Strategies for Neurorestoration: Growth Factors

Elena Posse de Chaves, PhD
928-MSB
Phone: 492-5966
Email: elena.chaves@ualberta.ca

Treatment of Neurodegenerative Diseases

• Most neurodegenerative diseases of the CNS are incurable

• Factors that contribute to lack of definitive cure are:
  – Poor knowledge of etiology
  – Difficult access of the brain

• Use of chemical compounds:
  – Bare capable of controlling the progression of brain diseases
  – Has numerous side-effects

Rationale for the Use of Neurotrophic Factors in Treatment of Neurodegenerative Disorders

• Reduced neurotrophic support plays a significant role in the pathogenesis of neurodegenerative diseases such as Alzheimer’s disease (AD), Parkinson’s disease (PD) and amyotrophic lateral sclerosis (ALS)
GROWTH FACTORS

• Natural chemical messengers that control the growth and function of cells

• They act by binding to receptors expressed on the surface of responsive cells

• Receptor binding triggers a biochemical cascade resulting in changes in gene transcription and cell function

• Many growth factors are versatile, while others are specific to a particular cell-type

Neurotrophic Factors in the CNS

Neurotrophic Factors and Their Receptors
**Neurotrophins**

- Members: NGF, BDNF, neurotrophin-3 (NT-3), neurotrophin-4 (NT-4 or NT-4/5)
- Biological actions result from activation of receptors Trks and p75NTR
- Regulate development and maintenance of the nervous system
- In the mature nervous system they affect:
  - neuronal survival
  - synaptic function and plasticity

**Distribution of the neurotrophins and their receptors in the brain (1)**

- **NGF**
  - expression varies during development depending on the region
  - highest levels of mRNA in the hippocampus and cerebral cortex
  - mRNA levels correlate with the degree of basal forebrain cholinergic innervations
- **BDNF**
  - expression is low in developing CNS and increases with maturation

**Distribution of the neurotrophins and their receptors in the brain (2)**

- **NT-3:**
  - most abundant neurotrophin in immature CNS
  - expression decreases with maturation
- **Receptors:**
  - p75NTR almost exclusively on basal forebrain cholinergic neurons
  - TrkA is also expressed on basal forebrain cholinergic neurons
  - TrkB and TrkC are much more widely distributed
- Adult hippocampus: BDNF>>NGF>>NT3 (mRNA)
Alzheimer’s disease

- Most common form of age-related dementia
- Characterized by:
  - progressive cognitive decline and dementia
  - accumulation of amyloid-β peptide in brain and CSF
  - loss of cholinergic neurons in the basal forebrain
- Degree of dementia correlates best with degeneration of basal forebrain cholinergic neurons

AD and NGF

- In AD, cholinergic neurons of the basal forebrain are lost or dysfunctional
- NGF is produced in innervation targets of BFCNs
- NGF receptors are synthesized by BFCNs
- NGF is transported retrogradely from the targets to BFCN cell bodies
- NGF promotes survival and differentiation of basal forebrain cholinergic neurons
- NGF mRNA levels in cortex of AD patients are normal
NGF and Down Syndrome

- Patients with DS over the age of 40 show neuropathological features of AD
- Normal NGF gene expression
- Normal binding of NGF to receptors
- Normal NGF signaling: BFCN cell bodies responded robustly to exogenous NGF
- The only deficiency in DS is in retrograde transport of NGF
- Defected transport is present before BFCNs degeneration

Defective Retrograde Trafficking in AD

- AD: Decreased expression of TrkA and p75NTR
- Consistent with disruption of retrograde transport of NGF
- NGF levels are:
  - unchanged or increased in hippocampus and cortex
  - decreased in the basal forebrain (CBs of BFCNs)
- Origin of the defect in NGF signaling is unknown
- Confirmation lacking:
  - *ngf* null mice die during the early postnatal period
  - Heterozygous NGF KO mice have no signs of AD (only 20% loss of BFCNs)

NGF Signaling in the Rodent BFCN-Hippocampal System

(a) Each of the steps needed for NGF signaling is shown, with the changes to these seen during BFCN degeneration indicated by arrows (or ND when not determined). (b) Dramatic decrease in the retrograde transport of NGF to the septum.

From "Traffic at the intersection of neurotrophic factor signaling and neurodegeneration: TRENDS in Neurosciences Vol. 26 No. 2 February 2003"
Schematic comparison of basal forebrain cholinergic neurons in aged controls and AD

The anti NGF AD11 Mouse

- Transgenic mice expressing anti NGF
  - levels of anti-NGF increase only in adult
- Mice have progressive neurodegeneration which resembles many features of AD
- Loss of cholinergic neurons in basal forebrain
- β-amyloid deposition occurs as a consequence of an altered processing of APP
- Marked tau hyperphosphorylation
- Behavioral deficits are severe, with impairment in working and spatial memory
The anti NGF AD11 Mouse (2)

• Delivery of NGF to brains of tg mice by intranasal injections rescue:
  – cholinergic deficit
  – beta amyloid deposition
  – tau phosphorylation
  – some cognitive functions (object recognition)

• AD-like neurodegeneration is due to alterations in NGF signaling
• Acetylcholinesterase inhibitors rescue BFCNs but do not ameliorate the tau-related phenotype
• AD-like phenotype in AD11 mice is not a mere consequence of atrophy of basal forebrain cholinergic neurons

NGF and AD: The New Perspective

• The major form of NGF accumulating in cerebral cortex and hippocampus of AD is pro-NGF
• pro-NGF preferentially binds to p75NTR receptor
• mAb αD11 neutralizes NGF but not pro-NGF
• AD12 mice: AD11 x p75NTR (-/-):
  – no β-amyloid intracellular deposits or extracellular plaques
  • Pro-NGF is involved in signaling pathways leading to AD

From “On the molecular basis linking Nerve Growth Factor to Alzheimer’s Disease” Cellular and Molecular Neurobiology, Vol. 26, Nos. 4-6, July/August 2006
BDNF in Alzheimer's disease

- BDNF decreases in AD hippocampus and temporal cortex
- Possible decrease of TrkB protein
- TrkB present in amyloid plaques of the hippocampus
- Maturation of TrkB and BDNF-inducible TrkB autophosphorylation is compromised in neurons lacking PS1
Huntington Disease

- Autosomal dominant neurodegenerative disease characterized by a progressive choreic movement disorder
- Neuropathology includes the loss of neurons particularly from the striatum and cerebral cortex
- In the striatum there is selective degeneration of medium-sized spiny projection neurons
- The protein huntingtin is mutated and forms toxic neuronal nuclear aggregates in the affected neurons

Huntington Disease and BDNF

- Striatal spiny neurons require BDNF for survival and differentiation
- Mutant huntingtin down-regulates BDNF production:
  - BDNF levels in cortical tissue are reduced by 45% in brains from HD patients
  - BDNF level is reduced in striatum and cortex of Tg mice overexpressing mutant huntingtin
- Overexpression of wild-type huntingtin protein in cell lines and 'knock-in' transgenic mice leads to increased levels of BDNF mRNA and protein

Parkinson’s Disease

- Slow, progressive disease
  - Peak onset after 60 (1% of people over this age)
- Characterized by:
  - rigidity and tremor of the limbs
  - postural instability
  - bradykinesia of the limbs and body
- Motor symptoms directly related to:
  - loss of pigmented cells in the substantia nigra
  - reduction dopamine in the striatum
- Hallmark: Lewy body containing the proteins ubiquitin and alpha-synuclein
**Parkinson’s Disease and Growth Factors (1)**

- **GDNF**: survival factor for mesencephalic dopaminergic neurons
  - improves motor deficits in animal models of PD
  - increases survival and differentiation of dopaminergic neurons in culture
  - promotes cell survival and fibre growth in nigral grafts
  - showed benefit from chronic delivery in non-human primate

**Parkinson’s Disease and Growth Factors (2)**

- **BDNF** increases number of dopaminergic neurons and dopamine release in cultures before grafting into rat models
  - Combined treatment of BDNF and GDNF are promising
- **NT4/5** in mesencephalic primary cultures increases:
  - survival of dopaminergic neurons
  - size of the neuronal soma
  - complexity of dendritic branching
- **NT-4/5** infusions can increase the efficacy of nigral grafts in rat models of PD

**Amyotrophic Lateral Sclerosis (ALS)**

- Most common form of adult onset motor neuron disease
- Motor neurons in the spinal cord, brain stem and motor cortex progressively die
- Limb and bulbar muscle weakness, usually begin in early to mid-50s
- Theories of the mechanism of pathology include: oxidative stress, neurofilament abnormalities, aberrant mitochondrial function and glutamate excitotoxicity
ALS and NTs

- Early stages:
  - NGF, BDNF, NT-3 and NT-4/5 increase in muscle
  - BDNF is strongly up-regulated
- Levels of NGF, NT-3 and NT-4/5 gradually increase during the course of the disorder
- In vitro NT-4/5 but not BDNF or NT-3 had neuroprotective effect on motoneurons
- Adenovirus-mediated intramuscular gene transfer of NT-3 in mouse models:
  - 50% increase in life span
  - reduced loss of motor axons
  - improved neuromuscular function

Challenges to the Clinical use of Neurotrophic Factors (NFs)(1)

- Short in vivo half-lives
- Poor pharmacokinetic profiles
- Pleiotropic effects following systemic application
- Difficulty to selectively target specific CNS sites
- Poor blood-brain barrier (BBB) permeability (most important)

Challenges to the Clinical use of Neurotrophic Factors (NFs)(2)

- Central administration routes have relied upon invasive techniques of administration:
  - unacceptably high costs
  - surgical risks and/or poor patient compliance
- Future clinical success of NFs depend on:
  - the development of targeted, noninvasive drug delivery strategies with optimized pharmacokinetic profiles
Routes of administration used to deliver neurotrophic factors to the CNS

A) Parenteral systemic administration
- Intravenous injection
- Intraperitoneal injection
- Intracerebroventricular injection

B) Central administration
- Direct injection/infusion
- Implantation of a polymer matrix preloaded with NFs
- Implantation of cells genetically modified to produce a given NF

Neurotrophic Factors: from animal models to primates and humans

1. Could adequate supplies of the human protein forms be produced?

2. Would NFs show the same promise in primates as they did in rats for treating CNS diseases
   - e.g. would NGF mediate protection of cholinergic neurons in primate models of AD as well as it did in rat models

3. How would the proteins be administered?
   - required over long time periods (months to years) and they do not cross the BBB

Current Experimental Efforts for the Therapeutic Use of NFs

- Indiscriminate 'flooding': obsolete therapeutic concept

- Current goal: Local (regulated) supply of NFs to specific populations of neurons:
  - Gene therapy:
    - In vivo: injection of viral vectors mediating the overexpression of NFs
    - Ex vivo: transplantation of engineered cells overexpressing NFs
  - Stem cells
In Vivo Gene Therapy

- Engineered replication-deficient viruses infect host brain cells
- Requirements:
  - cells easily accessible for infusion or injection of virus
  - integration and expression of transgene selectively in target cells at effective levels for extended time periods
- Viral vector systems used:
  - herpes simplex virus
  - adenovirus
  - lentivirus
  - monkey leukaemia virus

In Vivo Gene Therapy (2)

- Unregulated overexpression of NTs by viral vectors showed encouraging results
- Regulated overexpression using heterologous transcription factors regulated by low molecular weight ligands (tetracycline or steroids) are more promising

Ex Vivo Gene Therapy

- Retroviral vectors are used to transfect cells in a culture dish, and then these cells are transplanted
- Advantages:
  - Autologous cells from the graft recipient can be used.
  - Autograft implants minimize graft rejection
  - Fibroblasts engineered to produce NGF in phase 1 clinical trial for patients with AD
- Disadvantages:
  - level of the therapeutic protein diminishes greatly or is downregulated completely over time
  - not suitable for long-term neurodegenerative diseases
Use of NGF for AD Treatment (1)

- NGF given to BFCNs in vitro and in vivo results in increased survival and up-regulation of ChAT.

- Icv administration of NGF prevents:
  - degeneration of BFCNs caused by lesion of connection septum-hippocampus
  - cognitive deficits arising from these lesions

- Icv administration of NGF allows normal performance in memory tests in aged rats

Use of NGF for AD treatment (2)

- Icv administration of NGF in clinical trials
  - some improvements in a few of the cognitive tests
  - Important side-effects (loss of weight and appetite; pain)
  - dose-related Schwann cell hyperplasia in rats and primates; possibly also in humans-responsible for pain

- Other routes of administration
  - retroviral transfected fibroblasts, expressing NGF, rescue cholinergic neurons
  - NGF and BDNF gene transfer increases in ChAT immunoreactivity in septal neurons
  - NGF injected into the olfactory bulb of rats is retrogradely transported specifically to BFC nuclei
  - NGF can be transported into the brain following administration as nose drops
Use of NTs for treatment of HD

- Animal models: NTs may be of benefit in promoting the survival of striatal neurons
  - cells genetically engineered to release BDNF, NT-3, or NT-4/5 implanted into the striatum promote the survival of striatal projection neurones
  - BDNF is the most effective as a survival agent and NT-3 most successful at initiating differentiation
- Whether this therapeutic potential of the neurotrophins will extend to the clinic remains to be seen

Current Treatments for Parkinson’s Disease

- Anti-Parkinson drugs restore or mimic the actions of dopamine and improve motor symptoms
- Implantation of dopamine-rich tissue into the striatum aims to replace the lost nigral cells (results largely unsuccessful because of the low percentage of dopaminergic cells and low neuronal survival rate)
- Use of embryonic stem cells and embryonic neural tissue is under development

GDNF in PD Treatment

- Only in early stages
- Icv GDNF injections in one patient for over 1 year showed:
  - no restoration of dopaminergic cells
  - no improvement in symptoms
  - side-effects, including nausea and psychiatric symptoms
- GDNF administered directly into the parenchyma of the dorsal putamen in five PD patients after 1 year shows:
  - marked reduction of symptoms
  - no notable side-effects.
BDNF in PD Treatment

- Clinical trials have not bee successful
- Later study with recombinant human methionyl BDNF infused intrathecally:
  - no conclusions about the efficacy of the treatment (small number of patients)
  - Suggests BDNF can be given safely by this method

Challenges for Neurotrophic Factor Therapy

- Start the treatment at an early stage
- Improve procedures for regulated expression of NFs by stereotactic injection of viral vectors or transplantation of engineered cells
- Develop therapies to stimulate synthesis of endogenous NFs
- Induce transactivation of NF receptors with small molecules that penetrate the BBB
- Develop therapies that modify NFs signaling pathways selectively or exploiting methods of contemporary combinatorial chemistry

Bibliography

- Single articles that can be requested to me by email.