The Effects of Glucocorticoids on Myelination after Peripheral Nerve Injury

Morisaki, S; Fujiwara, H; Nishi, M; Oda, R; Kawata, M; Kubo, T

1 Department of Orthopaedics, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan
2 Department of Anatomy and Cell Biology, Nara Medical University, Nara, Japan
3 Department of Anatomy and Neurobiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan

morisaki@koto.kpu-m.ac.jp

INTRODUCTION:
Glucocorticoids are effective for improving the symptoms of peripheral nerve disorders, such as carpal tunnel syndrome and peripheral neuropathy. The effects of glucocorticoids are mainly anti-inflammatory, but the mechanisms of their effects in peripheral nerve disorders remain unclear. Schwann cells of the peripheral nerves express glucocorticoid receptors (GR), and glucocorticoids enhance the rate of myelin formation in vitro. Therefore, it is possible that the clinical improvement of peripheral nerve disorders by glucocorticoids is due, in part, to the modulation of myelination. In the present study, we used rats to examine the effects of glucocorticoids and GR on myelination. We performed adrenalectomy (ADX), followed by a daily injection of either low dose (1 mg/kg) or high dose (10 mg/kg) corticosterone (CORT). We then simulated a crush injury of the sciatic nerves.

MATERIALS AND METHODS:
Rats aged 6 weeks underwent a bilateral ADX, receiving intraperitoneal injections of CORT (at a dose of 0 mg/kg, 1 mg/kg, and 10 mg/kg body weight) dissolved in 200 µl of sesame oil daily. Three days after the ADX, we anaesthetized the animals again and subjected them to a crush injury. We then divided these rats into four groups, according to the type of treatment: (i) crush injury only with sham ADX operation; (ii) crush injury after ADX with vehicle treatment; (iii) crush injury after ADX with CORT (1.0 mg/kg/day) replacement, defined as the low-dose CORT replacement group; and (iv) crush injury after ADX with CORT (10 mg/kg/day) replacement, defined as the high-dose CORT replacement group. We performed immunohistochemical, biochemical, and morphological analyses for evaluating the effects of CORT on the myelination after peripheral nerve injury.

Real-time RT-PCR was analyzed by the Student t-test. Western blot was analyzed by one-way ANOVA, followed by post hoc Student t-test. Morphological assay was analyzed by one-way ANOVA, followed by post hoc Tukey-Kramer tests. Data in graphs are presented as mean ± the standard error of the mean (S.E.M.).

RESULTS:
Real-time RT-PCR analyses The levels of myelin basic protein (MBP) and P0 mRNA in the distal 10 mm segments of sciatic nerves were analyzed by real-time RT-PCR at three weeks after crush injury (n = 5 per group, Fig. 1). Three weeks after the crush injury, the MBP mRNA levels in the ADX with vehicle group are significantly lower than in the sham operation group and the low-dose CORT replacement group. The expression of P0 mRNA in the ADX with vehicle group is lower than that of the sham operation and the low- and high-dose CORT replacement groups. However, there are no significant differences among the experimental groups.

Western blot analyses The protein levels of MBP in the sham operation group and the low-dose CORT replacement group are significantly higher than in the ADX with vehicle group (n = 6 per group, Fig. 2).

Morphological analyses To examine morphological changes among the designed groups, we made semithin cross sections (2 µm thick) four weeks after the crush injury (Fig. 3, Table 1.). The results showed that the myelin diameter (D) was significantly greater in the sham operation group and the low-dose CORT replacement group than in the ADX with vehicle group. There were no significant differences among the four groups in the axonal diameter (d), suggesting that the ADX with CORT replacement did not influence the axonal regeneration but did affect myelination processes. The myelin thickness was significantly greater in the sham operation group and the low-dose CORT replacement group than in the ADX with vehicle group and the high-dose CORT replacement group.

DISCUSSION:
The present study reveals that: (1) endogenous glucocorticoids have a critical role in myelination processes, especially by increasing MBP levels; and (2) the effects of glucocorticoids are most prominent when the concentration of glucocorticoids is within a physiological level. The results show that, at three weeks after crush injury, the expression of MBP in the ADX with vehicle group decreased significantly, compared with the sham operation group; and that the expression of MBP was restored in both low- and high-dose CORT replacement groups.

Morphological analyses show that the myelin diameter is significantly greater in the sham operated group and the low-dose CORT replacement group than in the ADX with vehicle group. These results suggest that endogenous glucocorticoids have an important role in myelination after a peripheral nerve injury in vivo through the GR in Schwann cells.

REFERENCES:

Table 1 Summary of morphometric parameters

<table>
<thead>
<tr>
<th></th>
<th>fiber diameter (D) (µm)</th>
<th>axon diameter (d) (µm)</th>
<th>myelin thickness (D-d2) (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sham</td>
<td>*5.98 ± 0.507</td>
<td>4.001 ± 0.622</td>
<td>**1.057 ± 0.116</td>
</tr>
<tr>
<td>ADX</td>
<td>5.347 ± 0.464</td>
<td>3.695 ± 0.314</td>
<td>0.822 ± 0.105</td>
</tr>
<tr>
<td>low CORT</td>
<td>*5.910 ± 0.351</td>
<td>3.948 ± 0.304</td>
<td>**0.981 ± 0.113</td>
</tr>
<tr>
<td>high CORT</td>
<td>5.615 ± 0.282</td>
<td>3.990 ± 0.202</td>
<td>0.815 ± 0.069</td>
</tr>
</tbody>
</table>