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Myeloperoxidase predicts risk of vasculopathic events in hemizygous males with Fabry disease

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Abstract

Fabry disease results in a global vasculopathy leading to early-onset stroke and renal and cardiac failure. We found that random myeloperoxidase in serum and plasma was significantly elevated in 73 consecutive male patients with Fabry disease. Random serum myeloperoxidase level in men predicted the risk of a Fabry vasculopathy–related event in subsequent years. Long-term enzyme replacement therapy did not reduce myeloperoxidase level or eliminate the risk of vasculopathic events.

Fabry disease (OMIM 301500) is a progressive multisystem X-linked disorder[1] caused by a deficiency of α-galactosidase A that leads to failure to catabolize cellular lipids containing α-D-galactosyl moieties such as globotriaosylceramide (Gb_3). Patients exhibit a systemic vasculopathy with increased risk of stroke, cardiac dysfunction, and progressive renal disease.[1] The mechanism by which increased levels of intracellular glycolipids cause vascular disease is incompletely understood. In patients with Fabry disease, a prothrombotic state that includes increased expression of the integrin CD11b on monocytes has been described, suggesting leukocyte and endothelial activation.[2] Altered function of non-nitric oxide pathways, increased vascular staining for 3-nitrotyrosine and decreased vascular response to ascorbate infusion in patients with Fabry disease suggests increased production of reactive oxygen species (ROS) such as superoxide or hydrogen peroxide, the putative endothelium-dependent hyperpolarizing factor.[3] Furthermore, accelerated atherosclerosis has been found in both susceptible individuals and an animal model of Fabry disease.[4,5]

Elevated blood myeloperoxidase (MPO) is a predictor of acute cardiovascular events, fixed coronary stenosis, and endothelial dysfunction and is a determinant in the formation of the atherosclerotic plaque. We hypothesized that elevated blood MPO levels in this disorder is a risk factor for developing adverse events related to the vasculopathy of Fabry disease. We also sought to determine the effect of enzyme replacement therapy on circulating MPO levels.

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Methods

Subjects

Patients (table) participated in clinical research protocol approved by the Institutional Review Board (IRB) the National Institute of Neurological Disorders and Stroke, and all patients gave written informed consent. All male hemizygous patients were diagnosed using standard α-galactosidase A activity and all female heterozygotes had pathogenic mutations of the *GLA* gene. Three patients were of Hispanic ethnicity, and all others were of non-Hispanic white background. Baseline white blood cell count in male patients with Fabry disease was 5,900 ± 1,600 mm^3 (normal 3,300 to 9,600 mm^3) (only one patient had elevated neutrophil count), weight was 74.2 ± 16.8 kg, and height was 174.4 ± 16.2. Only two male patients were habitual smokers. C-reactive protein levels were normal in all but two of the patients and therefore were not useful to predict the complications related to the vasculopathy in this patient population (data not shown).

The patients were not selected for the events studied but represent the sample available at our institution enrolled in IRB-approved protocols to study the natural history and evaluation of enzyme replacement therapy (ERT) using genetically engineered α-galactosidase A (agalsidase alfa, Shire Human Genetic Therapies, Cambridge, MA). To avoid possible diagnostic bias, the blood specimens were processed in a blind fashion separated from the analysis of the clinical data. Anonymous age-matched normal control samples were obtained from Bioreclamation, Inc. (Hicksville, NY). As an additional internal quality control measure, additional controls were collected by the investigators under National Institute of Neurological Disorders and Stroke IRB-approved protocols. There was no significant difference in MPO levels between commercially acquired samples and the NIH control cohorts. There was no significant age difference between the patient and control groups. White matter lesion load on brain MRI was calculated as previously described and expressed in millimeters.[6]

MPO samples and assays

Serum and plasma samples were prospectively collected on all patients with Fabry disease seen at NIH and stored at −80 °C. During the ERT trial, samples were collected at baseline and at 2, 4, 6, 12, 18, 30, 48, and 55 months. At the time of blood collection, all patients were clinically stable and not experiencing acute vascular events. None of the patients had elevated leukocyte or neutrophil counts on the day of MPO sampling. Mean sample storage time until analysis was 45.5 months (range 1 to 8 years) with no effect on MPO levels. MPO levels were measured using an enzyme immunometric assay kit for human MPO (Assay Designs, Ann Arbor, MI) according the manufacturer's directions. MPO levels of samples were calculated using a four-parameter curve fit of standards with SoftMax Pro software.

Statistics

A Cox proportional hazard model with left truncation and right censoring with added covariates was fitted (see details of statistical method in appendix E-1 on the *Neurology* Web site at www.neurology.org). Events related to the vasculopathy of Fabry disease were defined as stroke, myocardial infarction, other vascular ischemic event, end-stage renal disease, and death directly related to Fabry disease. In addition to serum MPO level, other covariates were age at baseline, total blood cholesterol, mean arterial pressure, white matter ischemic lesion load observed on cerebral MRI, and use of medications such as statins, angiotensin-converting enzyme inhibitor, and angiotensin receptor blockers and residual α-galactosidase A activity. The presence of residual enzyme activity was included because residual activity is known to moderate the severity of a number of manifestations of Fabry disease.

Results

MPO levels in serum and plasma in patients not receiving ERT as well as in non-Fabry controls are described in the table. MPO levels in serum ($p < 0.0001$ by both *t* test and the nonparametric Mann-Whitney test [figure E-1] and in plasma ($p = 0.01$) were higher in the Fabry male patients vs controls. Exploratory data analysis indicated the (serum) MPO level had normal distribution (Shapiro-Wilks, W = 0.9654, $p = 0.086$ (0.09). The values of our healthy controls were similar to the previously published MPO levels using a similar method.[7] Using probability distribution functions, the median and mean of serum MPO in the normal population were 57.7 ng/mL and the third quartile of MPO was 96.93 ng/mL. By that estimate, 65.6% of the male patients had elevated serum MPO levels, whereas 57.5% had elevated levels by the mean ± 3 SD criterion. Mean serum MPO in female patients was increased but not significantly ($p = 0.09$).

Because the abnormality of MPO in serum had a higher event rate than plasma, we evaluated to what extent serum MPO levels predict the likelihood of developing vascular-related events in subsequent years. Complete follow-up data were available from 60 (of 73) consecutive male patients who were followed for 3 to 9 years (mean 6 ± 1.7) after the initial random serum MPO level. Events related to the vasculopathy of Fabry disease were stroke (n = 5), myocardial infarction (n = 5), toe infarct (n = 2), end-stage renal disease (n = 2), and death directly related to Fabry disease (n = 2).

The most parsimonious model (see appendix E-1) to fit the data using a Cox proportional hazard model was (3) event MPO + lesion. Likelihood ratio test (LRT) comparison between (3) and (1) indicated (3) to be a better fit ($p(>|\chi^2|) < 0.00005$), whereas LRT comparison between (3) and (2) gave $p(>|\chi^2|) < 0.6$, indicating (3) to be the more parsimonious model. This analysis indicated that a serum MPO parameter estimate of exp(MPO) ± SE = 1.0121 ± 0.00529 was significant ($p = 0.02$) or associated with a 12% increase in the event risk, whereas the parameter estimate for cerebral lesion load exp(lesion) ± SE = 1.0068 ± 0.00257 was also significant ($p = 0.009$) or associated with a 7% increase in the event risk. No other covariates were significantly associated with vascular events. The Schoenfeld residuals indicated a trend in the MPO hazard with time ($p = 0.071$), whereas the lesion residuals were nonsignificant ($p = 0.626$). Overall, the global test of Schoenfeld residuals (including both MPO and lesions) was nonsignificant ($p = 0.194$), indicating satisfactory compliance of the model (3) with the proportional hazard assumption.

We measured serum MPO in samples collected prospectively during a 6-month randomized, placebo-controlled trial of enzyme replacement therapy with agalsidase alfa and subsequent open-label continuation study in which all patients continued receiving enzyme infusions for up to a total of 55 months.[8] There was no significant reduction of serum MPO in these adult patients on agalsidase alfa (not shown). Seven of 23 patients (30%) with a long-term follow-up had vascular events while on ERT (five strokes and two peripheral artery ischemic events). Their baseline median MPO level was 187 (range 68 to 411), and 174 (range 51 to 274) at the latest time point; MPO levels decreased in four patients and increased in three.

Discussion

We found that randomly obtained serum and plasma MPO is significantly elevated in a large group of male patients with Fabry disease vs controls and further expands our earlier observation.[9] MPO levels in the serum of female heterozygotes were higher than those in controls, but the increase did not reach significance. We also found that an elevated serum MPO level on a single random sample is a significant risk factor for developing vascular-related events in subsequent years. Overall, MPO levels did not decrease with ERT of up to 55 months.

The mechanism of elevation of blood MPO in Fabry disease is not completely understood. Neutrophils, monocytes, and tissue-associated macrophages abundantly produce MPO in response to a number of activators including ROS. The more pronounced elevation of MPO in serum compared to plasma suggests that neutrophils in particular are primed in Fabry disease.[2] These findings suggest augmented inflammatory interactions and increased adhesion of circulating leukocytes to the vascular endothelial cells. This process is likely associated with priming of neutrophils. Increased production of ROS that likely occurs in Fabry disease may also stimulate neutrophils directly to produce MPO. Interestingly, Gb_3 itself has been associated with cell adhesion and apoptosis of B cells.

Recent studies have shown that increased MPO levels are associated with increased risk of cardiovascular disease or events, atherosclerosis, and endothelial dysfunction and have adverse effects on left ventricular remodeling and function. By contrast, patients deficient in MPO are significantly protected from these effects. MPO is thought to cause lipid peroxidation in vivo, catalyzing formation of many leukocyte-derived cytotoxic oxidants using H_2O_2 as substrate. These molecules interact with amino acids and lipoproteins such as high-density lipoprotein and low-density lipoprotein to promote atherosclerosis.[10] Therefore, MPO may be a mediator of the accelerated atherosclerosis and increased staining for 3-nitrotyrosine in blood vessels that is seen in both patients with Fabry disease and the animal model of Fabry disease.[5]

In recent years, ERT has been available for the treatment of Fabry disease. Although ERT shows some promise, strokes continue to occur.[8] This lack of reduction of MPO with ERT may be a correlate of continued occurrence of vascular events on this therapy. Therefore, it will be important to determine whether future therapies will be able to reduce serum MPO in parallel to reduced incidence of stroke in Fabry disease, thus making this marker a surrogate for this clinically important endpoint.

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Table

Myeloperoxidase (MPO) levels in serum and plasma of patients with Fabry disease and controls

	Male patients, n = 73	Male normal controls, n = 36	Female patients, n = 48	Female normal controls, n = 26
Age, y				
Mean	36 ± 12	38 ± 15	39 ± 8.4	37 ± 9
Range	12–64	18–85	19–52	20–48
Serum MPO, ng/mL				
Mean	139.4 ± 112	39 ± 24	97.6 ± 50	51 ± 35
Range	18–822	8.5–107	26–222	16.7–135
Plasma MPO, ng/mL				
Mean	42.4 ± 79.4	20 ± 8	ND	ND
Range	5.5–654	14–50		

ND = not done.