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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 h 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.

1. 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). MPO 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. MPO 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.

2. Materials and methods

2.1. 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}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 h after maximum exercise. The local medical ethics committee

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

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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.

2.2. 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 W (Watt) increasing every 2 min with 25 W. Endpoints for exercise were among others achievement of at least 85% of the age predicted heart-rate, 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.

2.2.1. 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, U.S.A.). 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.

2.3. Biochemical analysis

EDTA plasma samples were stored at −80 °C. MPO 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 h 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 h. 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 min. Wells were washed again. TMB substrate solution was added for 10 min 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 5223 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).

2.4. 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 logtransformed 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.

3. Result and discussion

3.1. 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.

Table 1
Clinical, biochemical and scintigraphic characteristics

Characteristics	Overall (n=126)	Ischemia (n=42)	No ischemia (n=84)	P value
Male gender	79 (62%)	32 76%)	47 (56%)	0.027
Age (years, mean±SD)	59 (11)	61 (11)	59 (11)	0.225
BMI (kg/m^2, mean±SD)	27±4.3	27±4.0	27±4.5	0.791
Hypertension	57 (45%)	16 (38%)	41 (49%)	0.255
Hypercholesterolemia	53 (42%)	23 (55%)	30 (36%)	0.030
Family history	69 (55%)	24 (57%)	45 (54%)	0.704
Current smoking	20 (16%)	6 (14%)	14 (17%)	0.730
Diabetes mellitus	34 (27%)	15 (36%)	19 (23%)	0.119
Documented coronary artery disease*	67 (53%)	30 (71%)	37 (44%)	0.004
Prior myocardial infarction	49 (39%)	25 (60%)	24 (29%)	0.001
Peripheral arterial disease	10 (8%)	4 (10%)	6 (7%)	0.641
Medication				
Aspirin	91 (72%)	33 (79%)	58 (69%)	0.261
Nitrates	49 (39%)	20 (48%)	29 (35%)	0.155
Calcium antagonists	37 (29%)	13 (31%)	24 (29%)	0.782
ACE inhibitors	32 (25%)	14 (33%)	18 (21%)	0.148
Statins	65 (52%)	25 (60%)	40 (48%)	0.207
Baseline MPO (μg/L), (median (interquartile range))	189 (155–237)	181 (154–253)	191 (155–231)	0.822
Duration of exercise (s, mean±SD)	511 (146)	513 (126)	511 (157)	0.945
Peak exercise (W, median (interquartile range))	125 (100–150)	125 (100–150)	125 (100–150)	0.660
Left ventricular ejection fraction (%, mean±SD)	56±12	49±14	58±12	<0.001
Summed difference score (median (interquartile range))	0 (0–4)	6 (4–7)	0 (0–0)	

Data are numbers (%), mean (±standard deviation (SD)), or median (25–75th percentile).
ACE, angiotensin converting enzyme; BMI, body mass index; MPO, myeloperoxidase.

3.2. 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 μg/L (154–253 μg/L) (median (interquartile range) to 260 μg/L (205–401 μg/L), and in patients without ischemia, MPO levels changed from 191 μg/L (155–231 μg/L) to 278 μg/L (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). Fig. 1 shows increases in circulating MPO in patients with (A) and without ischemia (B). There were no significant differences in MPO levels between patients with and without ischemia at any time point.

3.3. 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 μg/L (169–258 μg/L) versus 178 μg/L (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