 74.43 (37.12–87.49)</td>
<td>45.79 (14.52–77.07)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>mean Day 3 increases in calcium (mmol/L)</td>
<td>0.01 ± 0.12 (−0.03 to 0.05)</td>
<td>0.08 ± 0.09 (0.05–0.11)</td>
<td>0.07 (0.02–0.12)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

* Where appropriate, mean values are presented ± SDs.
† After repeated-measures ANOVA and Dunnett correction.

### Table 2

<table>
<thead>
<tr>
<th>Laboratory Results</th>
<th>No Severe Vasospasm (95% CI)</th>
<th>Severe Vasospasm (95% CI)</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean Day 3 increases in D-dimer (FEU mg/L)</td>
<td>0.41 ± 1.08 (0.04–0.77)</td>
<td>−0.69 ± 1.10 (−1.35 to −0.02)</td>
<td>−1.09 (−1.8 to −0.39)</td>
</tr>
<tr>
<td>mean Day 6 increases in D-dimer (FEU mg/L)</td>
<td>0.06 ± 0.98 (−0.30 to 0.41)</td>
<td>−1.12 ± 1.39 (−2.0 to −0.24)</td>
<td>−1.18 (−1.9 to −0.42)</td>
</tr>
<tr>
<td>mean Day 9 Mg (mmol/L)</td>
<td>0.85 ± 0.11 (0.82–0.89)</td>
<td>0.78 ± 0.07 (0.74–0.82)</td>
<td>−0.07 (−0.13 to −0.01)</td>
</tr>
<tr>
<td>mean Day 12 Mg (mmol/L)</td>
<td>0.87 ± 0.10 (0.83–0.91)</td>
<td>0.79 ± 0.13 (0.72–0.86)</td>
<td>−0.08 (−0.15 to −0.01)</td>
</tr>
</tbody>
</table>

* Where appropriate, mean values are presented ± SDs. p < 0.05 for all differences after repeated-measures ANOVA and Dunnett correction.
The serum glucose level was particularly related to patients having unfavorable outcomes. Patients who had unfavorable outcomes had significantly higher serum glucose levels from Days 0 to 6. The differences were 0.76 mmol/L (95% CI 0.66–1.46 mmol/L, p = 0.033), 1.10 mmol/L (95% CI 0.46–1.74 mmol/L, p = 0.001), and 0.99 mmol/L (95% CI 0.33–1.65 mmol/L, p = 0.004) for Days 0, 3, and 6, respectively, and were unrelated to the trial allocation group. C-reactive protein was particularly associated with early deaths and unfavorable outcome.

Serum Lipid Profile. No difference was found in the baseline lipid profile, but ALT levels continuously increased from Days 6 to 15 (p < 0.001; Fig. 3).

Within the entire study population, certain changes in serum biochemistry were associated with vasospasm, DIND, or poor outcome. Patients who developed vasospasm or severe vasospasm had more increases in CRP by 45.79 mg/L (95% CI 14.52–77.07 mg/L, p < 0.05) or corrected serum calcium by 0.07 mmol/L (95% CI 0.02–0.12 mmol/L, p < 0.05) on Day 3 (Table 1). Patients with Mg levels less than 0.8 mmol/L after Day 9 were more likely to suffer severe vasospasm or unfavorable outcome (Table 4). Moreover, patients who had an unfavorable outcome also had higher levels of glucose on Day 3 (p < 0.05) and higher levels of CRP on Days 3 to 9 (p < 0.001), but lower levels of calcium from Days 9 to 15 (p < 0.05).

The serum glucose level was particularly related to patient outcome, but the occurrence of vasospasm or DIND CI −2.49 to −0.68 × 10^9/L, p < 0.001) and again on Day 15 (−3.63 × 10^9/L, 95% CI −5.57 to −1.69 × 10^9/L, p < 0.001). However, the lymphocyte count moved in the opposite direction as it increased significantly on Day 9 (0.46 × 10^9/L, 95% CI 0.26–0.66 × 10^9/L, p < 0.001) and Day 15 (0.43 × 10^9/L, 95% CI 0.19–0.67 × 10^9/L, p < 0.001). The monocyte count was apparently reduced on Day 3 by 38.49 mg/L (95% CI 22.10–54.89 mg/L, p = 0.004) for Days 3 to 9 (p < 0.05) and again on Day 9 (0.43 × 10^9/L, 95% CI 0.17–1.28 FEU mg/L, p < 0.05).

Serum Biochemistry. No significant differences in serum biochemistry between baseline levels or subsequent changes (including ALT and muscle CPK) were found between the trial groups, except for the lipid profile.

In the whole study population, no significant changes were noted in serum glucose and Mg. Compared with baseline levels, levels of corrected serum calcium (the serum calcium level after adjustment according to the serum albumin level) were significantly increased from Days 3 to 15 (p < 0.001), and CRP was significantly higher than baseline on Day 3 by 38.49 mg/L (95% CI 22.10–54.89 mg/L, p < 0.001). There were gradual reductions in muscle CPK, with a maximum reduction occurring on Day 12 (−285.27 U/L, 95% CI −492.84 to 77.70 U/L, p < 0.05), but ALT levels continuously increased from Days 6 to 15 (p < 0.001; Fig. 3).

Within the entire study population, certain changes in serum biochemistry were associated with vasospasm, DIND, or poor outcome. Patients who developed vasospasm or severe vasospasm had more increases in CRP by 45.79 mg/L (95% CI 14.52–77.07 mg/L, p < 0.05) or corrected serum calcium by 0.07 mmol/L (95% CI 0.02–0.12 mmol/L, p < 0.05) on Day 3 (Table 1). Patients with Mg levels less than 0.8 mmol/L after Day 9 were more likely to suffer severe vasospasm or unfavorable outcome (Table 4). Moreover, patients who had an unfavorable outcome also had higher levels of glucose on Day 3 (p < 0.05) and higher levels of CRP on Days 3 to 9 (p < 0.001), but lower levels of calcium from Days 9 to 15 (p < 0.05).

The serum glucose level was particularly related to patient outcome, but the occurrence of vasospasm or DIND CI −2.49 to −0.68 × 10^9/L, p < 0.001) and again on Day 15 (−3.63 × 10^9/L, 95% CI −5.57 to −1.69 × 10^9/L, p < 0.001). However, the lymphocyte count moved in the opposite direction as it increased significantly on Day 9 (0.46 × 10^9/L, 95% CI 0.26–0.66 × 10^9/L, p < 0.001) and Day 15 (0.43 × 10^9/L, 95% CI 0.19–0.67 × 10^9/L, p < 0.001). The monocyte count was apparently reduced on Day 3 by 38.49 mg/L (95% CI 22.10–54.89 mg/L, p = 0.004) for Days 3 to 9 (p < 0.05) and again on Day 9 (0.43 × 10^9/L, 95% CI 0.17–1.28 FEU mg/L, p < 0.05).

Serum Biochemistry. No significant differences in serum biochemistry between baseline levels or subsequent changes (including ALT and muscle CPK) were found between the trial groups, except for the lipid profile.

In the whole study population, no significant changes were noted in serum glucose and Mg. Compared with baseline levels, levels of corrected serum calcium (the serum calcium level after adjustment according to the serum albumin level) were significantly increased from Days 3 to 15 (p < 0.001), and CRP was significantly higher than baseline on Day 3 by 38.49 mg/L (95% CI 22.10–54.89 mg/L, p < 0.001). There were gradual reductions in muscle CPK, with a maximum reduction occurring on Day 12 (−285.27 U/L, 95% CI −492.84 to 77.70 U/L, p < 0.05), but ALT levels continuously increased from Days 6 to 15 (p < 0.001; Fig. 3).

Within the entire study population, certain changes in serum biochemistry were associated with vasospasm, DIND, or poor outcome. Patients who developed vasospasm or severe vasospasm had more increases in CRP by 45.79 mg/L (95% CI 14.52–77.07 mg/L, p < 0.05) or corrected serum calcium by 0.07 mmol/L (95% CI 0.02–0.12 mmol/L, p < 0.05) on Day 3 (Table 1). Patients with Mg levels less than 0.8 mmol/L after Day 9 were more likely to suffer severe vasospasm or unfavorable outcome (Table 4). Moreover, patients who had an unfavorable outcome also had higher levels of glucose on Day 3 (p < 0.05) and higher levels of CRP on Days 3 to 9 (p < 0.001), but lower levels of calcium from Days 9 to 15 (p < 0.05).

The serum glucose level was particularly related to patient outcome, but the occurrence of vasospasm or DIND CI −2.49 to −0.68 × 10^9/L, p < 0.001) and again on Day 15 (−3.63 × 10^9/L, 95% CI −5.57 to −1.69 × 10^9/L, p < 0.001). However, the lymphocyte count moved in the opposite direction as it increased significantly on Day 9 (0.46 × 10^9/L, 95% CI 0.26–0.66 × 10^9/L, p < 0.001) and Day 15 (0.43 × 10^9/L, 95% CI 0.19–0.67 × 10^9/L, p < 0.001). The monocyte count was apparently reduced on Day 3 by 38.49 mg/L (95% CI 22.10–54.89 mg/L, p = 0.004) for Days 3 to 9 (p < 0.05) and again on Day 9 (0.43 × 10^9/L, 95% CI 0.17–1.28 FEU mg/L, p < 0.05).

Serum Biochemistry. No significant differences in serum biochemistry between baseline levels or subsequent changes (including ALT and muscle CPK) were found between the trial groups, except for the lipid profile.

In the whole study population, no significant changes were noted in serum glucose and Mg. Compared with baseline levels, levels of corrected serum calcium (the serum calcium level after adjustment according to the serum albumin level) were significantly increased from Days 3 to 15 (p < 0.001), and CRP was significantly higher than baseline on Day 3 by 38.49 mg/L (95% CI 22.10–54.89 mg/L, p < 0.001). There were gradual reductions in muscle CPK, with a maximum reduction occurring on Day 12 (−285.27 U/L, 95% CI −492.84 to 77.70 U/L, p < 0.05), but ALT levels continuously increased from Days 6 to 15 (p < 0.001; Fig. 3).

Within the entire study population, certain changes in serum biochemistry were associated with vasospasm, DIND, or poor outcome. Patients who developed vasospasm or severe vasospasm had more increases in CRP by 45.79 mg/L (95% CI 14.52–77.07 mg/L, p < 0.05) or corrected serum calcium by 0.07 mmol/L (95% CI 0.02–0.12 mmol/L, p < 0.05) on Day 3 (Table 1). Patients with Mg levels less than 0.8 mmol/L after Day 9 were more likely to suffer severe vasospasm or unfavorable outcome (Table 4). Moreover, patients who had an unfavorable outcome also had higher levels of glucose on Day 3 (p < 0.05) and higher levels of CRP on Days 3 to 9 (p < 0.001), but lower levels of calcium from Days 9 to 15 (p < 0.05).

The serum glucose level was particularly related to patient outcome, but the occurrence of vasospasm or DIND
line lipid profiles between the two trial groups. Subsequent measurements showed that patients in the pravastatin group had consistently lower LDL cholesterol levels from Days 3 to 12 ($p < 0.05$) and lower total/HDL cholesterol ratios from Days 3 to 15 ($p < 0.05$) (Fig. 4A and B).

In the pravastatin group, patients who developed vasospasm or severe vasospasm had had significantly lower baseline LDL cholesterol levels, with a difference of 0.74 mmol/L (95% CI 0.25–1.22 mmol/L, $p = 0.004$), and their reduction in LDL cholesterol levels appeared to be less extensive than in the other patients (Day 6 reduction: $-0.26 \pm 0.65$ mmol/L compared with $-0.90 \pm 0.84$ mmol/L in patients without vasospasm or severe vasospasm, respectively; $p = 0.040$; Fig. 4C and D). The pravastatin group also had fewer baseline platelets by $34.64 \times 10^9/L$ (95% CI 2.61–66.68 × $10^9/L$, $p = 0.034$), more leukocytes by $3.51 \times 10^9/L$ (95% CI 0.68–6.33 × $10^9/L$, $p = 0.016$), more neutrophils by $3.65 \times 10^9/L$ (95% CI 0.92–6.38 × $10^9/L$, $p = 0.010$), more increases in fibrinogen levels by 1.24 g/L (95% CI 0.33–2.15 g/L, $p = 0.009$), and more increases in CRP levels by 56.51 mg/L (95% CI 16.77–96.26 mg/L, $p = 0.007$) on Day 3 compared with those without vasospasm. Similar differences or changes were not significant in the placebo group.

In the placebo group, patients who developed vasospasm had higher baseline levels of D-dimer by 1.72 FEU mg/L (95% CI 0.83–2.61 FEU mg/L, $p < 0.001$) compared with those without vasospasm. These findings were not noted in the pravastatin group. Furthermore, the reduction in LDL cholesterol levels on Day 3 in the placebo group correlated with the duration of normal cerebral autoregulation on the ipsilateral side ($r = 0.52$, $p = 0.002$), which was not found on the contralateral side or in the pravastatin group.

In the placebo group, patients who later suffered DIND or an unfavorable outcome had higher baseline levels of D-dimer by 1.15 FEU mg/L (95% CI 0.11–2.20 FEU mg/L, $p = 0.032$) and CRP by 18.76 mg/L (95% CI 2.66–34.86 mg/L, $p = 0.024$), which were not noted in the pravastatin group.

In the entire group, negative correlations between levels of HDL cholesterol and CRP were noted from Day 3 to Day 12 ($r = -0.40$ to $-0.06$, $p < 0.05$). The correlation between increases in HDL cholesterol and hematocrit was significant from Day 0 to Day 15 ($r = 0.28$ to 0.47, $p < 0.05$).

**Discussion**

**Cholesterol-Independent Vascular Mechanisms of Statins**

Laboratory findings in this study were compatible with previous experimental studies and human trials on statin therapy in cardiology.21,22,28 Vascular effects of statins have been believed to be exclusively derived from upregulation of eNOS, and are therefore cholesterol-independent.10,37 The mechanisms of action of statins consist of oppressing induction of the major histocompatibility complex Class II
expression and suppressing major histocompatibility complex Class II–mediated T-cell activation and monocyte macrophages. Statins have also been shown to decrease expression of intercellular adhesion molecule-1 on cerebral endothelium and leukocytes; thus, the inflammatory cascade is not propagated. The antithrombogenic effect of statins occurs by increasing endogenous anticoagulants (such as plasminogen activators) and decreasing procoagulants (such as inhibitors of plasminogen activator).

In the context of the pathophysiology of aneurysmal SAH, immediate statin therapy therefore fits into the potential therapeutic window between the ictus and the development of cerebral vasospasm and related DIND.

These trial results have also identified those patients who benefited most from the acute statin therapy, that is, patients without septic conditions, in which systemic inflammatory cascades are not initiated. Patients who already have active inflammation, such as sepsis or ventriculitis, may also have increased expression of intercellular adhesion molecule-1 on cerebrovascular endothelial cells or leukocytes and are unable to obtain sufficient neuroprotection from statins. Therefore, determining the optimum statin doses for patients with aneurysmal SAH during septic conditions needs further investigation.

**Cholesterol-Dependent Vascular Mechanisms of Statins**

In this study, patients with higher baseline LDL cholesterol levels before pravastatin therapy or larger reductions of LDL cholesterol levels after pravastatin therapy were less likely to suffer vasospasm; therefore, it is possible that the neuroprotection of these medications may work partially through cholesterol-dependent pathways. Furthermore, in the placebo group, the baseline LDL cholesterol levels

<table>
<thead>
<tr>
<th>Variable</th>
<th>Interval</th>
<th>Mg &lt;0.8 mmol/L (95% CI)</th>
<th>Mg ≥0.8 mmol/L (95% CI)</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>severe vasospasm</td>
<td>Day 9</td>
<td>45.45 (24.65–66.26)</td>
<td>15.52 (6.20–24.84)</td>
<td>−29.94 (−52.74 to −0.07)</td>
</tr>
<tr>
<td></td>
<td>Day 12</td>
<td>53.33 (28.09–78.58)</td>
<td>16.92 (7.81–26.04)</td>
<td>−36.41 (−63.25 to −9.57)</td>
</tr>
<tr>
<td></td>
<td>Day 15</td>
<td>60.00 (29.64–90.36)</td>
<td>18.57 (9.4–27.68)</td>
<td>−41.43 (−73.13 to −9.73)</td>
</tr>
<tr>
<td>unfavorable outcome</td>
<td>Day 12</td>
<td>66.67 (42.81–90.52)</td>
<td>29.23 (18.17–40.29)</td>
<td>−37.43 (−63.73 to −11.14)</td>
</tr>
</tbody>
</table>

* Data for Mg values are presented as mean values. p < 0.05 for all differences after repeated-measures ANOVA and Dunnett correction.
correlated with the duration of impaired autoregulation, implying that the LDL cholesterol level may involve inducing cerebrovascular dysfunction. Moreover, studies on atherosclerosis-related carotid stenosis have identified a pivotal role played by the LDL cholesterol level, which can be converted into harmful oxidized products during vascular inflammation. Higher LDL cholesterol levels have been found to be associated with stimulating platelets to release thromboxanes, factors that have been known to enhance thrombogenesis, inflammation, induction of vasospasm, and proliferative angiopathy.

Subarachnoid blood can induce vascular inflammation, which subsequently catalyzes conversions of LDL cholesterol into oxidized LDL cholesterol. Leukocytes (particularly neutrophils) release these oxidized lipids, which deplete the eNOS-derived NO and cause dysfunction of endothelium and cerebral vasospasm. Thus, the LDL cholesterol that is reduced by statin therapy can decrease the substrate availability for generating reactive oxygen species and oxidized LDL in vascular endothelium. The neurovascular protection of statins may therefore function through both cholesterol-dependent and -independent mechanisms.

**Other Associated Neuroprotective Agents**

These trial results also identify correlations between Mg and DIND. An aggravating effect on cerebral vasospasm from a delayed reduction in serum Mg levels suggests anti-vasospastic and neuroprotective effects of Mg, although other studies that involved maintaining serum Mg levels in patients between 1.0 to 2.0 mmol/L or supplying certain doses of Mg showed only equivocal results. Magnesium has been discovered to play an essential role in regulating cholesterol synthesis by reversible inactivation of 3-hydroxy-3-methylglutaryl coenzyme A reductase via phosphorylation. Therefore, a higher level of serum Mg may enhance LDL cholesterol level reduction and provide indirect cerebrovascular protection.

High-density lipoprotein is not only an important antiatherogenic factor because it removes excess cholesterol from other cells into the liver, but it also protects vascular endothelial cells from inflammation and oxidative stress and plays a key role in protecting the cell membrane from oxidative damage. Moreover, elevated HDL cholesterol levels may improve cerebral blood flow by decreasing aggregation and improving deformability of erythrocytes, thus enhancing hemorheology and microcirculation. In contrast, LDL cholesterol and triglycerides have been shown to increase aggregation of erythrocytes and deteriorate microcirculation. Therefore, the reduction in the total/HDL cholesterol ratio in this study may have contributed to the neuroprotective effects of acute statin therapy in these patients with aneurysmal SAH.

This trial has also shown that the progressive hemodilution seems to be an ongoing pathophysiological process after aneurysmal SAH. It is unlikely to be exclusively iatrogenic, because only 27.5% of patients in this trial under-
Biological effects of statins after SAH

went triple-H therapy, and phlebotomy has never been used for aggressive hemodilution in our department. Anemia not only impedes systemic oxygen availability but also is associated with unfavorable outcome and deaths after aneurysmal SAH. The close association among inflammatory profile, hemodilution, and HDL cholesterol implies that adverse effects from systemic inflammation may have significantly deteriorated a patient’s outcome after aneurysmal SAH, in addition to cerebral vasospasm. Because the inflammatory process produces oxidative stress, resulting in the wearing off of the cell membrane, causing aggregation of erythrocytes and shortening their lifespan within the microcirculation, the progressive hemodilution may be the result of accelerated destruction of erythrocytes after aneurysmal SAH. Furthermore, HDL cholesterol has been discovered to repair the erythrocyte membrane by its antioxidant effect and removal of oxidized cholesterol, and a positive correlation between the hemoglobin and HDL cholesterol levels has been observed, implying that the lifespan of erythrocytes is dependent on the availability of HDL cholesterol.

Multicentered STASH Trial

Although this was a prospective, randomized, controlled trial, the sample size was relatively small and the study was performed at a single institution. Therefore, we have begun the SimvaStatin in Aneurysmal Subarachnoid Hemorrhage study in 1600 patients (STASH; International Standard Randomized Controlled Trial No. 75948817, http://www.stashtrial.com). This trial is designed to realistically reflect common clinical practice; exclusion criteria are minimal and the recent lifting of patent restrictions on pravastatin will ensure affordability and availability of the medication worldwide.

Conclusions

This study has demonstrated that effective cerebrovascular protection from a short treatment course of statins after aneurysmal SAH may function through cholesterol-dependent mechanisms, in addition to the cholesterol-independent pathway. Further confirmation from a multicenter Phase III trial (STASH trial) is justified.

References

5. Chien JY, Jeng JS, Yu C, Yang PC: Low serum levels of high-density lipoprotein cholesterol is a poor prognostic factor for severe sepsis. Crit Care Med 33:1688–1693, 2005
24. Moriarity PM, Gibson CA: Association between hematological
parameters and high-density lipoprotein cholesterol. *Curr Opin Cardiol* **20:**318–323, 2005


Manuscript submitted February 27, 2007. Accepted May 24, 2007. This project was sponsored by the Addenbrooke’s Foundation Hospital and the University of Cambridge. Dr. Tseng was supported by scholarships from the British Council and the Raymond and Beverley Sackler Studentship, University of Cambridge. Dr. Hutchinson is supported by the Academy of Medical Sciences/Health Foundation Senior Surgical Specialist Fellowship. Address correspondence to: Peter J. Kirkpatrick, F.R.C.S.(SN), Box 167, Department of Neurosurgery, Addenbrooke’s Hospital, Cambridge CB2 2QQ, United Kingdom. email: pkj21@medschl.cam.ac.uk.