

Enzyme Replacement Therapy With Elosulfase Alfa Decreases Storage of Glycosaminoglycan in White Blood Cells of Patients With Morquio A Syndrome

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Abstract

Mucopolysaccharidosis IVA (MPS IVA; Morquio A syndrome) is a lysosomal storage disorder caused by a deficient N-acetylgalactosamine-6-sulfate sulfatase activity, leading to cellular storage of undegraded keratan sulfate. Recently enzyme replacement therapy (ERT) was approved for MPS IVA, but some of ERT effects are still unknown. In the present study, we aimed to evaluate the efficacy of elosulfase alfa upon glycosaminoglycan (GAG) storage in peripheral blood white blood cells of patients with MPS IVA treated for 6 months, comparing samples from patients who received weekly infusions of enzyme (ERT-W) versus infusions every other week (ERT-EOW) versus placebo. A significant reduction in GAG storage was observed in both ERT-treated groups, with weekly ERT showing slightly better performance than ERT-EOW.

Keywords

Morquio A, mucopolysaccharidosis IVA, enzyme replacement therapy, elosulfase alfa

Introduction

Mucopolysaccharidosis IVA (MPS IVA; Morquio A syndrome) is a lysosomal storage disorder caused by deficient activity of N-acetylgalactosamine-6-sulfate sulfatase, leading to cellular storage of undegraded keratan sulfate (KS).¹ Recently, enzyme replacement therapy (ERT) was approved for treatment of MPS IVA (BMN110, approved by Food and Drug Administration [FDA] as Vimizin, BioMarin Pharmaceutical, Novato, California). Initial studies demonstrated an improvement in the 6-minute walk test as well as normalization of urinary KS levels. Also, the drug is considered safe, as no adverse events led to permanent treatment discontinuation.² Although the results are promising, there is still a lack of knowledge about the spectrum of effects of ERT on MPS IVA manifestations.

Patients with Morquio A syndrome typically present progressive bone and joint problems that lead to short stature and bone deformities.³ However, glycosaminoglycan (GAG) storage occurs in multiple organs, including the lung, aorta, heart valves, heart muscle, visceral organs, and bone marrow.⁴ An effective treatment of those alterations is very important, since these abnormalities can cause significant morbidity and even lead to death in these patients.⁵ Based on that, the goal of

the present work was to evaluate the effect of ERT upon GAG storage in peripheral blood white blood cells (WBCs) of patients with MPS IVA who participated in a phase III double-blind, placebo-controlled randomized clinical trial (Strive, sponsored by BioMarin Pharmaceutical), comparing samples from 3 different patient groups, that is, those who received weekly infusions of enzyme (ERT-W) versus patients who received infusions every other week (ERT-EOW) versus patients enrolled in the placebo (PLA) group.

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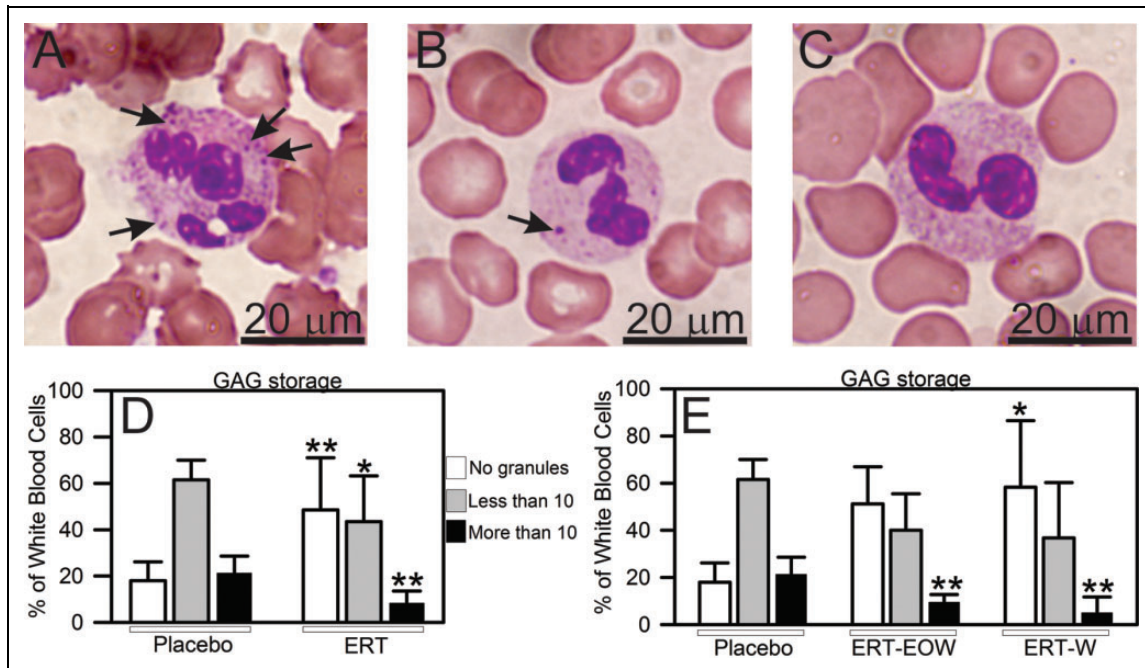


Figure 1. Effects of enzyme treatment upon GAG storage in MPS IVA white blood cells. A representative image of cells after May-Grunwald-Giemsa staining from (A) placebo; (B) MPS IVA cells from a patient treated every 2 weeks (ERT-EOW); and (C) MPS IVA cells from a patient treated every week (ERT-W); arrows indicate GAG granules. D, Results after counting cells without visible GAG storage (no granules), with less than 10 granules per cell (less than 10) and with more than 10 granules per cell (more than 10) in patients treated with placebo ($n = 6$) or ERT ($n = 10$), regardless the ERT regimen. E, Evaluation of GAG granules in patients, stratified by treatment regimen ($n = 4-6$ per group). * $P < .05$ and ** $P < .01$ compared with placebo group. Analysis of variance (ANOVA) and Tukey post hoc. ERT indicates enzyme replacement therapy; GAG, glycosaminoglycan; MPS IVA, mucopolysaccharidosis IVA.

Methods

Patients with confirmed diagnosis of Morquio A were enrolled in the clinical trial and assigned to 1 of the 3 treatment groups randomly. In the first group ($n = 4$), patients were treated weekly (ERT-W) with 2.0 mg/kg of recombinant human N-acetylgalactosamine-6-sulfatase. The second group ($n = 6$) received the same dose every other week (ERT-EOW). In the third group ($n = 6$), patients were infused with PLA. Peripheral blood was collected after 6 months of treatment, and blood smears were stained with May-Grunwald-Giemsa. One hundred WBCs were evaluated under a light microscope (Olympus BH-2 [Tokyo, Japan], magnification 2000 \times) for GAG granules by a blinded evaluator regarding treatment groups, and cells were classified as (1) cells with no visible GAG granules, (2) cells containing between 1 and 10 granules, or (3) cells with more than 10 granules in the cytoplasm. Statistical analysis was performed using SPSS version 18.0 with Tukey test as post hoc. A $P < .05$ was considered statistically significant.

Results

Placebo-treated cells presented several granules on the cytoplasm, which were reduced in the ERT-treated cells (Figure 1A-C). Comparing PLA versus both treated groups together, ERT increased the number of cells without observed GAG storage ($18.0\% \pm 8.1\%$ of cells with no storage observed

in PLA vs $48.5\% \pm 22.5\%$ in ERT treated, $P < .01$). The ERT was also able to reduce the number of cells with more than 10 inclusions in the cytoplasm ($21.3\% \pm 7.3\%$ in PLA vs $8.3\% \pm 5.2\%$ in ERT, $P < .01$), suggesting ERT was effective in reducing GAG storage (Figure 1D) in WBCs.

Another goal of the present study was to determine which of the regimens (if any) was more effective in reducing GAG storage in WBCs. When the treated groups were separated according to regimen, ERT-W patients had the biggest improvement (58.3% of cells with no storage, $P = .012$ vs PLA), while ERT-EOW had intermediate levels of cells with no GAG (51.2% , $P = .058$ vs PLA, not different from ERT-W). Also, cells presenting more than 10 granules reduced in both treated groups ($21.3\% \pm 7.3\%$ in PLA vs $9.5\% \pm 3.3\%$ in ERT-EOW and only $5.0\% \pm 6.7\%$ in ERT-W, $P < .01$ in both cases, without difference between treated groups). Cells presenting between 1 and 10 granules were reduced in both treated groups, but this was not statistically significant (Figure 1E). This last result can be possibly explained for 2 reasons: the small number of patients when we stratify the sample ($n = 4-6$ per group), and the fact that the high number of cells with more than 10 granules found in the PLA group probably shifted to the group of cells with less than 10 granules after treatment.

Conclusion

Our results support that treatment with elosulfase alfa decreases WBC GAG storage in patients with MPS IVA after

6 months. Although both regimens led to improvements, weekly ERT (as approved by the FDA) showed slightly better performance than ERT-EOW. Whether this cellular improvement could be translated to clinical benefits is something still to be evaluated. Also, future studies will focus on long-term correction, efficacy of therapy, and possible development of antibodies against the enzyme.

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Declaration of Conflicting Interests

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