AAV-vector Mediated Gene Delivery for Huntington's Disease: *An Investigative Therapeutic Study*

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Abstract

Progressive degeneration in the central nervous system (CNS) of Huntington's disease (HD) patients is a relentless debilitating process, resulting from the inheritance of a single gene mutation. With limited knowledge of the underlying pathological molecular mechanisms, pharmaceutical intervention has to-date not provided any effective clinical treatment strategies to attenuate or compensate the neuronal cell death. Attention has therefore turned to biotherapeutic molecules and novel treatment approaches to promote restoration and protection of selectively vulnerable populations of neurons in the HD brain.

Rapid advances in vectorology and gene-based medicine over the past decade have opened the way for safe and efficient delivery of biotherapeutics to the CNS. With numerous factors known to regulate the development, plasticity and maintenance of the mammalian nervous system many proteins have emerged as potential therapeutic agents to alleviate HD progression. This investigative study utilised gene delivery vectors derived from the non-pathogenic adeno-associated virus (AAV) to direct high-level expression of brain-derived neurotrophic factor (BDNF), glial cell-line derived neurotrophic factor (GDNF), Bcl-x_L or X-linked inhibitor of apoptosis protein (XIAP) within the rodent striatum. Maintenance of the basal ganglia and functional behaviour deficits were assessed following excitotoxic insult of the striatum by quinolinic acid (QA), a neurotoxic model of HD pathology.

Enhanced striatal expression of BDNF prior to QA-induced lesioning provided maintenance of the striosome-matrix organisation of the striatum, attenuating impairments of sensorimotor behaviour with a 36-38% increase in the maintenance of DARPP-32 / krox-24 expressing striatal neurons, reduced striatal atrophy and increased maintenance of striatonigral projections. Higher levels of BDNF however induced seizures and weight-loss highlighting the need to provide regulatable control over biotherapeutic protein expression. Continuous high-expression of BDNF or GDNF resulted in a downregulation of intracellular signal mediating proteins including DARPP-32, with AAV-GDNF not found to enhance the overall maintenance of striatal neurons. Neither of the anti-apoptotic factors provided significant protection of transduced striatal neurons but tended towards ameliorating QA-induced behavioural deficits, displaying behaviour – pathology correlations with the survival of parvalbumin-expressing neurons in the globus pallidus. The results of this thesis suggest BDNF as a promising putative biotherapeutic for HD, but emphasises the requirement to control expression following gene delivery, and for further elucidation of the physiological impact that enhanced expression of endogenous factors has on the host cells. Additionally the maintenance of neural networks beyond the caudate-putamen will be vital to ensuring efficient clinical outcomes for HD.

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Journal Publications

Research Articles

Kells AP, Henry RA, Connor B. (2007) **AAV-BDNF Mediated Attenuation of QA-Induced Neuropathology and Motor Function Impairment.** *Submitted to Gene Therapy.*

Kells AP, Henry RA, Connor B. (2007) **AAV-Mediated Expression of Bcl-x_L or XIAP Fails to Increase Neuronal Resistance against QA-induced Striatal Lesioning.** *Submitted to Experimental Neurology.*

Kells AP, Henry RA, Hughes SM, Connor B. (2006) **Verification of Functional AAV-mediated Neurotrophic and Anti-apoptotic Factor Expression.** *Journal of Neuroscience Methods.* 161(2): 291-300

Abstracts

Kells AP, Henry RA, Hughes SM, Faull, RLM, Connor B. (2007) **Attenuation of Functional Deficits in the QA Model of Huntington's Disease following AAV Vector Delivery.** 10th *Annual Meeting of the American Society of Gene Therapy, Seattle, WA, USA.* Molecular Therapy 15: S209

Kells AP, Henry RA, Hughes SM, Faull, RLM, Connor B. (2006) **Protection against Huntington's Disease Progression: AAV-mediated Delivery of Biotherapeutics.** *9th Annual Meeting of the American Society of Gene Therapy, Baltimore, MD, USA.* Molecular Therapy 13: S96

Kells AP, Henry RA, Hughes SM, Faull, RLM, Connor B. (2005) **Investigating the Protective Effect of GDNF and Bcl-x_L Gene Delivery in a Rat Model of Huntington's Disease.** *9th International Conference on Neural Transplantation and Repair, Taipei, Taiwan.*

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Abbreviations

3-NP	3-nitropropionic acid
AAV	Adeno-associated virus
ABTS	2,2-Azino-di-3-ethylbenzthiazoline sulfonate
Ad	Adenovirus
Amp	Ampicillin
ANOVA	Analysis of variance
A-P, M-L, D-V	Anterior-Posterior, Medial-Lateral, Dorsal-Ventral
BCA	Bicinchinonic acid
Bcl-x_L	Bcl-2-like protein long
BDNF	Brain derived neurotrophic factor
BSA	Bovine serum albumin
CAG	Cytosine-adenine-guanine
CBA	Chicken-β-actin
cDNA	Complementary DNA
ChAT	Choline acetyltransferase
CIP	Calf intestinal phosphatase
CMV	Cytomegalovirus
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
Ct	Cycle time
DAB	3-3 diaminobenzidine tetrahydrochloride
DARPP-32	Dopamine- and adenosine 3', 5'-monophosphate-regulated phosphoprotein of 32 kDa
DMEM	Dulbecco's Modified Eagle's Medium
DNA	Deoxyribonucleic acid
E14/15	Embryonic day 14/15
EDTA	Ethylenediaminetetraacetic acid
E. coli	***Escherichia coli***
FBS	Fetal bovine serum
GABA	Gamma-amino butyric acid
GDNF	Glial cell-line derived neurotrophic factor
GFRα1	GDNF family receptor α-1
GPe	Globus pallidus external segment
GPi	Globus pallidus internal segment
HA	Hemagglutinin
HD	Huntington's disease
Hdh	Huntingtin gene

HEK293	Human embryonic kidney 293 cells
HIAP	Human inhibitor of apoptosis
HSV	Herpes simplex virus
HT-1080	Human osteosarcoma cells
IAP	Inhibitor of apoptosis protein
IMDM	Iscove's Modified Dulbecco's Media
ITR	Inverted terminal repeats
LB	Luria-Bertani broth
Luc	Luciferase
LV	Lentivirus
MAPK	Mitogen-activated protein kinase
N171-82Q	Transgenic mice with 171aa N-terminal fragment of *Hdh* with 82 CAG repeats
NADPHd	Nicotinamide adenine diphosphate diaphorase
NAIP	Neuornal apoptosis inhibitor protein
NEB	New England Biolabs
NeuN	Neuronal nuclei
NGF	Nerve growth factor
NMDA	N-methly-D-aspartate
NOS	Nitric oxide synthase
NR2B	NMDA receptor 2B subunit
$p75^{NTR}$	p75 neurotrophin receptor
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
Pen	Penicillin
PNS	Peripheral nervous system
poly-Q	poly-glutamine tract
QA	Quinolinic acid
R6/2	Transgenic mice with exon 1 of *Hdh* containing ~150 CAG repeats
rh	Recombinant human
RM ANOVA	Repeated measures analysis of variance
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
ssDNA	Single-stranded DNA
Strep	Streptomycin
STS	Staurosporine
TE	Tris-EDTA buffer
TH	Tyrosine hydroxylase
WPRE	Woodchuck hepatitis post-transcriptional regulatory element
XIAP	X-linked inhibitor of apoptosis