Neuroregenerative Strategies in Intracerebral Hemorrhage

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Non-traumatic intracerebral hemorrhage

Incidence

12-15 / 100,000 / year
< 45 years: < 2 / 100,000 / year
> 80 years: 350 / 100,000 / year

Main risk factor

Chronic arterial hypertension
Non-traumatic intracerebral hemorrhage

Conservative treatment

Surgical treatment
### Early Surgical Treatment for Supratentorial Intracerebral Hemorrhage: A Randomized Feasibility Study

**Zuccarello et al., Stroke 1999**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Surgical</th>
<th>Conservative</th>
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</thead>
<tbody>
<tr>
<td>Bad Outcome (GOS 1-3)</td>
<td>44%</td>
<td>64%</td>
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<tr>
<td>Mortality</td>
<td>22%</td>
<td>27%</td>
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### Surgical Treatment for Intracerebral Hemorrhage (STICH): A Single-Center, Randomized Clinical Trial

**Morgenstern et al., Neurology 1998**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Surgical</th>
<th>Conservative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bad Outcome (GOS 1-3)</td>
<td>50%</td>
<td>69%</td>
</tr>
<tr>
<td>Mortality</td>
<td>24%</td>
<td>18%</td>
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Regeneration: Neurogenesis in the adult human brain

BrdU-immunostaining of the subventricular zone in adult human brain tissue

Eriksson et al., Nat Med 1998
Experimental concepts for the treatment of ICH

Transplantation of stem cells or neuronal precursors

Treatment with neuroprotective factors

Activation of endogenous neurogenesis
Transplantation of embryonic precursor cells

Limitations of neural transplantation

- Limited availability of embryonic tissue due to ethical and logistical problems

- Insufficient survival of the transplanted cells

- Suboptimal graft integration into the host brain
The Promise of Stem Cell Research

- Identify drug targets and test potential therapeutics
- Study cell differentiation
- Understanding prevention & treatment of birth defects

Cultured Pluripotent Stem Cells

- Tissues/Cells for Transplantation

- Toxicity Testing

- Bone marrow for leukemia & chemotherapy
- Nerve cells for Parkinson's disease
- Heart muscle cells for heart disease
- Pancreatic islet cells for diabetes

Adapted from Cell
Stem cell differentiation towards neuronal lineage
Vorläuferzelle
Regional spezifischer Vorläufer
Dopaminerger Vorläufer
Dopaminerger Neuroblast

Immortalisierung
Expansion in Neurosphären
Proliferierung

Neuronale Induktion (Nurr-1)
Differenzierung (Zytokine und konditionierte Media)
Differenzierung (Gliale Faktoren)

Differenzierung in glialer Co-Kultur

Dopaminerger Neuroblast

TRANSPLANTATION

Neuronal differentiation of mesenchymal bone marrow stem cells

Expression of neuronal marker proteins

NSE  tau  growth cone

Factors that influence the success of neuronal grafting

Predetermined factors
- Age and immunological state of the host

Technical factors
- Type of the transplanted cells
- Number of transplanted cells
- Storage and conditioning of the cells
- Location of the implantation site

Pretreatment
- Neural growth factors
- Nutritional factors
- Antioxidants
- Antiapoptotic substances
Creatine treatment
Creatine supplementation leads to an increase in cellular ATP reserves
The creatine-/phosphocreatine shuttle

Enhanced energy transfer at higher substrate concentrations
Higher cytoplasmic creatine levels inhibit the mitochondrial permeability transition

MPT inhibition by stabilization of mi-CK in the octameric state
Advantages of creatine for the treatment of CNS disorders

Endogenous substance
Specific transport across the blood-brain-barrier
High bioavailability
Low toxicity
No relevant side effects
Long term therapy is safe
Inexpensive drug
Effects of creatine on cultured neuronal precursor cells

- E14 embryo
- fetal rat brain
- fetal tissue
- mechanical dissociation
- multiwell plate
- control
- creatine
- roller-drum
CK expression in embryonic mesencephalic cell cultures

Andres et al., Neurosci 133:701-731 (2005)
Creatine protects dopaminergic neurons against MPP+ toxicity

Andres et al., Cell Transplant (2005)
Creatine protects neuronal morphology against MPP+ toxicity

Andres et al., Neurosci 133:701-731 (2005)
Creatine promotes survival of GABA-ergic striatal cells

Creatine protects GABA-ergic neurons against 3NP-toxicity

Effects of creatine: Summary

Creatine kinase isoenzymes are expressed in mesencephalic and striatal precursor cells and in the human brain.

Creatine is a potent endogenous neuroprotective factor for dopaminergic mesencephalic and GABA-ergic striatal neurons.

Elevated intracellular creatine levels promote the differentiation of neuronal precursors towards specific phenotypes.
Experimental model of deep striatal ICH

Mikroknife catheter

Barth et al., Restor Neurol Neurosci, in press (2006)
MR follow-up

Barth et al., Restor Neurol Neurosci, in press (2006)
Behavioral analysis
Transplantation of RN33B neuronal precursor cells
Cultured RN33B neuronal precursor cells
Intracerebral transplantation of RN33B precursor cells
Spontaneous ICH is a common clinical problem associated with severe residual neurological deficits.

Our experimental rat model of ICH allows the study of potential therapeutic concepts \textit{in vivo}.

In a pilot study, stem cell transplantation in ICH was technically feasible. The cells survived in the brain and showed neuronal differentiation.

The transplanted cells have the potential to replace the function of damaged neurons.

Treatment with the neuroprotective drug creatine may improve the outcome after ICH and influence the survival of transplanted cells.
Aims of the research project

Aim I
To show that neural progenitor cells transplanted into the perihematomal region are able to survive, differentiate, establish host connections and improve the functional outcome after ICH.

Aim II
To investigate the effects of experimental ICH and stem cell transplantation on endogenous neurogenesis in the injured brain.

Aim III
To demonstrate that creatine administration provides neuroprotection against ICH and improves neuronal survival as well as functional outcome after transplantation of RN33B cells.