

## **Anti-inflammatory Corticosteroid Reduces Mechanical Pain Behavior, Sympathetic Sprouting, and Elevation of Pro-inflammatory Cytokines in a Rat Model of Neuropathic Pain**

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Recent work suggests that inflammatory responses in the dorsal root ganglia (DRG) may play key roles in the development of chronic pathological pain states. This study examined the effects of corticosteroid on the pain behaviors and cellular correlates of the spinal nerve ligation (SNL) model of nerve injury-induced pain.

The SNL model was implemented in Sprague-Dawley rats via tight ligation of the L5 spinal nerve. Triamcinolone acetonide (1.5 mg/kg) or normal saline was injected 60 minutes prior to surgery and once per day for the first 3 days after surgery, or an osmotic minipump loaded with TA (250nM and a flow rate of .5uL/hr) was inserted with the catheter needle placed in the DRG. Mechanical and thermal pain behaviors were tested prior to surgery (3 measurements over 5 days), and on postoperative days (POD) 3 and 7. The content of 14 cytokines of the axotomized L5 DRG was measured using Bio-Plex System (Luminex). Sympathetic sprouting within the L5 DRG was determined with tyrosine hydroxylase immunostaining in 40- $\mu$ m sections, and quantified by counting the number of neuron somata surrounded by sympathetic fibers (basket).

Rats treated with steroid systemically showed significantly reduced mechanical and thermal pain behaviors on POD 3 and 7. Correlating with the reduction in pain behavior by systemic steroid treatment, it was observed that cytokines (GRO/KC, IFN, IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-18, and MCP-1) were selectively upregulated on POD 3 in the axotomized DRG. Steroid treatment partially reversed the increase in cytokine levels ( $p < 0.05$ , student's t-test). Sympathetic sprouting in the axotomized L5 DRG was significantly reduced by steroid treatment: the average number of sympathetic fiber baskets surrounding neurons on POD 8 decreased from  $14.0 \pm 1.6$  basket/mm<sup>2</sup> cellular area ( $n = 12$ ) in saline treated animals to  $2.8 \pm 0.4$  basket/mm<sup>2</sup> ( $n=13$ ) in steroid treated animals ( $p = 0.002$ , Mann-Whitney Rank Sum Test). The rats treated with local infusion of DRG with an osmotic minipump showed no significant difference in mechanical withdrawal threshold on POD 3 and 7, or sympathetic fiber baskets surrounding neurons. On POD 7 the density of the saline treated group was  $28.6 \pm 2.74$  basket/mm<sup>2</sup> cellular area ( $n=4$ ) while the density of the steroid treated group was  $33.6 \pm 3.86$  baskets/ mm<sup>2</sup> cellular area ( $n=4$ ). The observed increases in certain proinflammatory cytokines, and the marked behavioral and cellular effects of steroid treatment, suggest that the SNL nerve injury model has an important inflammatory component. It is of interest that some of the upregulated cytokines (e.g., IL-6, GRO/KC) have previously been reported to induce sympathetic sprouting or nerve growth. This is consistent with our finding that systemic steroid treatment markedly reduced levels of cytokines as well as of sympathetic sprouting (Supported by National Institute of Neurological Disorders and Stroke Grant# NS45594).