Targeting the vessel wall in cardiovascular prevention
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Chapter 12

Increases in myeloperoxidase levels after exercise in myocardial perfusion scintigraphy are not induced by myocardial ischemia

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ABSTRACT

Background: Increased systemic levels of myeloperoxidase (MPO) have been reported in patients with acute myocardial ischemia. We studied the association between exercise-induced myocardial ischemia measured by myocardial perfusion scintigraphy (MPS) and the magnitude and time course of changes in MPO levels in humans.

Methods: One hundred and twenty six patients underwent symptom limited exercise MPS. Myocardial ischemia was assessed semi-quantitatively. Plasma samples were taken before the start of exercise (baseline), at maximum exercise and every hour up to 6 hours after maximum exercise.

Results: Myocardial ischemia was present in 42 (33%) patients. MPO levels rapidly increased during exercise in patients with and without ischemia (to 131% (106-172%) and 145% (121-199%) of baseline, respectively). MPO levels and absolute changes in MPO did not differ between patients with and without ischemia at any time point. None of the patient characteristics, including presence of ischemia, was independently predictive of the absolute increase in MPO levels during exercise.

Conclusions: Exercise related immediate increases in MPO levels do not reflect myocardial ischemia.

Keywords: myocardial ischemia, myeloperoxidase, myocardial perfusion scintigraphy

Abbreviations: ACE, angiotensin converting enzyme; BMI, body mass index; ECG, electrocardiogram; LVEF, left ventricular ejection fraction; MPO, myeloperoxidase; MPS, myocardial perfusion scintigraphy; PMN, polymorphonuclear neutrophils; SDS, summed difference score; SPECT, single photon emission tomography
INTRODUCTION

We studied the association between the extent of exercise-induced myocardial ischemia measured by myocardial perfusion scintigraphy and the magnitude and time course of changes in MPO levels in humans.

Activation of neutrophils is considered to contribute to the pathogenesis of atherosclerosis through release of myeloperoxidase (MPO). Myeloperoxidase promotes oxidative damage by formation of free radicals and diffuse oxidants (1). Patients with coronary artery disease have increased systemic levels of MPO (2) and increased levels are associated with future adverse cardiac events in healthy individuals (3), patients with chest pain (4) and acute coronary syndromes (5-7).

The pathogenetic role of MPO in the development of cardiac events, however, remains unclear. Myeloperoxidase has been linked to plaque vulnerability (8), while various conditions of ischemia, often a result of atherosclerotic disease, can lead to activation of neutrophils, and hence MPO release, promoting further development of atherosclerosis. Exercise-induced muscle ischemia in claudication (9) and experimental myocardial ischemia and reperfusion lead to neutrophil activation (10). Systemic levels of MPO increase immediately after coronary stenting with transient myocardial ischemia (11), suggesting myocardial ischemia may lead to MPO release and may enhance progression of atherosclerosis.

MATERIALS AND METHODS

Study population
One hundred and twenty six patients, referred for the evaluation of the presence or absence of inducible myocardial ischemia and able to perform a bicycle exercise test, were included. Patients underwent symptom limited exercise myocardial perfusion scintigraphy according to a two-day stress/rest protocol using \(^{99m}\text{Tc-Tetrofosmin}\) and ECG gated single photon emission tomography (SPECT). Blood samples for analysis of MPO were taken before the start of exercise, at maximum exercise and subsequently every hour up to 6 hours after maximum exercise. The local medical ethics committee approved the protocol. All patients gave written informed consent before participation. Documented CAD defined as prior acute myocardial infarction, revascularization, or documented coronary artery stenosis (> 50%) on coronary angiogram.
Myocardial perfusion exercise protocol

Myocardial perfusion scintigraphy was performed according to the guidelines of the American Society of Nuclear Cardiology (12) using a two-day stress/rest protocol. A dose of 500 MBq $^{99m}$Tc-Tetrofosmin was administered at rest and at peak exercise. All patients were stressed with a bicycle ergo-meter with a starting workload of 50 Watt (W) increasing every 2 minutes with 25 W. Endpoints for exercise were among others achievement of at least 85% of the age predicted heartrate, recognizable chest pain, and > 2 mm ST-segment depression (13). All patients fasted both days and anti-anginal medication was discontinued before the exercise test and restarted after exercise.

Gated SPECT acquisition

Gated myocardial SPECT was performed with the patient in prone position using a three-headed gamma-camera (MultiSPECT-3, Siemens, Hoffman Estate, Illinois, USA). Acquisitions were gated for 16 frames per cardiac cycle. Estimates of left ventricular function (end-diastolic volume, end-systolic volume and left ventricular ejection fraction) were calculated using a completely automated algorithm, previously described and validated (14, 15). Stress and rest perfusion images were scored in consensus by two experienced nuclear medicine physicians (HJV and BLFvE-S) using a 5-point semi-quantitative score for each of 17 myocardial segments. Perfusion defect severity was classified as normal (0), equivocal abnormal (1), mildly abnormal (2), moderately abnormal (3) or severely abnormal (4). Subsequently summed stress score, summed rest score and the difference between those scores (summed difference score or SDS) were calculated. A SDS of three or greater was considered to indicate myocardial ischemia. Clinical parameters such as chest pain or electrocardiographic changes/abnormalities during exercise were not included in the definition of myocardial ischemia.

Biochemical analysis

EDTA plasma samples were stored at -80°C. Myeloperoxidase was measured using CardiomPO™ test (PrognostiX Inc., Cleveland, Ohio, U.S.A.). The CardiomPO Test™ is a sandwich enzyme linked immunosorbent assay (ELISA) approved by the Food and Drug Administration that uses a highly specific mouse monoclonal antibody and a polyclonal antibody. Calibrators of human MPO were used to establish a calibration curve to determine MPO concentration. Furthermore, three controls comprised of human MPO in a human plasma matrix were used to monitor and evaluate the precision and accuracy of the CardiomPO™ Test. Plates, coated with a mouse monoclonal antibody specific to MPO, were incubated with plasma samples for 1 hour at room temperature. After washing, a solution of a polyclonal rabbit anti-MPO antibody was added to bind to the MPO captured on the plate for 1 hour. A secondary goat anti-rabbit IgG antibody, labeled with the enzyme horseradish peroxidase (HRP), was added to each well after washing and incubated for 30 minutes. Wells were washed again. TMB substrate solution was added for 10 minutes resulting in the development of a blue color. The reaction was
stopped and absorbance was read spectrophotometrically at 450 nm. The absorbances of the calibrators were used to plot a standard curve of absorbance versus MPO concentration from which the MPO concentration in the controls or samples can be determined. The inter-assay and intra-assay variability were 2 and 6%. The lower detection limit was 13 pM, and the upper detection limit was 5,223 pM. Samples were analyzed in random order to avoid systemic bias, and in a blinded fashion. Normal control values for plasma MPO have been reported to be < 539 pM (95% upper percentile of middle-aged healthy population, n = 300, Prognostix).

Statistical analysis
The Student t-test, Mann-Whitney rank sum test and chi-square test were used when appropriate. All tests were two-tailed. For correlations between continuous variables, the Pearson correlation coefficient was calculated if both variables were normally distributed, and Spearman’s rho if otherwise. For uni- and multivariate analysis of correlations between patient clinical and biochemical characteristics and changes in MPO levels, changes in MPO levels were log-transformed to a normal distribution. Linear stepwise regression analysis was performed to assess independent determinants of changes in MPO level (criterion for entry and exit at 0.05 and 0.1). SPSS for windows release 12.0.1 (SPSS Inc., Chicago, IL) was used for analyses.

RESULTS

Patient characteristics and overall scintigraphic results
Clinical, biochemical and scintigraphic characteristics of patients separated by the presence or absence of ischemia are shown in Table 1. Patients with ischemia (as defined by the result of the myocardial scintigraphy, SDS ≥ 3, n = 42 (33%)) were more often male and more often had hypercholesterolemia, and a history of documented coronary artery disease or myocardial infarction. Baseline MPO levels were not significantly different between the two groups. Patients with ischemia showed larger diastolic and systolic volumes and lower left ventricular ejection fractions (LVEF) on post-stress images.

Changes in myeloperoxidase after exercise
Immediately after exercise absolute levels of MPO increased, peaking at maximum exercise (p < 0.001) both in patients with and without ischemia. In patients with ischemia, MPO levels changed from 181 (154-253) μg/L (median (interquartile range) to 260 (205-401) μg/L, and in patients without ischemia, MPO levels changed from 191 (155-231) μg/L to 278 (227-378) μg/L. The changes correspond with 131 (106-172) % and 145 (121-199) % of baseline levels in patients with and without ischemia, respectively. There was no significant difference in the absolute change from baseline to maximum exercise between patients with and patients
without ischemia ($p = 0.128$). Figure 1 shows increases in circulating MPO in patients with (Figure 1a) and without ischemia (Figure 1b). There were no significant differences in MPO levels between patients with and without ischemia at any time point.

**Determinants of baseline MPO and exercise-induced change in MPO**

Table 2 shows the univariate analysis of determinants of baseline MPO and of the change between baseline levels of MPO and levels at maximum exercise. Baseline MPO was increased only in hypertension patients (210 (169-258) μg/L vs. 178 (138-214) μg/L (median (interquartile range)). Changes to maximum exercise were lower in patients with previous myocardial infarction and patients using ACE inhibitors, whereas changes were higher in patients reaching peak exercise levels more than 125 W during exercise. In multivariate analysis, none of these parameters were independent determinants of the change in MPO level.
Table 2. Univariate analysis of baseline MPO levels and absolute changes in MPO levels, according to patient characteristic

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Baseline MPO (μg/L)</th>
<th>P</th>
<th>MPO change (μg/L)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>184 [153-223]</td>
<td>0.555</td>
<td>69 [22-143]</td>
<td>0.466</td>
</tr>
<tr>
<td>Female gender</td>
<td>196 [157-252]</td>
<td>0.761</td>
<td>49 [20-102]</td>
<td>0.120</td>
</tr>
<tr>
<td>Age &gt; 65 years</td>
<td>188 [162-232]</td>
<td>0.947</td>
<td>71 [29-155]</td>
<td>0.430</td>
</tr>
<tr>
<td>BMI &gt; 25 kg/m²</td>
<td>196 [151-237]</td>
<td>0.430</td>
<td>64 [20-175]</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>210 [169-258]</td>
<td>0.005</td>
<td>71 [30-158]</td>
<td>0.893</td>
</tr>
<tr>
<td>No hypertension</td>
<td>178 [138-214]</td>
<td>0.674</td>
<td>69 [22-171]</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>196 [156-268]</td>
<td>0.155</td>
<td>90 [30-175]</td>
<td>0.294</td>
</tr>
<tr>
<td>No hypercholesterolemia</td>
<td>179 [151-227]</td>
<td></td>
<td>69 [21-137]</td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td>174 [139-232]</td>
<td>0.051</td>
<td>71 [29-173]</td>
<td>0.488</td>
</tr>
<tr>
<td>No family history</td>
<td>200 [171-243]</td>
<td></td>
<td>69 [20-144]</td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td>196 [163-213]</td>
<td>0.708</td>
<td>96 [45-174]</td>
<td>0.277</td>
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<tr>
<td>No current smoking</td>
<td>185 [155-253]</td>
<td>0.483</td>
<td>69 [21-155]</td>
<td>0.195</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>197 [161-250]</td>
<td>0.155</td>
<td>51 [20-118]</td>
<td>0.195</td>
</tr>
<tr>
<td>No diabetes mellitus</td>
<td>186 [147-235]</td>
<td></td>
<td>72 [30-175]</td>
<td></td>
</tr>
<tr>
<td>Known coronary artery disease</td>
<td>187 [149-232]</td>
<td>0.552</td>
<td>59 [21-155]</td>
<td>0.356</td>
</tr>
<tr>
<td>No known coronary artery disease</td>
<td>196 [156-253]</td>
<td></td>
<td>75 [40-169]</td>
<td></td>
</tr>
<tr>
<td>History of myocardial infarction</td>
<td>191 [152-260]</td>
<td>0.797</td>
<td>38 [13-96]</td>
<td>0.003</td>
</tr>
<tr>
<td>No history of myocardial infarction</td>
<td>188 [155-237]</td>
<td></td>
<td>99 [46-178]</td>
<td></td>
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<tr>
<td>Peripheral arterial disease</td>
<td>182 [141-267]</td>
<td>0.760</td>
<td>45 [15-55]</td>
<td>0.119</td>
</tr>
<tr>
<td>No peripheral arterial disease</td>
<td>191 [155-237]</td>
<td></td>
<td>71 [27-169]</td>
<td></td>
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<tr>
<td>Aspirin</td>
<td>186 [149-235]</td>
<td>0.689</td>
<td>73 [21-173]</td>
<td>0.690</td>
</tr>
<tr>
<td>No aspirin</td>
<td>199 [166-240]</td>
<td></td>
<td>59 [37-131]</td>
<td></td>
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<tr>
<td>Nitrates</td>
<td>184 [154-222]</td>
<td>0.554</td>
<td>65 [22-139]</td>
<td>0.329</td>
</tr>
<tr>
<td>No nitrates</td>
<td>194 [154-243]</td>
<td></td>
<td>73 [30-175]</td>
<td></td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>199 [168-251]</td>
<td>0.220</td>
<td>77 [33-232]</td>
<td>0.340</td>
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<tr>
<td>ACE inhibitors</td>
<td>217 [167-291]</td>
<td>0.056</td>
<td>37 [18-108]</td>
<td>0.023</td>
</tr>
<tr>
<td>No ACE inhibitors</td>
<td>181 [149-218]</td>
<td></td>
<td>75 [34-176]</td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>198 [159-260]</td>
<td>0.169</td>
<td>71 [27-155]</td>
<td>0.921</td>
</tr>
<tr>
<td>No statins</td>
<td>181 [148-222]</td>
<td></td>
<td>65 [22-176]</td>
<td></td>
</tr>
<tr>
<td>Peak exercise ≤ 125 W</td>
<td>158 [135-337]</td>
<td>0.220</td>
<td>143 [55-248]</td>
<td>0.013</td>
</tr>
<tr>
<td>Peak exercise &gt; 125 W</td>
<td>196 [165-233]</td>
<td></td>
<td>59 [22-120]</td>
<td></td>
</tr>
<tr>
<td>Left ventricular ejection fraction ≤ 40%</td>
<td>196 [174-243]</td>
<td>0.404</td>
<td>40 [11-86]</td>
<td>0.066</td>
</tr>
<tr>
<td>Left ventricular ejection fraction &gt; 40%</td>
<td>188 [237-149]</td>
<td></td>
<td>74 [33-173]</td>
<td></td>
</tr>
<tr>
<td>Ischemia on perfusion scan</td>
<td>181 [154-253]</td>
<td>0.822</td>
<td>58 [19-136]</td>
<td>0.128</td>
</tr>
<tr>
<td>No ischemia on perfusion scan</td>
<td>191 [155-231]</td>
<td></td>
<td>79 [35-173]</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as median [interquartile range (IQR)]. Differences between patient characteristics were assessed with Mann-Whitney rank sum test. ACE, angiotensin converting enzyme; BMI, body mass index; MPO, myeloperoxidase.
DISCUSSION

Our study showed exercise-induced immediate increases in MPO levels. Increases in MPO, however, were not related to exercise-induced myocardial ischemia. These findings indicate that myocardial ischemia during exercise does not lead to measurable changes in MPO release.

The increase of MPO during exercise (31% and 45% in patients with and without myocardial ischemia) cannot be attributed to hemoconcentration only, which can be concluded from the 13% increase in IgM levels in a subset of the study patients undergoing the exercise protocol, as reported previously (16). The size of IgM prevents its passage into extravascular space, so the IgM antibody remains mainly intravascular. Therefore, short term changes in IgM concentration may serve as an indicator of water shift across the vessel wall, i.e. hemoconcentration. The increases found in our study were comparable to increases found in healthy athletes (17).
Release of MPO from neutrophils after exercise has been linked to release of glucocorticoid hormones (17, 18) and proteolysis (19). Furthermore, we found that increases in MPO were correlated with the extent of exercise performed. However, correlations between clinical parameters and MPO increases in our study could be subject to interference by multiple testing and should be regarded with caution.

Earlier studies showed exercise-induced immediate local increases of neutrophil activation in patients with peripheral arterial disease, rapidly followed by systemic neutrophil activation (9). Systemic changes which were higher in patients with claudiation patients than in healthy controls undergoing the same extent of exercise (20). We could not find increased MPO levels after exercise in myocardial ischemia, as measured by perfusion scintigraphy.

Myocardial perfusion scintigraphy has 87% sensitivity and 73% specificity in detection of significant coronary stenosis (> 50% stenosis) (21). However, MPS is a reflection of differences in radiofarmacon uptake compared with adjacent regions of myocardium, visualized in two- and three dimensional images. Although differences in uptake on myocardial perfusion scintigraphy are directly related to the flow through the coronary arteries, myocardial perfusion scintigraphy does not provide information on the actual plaque burden or actual coronary artery diameter stenosis. The relative low sensitivity and specificity of MPS compared with coronary artery diameter stenosis can in part be explained by the fact that these are a reflection of the discrepancy between a functional test (MPS) and actual anatomical information (coronary artery diameter stenosis). In clinical cardiology myocardial perfusion scintigraphy is essential for the assessment of myocardial ischemia and is often used in combination with anatomical information.

As baseline MPO levels were comparable between patients with and patients without exercise-inducible myocardial ischemia, myocardial preconditioning, which is known to decrease neutrophil activation (22, 23), is unlikely to be of influence on baseline MPO levels and absolute changes during exercise in our patients.

In line with a previous study showing the absence of increased systemic neutrophil activation in episodes of myocardial ischemia in acute coronary syndromes, stable and variant angina (24), we did not find increased systemic neutrophil activation in exercise induced myocardial ischemia. Holding predictive value for future adverse cardiac events (3-7), MPO is likely to be a marker of characteristics in the pathogenesis of atherosclerosis other than myocardial ischemia. MPO has been associated with vascular dysfunction (25-27), progression of atherosclerotic lesions (28, 29) and plaque instability (30). The latter hypothesis is supported be recent findings of increased MPO levels in 298 patients with acute coronary syndrome when compared to 382 stable angina and comparable extent of coronary artery disease (31).
Moreover, increased activation of neutrophils was seen in myocardial ischemia in unstable angina when compared to myocardial ischemia in variant angina (32). Hence, MPO may not be a marker of consequences of advanced atherosclerosis (i.e. myocardial ischemia), but may contribute to the pathogenesis of atherosclerotic plaques and plaque instability.

In conclusion, exercise related immediate systemic increases in MPO levels reflect exercise but not myocardial ischemia.

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REFERENCES