NEONATAL VERSUS ADULT ENZYME REPLACEMENT THERAPY IN MUCOPOLYSACCHARIDOSIS TYPE I MICE

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1. INTRODUCTION

Mucopolysaccharidosis type I (MPS I) is an autosomal recessive disorder characterized by deficiency of the enzyme alpha-L-iduronidase (IDUA) and storage of Glycosaminoglycans (GAGs), leading to multisystemic features. Since 2003, the FDA approved enzyme replacement therapy (ERT) for MPS I, which improve some parameters in the affected patients. Some anecdotic cases in the literature suggest that beginning the ERT as soon as possible lead to better outcomes, however this was never systematically investigated.

2. AIM

Since we have recently shown that storage of glycosaminoglycans (GAGs) occurs from birth in mucopolysaccharidosis (MPS) patients, this work aimed to test if there were benefits in starting the enzyme replacement (ERT) from birth compared to the adult period in MPS I mice.

2. METHODS

We compared four groups of male mice: in the first group, MPS I mice (knockout for the alpha-l-iduronidase gene) received ERT (Laronidase®, Genzyme) from birth (Neo-ERT, n=8) at 1.2 mg/kg intravenously every two weeks. The second group received the same treatment but started at 60 days of age (Ad-ERT, n=6). Those groups were compared to untreated MPS I (MPS, n=13) and normal mice (Nl, n=10). All animals were sacrificed at 6 months of age.

3. RESULTS

No obvious adverse reactions were observed in Neo-ERT or Ad-ERT. Both groups showed similar efficacy in the aspects studied, as indicated in figures 1 to 4 and table 1.

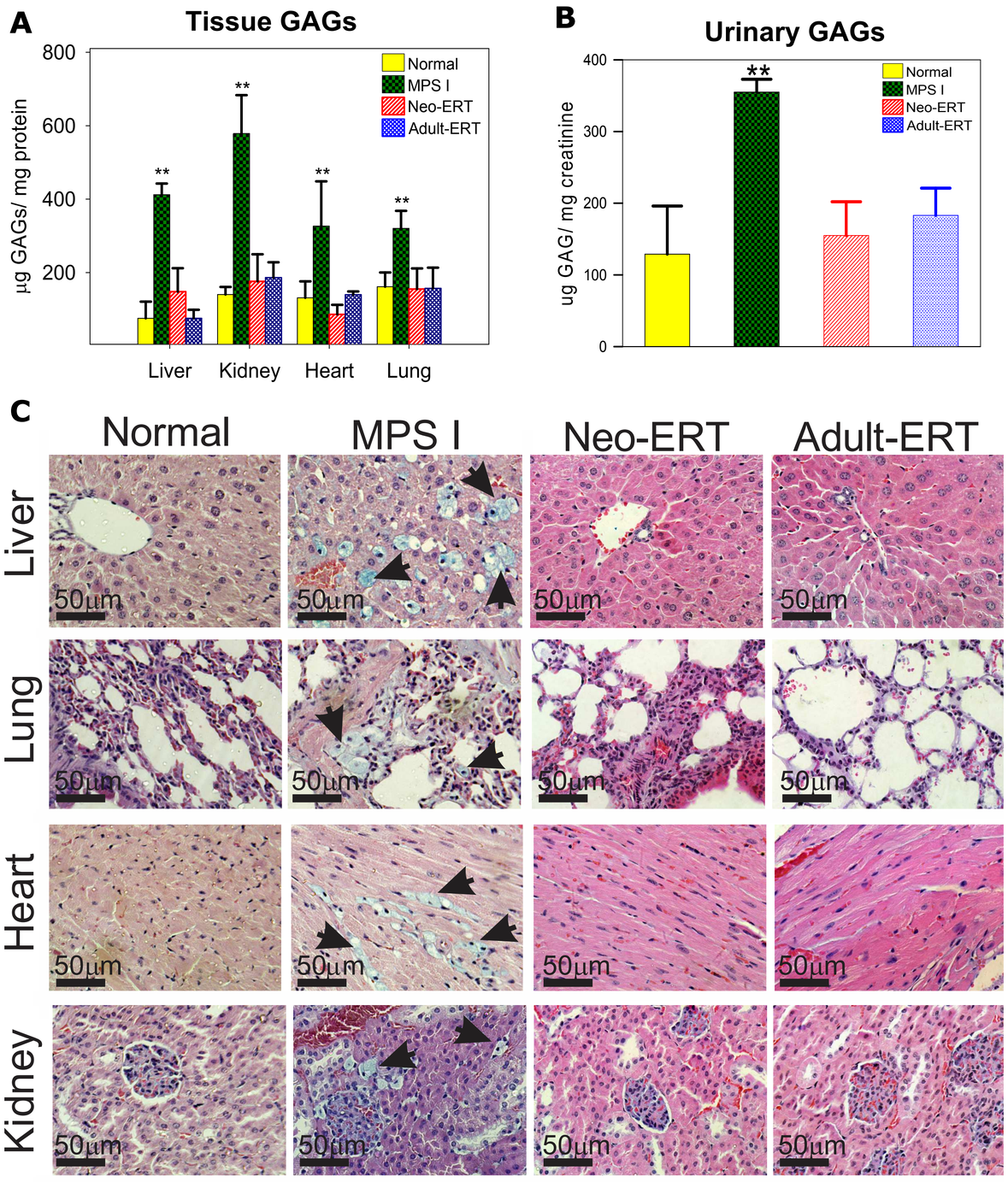


Figure 1: GAG analysis. a) Tissue GAG content analyzed by dymethyl blue test. B) Urinary GAGs at 6 months. C) Histological analysis of liver, lung, heart (myocardium) and kidney at 6 months. Arrows indicate storage in the MPS I group, which was completely cleared in the treated groups.

Table 1: ERT improves heart and valve function. Echocardiography was performed at 6 months. * p< 0.05, compared to normal mice. ANOVA and Tukey.

	Normal	MPS	ERT neo	ERT adult
Shortening Fraction (%)	38.08 ± 6.86	27.59 ± 5.93*	40.49 ± 7.99	36.72 ± 7.96
Ejection Fraction (%)	60.23 ± 8.6	48.53 ± 11.86*	59.11 ± 6.73	57.33 ± 7.6
Fractional area change	51.5 ± 10.4	41.3 ± 10.3*	52.6 ± 6.9	46.5 ± 7.8
AT/ET ratio for Pulmonary valve	0.25 ± 0.06	0.19 ± 0.04*	0.24 ± 0.04	0.25 ± 0.04

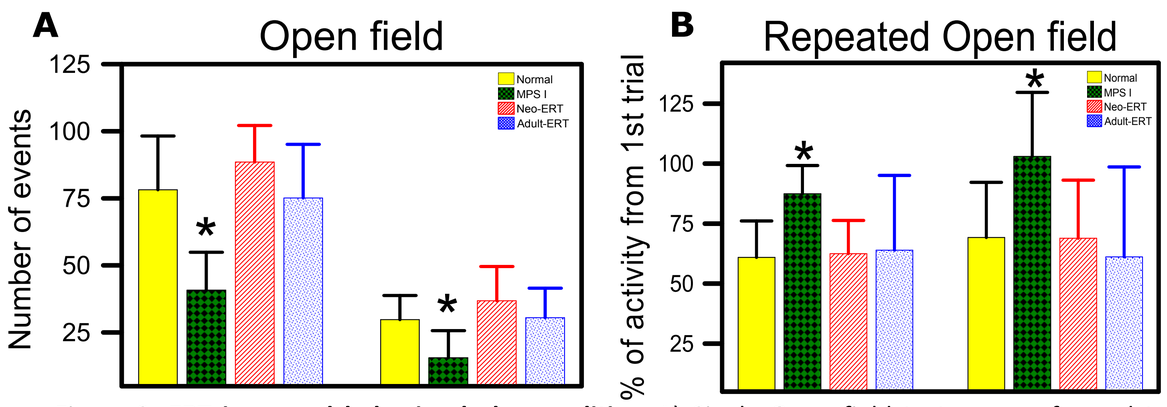
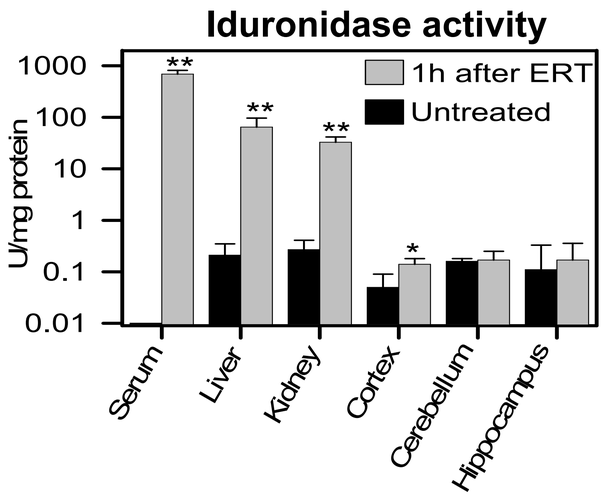


Figure 2: **ERT improved behavioral abnormalities**. A) Single Open field test was performed to evaluate locomotor activity and exploratory behavior for 5 minutes. B) Repeated open field test was performed as a measure of non-adversive memory in 3 consecutive 5-minute trials, with an interval of 30 min each trial. The activity at the 3rd trial was compared to the 1st trial. p< 0.05, compared to normal mice. ANOVA and Tukey.

Figure 3: **IDUA activity**. Four adult MPS I mice received the 1.2mg/kg of laronidase and were sacrificed 1h after injection. Organs were collected and enzyme activity was measured using a fluorescent substrate. *p<0.05; ** p<0.01 compared to untreated, Student t-test.



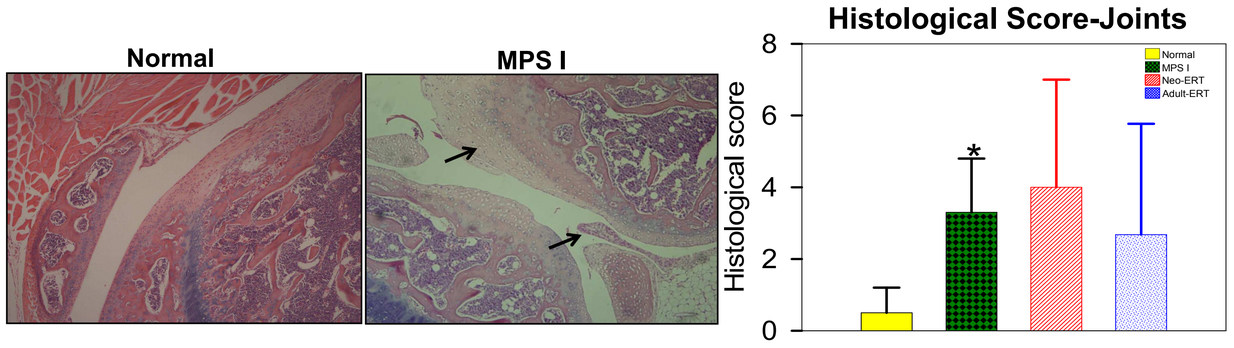


Figure 4: Joint disease is not corrected by ERT. A) Example of H-E staining of knee joints from normal (wild type) and B) MPS I mice (Magnification 10X). Arrows indicate alterations in the joint tissue, including pannus formation and irregular tissue structure. Based on those alteration, a score was created and slides were analyzed by a pathologist blinded to the treatemnt groups. C) Results from the pathology score in the joints, showing that both adult and neonatal ERT fail to correct this aspect of the disease. * p<0.05 compared to Normal, ANOVA and tukey.

CONCLUSION

Neo-ERT and Ad-ERT showed similar benefits in the aspects studied. More important, the ERT dose and regimen studied (chosen based on a trial published by our group - Mol Genet Metab 96:13-9, 2009) seem to cross the BBB in small quantities, which acts in favor of this regimen for patients instead of the currently used (0.6 mg/kg, weekly). Poorly-vascularized organs such as joints still prove hard to be corrected, which indicates the need of ancillary therapies. More experiments will verify if formation of antibodies against the enzyme occurs in Ad-ERT and neo-ERT.

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