Therapeutic Effects of Human Mesenchymal Stem Cells on Traumatic Brain Injury in Rats:

Secretion of Neurotrophic Factors and Inhibition of Apoptosis

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Cell death

- Programmed Cell Death
  - Oncosis-Necrosis
    - Caspase-dependent
      - Extrinsic Apoptosis
        - Anoikis
        - Pyroptosis
        - Cornification
    - Caspase-independent
      - Caspase-independent Intrinsic Apoptosis
        - Mitotic catastrophe
        - Autophagic Cell Death
          - Entosis
          - Netosis
          - Parthanatos
          - Necroptosis
  - Nonprogrammed Cell death
### Table 1: Characteristics of different types of cell death

<table>
<thead>
<tr>
<th>Type of cell death</th>
<th>Morphological changes</th>
<th>Biochemical features</th>
<th>Common detection methods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nucleus</strong></td>
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<tr>
<td>Apoptosis</td>
<td>Chromatin condensation; nuclear fragmentation; DNA laddering</td>
<td>Caspase-dependent</td>
<td>Electron microscopy; TUNEL staining; annexin staining; caspase-activity assays; DNA-fragmentation assays; detection of increased number of cells in subG1/G0; detection of changes in mitochondrial membrane potential</td>
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<tr>
<td>Autophagy</td>
<td>Partial chromatin condensation; no DNA laddering</td>
<td>Caspase-independent; increased lysosomal activity</td>
<td>Electron microscopy; protein-degradation assays; assays for marker-protein translocation to autophagic membranes; MDC staining</td>
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<tr>
<td>Mitotic catastrophe</td>
<td>Multiple micronuclei; nuclear fragmentation</td>
<td>Caspase-independent (at early stage) abnormal CDK1/cyclin B activation</td>
<td>Electron microscopy; assays for mitotic markers (MPM2); TUNEL staining</td>
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<tr>
<td>Necrosis</td>
<td>Clumping and random degradation of nuclear DNA</td>
<td>Increased vacuolization; organelle degeneration; mitochondrial swelling</td>
<td>Electron microscopy; nuclear staining (usually negative); detection of inflammation and damage in surrounding tissues</td>
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<tr>
<td>Senescence</td>
<td>Distinct heterochromatin structure (senescence-associated heterochromatin foci)</td>
<td>Flattening and increased granularity</td>
<td>Electron microscopy; SA-β-gal staining; growth-arrest assays; assays for increased p53, INK4A and ARF levels (usually increased); assays for RB phosphorylation (usually hypophosphorylated); assays for metalloproteinase activity (usually upregulated)</td>
</tr>
</tbody>
</table>

CDK1, cyclin-dependent kinase 1; MDC, monodansylcadaverine; MPM2, mitotic phosphoprotein 2; SA-β-gal, senescence-associated β-galactosidase; RB, retinoblastoma protein.
Traumatic brain injury

Austria

- Incidence: 303/100,000/year
- Mortality rate: 11/100,000/year

- Occurrence highest in:
  - in male teens
  - female octogenarians

- leading cause of death in people aged 15–45 year globally
Symptoms of TBI

Mild TBI

- Fatigue
- Headaches
- Visual disturbances, Sensitivity to light and sounds
- Memory loss
- Poor attention/concentration
- Sleep disturbances
- Dizziness/loss of balance
- Irritability-emotional disturbances
- Feelings of depression
- Seizure
- Nausea
- Loss of smell

http://www.traumaticbraininjury.com/symptoms-of-tbi/mild-tbi-symptoms/  last access on 25.11.17
Symptoms of TBI

Severe TBI

- not understanding the spoken word (receptive aphasia)
- difficulty speaking and being understood (expressive aphasia)
- partial or total loss of vision
- weakness of eye muscles and double vision (diplopia)
- loss or diminished sense of smell (anosmia)
- Convulsions
- Physical paralysis/spasticity
- Chronic pain Control of bowel and bladder
- Loss of stamina Appetite changes
- Regulation of body temperature

http://www.traumaticbraininjury.com/symptoms-of-tbi/severe-tbi-symptoms/ last access on 25.11.17
Materials and Methods

6-8 weeks old Sprague Dawley rats

Group 1:
- Sham control group (craniotomy, without TBI)

Group 2:
- Placebo group (TBI with saline injection)

Group 3:
- Treatment group (TBI with hMSC injection)
Materials and Methods

First set of animals (8 per subgroup)

- Neurological assessment
- ELISA
- Immunohistochemistry
- Sacrifice on day 2, 8, 15 and 29 (total n=96)

Second set of animals (4 per subgroup)

- Westernblot
- Sacrifice on day 2 and 8 (total n=24)
Experimental design

- Mononuclear cells from human bone marrow (posterior iliac crest)
- Differentiation in culture to human mesenchymal stem cells (hMSC)
- Intravenous application 24h after TBI
Traumatic brain injury

- Fixation in stereotaxic frame
- Drilling an 8mm hole
- Adjacent to central suture, between lambda and bregma
- Impacting right cortex with pneumatic piston
- 2mm of compression,
- Velocity of 3.2 m/sec

Methods

• Western blot

• Immunoprecipitation

• Immunofluorescent staining

• Cleaved caspase 3 (apoptosis)

• Neurological severity score/ Behaviour
Modified neurological Severity Score

Beam balance tests (normal = 0; maximum = 6)
0 = Balances with steady posture
1 = Grasps side of beam
2 = Hugs the beam and one limb falls down from the beam
3 = Two limbs fall down from the beam or spins on the beam (>60 s)
4 = Attempts to balance on the beam but falls off (>40 s)
5 = Attempts to balance on the beam but falls off (>20 s)
6 = Falls off; no attempt to balance or hang on to the beam (<20 s)

Reflexes absence and abnormal movements
1 = Pinna reflex (a head shake when touching the auditory meatus)
1 = Corneal reflex (an eye blink when lightly touching the cornea with cotton)
1 = Startle reflex (a motor response to a brief noise from snapping a clipboard paper)
1 = Seizures, myoclonus, myodystomy

Maximum points 18

One point is awarded for the inability to perform the tasks or for the lack of a tested reflex.
13-18 = severe injury; 7-12 = moderate injury; 1-6 = mild injury.

Raising the rat by the tail 3
1 = Flexion of forelimb
1 = Flexion of hindlimb
1 = Head moved >10° to the vertical axis within 30 s

Walking on the floor (normal = 0; maximum = 3)
0 = Normal walk
1 = Inability to walk straight
2 = Circling toward the paretic side
3 = Fall down to the paretic side

Sensory tests 2
1 = Placing test (visual and tactile test)
1 = Proprioceptive test (deep sensation, pushing the paw against the table edge to stimulate limb muscles)
Rotarod motor test
Results

Results

Results


Results

Results

Results

Results

Results

Discussion

- hMSC reach the lesional area

- Surrogate effect on tissue regeneration not sufficient

- Activation of neurotrophic factors

- Amelioration of neurological function due to paracrine effect