Elevated Endothelial Microparticles in Fabry Children Decreased After Enzyme Replacement Therapy

To the Editor:

Fabry disease (FD), an X-linked metabolic disorder, is caused by insufficient activity of the lysosomal enzyme alpha galactosidase A (α-gal A). This results in impaired catabolism of globotriaosylceramide ([Gb₃]) also called ceramidetrihexoside) and its subsequent accumulation leading to endothelial dysfunction and other anomalies.¹ At present, enzyme replacement therapy (ERT) is the only available therapeutic approach for patients with Fabry disease. However, in adult patients with advanced FD, ERT has its limitations.² Therefore, studies have been initiated to analyze the efficacy of ERT started at an earlier stage of FD.³,⁴ In general, the identification of markers that could serve as indicators of disease severity, prognosis, and possible effects of specific therapies is highly desirable in genetic disorders. Because it has been documented that elevated counts of endothelial cell derived membrane microparticles can be present in the circulation of patients with a variety of diseases with a vascular injury component, we explored whether microparticles (MPs) could serve as a potential marker.⁵

We investigated the presence of endothelial cell membrane-derived microparticles (EMPs) in peripheral venous blood samples of 10 pediatric Fabry patients (9 males and 1 female) before they were started on ERT and at the end of 6 and 12 months into the treatment regimen of infusions every other week with agalsidase alfa (Shire Human Genetic Therapies, Cambridge, Mass) at 0.2 mg/kg. Both the 6- and 12-month samples were collected 14 days after the last enzyme infusion, thus right before the next infusion. Median age of patients was 12 (range: 10 to 18); renal function, urinary protein excretion, and cardiac function and structure were normal. Carbamazepine or gabapentin were common medications. Patients were stable except for 1 who suffered recurrent symptomatic and lacunar ischemic strokes. Plasma from healthy age-matched volunteers (n=18) was used for comparison.

Venous blood samples, from both patients and controls, were collected using Vacutainer tubes with ACD-A. These blood samples were centrifuged for 15 minutes at 2700 g and 10°C to obtain platelet poor plasma (PPP). Subsequently, PPP was collected and centrifuged for 5 minutes at 2700 g to remove contaminating platelets. Aliquots of 1.5 mL of platelet free plasma (PPP) were thawed and centrifuged for 10 minutes at 20,000 g and 10°C. The sedimented MPs, 0.3 to 1.0 μm in size (mean 0.45 μm), were washed, incubated with saturating concentrations of selected antibodies simultaneously, washed again, resuspended, and analyzed by flow cytometry as previously described in great detail.⁶ Briefly, MPs were analyzed using a fluorescence-activated cell sorter (FACS) Calibur flow cytometer equipped with CellQuestPro software (Becton Dickinson) in a protocol with both forward scatter (FSC) and side scatter (SSC) in logarithmic mode. Standard beads 0.2 to 3.0 μm in diameter (Sigma; Molecular Probes) were used for FSC/SSC setting for MP acquisition. The flow rate was evaluated using

(Arterioscler Thromb Vasc Biol. 2007;27:e138-e139.)
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Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org
DOI: 10.1161/ATVBAHA.107.143511

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TruCount beads from Becton Dickinson analyzed as separate samples in parallel. EMPs were identified by antibodies to both endothelial antigen CD105 (endoglin) and the more specific CD144 (VE-cadherin). EMP counts per μL of plasma were calculated using double fluorescence plots. Nonparametric statistical analysis was used (Friedman repeated measures analysis, Mann–Whitney rank sum test, and Wilcoxon rank test where appropriate).6

We found that CD144+/CD105− EMPs were elevated in Fabry patients before ERT (median 442/μL; interquartile range 382 to 653/μL) compared with the control group (325/μL; 273 to 506/μL; P=0.031; Figure, A). It should be noted that the patient experiencing ischemic strokes was not an outlier. In contrast, there were no significant differences in red blood cell (CD235a+), leukocyte (CD45+), platelet (CD41a+) MP-counts between Fabry patients and the control group. Interestingly, we found a highly significant decrease (P=0.004) in EMP-counts after 6 months (338/μL; 315 to 588/μL) and 12 months (282/μL; 194 to 310/μL) of ERT (Figure, B), whereas platelet, leukocyte, and red blood cell MPs did not change significantly. The EMP counts after 12 months of ERT were below the EMP-counts of the control group; however, this difference was not significant. Our results suggest that ERT may influence the counts of circulating EMPs in pediatric Fabry patients. These findings warrant further studies to elucidate how circulating EMPs may correlate with severity and characteristics of endothelial injury in Fabry disease and whether EMP analysis could be used for monitoring the effects of ERT on endothelial functions. The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

Disclosures

None.

Monique P. Gelderman
CBER, FDA, Rockville, Md

Raphael Schiffmann
Developmental and Metabolic Neurology Branch
NINDS, NIH, Bethesda, Md

Jan Simak
CBER, FDA, Rockville, Md

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Arterioscler Thromb Vasc Biol. 2007;27:e138-e139
doi: 10.1161/ATVBAHA.107.143511
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/27/7/e138

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