THE ROLE OF TISSUE BIOMECHANICS IN PAINFUL INJURIES: MECHANICS, CELLULAR RESPONSES AND PAIN BEHAVIORS

Beth A Winkelstein (1), Joyce A DeLeo (2)

(1) Department of Bioengineering
University of Pennsylvania
Philadelphia, PA

(2) Department of Anesthesiology
Dartmouth College Medical School
Hanover, NH

INTRODUCTION

Pain affects as many as 50 million Americans and injuries leading to persistent pain syndromes make up a unique class of mechanisms which remain largely uncharacterized. A common painful injury results from mechanical loading of nerve roots, which occurs in both the low back and neck due to spinal injuries. Relationships have been demonstrated between tissue compression and behavioral hypersensitivity responses in animal models, with differential patterns of sensitivity depending on the nature of the mechanical insult [1,2]. Mechanical allodynia (MA) is an increased behavioral sensitivity to a non-noxious stimulus and is observed in the dermatome of the injured tissue. It can be measured by the frequency of paw withdrawals elicited by stimulation with normally non-noxious von Frey filaments [2]. Allodynia is a clinical measure of sensitivity and therefore provides a useful gauge of nociceptive responses.

A combination of neurologic, electrophysiological, biochemical, structural, and mechanical contributions produces persistent pain [3]. Edema and increased endoneurial pressure have been reported in compressed nerve roots [4,5]. Electrophysiologic responses are altered according to the magnitude of injury intensity [6], suggesting possible mechanisms by which differential clinical pain symptoms may be produced. However, while the relationships between mechanical injury and the aspects of the pain response are currently being delineated, no research has defined mechanical thresholds for the onset and maintenance of pain-related behaviors in these animal models.

A host of CNS neuroimmune changes contribute to persistent pain [7], including glial cell activation, and cytokine and chemokine upregulation and release. The role of CNS neuroinflammatory and neuroimmune activation responses in persistent pain is well documented and has been proposed as a potential mechanism through which central sensitization can occur [7]. While neuroimmune and electrophysiologic responses of the CNS likely work together to affect behavioral hypersensitivity, it is hypothesized that local biomechanics at the injury site modulates nociceptive cascades leading to pain.

Therefore, this study examines injury biomechanics for pain-behaviors in a rat lumbar radiculopathy (nerve root injury) model and examines neuroimmune responses in the CNS in the context of pain behaviors (MA) and injury mechanics. Using assessments of allodynia, mechanical thresholds for pain initiation and persistence are defined for nerve root compression in this injury model.

METHODS

Male Holtzman rats were divided into a sham (n=4) group with only nerve root exposure or a ligation group (n=18) with ligation of the left L5 nerve roots using 6-0 silk suture. Using image analysis, *in vivo* nerve root compressive strains were calculated at injury [1], using the nerve root boundaries in the unloaded and loaded (injured) conditions. Following injury, behavioral hypersensitivity was measured by recording allodynia responses postoperatively on days 1,3,5,7,10,14, using a 12-gm von Frey filament.

Compressive thresholds for pain onset and maintenance were determined using total allodynia responses, for each animal postoperatively. The onset of pain was defined as the highest allodynia response of any sham animal. Each ligated rat was assigned a pain score of 1 or 0 based on having a total allodynia sum: (1) above this limit or (0) equal to or below it. Likewise, the mean total allodynia for the ligation group was used to define a behavioral persistent pain response. In this case, a persistent pain score was assigned 1 for those responses above the mean and 0 for those below it. Logistic regression was used to determine tissue strain threshold values for pain behaviors due to nerve root compression.

In a subset of rats, lumbar spinal cord tissue was harvested on postoperative days 1,3,7,14 (5 rats/time point) for immuno-histochemical (IHC) analysis of glial activation using OX-42 for microglial activation and GFAP for astrocytic activation [2].

To provide mechanical context for the injury strains, L5 spinal nerve roots (n=12) were harvested from matched rats for *ex vivo* mechanical compression testing. Displacements were applied incrementally using a translation micrometer. Compressive load was recorded until a maximum force of 1.35 N was reached. Load, displacement and image data were acquired at each increment, following a 30 second relaxation time. Using specimen geometry, stress-strain responses were determined for each specimen and the no-load strain was calculated by a linear fit of these stress-strain data.

RESULTS

For all shams, the mean calculated strain from image analysis was 0.32 ± 0.79%, which is within the range of analysis errors, indicating no measured tissue injury in shams. The mean applied strain for the ligations was 19.7 ± 10.6%. All animals undergoing nerve root ligation exhibited allodynia following injury (Figure 1). The typical behavioral response exhibited a robust initial increase in MA which was maintained with only minor decreases over time. Allodynia following ligation injuries was elevated over shams ($p<0.001$). For the ligations, allodynia responses displayed significant correlation ($p<0.05$) with the degree of applied compression (Figure 2).

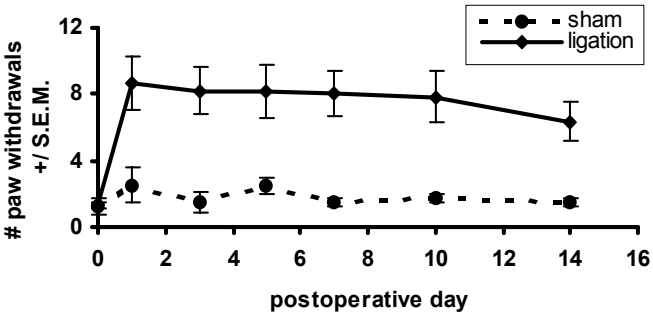

Figure 1. Mean allodynia is depicted postoperatively. There is a significantly increased MA for ligations compared to shams.

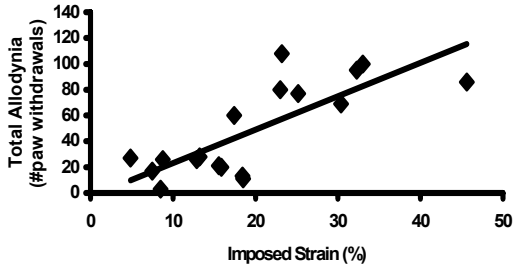

Figure 2. Overall allodynia is significantly correlated with applied compression strain (r= 0.776).

The threshold for nerve root deformation initiating a pain response, while not necessarily a maintained response, was determined to be a nerve root compression of 8.4%. Strain of 22.2% was predicted to produce persistent allodynia in this model for 95% of animals. Likewise, the average no-load strain for the nerve root during isolated compression testing was determined to be 47.8 ± 11.5%.

The degree of microglial activation in the spinal cord showed a direct relationship with the magnitude of initial tissue injury (Figure 3), which was maintained at all postoperative time points. However, astrocytic activation was not related to injury magnitude.

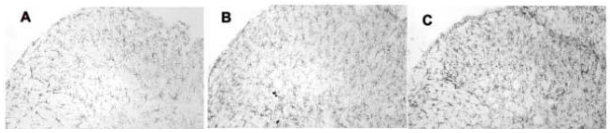

Figure 3. Microglial activation (using OX-42) in the L5 spinal cord dorsal horns at day 7 for a normal (A), 21.4 ± 5.4% strain (B) and 39.1 ± 7.3% strain (C) animal, showing graded activation with injury magnitude of compression.

DISCUSSION

These results provide specific mechanical thresholds for initiating and maintaining persistent behavioral hypersensitivity as measured by allodynia. While values suggest tissue tolerances for eliciting MA for nerve root ligation, they represent only one aspect of the nociceptive responses of pain. For example, this work does not define those thresholds for loading above which specific physiological responses are initiated; they do not indicate when production and/or release of spinal cytokines is elevated, when glial activation occurs, or when electrophysiologic changes occur. The IHC findings here, and previously reported relationships between compression and cytokine upregulation [1], point to a modulatory role for injury biomechanics in elements of the nociceptive neuroimmune cascade for persistent pain.

The pain thresholds for tissue deformation are less than one-half those of the no-load strain for the rat nerve root in compression, suggesting that the tissue deformations eliciting persistent pain may actually be much less than those deformations at which nerve roots sustain load. This, along with the physiologic findings, highlights the need for continued research focused on understanding this unique class of injuries where physiologic dysfunction may precede that of any mechanical failure.

REFERENCES

1. Winkelstein, B, Rutkowski, M, Weinstein, J, DeLeo, J, 2001, "Quantification of Neural Tissue Injury in a Rat Radiculopathy Model: Comparison of Local Deformation, Behavioral Outcomes, and Spinal Cytokine mRNA for Two Surgeons," J Neurosci Meth,111:49-57.
2. Colburn, R, Rickman, A, DeLeo, J, 1999, "The Effect of Site and Type of Nerve Injury on Spinal Glial Activation and Neuropathic Pain Behavior," Exp Neurol,157:289-304
3. Wall, P, and Melzack, R, 1994, *Textbook of Pain,* 3rd Edition, London: Churchill Livingstone.
4. Lundborg G, Myers R, Powell H, 1983, "Nerve compression injury and increased endoneurial fluid pressure: a "miniature compartment syndrome,"" J Neurol, Neurosurg & Psych, 46:1119-24.
5. Olmarker K, Rydevik B, Holm S, Bagge U, 1989, "Effects of experimental graded compression on blood flow in spinal nerve roots: A vital microscopic study on the porcine cauda equina," J Orthop Res, 7:817-23.
6. Pedowitz, R, Garfin, S, Massie, J, Hargens, A, Swenson M, Myers, R, Rydevik, B, 1992, "Effects of Magnitude and Duration of Compression on Spinal Nerve Root Conduction," Spine,17:194-9.
7. DeLeo J, Yezierski R, 2001, "The role of neuroinflammation and neuroimmune activation in persistent pain," *Pain,*91:1-6.

ACKNOWLEDGEMENTS

Grant support from NINDS NS11161, NIAMS AR47564-01, Bristol-Myers Squibb/Zimmer Orthopaedic Foundation.