Apoptosis and autophagy in therapeutic hypothermia after spinal cord injury in rat

Jun-Yeong Seo, MD¹,
Young-Hoon Kim, MD², Kee-Yong Ha, MD².

Department of Orthopaedic Surgery,
¹Jeju National University Hospital,
²Seoul St. Mary’s Hospital,
Republic of Korea
Purpose

- This study investigated autophagy and apoptosis in therapeutic moderate systemic hypothermia (TH) with methylprednisolone sodium succinate (MP) treatment after an acute spinal cord injury (SCI) in a rat model.
Sprague-Dawley rats

- Control group: SCI only (n=23)
- Group 1: MSH for 4 hours immediately after SCI (n=23)
- Group 2: High-dose methylprednisolone treatment after SCI (n=23)

Spinal cord injury

- Laminectomy T8~10
- Cord contusion using MASCIS impactor
- 25 g-cm lesion (10g rod fall from 2.5cm height)
Methods

• Methylprednisolone (MP)
  – National Acute Spinal Cord Injury Study II protocol
  – 30 mg/kg intraperitoneally
  – 1 hour interval
  – 124.2mg/kg (5.4 mg/kg/hr x 23hr) subcutaneously

• Therapeutic hypothermia (TH)
  – External cooling (ice cube).
  – Target rectal temperature: 30 ~ 32 °C (MSH)
  – Reached within 15 min, maintained for 4 hours (h)
  – Rewarming(2h): heating light
### Methods

- Rats were killed after 2 and 7 days

<table>
<thead>
<tr>
<th>Test</th>
<th>Apoptosis</th>
<th>Autophagy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunohistochemistry</td>
<td>TUNEL stain</td>
<td>LC3β</td>
</tr>
<tr>
<td>Western blotting</td>
<td>FasL</td>
<td>LC3β Beclin 1</td>
</tr>
<tr>
<td></td>
<td>Caspase 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caspase 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caspase 3 BID</td>
<td></td>
</tr>
<tr>
<td>Transmission electron microscope (TEM)</td>
<td>n/a</td>
<td>Identification of autophagosome</td>
</tr>
</tbody>
</table>
• Behavioral Assessments
  – BBB (Basso, Beattie, Bresnahan) locomotor rating scale
  – Weekly locomotor testing for 6 weeks
  – By two observers, no information about rats
Results

[Images of fluorescence microscopy images showing the distribution of DAPI, TUNEL, LC3, and merged images for 2DAY and 7DAY samples of Control, MP, and TH conditions.]

Bar graphs showing the number of TUNEL and LC3 positive cells for each condition and time point (2 Day and 7 Day) compared to control. Asterisks indicate statistical significance.
Results

**FasL**
- 2 day: Membrane, Soluble
- 7 day: Membrane, Soluble

**Caspase 8**
- 2 day: Procaspase 8, Active caspase 8
- 7 day: Procaspase 8, Active caspase 8

**BID**
- 2 day: BID, 1BID
- 7 day: BID, 1BID

**Caspase 9**
- 2 day: Procaspase 9, Active caspase 9
- 7 day: Procaspase 9, Active caspase 9

**Caspase 3**
- 2 day: Procaspase 3, Active caspase 3
- 7 day: Procaspase 3, Active caspase 3
Results

LC3

2 day
- LC3 I
- LC3 II
- β-actin

7 day
- LC3 I
- LC3 II
- β-actin

Beclin 1

2 day
- Beclin 1
- β-actin

7 day
- Beclin 1
- β-actin

Relative optical density

Control | MP | TH
---|---|---
2 Day
- 4.51
- 3.0
- 1.57

7 Day
- 0.22
- 0.18
- 0.14

Control | MP | TH
---|---|---
2 Day
- 0.54
- 0.32
- 0.21

7 Day
- 0.39
- 0.37
- 0.37
Results

Autophagosome
Lysosome
Multivesicular body

Scale bars, 0.2 μm
Conclusions

- Both TH and MP treatment have neuro-protective effects at the injured spinal cord by inhibiting apoptosis and autophagy.
- Those treatments reduced the extrinsic and intrinsic apoptotic pathways two days after injury.
- The application of MSH is thought to be effective treatment in acute SCI.
- Further investigation how to apply this safely to the human is needed.
The authors have declared that no competing Interests exist.

This work was supported by the research grant of Jeju National University Hospital.