The spectrum of premature atherosclerosis: from single gene to complex genetic disorder
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Hs-CRP reduction with aggressive statin therapy is associated with regression of carotid IMT in patients with Familial Hypercholesterolemia

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Abstract

Background: High sensitive C-reactive protein (hs-CRP) has emerged as the best studied and most promising marker of inflammation in atherosclerotic vascular disease.

Materials and methods: The ASAP (effects of Atorvastatin versus Simvastatin on Atherosclerosis Progression) study was a 2-year randomised, double-blind trial with 325 Familial Hypercholesterolemia patients, treated with atorvastatin 80mg or simvastatin 40mg. Intima media thickness (IMT) of carotid artery segments and hs-CRP levels were determined at baseline, 1 and 2 years.

Results: Baseline median hs-CRP values were 2.1mg/l (interquartile range (IQR) 0.9-5.2) and 2.0 mg/l (IQR 0.8-3.0) and after 2 years these levels decreased to 1.1 mg/l (IQR 0.6-2.4) and 1.5 mg/l (IQR 0.6-3.0) in the atorvastatin 80mg and simvastatin 40mg group, respectively. These changes were significant within as well as between the two groups. No correlations were observed between change in hs-CRP after 2 years and change in lipids. A significant correlation was found in univariate analysis between the decrease of hs-CRP and the reduction of IMT.

Conclusions: Our results show that atorvastatin 80mg reduces hs-CRP levels to a greater extent than simvastatin 40mg. Furthermore, we show that the extent of hs-CRP reduction is associated with the progression rate of the atherosclerotic process as measured by IMT.

Introduction

In recent years a wealth of evidence has indicated that inflammatory mechanisms play a central role in atherogenesis and its clinical sequelae. High sensitive C-reactive protein (hs-CRP) has emerged as the best studied and most promising marker of inflammation in relation to atherosclerotic vascular disease. Its levels predict the risk of atherosclerotic events in apparently healthy men and women and also in patients with both stable and unstable angina, as well as after a coronary event. Hs-CRP levels combined with standard lipid screening appeared to improve the detection of subclinical atherosclerosis. Recent evidence suggests that hs-CRP might negatively effect the atherosclerotic process directly at the endothelial level by inducing MCP-1 production and indirectly by promoting uptake of oxidized low density lipoprotein by macrophages.

The key question, therefore, becomes whether a therapeutic modality could reduce
levels of hs-CRP and more importantly whether lowering of these levels can reduce cardiovascular risk.

Data from the CARE (Cholesterol and Recurrent Events) trial have shown that statin therapy reduces hs-CRP levels significantly. However, in this and other studies, the change in hs-CRP attributable to statin therapy was unrelated to low density lipoprotein cholesterol (LDL-c) lowering. Another promising marker for the extent of atherosclerotic vascular disease is the measurement of intima media thickness (IMT) of peripheral arteries. Cross-sectional studies indicate an association between IMT and cardiovascular risk factors, and the prevalence of cardiovascular disease (CVD). Furthermore, in prospective studies carotid IMT was able to predict coronary artery disease (CAD). We recently reported that LDL-c reduction with atorvastatin 80mg over a two year period in patients suffering from heterozygous familial hypercholesterolaemia (FH) was accompanied by a striking regression of carotid IMT. The ASAP (effects of Atorvastatin versus Simvastatin on Atherosclerosis Progression) study protocol was also prospectively designed to assess the effects of both intervention modalities (simvastatin 40 mg and atorvastatin 80 mg) on hs-CRP levels. This provided us with the unique opportunity to study prospectively the long-term effects of statin therapy on hs-CRP in a randomised double-blind trial and assess for the first time whether these changes were related to the changes in the primary outcome parameter, carotid IMT.

Methods

Protocol

The design and main results of the ASAP study have been reported previously. In short; ASAP was a 2-year, two centre, randomised, double-blind study to assess whether treatment with atorvastatin 80 mg or simvastatin 40 mg could retard atherosclerosis progression in FH patients. After an 8 week placebo run in, baseline measurements of lipoprotein parameters, hs-CRP and IMT were performed. These measurements were repeated after 1 and 2 years. The Institutional Review Boards of both centres approved the protocol and written informed consent was obtained.

Laboratory

Lipoprotein parameters included total cholesterol (TC), (calculated) LDL-c, high density lipoprotein cholesterol (HDL-c) and triglycerides (TG) and were analysed as


Blood for hs-CRP analyses was stored at -70°C after collection and measurements were performed in one batch at a central laboratory after finishing the study (TNO Gaubius Laboratory, Leiden, The Netherlands). Hs-CRP was measured in citrated plasma by an enzyme-immunoassay using polyclonal antibodies (Dako, Copenhagen, Denmark). Measurements were performed twice (coefficient of variation less than 6%). The final result represents the mean of the two measurements.

**IMT**

IMT measurement procedures have also been reported before. In short, ultrasound examinations were performed using a Biosound Phase-2 real time scanner (Biosound Esaote, USA) equipped with a 10 MHz transducer. Three 10 mm segments were scanned bilaterally: the distal portion of the common carotid artery (CCA), the carotid bifurcation (BUL) and the proximal portion of the internal carotid artery (ICA). The mean IMT represents the average over anterior and posterior walls in the CCA, the BUL and the posterior wall of the ICA, bilaterally. IMT measurements were performed of both anterior and posterior walls of the CCA and BUL and posterior wall of the ICA. Images were analyzed with a semi-automatic software program (Eurequa; TSA company, Meudon, France). The ultrasound scannings were made by well-trained ultrasonographers in the two centers. The images were stored on disk and read by independent readers blinded to any information on the patients. The intra-observer and inter-observer coefficients of variation were <5%. During the study reproducibility was checked at regular time points.

**Statistical analysis**

For lipoproteins, the percent change from baseline was calculated after 2 years. The primary efficacy parameter was change in IMT in millimeters after 24 months. Treatment differences in the percent changes from baseline of lipid and IMT were analyzed with ANCOVA, using the change in mean IMT as the dependent variable and treatment group, centre and baseline mean IMT as the independent variables, neither IMT nor CRP levels were adjusted for baseline in the statistical analysis. Because of the skewed distributions of hs-CRP, median concentrations were used for analyses. Both absolute and relative change in hs-CRP after one and two years were calculated. The signed rank Wilcoxon test was used to evaluate the significance of any difference in median hs-CRP changes over time; in each treatment group and between treatment groups. Spearman correlation coefficients
were calculated to assess evidence of association between the change of hs-CRP and the following parameters: body mass index (BMI), hs-CRP levels and IMT at baseline, mean change in IMT, LDL-c, HDL-c and TG after 2 years of treatment. Statistical analyses were performed using SAS (version 6.12, SAS Institute Inc.).

Results

Patients and primary results

Of the 325 patients in the original intention to treat population, 45 patients did not complete the study (13.8%). Furthermore, 12 patients had missing hs-CRP data on either baseline or at 2-years. FH patients who did not complete the study did not differ significantly, in terms of demographic and laboratory parameters, from FH patients who received treatment for 2 years. One hundred and sixty vs. 165 patients were allocated to either atorvastatin (66 men, 94 women) or simvastatin (62 men, 103 women), respectively. At baseline, no significant differences between treatment groups were found in either lipid or lipoprotein levels, nor in hs-CRP or mean IMT. IMT and hs-CRP were markedly increased at baseline. The baseline characteristics are summarized in Table 1.

<table>
<thead>
<tr>
<th>Table 1: Baseline characteristics of the patients with hs-CRP measurements in the ASAP study</th>
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<tr>
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<tr>
<td>Atorvastatin 80 mg (n= 135)</td>
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<td>------</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Gender (female/male)</td>
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<tr>
<td>Smoking (%)</td>
</tr>
<tr>
<td>CVD (%)</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
</tr>
<tr>
<td>BP (mmHg)</td>
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<tr>
<td>Mean IMT (mm)</td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
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</tbody>
</table>

Values are means (SD), except hs-CRP which is given as median (interquartile range). CVD = cardiovascular disease, BMI = body mass index, BP = blood pressure, hs-CRP = high sensitive C-reactive protein.
Table 2 shows baseline and 2 year levels of TC, LDL-c, HDL-c, TG and hs-CRP. As previously described TC, LDL-c and TG levels were lowered significantly within each treatment arm (p<0.0001 for all parameters), whereas atorvastatin reduced TC, LDL-c and TG levels significantly more than simvastatin. The changes in IMT have also been described previously. In summary, after 2 years, IMT in the atorvastatin group decreased by 0.031 mm (p=0.0017) and increased by 0.036 mm in the simvastatin group (p=0.0005). This difference between the two treatments was significant (p<0.001).

**Hs-CRP**

Data on hs-CRP are presented in table 2. The median hs-CRP at baseline was 2.1 mg/l and decreased to 1.1 mg/l after 2 years in the atorvastatin 80 mg group (p<0.001). In the simvastatin 40 mg group median hs-CRP was 2.0 mg/l at baseline and decreased to 1.5 mg/L after 2 years (p=0.002). The difference between the two treatment arms after 2 years was significant (p=0.02). The median

| Table 2. Plasma levels of lipids, lipoproteins and hs-CRP at baseline and after 2 years of treatment with Simvastatin 40 mg or Atorvastatin 80 mg. |
|-----------------|------------|-------------|-------------|-------|-------|
| Satin | Baseline | 2 years | % change* | p† | p‡ |
| TC | S | 10.20 (1.89) | 6.45 (1.15) | -35.9 | <0.001 | <0.001 |
| A | 10.04 (1.87) | 5.59 (1.09) | -43.5 | <0.001 | |
| LDL-c | S | 8.22 (1.90) | 4.53 (1.15) | -44.0 | <0.001 | <0.001 |
| A | 8.06 (1.83) | 3.67 (1.07) | -52.6 | <0.001 | |
| HDL-c | S | 1.16 (0.29) | 1.32 (0.35) | 13.8 | <0.001 | 0.8541 |
| A | 1.18 (0.33) | 1.33 (0.40) | 14.3 | <0.001 | |
| TG | S | 1.79 (0.90) | 1.37 (0.77) | -19.0 | <0.001 | <0.001 |
| A | 1.84 (1.07) | 1.16 (0.64) | -31.1 | <0.001 | 0.0023 |
| hs-CRP | S | 2.0 (0.8-3.0) | 1.5 (0.6-3.0) | -19.7 | 0.002 | <0.001 |
| A | 2.1 (0.9-5.2) | 1.1 (0.6-2.4) | -40.1 | 0.02 |

Values are means (SD), except hs-CRP which is given as median (interquartile range). TC= Total Cholesterol, LDL-c= Low density lipoprotein, HDL-c= High density lipoprotein, TG= Triglycerides (all given in mmol/L), hs-CRP= High sensitive C-reactive protein (in mg/l), S= Simvastatin 40 mg, A= Atorvastatin 80 mg, % change* = percentage change of median hs-CRP after 2 years of treatment, p† = p value within one treatment group for the absolute change, p‡ = p value between atorvastatin and simvastatin group for the absolute differences.
percentage decrease in hs-CRP after one year of treatment was 44.9% in the atorvastatin 80 mg group versus 14.0% in the simvastatin 40 mg group (p<0.001). After 2 years the percentage decrease was 40.1% in the atorvastatin 80 mg group versus 19.7% in the simvastatin 40 mg group (p=0.02). The relative changes after 1 and 2 years in the atorvastatin 80 mg group were highly significant (p<0.001 and p<0.001, respectively). In the simvastatin 40 mg group this was no longer significant (p=0.56 and p=0.15, respectively). The difference between the two treatment arms after 1 and 2 years remained significant (p<0.001 and p=0.021, respectively) (Figure 1).

In the atorvastatin 80 mg group almost 72% of patients experienced a decrease of hs-CRP after 2 years of treatment, compared with 59% in the simvastatin 40 mg group (Table 3). Although there were no significant differences between the two treatment arms (p=0.06), when the group of patients with no change in hs-CRP were combined with the patients with an increase in hs-CRP the differences between the two treatment arms became significant (p=0.03). Change in hs-CRP levels was correlated with hs-CRP at baseline and BMI ($r^2=0.70$; Figure 1. Percentage change of hs-CRP levels in the Simvastatin 40 mg (Simvastatin) and Atorvastatin 80 mg (Atorvastatin) treatment groups at baseline, one and two years.

Figure 1. Percentage change of hs-CRP levels in the Simvastatin 40 mg (Simvastatin) and Atorvastatin 80 mg (Atorvastatin) treatment groups at baseline, one and two years.

$Hs$-CRP= high sensitive C-reactive protein, p= p-values between simvastatin and atorvastatin after 1 and 2 years of treatment
Table 3. Percentage of patients with a decrease, no change or increase of hs-CRP levels in the treatment groups after 2 years.

<table>
<thead>
<tr>
<th></th>
<th>Decrease</th>
<th>No change</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acorvastatin 80 mg</td>
<td>71.8</td>
<td>1.5</td>
<td>26.7</td>
</tr>
<tr>
<td>(N=135)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simvastatin 40 mg</td>
<td>59.4</td>
<td>4.5</td>
<td>36.1</td>
</tr>
<tr>
<td>(N=133)</td>
<td></td>
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hs-CRP= high sensitive C-reactive protein, missing data in the atorvastatin group is 25 patients vs in the simvastatin group 32 patients.

p=0.001). Baseline hs-CRP levels were not correlated with baseline IMT or the presence of CVD (r=0.02; p=0.67 and r=0.08; p=0.17, respectively). There was no difference in hs-CRP at baseline or hs-CRP change between smokers and non-smokers. Change in hs-CRP was not correlated with change in LDL-c (r=0.02, p=0.81), HDL-c (r=-0.08, p=0.18), triglycerides (r=0.11, p=0.08) or total cholesterol (r=0.03, p=0.62).

However, a significant correlation was found between the decrease of hs-CRP and the reduction of IMT, as seen in all patients combined (r=0.13; p=0.03). In particular, patients in the highest tertile of CRP change (median hs-CRP reduction 3.28 mg/l; mean reduction IMT 0.016 mm/2years), compared to the bottom tertile (median hs-CRP increase 0.57 mg/l; mean increase IMT 0.032 mm/2years), exhibited a twofold greater reduction of mean carotid IMT. As expected statistical significance was lost in multivariate analysis with treatment per se as an important confounder. In a stepwise regression model age, baseline IMT and drug were the most important variables (together: r²= 0.31; p<0.001).

Discussion

We have reported previously that lipid lowering therapy with high dose atorvastatin over a 2-year period led to regression of carotid IMT in FH heterozygotes.18 The current analysis shows that atorvastatin 80 mg also reduces hs-CRP levels to a greater extent than lipid lowering with simvastatin 40 mg. Furthermore, we show that those patients with the largest hs-CRP reduction might exhibit a twofold
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greater IMT decrease in univariate analysis. These findings may indicate that the extent of hs-CRP reduction is associated with the progression rate of the atherosclerotic process as measured by IMT. Therefore a reduction of inflammatory parameters by statin therapy, in addition to LDL-C lowering, could be important in the prevention of premature CAD.

Inflammation plays a major role in atherothrombosis and hs-CRP seems one of the best markers related to this process. Hs-CRP might play both a direct and indirect role in atherogenesis and this consequently has raised the possibility of hs-CRP being a potential target for therapy itself. Although limitations, inherent to inflammatory screening remain, available data suggest that hs-CRP has the potential to play an important role as an adjunct for global risk assessment in primary prevention of cardiovascular disease.\textsuperscript{22}

Previous trials with different statins demonstrated the CRP lowering ability of these compounds. In the CARE trial, patients were treated with either placebo or pravastatin. Hs-CRP was lowered by approximately 17% in the pravastatin group, whereas patients on placebo showed a modest increase of hs-CRP levels of approximately 4%.\textsuperscript{11}

The recent PRINCE (Pravastatin Inflammation/CRP Evaluation) trial conducted with pravastatin in 2884 men and women showed a 13.1% decrease in hs-CRP after 24 weeks.\textsuperscript{23} In an 8-week prospective trial with both 0.4 and 0.8 mg cerivastatin, hs-CRP was lowered by 11.1% and 13.3%, respectively. However, this difference observed between low and high dose cerivastatin was not statistically significant.\textsuperscript{12}

More recently, another short-term study compared pravastatin 40 mg/day, simvastatin 20 mg/day and atorvastatin 10 mg/day.\textsuperscript{10} All three statins lowered hs-CRP; by 20.3%, 22.8% and 28.3%, respectively. There were no significant differences in the median percent reduction between these statins. None of the four mentioned studies found a clear correlation between reduction in hs-CRP and any change in lipids or lipoproteins.\textsuperscript{10-12} While the three most recent studies did not include clinical endpoints, a subgroup analyses of the CARE trial showed a statistically significant association between evidence of inflammation and coronary events in patients assigned to placebo. However, this association was no longer significant in the group treated with pravastatin. Patients with evidence of ongoing inflammation as detected by high levels of hs-CRP appeared to have a greater relative risk reduction in subsequent coronary events attributable to pravastatin than those without.\textsuperscript{24}

In the Airforce/Texas Atherosclerosis Progression Study (AFCAPS/TexCAPS)
Lovastatin lowered hs-CRP by 14.8% after one year of treatment. Patients with high levels of hs-CRP at baseline showed increased rates of coronary events. Lovastatin was effective in patients with a high TC to HDL ratio. Furthermore, lovastatin was also effective in patients with a ratio TC to HDL lower than the median and a hs-CRP level higher than the median, but ineffective in patients with a ratio TC to HDL and hs-CRP level both lower than the median. So, the authors concluded that statin therapy may be effective in the primary prevention of coronary events among subjects with relatively low lipid levels but with elevated levels of hs-CRP.

Our study differs in important aspects from those previously carried out. First, our patient population was different. FH heterozygotes have severely and longstanding elevated LDL-c levels, which usually result in extensive atherosclerosis. FH patients are therefore increasingly used as a model for atherosclerosis as reflected in our patient group with hs-CRP values above 2.0 mg/l. Second, the follow-up period in the ASAP study was 2 years versus only 24 weeks in the PRINCE trial, 8 weeks in the cerivastatin study and only 6 weeks in the study comparing pravastatin, simvastatin and atorvastatin. Furthermore, the ASAP study used high-dose lipid-lowering therapy (atorvastatin 80 mg) versus more commonly used dosages in the other studies.

The association between hs-CRP decrease and IMT change in our study was significant in the univariate analysis. As expected this significance was lost in the multivariate analysis, including age, baseline IMT and treatment per sé. Recently, two articles have been published in which hs-CRP and atherosclerosis as measured by IMT were not independently associated or only in males with advanced carotid plaques. A potential limitation of our study and the previous mentioned studies is the use of IMT versus clinical endpoints. However, IMT measurements are now widely accepted as a surrogate marker for future cardiovascular events. Prospective studies with hard clinical endpoints will still be required for a more definitive answer concerning the effect of hs-CRP lowering by statins and the consequences on cardiovascular events. The IDEAL-study addresses this question, since it is a clinical end-point trial in patients with CVD, also treated with either atorvastatin 80mg or simvastatin 40mg.

Our study clearly shows a decrease of hs-CRP levels in FH patients by simvastatin and atorvastatin and an association between this decrease and IMT change. The results of our study might implicate that the benefits of high dose atorvastatin therapy are a consequence of the reduction of both LDL-c and hs-CRP levels as well. However, the association between hs-CRP decrease and IMT is small, but significant and so further prospective studies to elucidate the role of hs-CRP are still needed.
Literature


