Statins and cardiovascular diseases: the multiple effects of lipid-lowering therapy by statins

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Abstract

Cholesterol lowering involving different therapies improves the clinical outcome of patients. To define the underlying pathomechanism, we studied whether treatment with statins was associated with changes in blood thrombogenicity, endothelial dysfunction and soluble adhesion molecule levels. Fifty hypercholesterolemic patients were treated with pravastatin (40 mg/day, n = 24) or simvastatin (20 mg/day, n = 26). Lipid profile and blood thrombogenicity were assessed in all patients before and after 3 months of cholesterol reducing therapy. Blood thrombogenicity was assessed as thrombus formation, perfusing non-anticoagulated blood directly from the patients' vein through the Badimon perfusion chamber (shear rate 1690/s). Endothelial-dependent vasomotor response was tested by laser-Doppler flowmeter. Soluble adhesion molecule level were measured by ELISA. Total and LDL cholesterol were reduced in the two treatment groups by statin therapy. Statin therapy was associated with a significant reduction in blood thrombogenicity and endothelium-dependent vasoresponse. No differences were observed between simvastatin or pravastatin treatment. Lipid lowering by statins had no effect on plasma levels of fibrinogen, sL-selectin, sP-selectin and sICAM-1 antigen. Cholesterol lowering by both statins reduced the increased blood reactivity and endothelial dysfunction present under hypercholesterolemia. The multiple effects of lipid lowering therapy by statins may explain the benefits observed in recent epidemiological trials. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Statin; Hyperlipidemia; Thrombosis; Endothelium; Atherosclerosis

1. Introduction

Hypercholesterolemia — the major risk factors for the development of atherosclerotic disease [1–4] is associated with increased deposition of lipids and monocytes/macrophages within the arterial wall, endothelial dysfunction and vasoconstriction, enhanced platelet reactivity, and hypercoagulability [5,6]. Reduction of serum cholesterol levels by different interventions including diet, exercise, lifestyle changes or pharmacological approaches is associated with a significant reduction in cardiovascular mortality and morbidity [3–6]. Recently, the effectiveness of a new class of powerful hypolipidemic agents, the statins, has been tested in several large clinical trials. These trials showed significant improvement in clinical outcomes of patients with and without coronary artery disease [7–12]. These observations were applicable to primary and secondary prevention of coronary events in both, hyper- and normocholesterolemic populations [4,6–12]. Statin treatment was associated with a consistent reduction in coronary events of ∼30–40% [13,14].

Angiographic trials assessing the effect of lipid lowering treatment on coronary atherosclerosis showed only a small reduction in coronary stenosis [14,15]. The clinical benefits observed in these trials significantly
exceed the expectations based on alterations in vessel lumen diameter. This suggested that lipid-reducing therapy might have additional effects that would explain the discrepancy between clinical benefits and lack of significant lesion regression [3–6]. Experimental and clinical studies have indicated a relationship between hyperlipidemia and increased blood thrombogenicity [15–21]. It was suggested that correction of hypercholesterolemia by pravastatin could normalize blood thrombogenicity [20]. However, conflicting findings on the effect of different statins on thrombosis have been reported by other authors [22,23]. In addition, hyperlipidemia has been associated with an impaired endothelium-dependent vasoresponse [24] and cholesterol lowering by the use of specific statins has been reported to improve endothelial dysfunction [3,24]. The goal of our study was to delineate whether lipid lowering by statins, irrespective of the specific statin used, was associated with changes in blood thrombogenicity and endothelial dysfunction in the same hyperlipidemic patient population. Furthermore, we studied whether changes in thrombogenicity and endothelial function were associated with alterations in circulating soluble adhesion molecule levels.

The working hypothesis of this study was that (1) the reduction of plasma lipid levels reduces blood thrombogenicity and endothelial dysfunction and (2) that the decreased thrombogenicity of blood and endothelium is associated with a reduction in circulating soluble adhesion molecule levels. Given the number of pathogenic processes modulated by plasma lipid levels, we further hypothesized that the significant benefits observed in clinical trials with statins might rather be a result of the significant reduction of plasma cholesterol level than a specific effect associated with the administration of one specific statin. To prove this hypothesis, hyperlipidemic patients were randomized into two groups receiving one of two most frequently prescribed statins for lipid reduction.

This study demonstrates multiple effects of cholesterol reducing therapy by the statin class on blood thrombogenicity and endothelial dysfunction in the same patient group under hyperlipidemic conditions. The combined reduction of blood thrombogenicity and endothelial dysfunction observed after lipid lowering by statins may be responsible for the clinical improvements reported in several large epidemiological trials.

2. Material and methods

2.1. Patient population

The study population included hypercholesterolemic patients (n = 50). Hypercholesterolemia was defined as LDL-cholesterol level > 120 mg/dl. Twenty-six of the patients had documented coronary artery disease and/or peripheral artery disease. The presence of coronary artery disease was documented by coronary angiography (stenosis > 50%), history of previous myocardial infarction or typical angina pectoris with typical changes in treadmill ECG. Exclusion criteria included malignant diseases, hematological disorders, severe hypertension, heparin or warfarin therapy and exposure to any lipid lowering therapy within the 8 weeks prior to randomization. The selected patients were instructed to follow a low cholesterol, low fat diet and then randomized into two treatment groups. Group I was assigned to receive pravastatin, 40 mg per day and Group II simvastatin, 20 mg per day for a period of 3 months. All patients were advised to take the medication at evening time. Other drugs the patients were currently receiving remained unchanged. The study was approved by the Institutional Review Board, and all patients signed informed consent.

The following routine laboratory parameters were determined at baseline and at the follow-up visits: lipid profile, white blood cell count, platelet count, partial thromboplastin time (aPTT), and fibrinogen level. In addition, citrated plasma samples, obtained pre and post treatment with simvastatin or pravastatin, were aliquoted and frozen for the analysis of sL-selectin, sP-selectin and sICAM-1 (R&D systems, Minneapolis, MN). The coefficient of variation for all these parameters was < 6% in all instances. All assays were performed according to the manufacturer’s instructions.

2.2. Assessment of blood thrombogenicity

Blood thrombogenicity was evaluated at baseline and 3-months after lipid-lowering therapy in both groups of patients. This experimental design allows for each patient to serve as his/her own control. Effect of lipid lowering on blood thrombogenicity was assessed as changes in the thrombus area formed in an ex-vivo perfusion chamber [25]. The ex-vivo perfusion chamber mimics the in vivo local rheologic conditions that develop in a mildly stenosed coronary artery. In this perfusion system, thrombus formation is triggered by the exposition of components of the subendothelial vessel wall to the flowing blood. Blood from hypercholesterolemic patients, prior to and after statin treatment, was allowed to circulate through the perfusion system. Blood reactivity was assessed by morphometric analysis of thrombus formed on the thrombogenic substrate. The description, validity and reliability of the Badimon perfusion system to study thrombus formation on defined thrombogenic substrates under stable rheological conditions have been previously reported [25,26].

Perfusion chamber experiments and blood sampling were performed in the mornings, in fasting patients. In
brief, a 19-gauge needle was carefully inserted into an antecubital vein. After discarding the first 5 ml of blood, the needle was connected to three Badimon perfusion chambers placed in series to minimize blood loss. Native, non-anticoagulated, blood was directly perfused from the antecubital vein through the perfusion chambers at a constant flow maintained by using a peristaltic pump (Masterflex, Cole-Parmer Instruments) distal to the chambers. The perfusion chamber consists of a cylindrical flow channel (1 mm diameter and 2.5 cm length) that allows the blood to flow over the thrombogenic substrate. All the perfusions were performed for a period of 5 minutes at a flow rate of 10 ml/min (calculated shear rate of 1690/s; Reynolds number 60; average blood velocity 21.2 cm/s). The selected dynamic conditions modeled the local rheology developing on mildly stenosed coronary arteries. Our previous work demonstrated that these rheologic conditions result in consistent levels of platelet deposition.

To mimic the in vivo situation of severe arterial injury associated with coronary interventions, porcine aortic tunica media was used as the substrate to trigger thrombus formation. Segments of porcine aorta were cut into segments (2.8 x 0.8cm) and surgically prepared to expose the deeper components of the arterial wall as previously described [25,26].

The histological assessment of thrombus formation was performed by histomorphometry as previously described [25]. In brief, the perfused media segments were fixed in 4% paraformaldehyde. Six 2 mm cross sections were removed from the perfused media segment: two in the proximal, middle, and distal thirds of the exposed vessel segments. These tissue sections were then embedded in paraffin and processed for histology. Sections (5 μm) were cut and stained with a combined Masson’s trichrome-elastin to visualize the total thrombus formed on the exposed substrates.

Morphometric analysis of thrombus was conducted at 400-fold magnification. Images were digitized with a Sony DKC-5000 camera using Adobe Photoshop 4.0 software on a PowerMacintosh 8500 computer. Thrombus area on each section was measured by computerized planimetry using NIH Image 1.6 software. The results are given as the average of the analyzed sections per perfused media segments, and the results from the three perfusion chamber were averaged to obtain the overall thrombus formation for each patient. The measurements were independently done by two of the investigators, who were blinded to the therapy assignment.

2.3. Endothelium-dependent vasomotor response

Skin blood flow is controlled both by reflexes and local factors. Cutaneous vascular response to local warming is an endothelium-mediated mechanism [27]. Therefore, measurements of microcirculatory blood flow to heat have been used as marker of endothelium function. Vascular responses to local heat were recorded in a subgroup of 11 hyperlipidemic patients at baseline and after 3-months on lipid lowering therapy. A laser-Doppler flowmeter with a combined laser Doppler and thermostatic probe (Perimed, model PF 4001 Master, Perimed, Järfalla, Sweden) was used to assess the microcirculatory blood flow [28]. Measurements were made on the dorsum of the hand with the probe fixed to the skin by a double-sided adhesive ring. This technique shows an intraindividual variability of <2%. Results are expressed as arbitrary perfusion units (PU) and are an index of the blood flow [29].

All measurements were made in a temperature controlled room (19–22°C) with the patients lying in supine position. Skin blood flow was continuously recorded during a baseline period with the probe set to 33°C (neutral skin temperature) and during local skin heating to 44°C. After 3 min of monitoring baseline perfusion, blood flow was measured for 10 min over the heating period. The typical response is a maximum vasodilatation response after 3–5 min of skin heating over 42°C. Comparisons were made between the mean baseline flow and the area under the curve of the time course recorded during 3 min at 33°C (baseline perfusion) and 5 min of heating over 42°C (endothelium-dependent vasomotor response to heat) after 5 min heating period. Results (mean ± SEM) are expressed as perfusion units (PU) and perfusion units x min.

2.4. Data analysis

Differences between the treatment groups were assessed using ANOVA. Comparisons pre and post therapy within the same group were analyzed using Student’s paired t-test. If not otherwise indicated, data are given as mean ± SD for independent measurements. The two tailed significance threshold was set at P < 0.05.

3. Results

3.1. Study population

No significant differences between both groups were present in the anthropometric, routine laboratory and other clinical risk factors at baseline (Table 1). The medication profile pre-treatment was also comparable in the two groups of patients. The serum lipid profile comprising total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides was similar in both groups at baseline. Total cholesterol and LDL cholesterol level were significantly reduced in both groups of patients after 3-months treatment with either simvastatin or pravastatin. HDL cholesterol and triglyceride levels were not affected by statins therapy (Table 2).
3.2. Blood thrombogenicity

Baseline blood thrombogenicity, evaluated by the thrombus area formed on the perfused substrates, was similar in both groups. A representative photomicrograph of thrombus formation at baseline and 3-months after statin treatment is shown in Fig. 1. The simvastatin group showed a significant reduction in thrombus formation (11 067 ± 980 vs 8276 ± 728 μm²/mm baseline and post-treatment, respectively; P < 0.05). Similar reduction in thrombus formation took place in the pravastatin group (12 648 ± 1023 vs 9411 ± 992 μm²/mm at baseline and 3-months post treatment, respectively; P < 0.05). No significant difference in thrombus formation was found between the two groups.

<table>
<thead>
<tr>
<th>Patient population</th>
<th>Group I</th>
<th>Group II</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>62 ± 2</td>
<td>58 ± 2</td>
</tr>
<tr>
<td>Hypertension</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Smoking</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Family history CHD</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Cerebral ischemic disease</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Peripheral artery disease</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>CAGB</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Angioplasty</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

| Table 2 | Plasma lipid profile and plasma levels of fibrinogen, sP-selectin, sL-selectin, CAM-1 of the two patient groups at baseline and after 3-months with statins*
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sinvastatin group</td>
</tr>
<tr>
<td></td>
<td>Baseline          Post treatment</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>251 ± 10</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>141 ± 11</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>40 ± 1.9</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>184 ± 10</td>
</tr>
<tr>
<td>Total/HDL cholesterol ratio</td>
<td>6.6 ± 0.4</td>
</tr>
</tbody>
</table>
| LDL/HDL cholesterol ratio  | 4.8 ± 0.3 | 2.8 ± 0.2* | 4.4 ± 0.3 | 3.0 ± 0.2*
| Fibrinogen (mg/dl)         | 255 ± 13 | 263 ± 10 | 243 ± 16 | 239 ± 19 |
| sP-selectin (ng/ml)        | 105 ± 17 | 115 ± 15 | 130 ± 30 | 129 ± 22 |
| sL selectin (ng/ml)        | 834 ± 42 | 778 ± 32 | 753 ± 30 | 738 ± 31 |
| sICAM-1 (ng/ml)            | 54 ± 3   | 48 ± 3  | 47 ± 5  | 53 ± 5  |

* Data is expressed as mean ± SD.
* P < 0.05 versus baseline values.

Fig. 1. Representative histological sections corresponding to baseline (top) and 3-months post treatment (bottom).

Fig. 2. Values of thrombus formation corresponding to the Simvastatin group. A. All patients. B. Subgroup of patients with cardiovascular disease and. C. Subgroup of patients without cardiovascular disease. Data expressed as μm²/mm².

Statin-treated groups. The antithrombotic effect of lipid reduction was still present when the groups were subdivided into patients with and without cardiovascular disease (CVD) for both groups (Figs. 2 and 3). The group of patients with CVD in the Simvastatin group (n = 14) showed values of 11 373 ± 1087 μm²/mm at baseline and 8368 ± 1061 μm²/mm at 3-months, respectively, while the group of patients without CVD (n = 12) had 10 710 ± 1755 μm²/mm at baseline and 8170 ± 1027 μm²/mm at 3-months, respectively. Values of thrombus formation within the pravastatin group were 13 607 ± 1444 and 9573 ± 1447 μm²/mm at baseline and 3-months, respectively for the CVD group; and 11 689 ± 1457 μm²/mm at baseline and 9251 ± 1419 μm²/mm at 3-months in the non-CVD group. The lipid lowering effect of the two statins used in our study was associated with an ≈ 20–25% reduction in thrombus formation as compared with the baseline values.

A correlation between thrombus formation and total cholesterol and blood thrombogenicity. A significant relationship between amount of thrombus and total cholesterol/HDL cholesterol ratio was found considering the measurements pre and post lipid lowering treatment (correlation coefficient r = 0.410, P ≤ 0.001, Fig. 4).

3.3. Endothelium-dependent vasomotor response (see Table 3)

Hyperlipidemia has been associated with an impaired endothelium-dependent vasoresponse. Using a laser doppler flow meter evaluation of blood flow as an indicator of endothelium-dependent vasoresponse, we observed similar findings.[27] Hyperemic reaction induced by local warming is an endothelial-dependent mechanism and thus, measuring blood flow changes would be an indicator of endothelial function. The effect of lipid lowering was found to be significant when measuring heat-mediated blood flow (49.7 ± 5.7 PU vs 81.5 ± 12.3 PU; at baseline and 3-months post-treatment, respec-
tively; \( P \leq 0.01 \)). These observations suggest an improvement of the endothelium-dependent vasoresponse by the hypolipidemic treatment. Similar observations were obtained when analyzing the areas under the curve at the different time/points and conditions.

3.4. Soluble adhesion molecule levels

Treatment with statins did not induce any significant changes in the plasma levels of sP-selectin, sL-selectin or sICAM-1 between baseline and post-treatment measurements (Table 2).

In addition, the treatment with statins was not associated with any significant effect on white blood cell count, platelet count, activated partial thromboplastin time (aPTT) or plasma fibrinogen.

4. Discussion

This study demonstrates that the reduction of plasma cholesterol levels by statins reduces the high blood thrombogenicity observed in the same group of patients under hyperlipidemic conditions. We conclude that the reduction of blood thrombogenicity and endothelial activity may be mechanisms by which cholesterol lowering through statins accounts for the beneficial effects on CHD observed in several large epidemiological trials.

Epidemiological evidence has shown the relationship between plasma total cholesterol and mortality rate from CHD [2–12]. A variety of mechanisms have been proposed to understand the effect of hypercholesterolemia on atherosclerosis and thrombosis [2–6]. Acute thrombus formation depends not only on the
Fig. 4. Correlation between total cholesterol/HDL cholesterol ratio and amount of thrombus formation. Lipid levels and thrombus formation were measured pre and post lipid reducing therapy (n = 100 measurements for 50 patients, respectively). R, correlation coefficient.

Table 3
Effect of lipid lowering by statins on endothelial activity at baseline and after 3-months with statins

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>3-month statins</th>
</tr>
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<tbody>
<tr>
<td>Hyperlipidemic patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal values</td>
<td>6.5 ± 0.9</td>
<td>8.1 ± 0.9</td>
</tr>
<tr>
<td>Heat induced</td>
<td>49.7 ± 5.7*</td>
<td>81.5 ± 12.3**</td>
</tr>
</tbody>
</table>

* P<0.05 versus basal values.
** P<0.05 versus pre-treatment.
* Results expressed as PU.

local alterations of the arterial wall, but it is also modulated by the thrombogenicity of the circulating blood [30–33]. Our group and others have recently shown that the circulating platelets are activated and hyperactive in hypercholesterolemia [16–20]. In addition, it has been reported that reducing cholesterol level by pravastatin decreases thrombus formation on deeply injured arterial wall [20]. Whether this antithrombotic effect was a result of the cholesterol reduction or a specific effect of the specific statin used was questioned [21,22]. On the other hand, it has recently been demonstrated that lipid reducing therapy by simvastatin markedly depressed thrombin generation and that this effect on thrombin generation was not dependent on the presence of aspirin in patients with hypercholesterolemia [34].

This study revealed that the blood thrombogenicity at hyperlipidemic baseline conditions is significantly reduced after reducing cholesterol in the same patient population. The reduction in thrombus formation was achieved by the use of either simvastatin or pravastatin as lipid lowering agent. The effect of placebo treatment on cholesterol level and thrombus formation has also been tested in the same ex vivo model system and shear stress conditions that we used here [35]. Lipid plasma levels and thrombus formation were not altered when a placebo instead of pravastatin was given [35]. In our study, both statins, simvastatin and pravastatin, induced a significant hypolipidemic effect that was associated with similar reduction of blood thrombogenicity in the respective group. Therefore, we conclude that the reduction of blood thrombogenicity could be one of the mechanisms by which cholesterol lowering by statins reduces the incidence of acute thrombotic events.

It has been suggested that endothelial dysfunction plays a major role, not only in the pathogenesis of atherosclerosis but also in its ischaemic manifestations. Therefore, normalization of the dysfunctional endothelium may restore its antiatherogenic properties. There are evidences that endothelial dysfunction is often improved by cholesterol lowering [24,36] and it seems to be mediated by an increased bioavailability of nitric oxide [37]. To define whether the described lipid lowering effect of statins was the only mechanism responsible for the observed antithrombotic effect, we studied the effects of lipid reduction by statins on endothelial dependent vasoresponse in the same patient population. As described by others, we observed a significantly impaired endothelium-dependent vasoresponse in our hyperlipidemic population after lipid lowering therapy compared to baseline. Furthermore, a marked improvement of the endothelial-dependent vasoresponse was associated to the lipid lowering effect induced by the statins.

An additional objective of the study was to assess whether the observed normalization of blood thrombogenicity and endothelial function by statin therapy would be associated with changes in plasma levels of adhesive proteins involved in the interactions between blood cells and endothelium. We determined plasma levels of sP-selectin, sL-selectin and sICAM-1 at baseline and 3-months after statin administration. Treatment with statins did not modify levels of adhesive proteins in the circulating blood. We speculate that the study population tested here may have been to small to identify differences. Since only the circulating soluble antigen levels were determined in this study, it cannot be excluded that surface-associated selectin and ICAM-1 expression on endothelial cells and/or circulating blood cells may have been affected by statin therapy.

As indicated by Fig. 5, hyperlipidemia is associated with an increased platelet reactivity, endothelial dysfunction and plaque progression, altogether leading to an increased risk of acute thrombotic events. Our data on blood thrombogenicity and endothelial function were simultaneously obtained from the same individuals before and after lipid lowering therapy with two different statins. Our data and the observations of
others suggest that multiple biological systems may be affected by lipid lowering therapy with statins. This study strongly supports the hypothesis that the pleiotropic effects of lipid lowering therapy by statins may explain the significant clinical benefits of statin therapy observed in several large epidemiological trials [4–6,38–41]. Thus, the observed improvement in clinical outcome after statin therapy seems rather to be a result of interfering with multiple pathogenic parameters present under hypercholesterolemic conditions (Fig. 5) than the intrinsic effect of one specific statin. The major limitation of our study is that we can not rule out whether all statins as a drug class have a direct effect on thrombogenicity and endothelial function or whether the observed changes are mediated by the lipid reduction only.

In summary, we conclude that the reduction of blood thrombogenicity and endothelial activity may be mechanisms by which cholesterol-reducing therapy with statins improves the clinical outcome of patients with hypercholesterolemia.

Acknowledgements

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