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ScienceDirect
Molecular Genetics and Metabolism 93 (2008) 411–418
Molecular Genetics and Metabolism
www.elsevier.com/locate/ymgme

Lentiviral-mediated gene therapy for murine mucopolysaccharidosis type IIIA

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Received 14 November 2007; accepted 14 November 2007
Available online 8 January 2008

Abstract

Mucopolysaccharidosis type IIIA (MPS IIIA) is a heritable glycosaminoglycan (GAG) storage disorder which is characterised by lysosomal accumulation of heparan sulphate, secondary to a deficiency of sulphamidase (heparan-*N*-sulphatase, *N*-sulphoglucosamine sulphohydrolase, EC No. 3.10.1.1.). There is currently no treatment for affected individuals who experience progressive CNS deterioration prior to an early death.

As a first step towards developing gene therapy as a treatment for MPS IIIA, an MPS IIIA mouse model was used to examine the efficacy of intravenous lentiviral-mediated gene therapy. Five-week-old mice were injected with virus expressing murine sulphamidase and analysed 6 months after treatment. Transduction by the lentiviral vector was highest in the liver and spleen of treated animals, and sulphamidase activity in these tissues averaged 68% and 186% of normal, respectively.

Storage was assessed using histochemical, chemical and mass spectrometric analyses. Storage in most somatic tissues was largely normalised, although chondrocytes were an obvious exception. Histologically, improvement of lysosomal storage within the brain was variable. However, β-hexosaminidase activity, which is abnormally elevated in MPS IIIA, was significantly reduced in every treated tissue, including the brain. Total uronic acid was also significantly reduced in the brains of treated mice. The level of a disaccharide marker (hexosamine-*N*-sulphate[α-1,4]hexuronic acid; HNS-UA) of heparan sulphate storage was also decreased in the brains of treated mice, albeit non-significantly.

These results suggest that lentiviral-mediated somatic gene transfer may affect not only the somatic, but possibly also the CNS pathology, found in MPS IIIA.

Keywords: HIV-1; Vector; MPS IIIA; Mice; Correction

MPS III, otherwise known as Sanfilippo syndrome, is the most common of the MPS disorders [1], and has an incidence in Australia of approximately 1 in 55,000 live births [2]. MPS III results from the storage of heparan sulphate and has four recognised subgroups, A, B, C and D, each of which are distinguished by a specific enzyme deficiency. MPS IIIA is the most prevalent subtype in Australia [2] and results from a deficiency of the lysosomal enzyme sulphamidase (heparan-*N*-sulphatase, *N*-sulphoglucosamine sulphohydrolase, EC No. 3.10.1.1.).

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doi:10.1016/j.ymgme.2007.11.008

Initially, MPS IIIA children appear normal. Disease symptoms present at around 2–6 years of age, and include hyperactivity, aggressive behaviour, delayed development, coarse hair, hirsutism, sleep disorders and hepatosplenomegaly [1]. By 6–10 years of age, social and adaptive skills rapidly decline as a result of severe neurological degeneration, resulting in a reduced quality of life and a premature death. There are currently no therapies available to slow or stop the progression of MPS IIIA and consequently most patients die within their second decade of life.

Current therapies in use to treat other MPS disorders provide exogenous enzyme to enzyme deficient cells, either directly by enzyme replacement therapy (ERT) [4], or indirectly *via* donor cells (bone marrow transplant; BMT) [1]. Both approaches rely primarily on the receptor mediated endocytosis of exogenous enzyme, supplied either by infusion (ERT) or by secretion from haematopoietic cells (BMT), *via* the mannose-6 phosphate targeting signal [3] that is also responsible for targeting the enzyme to the lysosome. Non-receptor mediated internalisation of lysosomal enzymes may also occur, albeit at a much lower level [3].

However, ERT with the dose of replacement enzyme used in conventional clinical trials is too low to have therapeutic effects in the central nervous system (CNS), and hence is not useful for MPS IIIA. In addition, while trials of BMT have shown variable success in other MPS (predominantly MPS I) [1], for MPS III patients, no preservation of intellectual function following BMT has been seen [5].

Due to the limitations of ERT and BMT, alternative therapies for MPS IIIA are urgently required. Gene therapy provides a means of supplying patients with a potentially permanent source of corrective levels of enzyme [6]. Gene therapy is also potentially cheaper and safer than ERT and BMT, respectively. Three broad approaches to gene therapy for MPS CNS disease have been developed, systemic delivery of vector, transduction of haematopoietic stem cells, and targeted gene delivery to the CNS [6].

Systemic gene delivery may influence CNS pathology by maintaining a level of circulating enzyme that is sufficient to deliver significant enzyme through the blood–brain barrier, which shows measurable, albeit low, M6P-mediated transport of lysosomal enzymes [7,8]. Haematopoietic stem cell mediated gene therapy also delivers enzyme to the brain, in this instance *via* transmigration of gene corrected cells into the CNS [6]. In a study performed in MPS IIIB mice [9] donor bone marrow cells were retrovirally modified to over-express therapeutic enzyme. Subsequent transplantation of these cells into affected mice resulted in a widespread reduction of brain pathology. A CNS targeted AAV-mediated gene therapy approach for MPS IIIA has also recently been reported [10].

Lentiviral vectors appear well suited to gene therapy for inherited disorders as they can transduce non-cycling cells, provide a stable genetic modification of the target cell, evoke little or no immune response, are easily pseudotyped to allow for broad cellular tropism and can be produced in relatively high titres [11]. We have previously shown that intravenous administration of a lentiviral vector expressing murine sulphamidase to MPS IIIA mice results in a rapid normalisation of urinary GAG, and over time, normalises the abnormal weight gain seen in these animals [12]. This manuscript presents data that was collected from the same animals after sacrifice.

Materials and methods

Animals and gene delivery

The MPS IIIA mouse model is a naturally occurring model that results from a missense mutation in the sulphamidase gene [13]. The treated mice used in this study have been described elsewhere [12]. Briefly, six 5-week-old male MPS IIIA mice were treated with 50 μg p24 equivalent of a lentiviral vector, which expresses murine sulphamidase under the transcriptional control of the murine phosphoglycerate kinase (PGK) gene promoter, *via* injection into the tail vein. Three mice were pre-treated by intravenous injection of hyperosmotic mannitol (200 μl of 25% (w/v) mannitol in saline) 5 min prior to the administration of the vector in an attempt to achieve vector delivery to the CNS. Control mice (a total of four untreated MPS IIIA and four unaffected animals) were from the same colony. All animals were killed by CO_2 asphyxiation prior to the removal of liver, spleen, heart, kidney, knee joint and brain. All studies using animals had the approval of the Children, Youth and Women's Health Service animal Ethics Committee.

Real-time PCR analysis

Real-time PCR for determination of vector copy number was performed as previously described [12].

Sulphamidase and β-hexosaminidase activity

Tissues were homogenised in 0.1% Triton X-100 using a manual Teflon to glass tissue homogeniser. One microliter volumes of homogenate were assayed for sulphamidase activity as previously described [14]. Radiolabelled products and residual substrates were separated using a dedicated high pressure liquid chromatography (HPLC) system and counted by liquid scintillation (Hewlett Packard series 1100). Results were collected using Ginastar HPLC software and converted from net % substrate conversion to rate of conversion ($\text{pmol min}^{-1}\,\text{mL}^{-1}$) using the formula; (Net % conversion/100) × (pmol substrate in incubation/time of incubation (min)) × (1/mg protein per incubation). The limit of detection of the sulphamidase assay was defined as corresponding to turnover of substrate 0.5% above the background value (5.6 pmol/min/ml). β-Hexosaminidase activity was determined as previously described [15]. Enzyme activities were normalised to total protein as determined using the BioRad protein assay according to the manufacturer's instructions.

Uronic acid assay

Total uronic acid in tissue homogenates was determined using the hydroxydiphenyl method [16] and normalised to total protein.

Preparation of samples for electrospray ionization tandem mass spectrometry (ESI-MS/MS)

Tissues homogenised in 0.1% (v/v) Triton X-100 were clarified by centrifugation. Oligosaccharides in the supernatant were derivatized with 1-phenyl-3-methyl-5-pyrazolone and analysed by ESI-MS/MS on a PE Sciex API 3000 triple-quadrupole mass spectrometer as previously described [17]. Data were collected using Analyst 1.1 software and the relative amounts of hexosamine-*N*-sulphate[α-1,4]hexuronic acid (HNS-UA) were determined by relating the peak height to that of the internal standard;