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Journal of the Neurological Sciences 255 (2007) 7 – 16

Journal of the Neurological Sciences

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Metabolic correction in oligodendrocytes derived from metachromatic leukodystrophy mouse model by using encapsulated recombinant myoblasts

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Received 24 July 2006; received in revised form 19 December 2006; accepted 9 January 2007

Abstract

In an effort to develop an encapsulated cell-based system to deliver arylsulfatase A (ARSA) to the central nervous system of metachromatic leukodystrophy (MLD) patients, we engineered C_2C_{12} mouse myoblasts with a retroviral vector containing a full-length human ARSA cDNA and evaluated the efficacy of the recombinant secreted enzyme to revert the MLD phenotype in oligodendrocytes (OL) of the $As2^{-/-}$ mouse model. After transduction, C_2C_{12} cells showed a fifteen-fold increase in intracellular ARSA activity and five-fold increase in ARSA secretion. The secreted hARSA collected from transduced cells encapsulated in polyether-sulfone polymer, was taken up by enzyme-deficient OL derived from MLD mice and normally sorted to the lysosomal compartment, where transferred enzyme reached 80% of physiological levels, restoring the metabolism of sulfatide. To evaluate whether secreted enzyme could restore metabolic function in the brain, encapsulated cells and secreted ARSA were shown to be stable in CSF *in vitro*. Further, to test cell viability and enzyme release *in vivo*, encapsulated cells were implanted subcutaneously on the dorsal flank of DBA/2J mice. One month later, all retrieved implants released hARSA at rates similar to unencapsulated cells and contained well preserved myoblasts, demonstrating that encapsulation maintains differentiation of C_2C_{12} cells, stable transgene expression and long-term cell viability *in vivo*. Thus, these results show the promising potential of developing an ARSA delivery system to the CNS based on the use of a polymer-encapsulated transduced xenogenic cell line for gene therapy of MLD.

Keywords: Gene therapy; Arylsulfatase A; MLD mouse model; Oligodendrocytes; Cell encapsulation

1. Introduction

Metachromatic leukodystrophy (MLD) is an inherited autosomal recessive disorder due to deficient activity of the lysosomal enzyme arylsulfatase A (ARSA; EC 3.1.6.8). ARSA catalyzes the degradation of galactosyl-3-sulfate ceramide (sulfatide), a major component of myelin sheaths. In ARSA deficiency, sulfatide accumulates within lysosomes

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doi:10.1016/j.jns.2007.01.010

of oligodendrocytes and Schwann cells, the myelin-forming glia, and is thought to kill them, leading to myelin degeneration in the nervous system [1,2]. Different forms of MLD are distinguished by age of onset and severity. The majority of cases are present within the first two years of life and rapidly progress over the next two years to include ataxia, seizures, quadriplegia, and severe mental retardation culminating in death in early childhood [1,2]. Currently, no specific treatment to halt the progression of MLD or reverse its fatal outcome exists. Enzyme replacement therapy (ERT) by direct intravenous infusion of normal or modified enzyme has not provided adequate benefit in patients affected by LSD with central nervous system (CNS) involvement [3]. Allogeneic bone marrow transplantation (BMT) can reverse or stop demyelination when performed in MLD patients with high residual activity or in presymptomatic stage of late-infantile form, whereas the infantile form progresses steadily despite normalization of circulating enzyme levels, suggesting a requirement for early treatment of the CNS. [4,5].

A mouse model of MLD, generated by targeted disruption of the mouse ARSA gene, As2 exists [6] and it has been used to investigate the pathogenesis and treatment of this disease. The fibroblasts isolated from $As2^{-/-}$ mice are completely deficient in ARSA mRNA and no turnover of sulfatide is detected. Although the animals do not show the severity of symptoms of the human disease, $As2^{-/-}$ mice cannot metabolize sulfatide and starting at the age of 9–10 months develop typical inclusions throughout the white matter of the CNS, comparable to those of MLD patients. As a consequence, neuronal alterations develop with associated behavioural deficits [6]. Protection from the development of tissue damage as been shown in MLD mice after ARSA-lentiviral vector transduced hematopoietic stem cells (HSC) transplantation [7]. A major drawback of this approach is represented by the long interval from the transplantation to the development and peripheralization of a significant proportion of transplanted cells. Matzner et al. [8] recently reported that MLD mice receiving weekly intravenous injection of recombinant ARSA show improvements in the histopathology of peripheral tissues and CNS. Although protein therapy may represent a powerful approach to replace the missing enzyme in affected tissues of MLD patients, several shortcomings of this type of strategy have been noted for other LSDs such as Gaucher syndrome and mucopolysaccharidosis type I [9–12]: a) not all patients are suitable for protein therapy; b) some organs and tissues are corrected more readily than others; c) there are problems with gauging efficacy in these highly variable disorders; and d) the therapies are expensive, limiting access to patients from countries able to afford expensive health care. Therefore, alternative strategies for the treatment of these devastating diseases are required.

It is widely accepted that, for many inborn errors of metabolism, an early treatment is critical to the prevention of long-term developmental defects. Intracerebroventricular (ICV) administration may allow rapid onset and long-term expression of the missing proteins within the nervous system, overcoming the barriers that limit their diffusion from the circulation. Animal experiments have shown that direct parenchymal enzyme administration by using viral vectors results in long-term sustained expression of the missing protein and therapeutic benefit in several diseases, including the MLD [13–15]. Direct localized CNS delivery is achievable by mechanical means, or by gene therapy or cell grafting. An alternative approach to direct CNS delivery is represented by implantation of encapsulated cells into the intrathecal space. This strategy has been used to deliver dopamine and neurotrophic factors for the treatment of Parkinson disease [16,17], β-endorfin for pain [18], and ciliar neurotrophic factor for Huntington disease and ALS directly into the CNS [19,20]. The main advantages of this strategy include the fact that cells are encapsulated in a protective immunoisolation barrier, thus overcoming the host immune response against transplanted cells, and that the gene product is widely distributed along the neuroaxis by the CSF. Encapsulated cells can survive for several months and release the recombinant product constitutively [21]. Beside chronic delivery, the encapsulation-mediated ARSA delivery strategy also enables the treatment to be arrested in the event that side effects should appear, as capsules are retrievable by a simple surgical procedure.

Recently, a similar technique using alginate microcapsules has been successfully applied to pre-clinical treatment of MPSVII ad MPS II mice [21,22].

In this work, we have chosen the strategy that use genetically engineered C_2C_{12}, encapsulated in hollow fibers, to test their ability to transfer ARSA expression in oligodendrocytes derived from MLD mice and their survival for long-term secretion after *in vivo* implantation. Here we show that hARSA-transduced C_2C_{12} cells over-express and secrete high levels of ARSA. After encapsulation in hollow fibers, the cells are able to produce recombinant enzyme, which can subsequently be taken up by cultured mouse $As2^{-/-}$ OL restoring the metabolism of sulfatide. The encapsulated transduced cells also survive for at least 4 weeks when the capsules are implanted into allogenic recipient mice.

2. Materials and methods

2.1. Vector construction and viral production

A human wild-type ARSA cDNA [23] was kindly provided by V. Gieselmann (University of Bonn). A recombinant retrovirus was prepared by cloning the EcoRI fragment (nucleotides −62 to +1611 relative to ATG initiation codon) into the EcoRI site of the pLXTN retroviral expression vector [24,25]. Plasmid LARSATN was transfected into ecotropic packaging cell line E-86 by calcium phosphate co-precipitation. After 48 h, culture medium was harvested and filtered through a 0.2 μm filter (Nalgene, Rochester, NY) and used to transduce Am12 amphotropic packaging cell line. Twenty-four hours after transduction, Am12 cells were