Targeting the vessel wall in cardiovascular prevention
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Myeloperoxidase levels are not associated with carotid atherosclerosis progression in patients with familial hypercholesterolemia

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ABSTRACT

Introduction: Myeloperoxidase (MPO), an antimicrobial enzyme of the innate immune system, has been proposed to exert a wide array of pro-atherogenic effects throughout all stages of the atherosclerotic process. In view of the potent anti-inflammatory effects of statins in vitro, we evaluated the impact of statin therapy on plasma MPO levels in patients with heterozygous familial hypercholesterolemia (FH), treated with either intensive or conventional lipid-lowering therapy. Furthermore, we evaluated the relation between MPO levels and atherosclerosis progression, as determined by intima media thickness (IMT).

Methods: We measured plasma MPO levels, lipoprotein profiles, high sensitivity-C-reactive protein (hs-CRP) as well as IMT of carotid artery segments in 122 FH patients at baseline and after 2-year treatment with atorvastatin 80 mg or simvastatin 40 mg QD.

Results: Baseline median MPO values were 147 pM (interquartile range (IQR) 122–217) and 144 pM (IQR 118–216) and these increased significantly to 221 pM (IQR 144–290) and 255 pM (IQR 152–324) during 2-year follow-up in both the atorvastatin 80 mg and simvastatin 40 mg group, respectively. There was no correlation between MPO levels and IMT progression, change in lipoproteins or hs-CRP.

Conclusion: In FH patients, statins do not prevent an increase in MPO levels during follow-up. Moreover, MPO levels are not associated with atherosclerosis progression in these patients.

Keywords: Myeloperoxidase, statin, intima media thickness, atherosclerosis, familial hypercholesterolemia

Abbreviations: ASAP, effects of atorvastatin versus simvastatin on atherosclerosis progression; BMI, body mass index; FH, familial hypercholesterolemia; HDL-c, high-density lipoprotein-cholesterol; Hs-CRP, high sensitivity C-reactive protein; IMT, intima media thickness; LDL-c, low-density lipoprotein-cholesterol; MPO, myeloperoxidase; TC, total cholesterol; TG, triglycerides
INTRODUCTION

Statin therapy reduces cardiovascular event rate by 30% (1). Whereas its principal mechanism of action pertains to its low-density lipoprotein-cholesterol (LDL-c) lowering effect, a variety of experimental and clinical studies have highlighted that statins may also exert distinct pleiotropic effects (2, 3). In particular, statins have potent anti-inflammatory effects, ranging from decreasing leukocyte activation to lowering production of acute phase reactants. However, these effects have been predominantly observed in in vitro experiments. The impact of the pleiotropic effects of statins in vivo is not well established. In this respect, the PROVE IT-trial lends support to the potential relevance of anti-inflammatory effects of statins. It showed that atorvastatin was associated with a decrease in C-reactive protein (CRP) levels and improved cardiovascular outcome, independent from LDL-c lowering (4, 5).

In the inflammatory cascade, myeloperoxidase (MPO) has emerged as an important mediator of pro-atherogenic changes within the vasculature. MPO can be released by activated neutrophils, monocytes and macrophages. Beyond its role as antimicrobial enzyme (6), MPO also mediates oxidation of LDL-c (7, 8) and apolipoprotein (apo) A1 (9). This results in even more pro-atherogenic LDL-c and possibly in impaired reverse cholesterol transport by high-density lipoprotein-cholesterol (HDL-c) respectively (7-10). Furthermore, it reduces nitric oxide availability (11).

Increased levels of MPO and its products have been detected in plasma as well as in plaques from patients with cardiovascular disease (12-15). We recently showed that serum MPO levels are associated with the future risk of coronary artery disease (CAD) in healthy individuals (16). In line, a study showed that MPO levels correlate with the severity of coronary artery disease (CAD) assessed during angiography (17). However, this could not be confirmed in a later study (18). Finally, MPO also has clear predictive value for future cardiovascular events in patients with acute coronary syndrome or chest pain (19, 20). Hence, MPO has been put forward as a potential biomarker and future target for prevention of cardiovascular disease.

The effect of statins on MPO and the impact of MPO on atherosclerosis progression are as yet unknown. Therefore, we set out (i) to evaluate the effect of treatment with intensive (atorvastatin 80 mg) versus conventional (simvastatin 40 mg) on plasma MPO levels in patients with familial hypercholesterolemia (FH) and (ii) to assess the relation between (changes in) plasma MPO levels and atherosclerosis progression as measured by intima media thickness (IMT) and other cardiovascular risk markers.
MATERIALS AND METHODS

Study design
We determined plasma MPO levels in samples of a subgroup of 122 patients out of 325 patients included in the ASAP study (effects of Atorvastatin versus Simvastatin on Atherosclerosis Progression), depending on the availability of plasma samples. The design and main results of the ASAP study have been reported previously (21, 22). 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 patients with heterozygous FH. After an 8-week placebo run-in, baseline measurements of lipoprotein parameters, high sensitivity-C-reactive protein (hs-CRP) and IMT were performed. These measurements were repeated after 2 years. The Institutional Review Boards of both centres approved the protocol and written informed consent was obtained. The primary endpoint was carotid atherosclerosis progression defined as change in IMT measured by quantitative B-mode ultrasound (22).

Laboratory parameters
Total cholesterol (TC), (calculated) LDL-c, HDL-c, triglycerides (TG), and hs-CRP were determined as described previously (22, 23). Blood was stored at −70 °C after collection. MPO levels were measured in heparin plasma samples taken at baseline and after 2 years of 61 patients who received simvastatin 40 mg and 61 patients who received atorvastatin 80 mg using a commercially available MPO-ELISA kit (CardioMPO™ Test, Prognostix, Cleveland, OH, USA. The CardioMPO blood test is based on research studies performed by S. Hazen, M. Penn, and M.L. Brennan at the Cleveland Clinic.). Duplicate measurements were performed (coefficient of variation less than 5%). The final result represents the mean of these measurements.

Carotid IMT
IMT measurements were performed at baseline and after 2 years of treatment. In short, ultrasound examinations were performed using a bio-sound 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 analysed with a semi-automatic software program (Eurequa, TSA company, Meudon, France). The intra-observer and inter-observer coefficients of variation were less than 5%.
Statistical analysis
Since the distributions of MPO, hs-CRP and TG were skewed, data were log-transformed prior to further analysis or analysed using non-parametric tests. In tables, median and interquartile ranges were used. Both absolute and relative changes in MPO after 2 years were calculated. The Wilcoxon signed-rank test and Wilcoxon rank-sum test were used to evaluate the significance of MPO changes over time in each treatment group and the difference between treatment groups. Spearman correlation coefficients were calculated to assess evidence of association between MPO levels and the change of MPO and the following parameters: IMT at baseline, change in IMT, hs-CRP, leukocyte count, TC, LDL-c, HDL-c, TG, and body mass index (BMI) at baseline and after 2 years of treatment. The association between IMT (progression) as well as MPO and risk factors was also explored using a multivariate regression model, including the following factors: age, gender, statin therapy, history of cardiovascular disease (yes/no), smoking (yes/no), body mass index (BMI), LDL-c, HDL-c, TG, hs-CRP and leukocyte count. Statistical analyses were performed using SPSS (version 12.0, Chicago, IL, USA). A p value < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics
Demographic and baseline characteristics of the study population are listed in Table 1. FH patients in the subgroups did not differ significantly, in terms of age, gender, history of cardiovascular disease, smoking habit and laboratory parameters at baseline, from the total cohort (supplemental data, Tables 1 and 2) (22, 23). Patients were allocated to either atorvastatin 80 mg or simvastatin 40 mg. At baseline, no significant differences between treatment groups were found in lipoprotein levels, hs-CRP, mean IMT or MPO. IMT and hs-CRP were markedly increased at baseline compared to age-matched, normocholesterolemic controls (22, 23). Median plasma MPO at baseline was 147 pM (interquartile range (IQR): 122–217) in the atorvastatin 80 mg group (p = 0.01) and 144 pM (IQR: 118–216) in the simvastatin 40 mg group (ns) (Table 2).

MPO levels increase during 2-year statin therapy
Median MPO levels increased to 221 pM (IQR: 144–290) in the atorvastatin group and to 255 pM (IQR 152–324) in the simvastatin group after 2 years (increase within group, p < 0.001, between groups, P = 0.2) (Table 2). Sixty-seven percent of patients in the atorvastatin 80 mg group versus 70% in the simvastatin 40 mg group experienced an increase of MPO after 2 years of treatment (Table 3). The median percentage increase in MPO after 2 year of treatment was 36% (IQR: −15–108) in the atorvastatin 80 mg group versus 50% (IQR: −7–155) in the simvastatin 40 mg group. As previously described TC, LDL-c, TG levels and hs-CRP were
lowered significantly within each treatment arm, and in line with expectations atorvastatin reduced TC, LDL-c, TG and hs-CRP levels more potently than simvastatin (22, 23). The changes in IMT have been described in detail previously. After 2 years, the IMT increase was higher in
the simvastatin compared to the atorvastatin group (p < 0.001 in total cohort, p = 0.2 in our subgroup).

**MPO levels do not correlate with IMT**

Baseline MPO, MPO after 2-year treatment and change in MPO levels were not correlated with age, LDL-c, HDL-c, TG, TC or hs-CRP (Table 4). MPO modestly correlated with leukocyte count at 2-year treatment (Spearman’s rho 0.23, p = 0.01). Baseline MPO levels were not correlated

**Table 4. Correlation of baseline MPO and change in MPO with age, IMT, lipoprotein profile, hs-CRP and leukocyte count**

<table>
<thead>
<tr>
<th>Spearman's rho Baseline MPO with baseline values of</th>
<th>MPO after 2 years with values after 2 years</th>
<th>Change in MPO with change in values of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>−0.14</td>
<td></td>
</tr>
<tr>
<td>IMT</td>
<td>−0.09</td>
<td>−0.02</td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>0.12</td>
<td>0.05</td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>0.14</td>
<td>0.23*</td>
</tr>
<tr>
<td>TC</td>
<td>0.07</td>
<td>0.04</td>
</tr>
<tr>
<td>LDL-c</td>
<td>0.12</td>
<td>0.08</td>
</tr>
<tr>
<td>HDL-c</td>
<td>−0.23</td>
<td>−0.18</td>
</tr>
<tr>
<td>TG</td>
<td>−0.04</td>
<td>0.12</td>
</tr>
</tbody>
</table>

IMT, intima media thickness; hs-CRP, high sensitivity-C-reactive protein; MPO, myeloperoxidase; TC, total cholesterol; TG, triglycerides.

* P < 0.05.

**Figure 1.** Change in IMT in patients with increasing or decreasing plasma myeloperoxidase levels

Patients were divided in two groups, based on MPO increase or decrease. There was no difference in the progression of IMT during 2 years (MPO increase: IMT −0.02 mm vs. MPO decrease: IMT + 0.05 mm, P = 0.3).
with baseline IMT, neither were baseline MPO levels or the change in MPO correlated with the change in IMT. Also, when dividing the patients in two groups, based on MPO increase or decrease, there was no difference in the progression of IMT (MPO increase: IMT −0.02 mm vs. MPO decrease: IMT +0.05 mm, p = 0.3, Figure 1). Multivariate analysis, similarly, did not reveal a relation between MPO levels and IMT. There was no difference in MPO at baseline or change in MPO between men and women, or patients with or without history of cardiovascular disease (baseline: 149 (113–216) vs. 145 (119–218) pM). There was a trend towards a larger increase of MPO in smokers than in non-smokers (mean change (S.D.): 177 (64) vs. 60 (22), p = 0.058). Change in MPO levels correlated with MPO at baseline (Spearman's rho 0.47, p < 0.001).

DISCUSSION

Our study shows that plasma MPO levels are neither related to IMT, nor to IMT progression rate during a 2-year follow-up period in FH patients. In spite of a clear reduction in IMT progression rate during atorvastatin compared to simvastatin therapy, MPO levels increased equally in both treatment arms.

MPO at baseline

Plasma MPO is predominantly derived from excretion by neutrophils and monocytes into the blood, as attested to by the positive correlation between leukocyte count and MPO levels (16, 24). Although no normocholesterolemic control group was included in the present analysis, plasma MPO levels were similar to those reported in healthy volunteers in a recent study by Tang et al. (median 172 pM, IQR 125–225) (25). Normal control values for plasma MPO have been reported to be < 539 pM (95% upper percentile of middle-aged healthy population, n = 300, Prognostix).

MPO increase during statin therapy

In our study, statin therapy with simvastatin as well as atorvastatin was accompanied by an increase in MPO levels during 2-year follow-up. In contrast, intensive statin therapy was associated with profound LDL reduction as well as a significant reduction in IMT progression rate (22). This finding appears inconsistent with several previous studies. First, statins have been shown to inhibit MPO mRNA expression in macrophages in vitro (26). Second, Zhou et al. reported a larger decrease in serum MPO levels in patients with acute coronary syndrome after 1 week of atorvastatin therapy compared to conventional treatment without a statin (27). Third, Shishehbor et al. observed a significant decrease in chlorotyrosine, a specific product of MPO activity (28, 29), in hypercholesterolemic patients after 12 weeks treatment with atorvastatin 10 mg (30).
With respect to this apparent contradiction, several options might be considered. First, it is unclear whether the increase in MPO is statin-related. Since it is unethical to include placebo therapy in high-risk patients such as those with familial hypercholesterolemia, we cannot exclude that without statin use MPO levels would have increased even further during 2-year follow-up. Moreover, MPO levels may have increased during follow-up due to statin-unrelated factors. However, we found no relation between MPO and age, or with duration of sample storage (data not shown). Our findings are supported by a study by Vita et al. in which MPO levels were also significantly higher in individuals on cardiovascular medications, including statins. This was attributed to confounding by indication bias, but could also be a statin-effect (31). Second, there may be a discrepancy between various measurements of MPO. Thus, products generated by MPO, such as chlorinated tyrosine residues, or serum MPO levels may be better indicators of MPO-mediated vascular damage than plasma MPO levels (28, 29). Finally, it is also possible that statins counteract MPO-mediated damage by their antioxidative capacities (32, 33), rather than counteract MPO production and release in vivo.

MPO and IMT progression

MPO has emerged as a potential pro-atherogenic mediator both in animal models (34) as well as in observational studies in patients with advanced cardiovascular disease (17, 19, 25). Most importantly, expression of human MPO in macrophages promoted atherosclerosis in LDL-receptor knockout mice (34). However, we did not find a correlation between MPO levels and IMT or IMT progression rate. Similarly, Exner et al. only found an association between MPO and progression of carotid stenosis in a subset of patients (35). In line, Baldus et al. reported comparable baseline plasma MPO levels between patients with or without angiographically detectable coronary artery disease (18). The lack of relation between MPO and IMT (progression) may point towards the impact of high LDL-c levels in patients with FH, which would overrule the inflammatory pathways. In this respect, hs-CRP levels, although increased, only weakly correlated with IMT ($r = 0.13$) in the ASAP study (23). In contrast, MPO levels are higher in patients with coronary artery disease (CAD) and can predict future cardiovascular events in these patients and patients with chest pain (17, 19, 20). In this scenario, MPO might be a better marker of plaque instability, rather than of progression. In order to settle this issue, more data on the predictive value of MPO in primary prevention settings are eagerly awaited (16).

Study limitations

Plasma levels of MPO were determined in two samples. Variation over time, e.g. temporarily increased levels due to infection, could have affected these variables. In this respect, measurement of specific MPO products, such as chlorotyrosine in HDL, may provide a better indication of MPO activity and MPO-induced damage (7-10, 29). Furthermore, it has recently
been suggested that MPO levels after heparin administration are a better reflection of subendothelial MPO (18). However, these analyses could not be performed in the present study.

CONCLUSION

In FH patients, statins do not prevent an increase in MPO levels during follow-up. Moreover, MPO levels are not associated with atherosclerosis progression. These data do not support an important role for pleiotropic effects of statins in terms of leukocyte activation. Furthermore, our study implies that MPO release is not a principal factor mediating IMT progression in patients with FH and not suitable as a biomarker for atherosclerotic burden or progression in these patients.

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DISCLOSURE

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REFERENCES


