P38 Mitogen activated protein kinase inhibitor ameliorates motor function by inhibition of chondroitin sulfate proteoglycans after spinal cord injury in rat

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Axonal regeneration inhibitory factors

- **Astrocyte-derived axon growth inhibitors**
  - Chondroitin sulfate proteoglycans (CSPGs)
    - a, neurocan
    - b, brevican
    - c, phosphacan
    - d, versican etc...

(axel sandvig et al. glia 46: 225-251 (2004))
MAPK signaling (mitogen-activated protein kinase)

**Trophic factors, Stress, Cytokines, inflammatory**

Cell membrane

- MAPKK
- MAPK
- MEK1/2
- MEK4/7
- MEK3/6
- ERK1/2
- JNK
- p38

Nucleus

- Proliferation, differentiation
- Inflammation, Apoptosis, Growth, Differentiation

SB203580: p38 MAPK inhibitor
Spinal cord injury model
Wister rat (female; 4-6W; 250-300g)
contusion by MASCIS impactor 10g x 25mm (Th11)
Immediately after SCI vehicle group SB20474 (10µg/100µL in PBS)
SB group SB203580 (10µg/100µL in PBS)
Intrathecal administration (from L4/5)

Behavior
BBB score
3day, 1W, 2W after the SCI (n=8)

Quantity of CSPGs (2 weeks after the SCI; n=6)
Western blot analysis
-- CSPGs: anti-proteoglycan (3B3 ∆di-6-sulfate)
-- neurocan: anti-neurocan (1G2)

Pathological examination (2 weeks after the SCI)
10µm thickness frozen section; double immunofluorescence staining
1. CSPGs: anti-proteoglycan (3B3 ∆di-6-sulfate); FITC
2. neurocan: anti-neurocan (1G2); FITC
3. astrocyte: anti-GFAP; Cy3
1+ 3 and 2 + 3
Result (behavior)

(BBB score)

(mean ± SEM; n=8)

** (p<0.01)  (Wilcoxon rank sum test)

Vehicle

SB

10.6 ± 0.86

14.8 ± 0.5

3W

4W

5W

6W

3day

1W

2W

3W

4W

5W

6W

(after treatment)
Result (amount of CSPGs)

vehicle SB

3B3

\[\text{vehicle: } 100 \pm 24.1 \quad \text{SB: } 64.0 \pm 4.9\] (ANOVA Fisher’s PLSD; \(p=0.049\))

(mean ± SEM; \(n=6\))

\(\rightarrow 220\text{kD}\)
Neurocan

Result (amount of Neurocan)

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<thead>
<tr>
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<th>vehicle</th>
<th>SB</th>
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<tbody>
<tr>
<td>270kD</td>
<td>100 ± 24.5</td>
<td>39.4 ± 4.0</td>
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<tr>
<td>130kD</td>
<td>(intact neurocan)</td>
<td>(neurocan-C)</td>
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(mean ± SEM; n=6)

* (ANOVA Fisher’s PLSD; p=0.024)
Result (CSPGs staining)

SB

CSPGs FITC

Astrocyte Cy3

vehicle

CSPGs FITC

Astrocyte Cy3

marge

cavity

cavity
Result (neurocan staining)

SB

Neurocan FITC

Astrocyte Cy3

vehicle

Neurocan FITC

Astrocyte Cy3

cavity

marge
Administration of p38 MAPK inhibitor (intrathecal) after SCI

Inhibition of GSPGs including neurocan (axonal inhibitory factors)

Regeneration of damaged axon

Create re-circuit of neural networks

Conclusions
Disclosure Information

The authors have no conflict of interest to report.