GENE TRANSFER IN MURINE MPS IIIA USING CANINE ADENOVIRAL VECTORS

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LIST OF ABBREVIATIONS

AAV	Adeno-Associated Virus
Ad	Human Adenovirus
BBB	Blood-Brain Barrier
BME	Basal Medium Eagle
bp	Base Pair
CAR	Coxsackie Adenovirus Receptor
CAV-2	Canine Adenovirus Serotype 2
CAV-GFP	Canine Adenoviral Vector Expressing Green Fluorescent Protein
CAV-NS	Canine Adenoviral Vector Expressing Recombinant Human Sulphamidase and Green Fluorescent Protein
CHO	Chinese Hamster Ovary
CMV	Cytomegalovirus
CNS	Central Nervous System
CTL	Cytotoxic T Lymphocyte
Cy3	Indocarbocyanine
CYWHS	Children, Youth and Women's Health Service, Adelaide, Australia
DAPI	4',6-Diamidino-2-Phenylindole
DELFIA	Dissociation-Enhanced Lanthanide Fluorescent ImmunoAssay
DK	Madin-Darby Canine Kidney
DMSO	Dimethylsulphoxide
DMEM	Dulbecco's Modified Eagle's Media
DNA	Deoxyribonucleic Acid
dNTP	DeoxyNucleotide Triphosphates
ΔE1	E1-Deleted
EGFP	Enhanced Green Fluorescent Protein
ELISA	Enzyme Linked ImmunoSorbent Assay
EM	Electron Microscopy
ERT	Enzyme Replacement Therapy
FACS	Fluorescence Activated Cell Sorting
FCS	Foetal Calf Serum
FITC	Fluorescein Isothiocyanate
FISH	Fluorescent In Situ Hybridisation
g	Gravitational force
g	Grams
G	Gauge
GAG	Glycosaminoglycan(s)
GFAP	Glial Fibrillary Acidic Protein
GFP	Green Fluorescent Protein
HD	Helper-Dependent
hr	Hours
HNS-UA	Hexosamine-*N*-Sulphate [α-1,4] Hexuronic Acid
IFN	Interferon
IGMM	Institut de Génétique Moléculaire de Montpellier, France

IL	Interleukin
IRES	Internal Ribosomal Entry Site
ITR	Inverted Terminal Repeat
kb	Kilobase Pair
kDa	Kilodalton
LB	Luria Bertani
LDRU	Lysosomal Diseases Research Unit, Adelaide, Australia
LSD	Lysosomal Storage Disorder(s)
M	Molar Concentration
M6P	Mannose-6-Phosphate
MCS	Multiple Cloning Site
MHC	Major Histocompatibility Complex
min	Minutes
mL	Millilitre
MPS	Mucopolysaccharidosis
MPS IIIA	Mucopolysaccharidosis Type IIIA
mRNA	Messenger RNA
MWCO	Molecular Weight Cut-Off
NDS	Normal Donkey Serum
NS	Sulphamidase
OCT	Optimal Cutting Temperature
p	Statistical p-Value
pI	polypeptide I, etc.
PBS	Phosphate Buffered Saline
PC	Physical Containment Level
pCAV-NS	Plasmid DNA Encoding the CAV-NS Viral Vector
PCR	Polymerase Chain Reaction
PFA	Paraformaldehyde
rhNS	Recombinant Human Sulphamidase
RSV	Rous Sarcoma Virus
S	Sulphate
SDS	Sodium Dodecyl Sulphate
sec	Seconds
SUMF1	Sulphatase Modifying Factor-1
SV40	Simian Virus 40
T_M	Memory T Lymphocyte
TNF	Tumour Necrosis Factor
U	Units
μL	Microlitres
VA	Viral-associated
v/v	Volume per volume
wk	Weeks
w/v	Weight per volume

THESIS ABSTRACT

Mucopolysaccharidosis type IIIA (MPS IIIA) is an autosomal-recessively inherited disorder caused by the deficiency of lysosomal sulphamidase (NS) enzyme activity, resulting in the accumulation of the glycosaminoglycan (GAG) heparan sulphate (HS). MPS IIIA patients experience progressive and severe neurological deterioration with death usually occurring in the mid-late teenage years. A naturally-occurring mouse model of MPS IIIA has been characterised and the biochemical, histological and behavioural changes closely parallel the human condition. In order to treat the neurological effects of MPS IIIA, it is anticipated that a continual supply of replacement enzyme to affected cells will be required. Consequently, this study aimed to evaluate the efficacy, longevity and safety of gene therapy as a potential treatment for MPS IIIA.

Canine adenoviral vectors (CAV-2) were selected on the basis of several important properties. They are non-integrating, are predicted to be less immunogenic in humans than human-derived viral vectors and mediate transgene expression for at least 1 year *in vivo*. An E1-deleted (ΔE1) CAV-2 vector, CAV-NS, co-expressing recombinant human NS (rhNS) and Green Fluorescent Protein (GFP) was constructed and purified. *In vitro* testing revealed rhNS produced by CAV-NS significantly decreased sulphated GAG storage in human MPS IIIA fibroblasts in a mannose-6-phosphate-dependent manner.

Preliminary studies in young adult guinea pigs with CAV-GFP demonstrated widespread GFP expression in the absence of a humoral response. In contrast, minimal GFP expression was found in CAV-injected adult mice due to formation of neutralising antibodies against the CAV-2 capsid. Consequently, intraventricular delivery of CAV-NS was evaluated in newborn mice at various doses. Widespread and dose-dependent GFP expression was observed and the optimal dose for large-scale studies was determined to be 10^9 CAV-NS particles/hemisphere. Antibodies against CAV-2, rhNS or GFP were not detected.

Concurrently, the cognitive function and anxiety-related behaviours of unaffected and MPS IIIA mice were evaluated. MPS IIIA mice had significantly impaired memory and spatial learning in the Morris Water Maze (16-wks) and reduced anxiety in the Elevated Plus Maze (18-wks) when compared to unaffected animals.

In a large therapeutic assessment trial, newborn MPS IIIA or unaffected mice received 10^9 particles of CAV-NS, saline or remained uninjected. GFP expression was visualised for at

least 20-wks post-injection. Reductions in the vacuolation of ependymal and choroidal cells of the lateral ventricle and the cerebral cortex of treated MPS IIIA animals were observed in some GFP-positive (and presumably rhNS-expressing) regions. Furthermore, improvements in reactive astrogliosis, but not in the number of activated microglia, were measured in CAV-NS-treated MPS IIIA mice. However, insufficient CAV-NS-mediated rhNS expression was generated to improve functional changes as assessed by a behavioural test battery (motor function, open field activity, Elevated Plus Maze, Morris Water Maze), potentially due to chronic inflammatory responses against the CAV-2 vector.

Collectively, these data suggest that early intervention with ΔE1 CAV-NS gene therapy was able to improve several components of neuropathology in MPS IIIA animals but was unable to significantly alter the clinical progression of murine MPS IIIA.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available in all forms of media, now or hereafter known.

SIGNED ..... DATE .....

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