Mapping Severities of Primary Spinal Cord Injury due to Cervical Spine Dislocation in Rats

1Xu, Z; 1Huang, Z P; 1Wu, X H; 1Cheng, J T; +2Zhu, Q A
+1 Southern Medical University, Guangzhou, China
qinganzhu@gmail.com

INTRODUCTION
The character of spinal cord injury (SCI) due to fracture-dislocation has been demonstrated different to SCI caused by contusion and distraction either in primary injury or secondary injury at 3 hours[1-3]. However, in the previous studies, the dislocation was 2.5 mm at C4-C5 level, shearing the spinal cord as high as 90%. The rats were injured too severe to survive for studying the secondary SCI at a longer time. There is a need to investigate this innovative SCI model at different lower levels of dislocation associated with mild or moderate SCI. The objective of this study was to evaluate severities of primary SCI with 3 dislocation displacements at C4-C5 level using Sprague-Dawley rats, specifically to measure hemorrhage volume in the grey and white matter, the force and displacement during the dislocation, as well as to observe fracture of the cervical spine column.

METHODS
All procedures were approved by our institution’s Animal Care Committee. A SCI device leading to cervical spinal dislocation fracture was developed based on cervical vertebrae anatomy of Sprague-Dawley rats[11]. A rostral clamp connected to a stereotoxic apparatus held C3 and C4 and kept stationary during injury, while a caudal clamp held C5 and C6 and was connected to a material testing machine. The caudal clamp was driven dorsally at a constant speed and returned to the original position, producing C4-C5 dislocation and spinal cord injury (Figure 1). The displacement and force were recorded continuously during injury. 24 male Sprague-Dawley rats (mean weight 305g) were divided into 4 groups. The rats were anesthetized during the injury. The C4-C5 dislocation was produced in 3 groups up to 1.3mm, 1.6mm and 1.9mm at 2mm/sec, respectively, while 1 group, as a sham group, was subjected to the same procedure without dislocation. The rats were perfused intracardially right after injury. The fracture at C4-C5 was verified with microscopic observation. A 1.5-cm segment of the spinal cord centered at the C4 and C5 was harvested. A series of parasagittal sections of the cord were cut at a 20µm increment. One set of sections from each cord was stained with hematoxylin and eosin. Total volume of hemorrhage in the grey and white matter was calculated, respectively. The maximal force and displacement were determined at the peak on the force-displacement curve.

RESULTS
All C4-C5 intervertebral disks was ruptured in experimental groups, at C4 inferior endplate with an average range of displacement of 1.00±0.17mm and the maximal force of 12.7±5.1N (Table 1). Two symmetrical hemorrhage strips or points were observed on all spinal cords in the 1.6mm and 1.9mm groups (Figure 2). The hemorrhage was mainly located in the gray matter and scattered in the white matter more dorsally than ventrally (Figure 3). More hemorrhage was observed dorsally, as well as in the grey matter. The hemorrhage in the grey matter is more in the 1.9mm group than the 1.6mm group (Table 1, P<0.05).

Table 1. The mean and SD of force and displacement at rupture during the fracture-dislocation, as well as mean and SD of hemorrhage volume in the spinal cord

<table>
<thead>
<tr>
<th>Groups</th>
<th>Max. Force (N)</th>
<th>Displacement (mm)</th>
<th>Hemorrhage in White Matter (mm³)</th>
<th>Hemorrhage in Grey Matter (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.30mm</td>
<td>14.7±5.5</td>
<td>0.99±0.20</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>1.60mm</td>
<td>11.3±1.6</td>
<td>0.99±0.10</td>
<td>0.12±0.09</td>
<td>0.82±0.22</td>
</tr>
<tr>
<td>1.90mm</td>
<td>12.7±5.1</td>
<td>1.00±0.17</td>
<td>0.79±0.63</td>
<td>1.63±0.64</td>
</tr>
</tbody>
</table>

DISCUSSION:
The present result suggested that cervical spine fracture occurred with dislocation displacement over 1.3mm, while the spinal cord injury was observed with the displacement greater than 1.6mm. The SCI severity seems increase with the dislocation. There is more hemorrhage in the grey matter, and more injury to the cord seen dorsally. The injury to neural structures should be investigated in future.

SIGNIFICANCE:
This study identified SCI severity caused by cervical spine fracture-dislocation. A mild and moderate SCI with this model could be used in secondary SCI study.

ACKNOWLEDGEMENTS:
This research is funded by the Research Fund of Guangdong Advanced Education Ministry (2009)

REFERENCES:

Figure 1. Photographs of set-up of fracture-dislocation of rat cervical spine. The rostral clamp hold the C3 and C4 stationary, while the caudal hold the C5 and C6 connected the actuator (A-C). The caudal clamp was driven dorsally up to 1.9mm at speed of 2mm/s.

Figure 2. Photographs of cervical spinal cords harvested from the perfuse rat. The scale grid is 1 mm.

Figure 3. H & E-photomicrographs showing that primary hemorrhage was concentrated in the grey matter in the 1.6mm and 1.9mm groups. There was no hemorrhage in the 1.3mm and sham groups. Bar=5µm.