Intracellular GAG Level in Leukocytes is a Promising Pharmacodynamic Biomarker for MPS VI

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1–INTRODUCTION

MPS are caused by the intracellular accumulation of GAGs due to their inefficient degradation leading to cell dysfunction, tissue impairment and subsequent clinical manifestations. One can expect from an effective treatment of MPS that it reduces the intracellular GAG storage. Quantitative methods to measure total GAG levels or component GAG (i.e. CS, DS, HS, KS) levels are described in body fluids such as urine, CSF and serum but not in cells. This study aims to develop a robust quantitative method to measure component GAG levels in leukocytes and to demonstrate that this analysis can provide a reliable treatment pharmacodynamics marker in MPS VI patients' leukocytes and to demonstrate that this analysis can provide a reliable biomarker for MPS VI.

2–RESEARCH OBJECTIVES

- Devise a MS/MS methodology to quantify intracellular levels of three GAG components (Chondroitin Sulfate (CS), Dermatan Sulfate (DS), and Heparan Sulfate (HS)) in leukocytes, and to ensure its robustness (data not presented in this poster) and suitability for use in a clinical trial setting.
- Determine GAG levels in circulating leukocytes (leukoGAGs) in healthy volunteers and patients with mucopolysaccharidosis type VI receiving enzyme replacement therapy (ERT).
- Assess the comparison of leukoGAG levels with arylsulfatase B enzyme (ASB; N-acetylgalactosamine 4-sulfatase) activity in circulating leukocytes

3–METHODOLOGY

- Non-interventional study
- Patients and controls: 6 MPS VI patients treated with ERT (Naglazyme™) and 6 aged-matched healthy controls
- Endpoints:

Total GAG and GAG components content

  - In urine (first morning void)
  - In leukocytes (intracellular) isolated from peripheral blood*

ASB activity

  - In leukocytes isolated from peripheral blood*

** For MPS VI patients blood was collected before ERT infusion and 1 hour after ERT infusion completion*

- Sample analysis method
  - **Total GAG:** colorimetric method (1,9-dimethylmethlyene Blue (DMB) staining)**
  - **GAG components (CS, DS, HS):** quantitative ultra-performance liquid chromatography-tandem mass spectrometric (UPLC-MS/MS) method
  - **ASB activity**: colorometric method using p-Nitrocatechol sulfate substrate

*** Total GAG and GAG components are normalized with creatinine levels in urine and with protein levels in leukocytes*

4–SUBJECT DEMOGRAPHICS AND OTHER CHARACTERISTICS

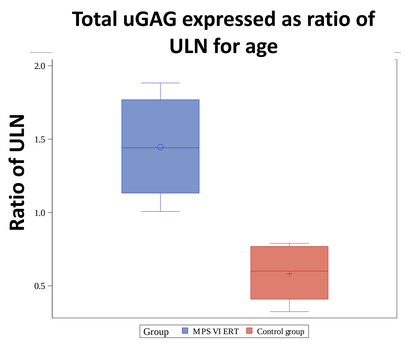
Control not affected subjects group		
Variable		Outcome
Gender		
Male	Number (%)	5 (83.3%)
Female	Number (%)	1 (16.7%)
Age		
Age (years)	Mean (SD)	17.15 (7.42)
	Median	15.87
	Min - Max	8.29 – 26.48
7-15 years	Number (%)	3 (50%)
≥ 16 years	Number (%)	3 (50%)

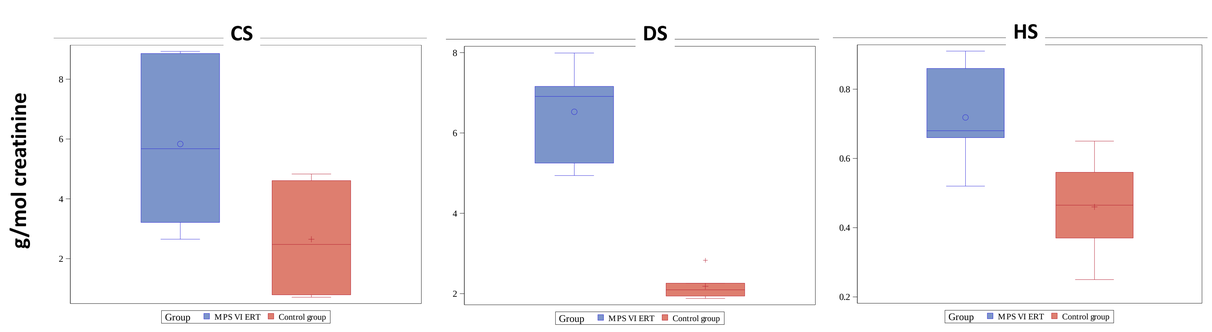
MPS VI patient group		
Variable		Outcome
Gender		
Male	Number (%)	4 (66.7%)
Female	Number (%)	2 (33.3%)
Age		
Age (years)	Mean (SD)	16.99 (6.71)
	Median	16.76
	Min - Max	10.12 – 25.38
7-15 years	Number (%)	3 (50%)
≥ 16 years	Number (%)	3 (50%)
Age at diagnosis (years)	Mean (SD)	3.78 (5.32)
	Median	1.71
	Min - Max	0.50 – 14.34
Treatment		
On ERT at enrolment	Number (%)	6 (100 %)
Age at first infusion (years)	Mean (SD)	6.99 (5.58)
	Median	6.12
	Min – Max	0.81 – 14.53
ERT exposure time (years)	Mean (SD)	**10.08** (3.03)
	Median	9.99
	Min - Max	6.16 – 13.61

- Demographic data of healthy controls and MPS VI patients are comparable
- MPS VI patients included in the study have been exposed to ERT for an average of 10 years [from 6.2 to 13.6 years]

5–URINARY GAG (uGAG) LEVELS

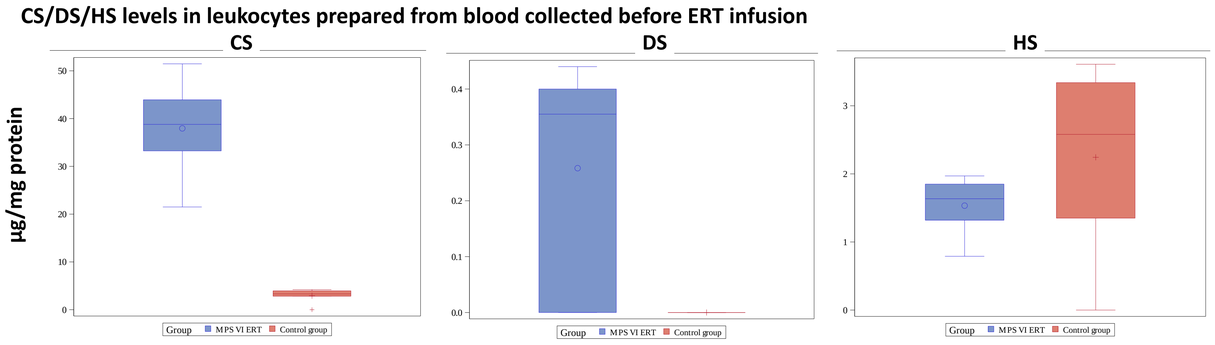
- All healthy controls have total uGAG levels below aged matched upper limit of normal (ULN)
- Despite treatment, all MPS VI have uGAG above aged matched ULN
- MPS VI patients have increased urinary CS and DS levels compared to basal levels (healthy controls)





6–LEUKOCYTES GAG (leukoGAG) LEVELS

CS/DS/HS levels in leukocytes prepared from blood collected before ERT infusion



- Contrarily to uGAG levels, leukoGAG levels (total and components) are not correlated (decrease) with age – not shown
- In healthy volunteers, CS and HS levels are similar (2.9 ± 1.5 and 2.2 ± 1.4 µg/mg protein, respectively), whereas DS levels are below the limit of detection (0.1 µg/mg protein)
- Before ERT weekly infusion, MPS VI patients have increased CS (12 fold) and DS levels in leukocytes compared to basal levels (healthy controls). HS levels are unchanged.

ASB activity and CS/DS/HS levels in leukocytes prepared from blood collected before or 1 hour after ERT infusion

Time of blood collection	ASB activity (nmol/hr/mg)		CS levels (µg/mg protein)		DS levels (µg/mg protein)		HS levels (µg/mg protein)	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Prior infusion	4	12.66 ± 3.66	5	37.14 ± 13.75	5	0.37 ± 0.08	5	1.77 ± 1.04
1h after infusion	4	123.20 ± 25.99	5	34.94 ± 12.44	5	0.26 ± 0.16	5	2.39 ± 1.54

- One hour after ERT infusion, ASB activity in leukocytes is increased by 10-fold
- Despite the increased ASB activity in leukocytes post infusion, no impact on intracellular CS and DS (and HS) levels in leukocytes was observed.

7–CONCLUSION

The MS/MS methodology developed to quantify intracellular levels of three GAG components (CS/DS/HS) in leukocytes is suitable for use in a clinical trial setting.

The results show that all MPS VI patients receiving ERT have elevated total GAGs, DS and CS in urine compared to values in age-matched control subjects. Total uGAGs in MPS VI patients are 1.5 - fold higher than the normal upper limit of normal. In leukocytes from healthy volunteers, both CS and HS are present (DS are below detection limit). CS levels are elevated in all MPS VI patients receiving ERT compared to control subjects. Leukocyte ASB activity in samples collected 1 hour post-ERT infusion is nearly eight-fold higher than in samples collected pre-infusion. However despite this increase, CS content in leucocytes remains more than 12-fold higher than control subjects.

In conclusion, MPS VI patients treated with ERT, maintained a high level of CS in leukocytes compared to age-matched healthy volunteers demonstrating potential room for improvement and suggesting the possibility to further reduce this level with a new treatment such as odiparcil, an oral GAG clearance therapy developed by Inventiva and currently in clinical trials in MPS VI patients (Phase IIa iMProveS trial; Clinical-Trials.gov #NCT03370653).