408. Safety of Lysosomal Enzymes Over-Expression in HSC for Gene Therapy of Storage Disorders

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In the past years we worked extensively on the development of HSC gene therapy for two fatal demyelinating lysosomal storage disorders, Metachromatic (MLD) and Globoid Cell (GLD) leukodystrophies. We recently demonstrated that transplantation of lentiviral vector-transduced Hematopoietic Stem Cells (HSC) prevented and corrected functional and pathological manifestations of MLD in the mouse model (Biffi A. et al., JCI 2004; Biffi A. et al, submitted for publication). Moreover, we proved the critical role of Arylsulfatase A (ARSA) over-expression to attain therapeutic efficacy. Similarly, promising preliminary results were obtained in the murine model of GLD. ARSA and galactocerebrosidase (GALC), the defective enzymes in MLD and GLD, respectively, catalyze two consecutive steps of sulfatide metabolism, leading to ceramide production. Both enzymes are poorly expressed in most tissues. In the perspective of future clinical applications of the HSC gene therapy, we assessed the safety of ARSA and GALC over-expression. We challenged murine and human HSC with LV encoding ARSA or GALC and tested their long-term repopulating potential and differentiation. Both human and murine HSC after gene transfer over-expressed ARSA up to 10-15 fold above normal levels and retained their capability to proliferate and differentiate in vitro, as assessed by CFC and LT-CIC assays. Murine cells efficiently repopulated transplanted hosts long-term. Interestingly, when transplanted in xenograft models (NOD-SCID and γchain-/-RAG-/- mice), human CD34+ HSC over-expressing ARSA repopulated long-term chimeric mice, both primary and secondary recipients, in which transduced cells were detected up to 20 weeks after the transplant, and showed a normal differentiation in B, T cells and monocytes. On the contrary, when GALC over-expressing murine HSC were transplanted into both wild type and homozygous defective irradiated mice, they failed to rescue transplanted animals from lethal conditioning. Transduced cells demonstrated a significantly reduced capability to proliferate and differentiate in vitro, as assessed by CFC assay, and underwent apoptosis, as assessed by TUNEL and activated caspase III stainings. Interestingly, these findings were completely reverted upon treatment of transduced cells with anti-apoptotic molecules, such as IGF1. Preliminary quantification of ceramide in both ARSA and GALC transduced HSC demonstrated an increase only upon GALC over-expression, indicating the critical role of this enzyme in controlling ceramide production and intracellular homeostasis. Overall, these data underline the safety of ARSA over-expression for future clinical testing and the requirement of regulated GALC expression for efficacious and safe HSC gene therapy, thus indicating a dramatic difference in the safety profile of these lysosomal enzymes.

409. Complete Correction of Enzymatic Deficiency and Lysosomal Storage throughout the Brain in a Mouse Model of GM1-gangliosidosis after Neonatal Intracerebroventricular Injection of an AAV1 Vector Encoding Lysosomal Acid Beta-galactosidase

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GM1 gangliosidosis is a glycosphingolipid (GSL) lysosomal storage disease caused by autosomal recessive deficiency of lysosomal acid β-galactosidase (βgal). This disease is characterized by accumulation of GM1-ganglioside and its asialo-form (GA1) in the CNS. In this study we examined the effect of neonatal intracerebroventricular injection of an adeno-associated virus (AAV) vector (serotype 1) encoding mouse βgal under control of a CBA promoter (AAV1-CBA-βgal), on bgal enzyme activity and brain GSL content in adult GM1 gangliosidosis (βgal-/-) mice. A total of 4 µl of AAV1-CBA-βgal vector with a titer of $4.3x10^{13}$ gc/mL was injected into the lateral ventricles (2 µl each) of P0 βgal-/- mice. At 3 months we analyzed one brain hemisphere for enzyme distribution by X-gal staining at pH 5.0. βgal was expressed throughout the brain at higher levels than those found in wild type controls or untreated βgal-/- mice. The highest levels of enzymatic activity were associated with allocortical areas such as the hippocampal formation and olfactory bulb, and periallocortical areas. Five regions in the other hemisphere were analyzed for enzymatic activity and GSL content. AAV1-treated βgal-/- mice presented enzymatic activities between 7- and 65-fold higher (depending on the region) than in wild type controls. Total brain ganglioside and GM1 content (µg of sialic acid/100 mg dry weight) was reduced from 824 ± 65 µg and 476 ± 42 µg in non-treated βgal -/- mice to 444 ± 21 µg and 46.9 ± 2.1 µg in AAV1-treated βgal -/- mice, respectively. These GSL values in treated mice were statistically identical to those measured in wild type controls (412 ± 17 µg and 53.2 ± 1.1 µg, respectively). Furthermore, GA1 storage was completely eliminated. Thus GSL levels in the brains of AAV-treated βgal-/- mice were restored to normal levels. Analysis of GM1-ganglioside storage in the brain by staining with subunit B of cholera toxin (CTX-B) showed that AAV-treated βgal-/- mice were identical to wild type controls. Interestingly, fiber tracts in untreated βgal-/- mice stained weakly with CTX-B or not at all. This may be the mouse equivalent of delayed myelination reported in type I GM1-gangliosidosis in humans and dogs. Filipin staining of brains for unesterified cholesterol, which accumulates in the brain in glycosphingolipidoses, showed that AAV1-treated βgal-/- mice were indistinguishable from wild type mice, while untreated βgal-/- mice displayed strong storage throughout the brain. Altogether the biochemical and histological evidence in this study show that brain metabolism in AAV-treated βgal-/- mice was normal. Thus we conclude that neonatal AAV-mediated gene delivery of lysosomal acid β-galactosidase to the brain is an effective approach for treatment of GM1 gangliosidosis.