

Peridural Fibrosis Associated with Postoperative Allodynia, but not Neurological Dysfunction in a Rodent Model

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Abstract

Failed back surgery syndrome (FBSS) is a prevalent, debilitating condition that affects the quality of life of many patients. The role of peridural fibrosis in FBSS continues to be a source of controversy. The authors report an association between peridural fibrosis and tactile allodynia, but not neurological dysfunction in a rodent cauda equina crush injury model. Methods Twenty-one rats were equally divided into three different treatment arms: 1) sham, 2) laminectomy-alone, and 3) laminectomy plus cauda equina crush injury. Tactile allodynia was tested and open field locomotor testing was completed according to a predetermined schedule. Animals were sacrificed on postoperative day 42. Histological sectioning was used to determine the degree of peridural fibrosis present in each group of animals. Results Animals who underwent greater surgical manipulation showed greater degrees of peridural fibrosis on histological analysis. Animals in group 3 showed significantly more allodynia at all time points during the study. Laminectomy animals showed more allodynia than sham animals, but less than crush injury animals. Locomotor function was worse in laminectomy-only animals on post-operative days 8 and 15, compared with sham animals. Locomotor scores improved thereafter to that of sham animals. Crush injury animals remained functionally impaired through the duration of the study. Histological analysis demonstrated greater degrees of peridural fibrosis in laminectomy and crush injury animals, as compared with sham. Conclusions Greater degrees of tactile allodynia were demonstrated in rodents with more surgical manipulation. Alternatively, neurological function, as measured by open field locomotor testing, was unaffected in laminectomy-only animals, though tactile allodynia remained worse compared with sham animals. Histological analysis qualitatively demonstrated an association between degree of surgical manipulation and peridural fibrosis. Peridural fibrosis, although associated with greater degrees of allodynia, did not appear related to neurological injury.

Keywords: Peridural fibrosis; Failed back surgery syndrome; Allodynia; Cauda equina injury

Introduction

Peridural fibrosis is one of many potential etiologies of the failed back surgery syndrome (FBSS). With a reported prevalence of up to 24% of patients [1], FBSS represents a complex constellation of symptomatology with various etiologies that ultimately impair quality of life, prompt numerous office visits and drive up healthcare expenditure. As such, there have been considerable efforts to find ways to mitigate the degree of peridural fibrosis in hopes of improving neurological outcome and quality of life in this patient population.

While various preclinical studies have demonstrated an association between peridural fibrosis and post-operative allodynia, few have demonstrated a significant benefit from therapeutics intended to curb the fibrotic response [2-8]. Furthermore, few groups have objectively measured motor function postoperatively to exclude occult neurological injury as a source of persistent pain, something that is routinely performed in clinical practice. We examined the association between peridural fibrosis, post-operative allodynia, and post-operative locomotor function in a cauda equina crush injury rodent model. Open-field locomotor testing was performed to rule out occult iatrogenic injury as an etiology for poor neurological outcome and post-operative allodynia in surgically manipulated animals, an endpoint that has seldom been reported [9].

Methods

All experimental protocols were approved by the UC Davis Institution's Animal Care and Use Committee. Twenty-one adult male Sprague-Dawley rats were randomized into three treatment groups (n=7/group): (1) sham, (2) laminectomy alone, and (3) laminectomy plus cauda equina crush injury. A high-powered microscope was

used to perform all surgical procedures. Group 1 had L3-6 laminae exposed without further manipulation. Group 2 had L5 micro laminectomy and Group 3 had L5 microlaminectomy with aneurysm clip application across the thecal sac and cauda equina for 3 seconds. All animals received carprofen (1 mg/kg) and Ringer's solution for 3 days postoperatively.

Allodynia testing

Tactile allodynia was tested using the von Frey hair technique. Graded von Frey filaments ranging in intensity from 6.9 mN to 288.4 mN were applied to the plantar surface of the hind paws. Withdraw thresholds were recorded in each experimental group according to the following schedule: post-operative days 3, 7, and weekly thereafter until postoperative day 42. Results in each group were pooled and reported as percentage of maximum potential effect (%MPE).

Locomotor testing

Open field locomotor testing was performed on each group of animals on post-operative days 4, 8, and weekly thereafter until post-operative day 43. Gait, coordination, and stepping behavior were

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evaluated by an independent observer blinded to the animal's treatment group according to the methods described in the BBB locomotor grading scale [10].

Histological analysis

Animals were euthanized on post-operative day 43. Lumbar spinal columns were harvested en-bloc and embedded in paraffin for sectioning and immunohistochemistry. Histological sectioning was obtained through the surgical site of interest. A modified Goldner's trichrome stain [11] was used to grossly quantify the degree of peridural fibrosis in each group.

Results

Allodynia testing

As compared to the sham group, animals in the surgically-manipulated groups demonstrated differences in mechanical allodynia that corresponded directly to the degree of surgical manipulation (i.e, those with cauda equina crush injury had more allodynia compared with laminectomy-only animals). The data were analyzed using the percentage of maximum potential effect (%MPE), which takes into account different baseline thresholds between animals and standardizes the results as a percentage. Animals in the cauda equina crush injury group exhibited significant allodynia at all-time points (Figure 1). Animals in the laminectomy group exhibited significantly lower %MPE values as compared with the Sham group, however these values were higher than in the cauda equina injury group. Thus, animals with cauda equina crush injury showed the lowest %MPE response to von Frey testing among the three groups (Figure 1).

Locomotion testing

Open-field locomotor testing was performed and graded according to the method of Basso, Beattie, and Bresnahan (BBB) as previously described [10]. Laminectomy animals performed significantly worse in comparison to sham animals on post-operative days 8 and 15 with scores of 18 (Figure 2). A BBB score of 18 corresponds to consistent fore and hind limb coordination during gait with predominant paw position rotated at lift off. After post-operative day 15, locomotor function improved and approached that of sham animals, with a score of 20-21, indicating normal gait. Animals with cauda equina crush injury had significantly lower locomotor scores at all-time points in the study, in the range of 15-16. A score of 15-16 indicates an animal with consistent front-hind limb coordination, with rotated paw position and without consistent toe clearance. Neurological function in these animals never recovered to baseline or approached that of laminectomy-only animals.

Peridural fibrosis histological analysis

Serial sections through the sham/surgical site demonstrated degrees of peridural fibrosis that were associated with the amount of surgical manipulation, though this was not volumetrically quantified (Figure 3). Sham animals showed preservation of neural and bony elements with limited fibrotic responses along the periosteal surfaces where the laminae were exposed. Collagen is represented as a blue-green hue with the trichrome stain. Laminectomy-only animals demonstrated a greater fibrotic response between the paraspinous muscles and in the epidural space with mild distortion of the dura (Figure 3). Cauda equina morphology was no different compared to sham animals. Exiting nerve roots in sham animals were preserved. Laminectomy-only animals showed evidence of scar formation in the epidural space and tethering of the exiting nerve roots. Crush injury animals had an overwhelming fibrotic response in the epidural space with thick epidural bands of

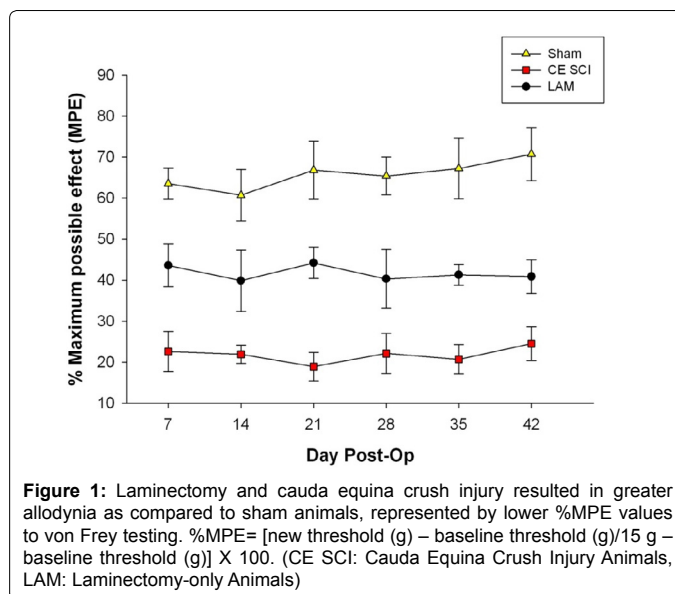


Figure 1: Laminectomy and cauda equina crush injury resulted in greater allodynia as compared to sham animals, represented by lower %MPE values to von Frey testing. %MPE= [new threshold (g) – baseline threshold (g)]/15 g – baseline threshold (g) X 100. (CE SCI: Cauda Equina Crush Injury Animals, LAM: Laminectomy-only Animals)

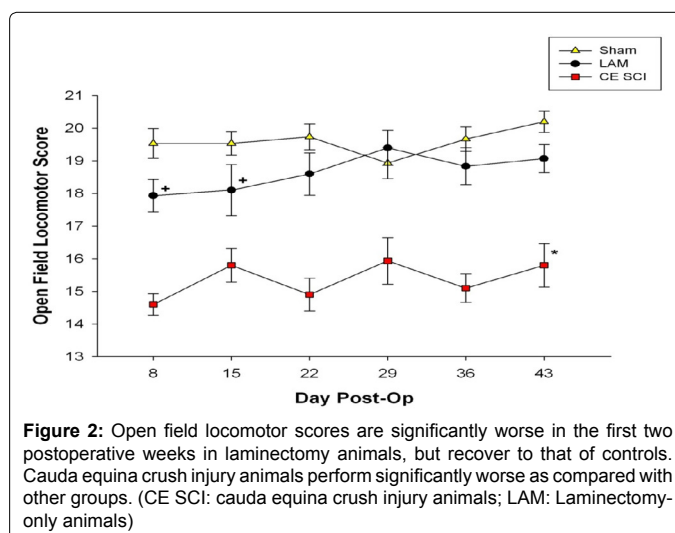


Figure 2: Open field locomotor scores are significantly worse in the first two postoperative weeks in laminectomy animals, but recover to that of controls. Cauda equina crush injury animals perform significantly worse as compared with other groups. (CE SCI: cauda equina crush injury animals; LAM: Laminectomy-only animals)

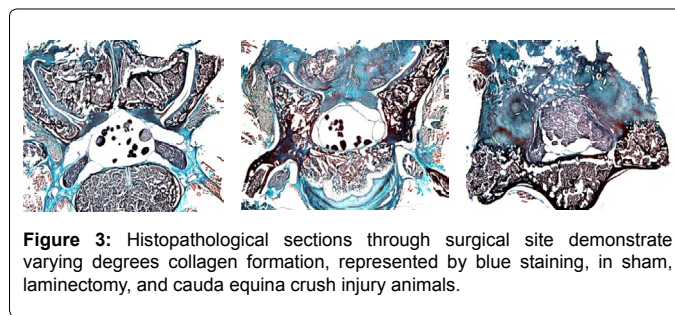


Figure 3: Histopathological sections through surgical site demonstrate varying degrees collagen formation, represented by blue staining, in sham, laminectomy, and cauda equina crush injury animals.

fibrosis, thecal sac deformation, and nerve root swelling in response to crush injury. Additionally, dense peridural scar is seen along the lateral gutters and in the region of the swollen exiting nerve roots. No significant scarring was observed within the thecal sac.

Discussion

Failed back surgery syndrome is a debilitating clinical entity, with a constellation of symptoms attributable to various etiologies.

Peridural fibrosis remains mostly a diagnosis of exclusion when recurrent pathology cannot be found. Peridural fibrosis remains a controversial source of back pain in the failed back surgery syndrome with some clinical studies showing no significant association between peridural fibrosis and clinical outcome [1,12], while others demonstrate significant improvement in postoperative pain and quality of life measures with treatments that limit peridural scar formation [13-15].

Perhaps the largest multicenter randomized controlled trial of ADCON-L showed that although patients with extensive peridural scar were significantly more likely to experience recurrent radicular pain, there was no demonstrable association between pain scores and peridural scar formation [16]. A second clinical European two-year follow up study using ADCON-L also showed no significant association between peridural scar and clinical outcome [1]. One explanation is that a relatively small percentage of patients (16.7%) with extensive peridural scar formation manifested recurrent radiculopathy [16]. In laminectomized rabbits, ADCON-L and Sefrafilin both reduced peridural fibroblast and inflammatory cell density, though this was not statistically significant [5]. Despite this encouraging data, evidence of delayed CSF leak and impaired healing from the use led to the eventual withdrawal of ADCON-L from the market [17,18].

Recent reports of biodegradable composite lamina and absorbable cement have had some success [19,20] in preclinical models. Additional treatment strategies that modulate the fibrotic response on a cellular level have also been effective at reducing peridural fibrosis in animal models [2,4-8]. Oxiplex gel showed promising results [13-15] and also demonstrated that the subset of patients with severe leg pain and weakness derived the most long-term benefit. Low-dose irradiation, interestingly, may also help to improve results in patients undergoing revision surgery for radiculopathy [21].

Delineating a causal relationship between scar formation and pain has been difficult. Massie et al. showed that reduced scar formation in animals treated with a high molecular weight hyaluronic acid gel was associated with less radiculopathy, but results lacked statistical significance. Another study showed reduced mechanical, but not thermal hyperalgesia in laminectomized animals treated with epidural low and high-dose hyaluronic acids [6]. However, results failed to demonstrate a direct correlation between peridural fibrosis and pain behavior. Conversely, studies using low-dose pre- and postoperative radiation showed improvement in both the amount of peridural fibrosis and pain outcomes [9,21].

Our results show that laminectomy and cauda equina crush injury produce a profound fibrotic response in the epidural space that is not seen in sham animals. Qualitative histological analysis was performed through the laminectomy defect to assess the degree of peridural scar formation. Detailed three-dimensional quantitative analysis would have been difficult due to inherent variability configuration and size and shape of the neural structures of interest and epidural space among animals. Although there appears to be a relationship between the degree of peridural scar and pain behavior, it is unlikely that the fibrotic response is the only contributor.

Direct injury to the cauda equina undoubtedly contributes to postoperative pain in crush injury animals. However, even laminectomy-only animals demonstrated allodynia compared with sham animals. Since occult neurological injury could explain post-operative allodynia, we controlled for this variable by using a high-powered microscope for all surgeries. The surgical microscope provides superior illumination and yields a greater degree of detail in small spaces; qualities that have been purported to minimize the chance of inadvertent injury

to important neural structures, minimize blood loss, and decrease postoperative pain, which all would theoretically lead to shorter hospital stays in the clinical population. A prospective randomized single-surgeon study showed no significant differences in those undergoing microdiscectomy versus standard open discectomy in postoperative pain control and pre- or postoperative visual scores for sciatica [22]. However, they did demonstrate differences in surgical bleeding, hospital length of stay, and postoperative lumbar pain that favored the microdiscectomy group. A Cochrane review analyzed data from three trials and found no significant difference in complications, length of in-patient stay, or formation of scar tissue between the microdiscectomy and traditional open discectomy groups [23]. An important distinction between these studies and ours is the population being studied. Human specimens are large enough to safely perform these operations using loupe magnification alone. Rodents are several orders of magnitude smaller and inadvertent injury could easily occur without being able to clearly visualize the surrounding neural structures.

Another method of evaluating the impact of inadvertent surgical injury is by testing postoperative neurological function via an open field test. Few studies have routinely incorporated this into their experimental design. Su et al. showed no significant difference in walking track performance, Sciatic Function Index, or Peroneal Function Index in any of three groups of laminotomized rats treated with low-dose radiation to the cauda equina, [9] demonstrating that no significant degree of neurological injury had occurred in these animals. While we expected to see a significant degree of motor dysfunction in the crush injury group, we did not anticipate the differences in locomotion seen during the first two weeks in the laminectomy-only animals. Had the animals sustained neurological injury during surgery, locomotor function would not have improved with time. We hypothesize that transiently lower open field scores in the laminectomy animals reflected behavior that was possibly related to pain and muscle spasm. %MPE values in the laminectomy and crush injury groups were immediately lower and remained so throughout the study. Allodynia could have been related to inadvertent neurological injury during surgery, but results from open field testing do not support this. Since fibrosis is a progressive mechanism it would be expected that allodynia results reflect this trend if peridural fibrosis is the only contributor. We did not find this to be the case. We hypothesize that liberation of cytokines and other immune-related factors in the vicinity of the neural elements may have contributed to sustained allodynia in laminectomy and crush injury animals. Most importantly, these results did not mirror those seen with open-field locomotor testing. Therefore, we would surmise that neurological injury did not cause allodynia in these animals.

Limitations of our study include the subjective histological analysis of peridural scar formation, which could be improved by using an objective classification scale similar to that used in other studies [1,8,16]. Furthermore, employing three-dimensional quantitative analysis or blinded radiographical interpretation of peridural fibrosis at different time points may help to strengthen the association between allodynia and extent of postoperative fibrosis.

Conclusion

This randomized study examined the effects of peridural fibrosis on postoperative allodynia in a rodent cauda equina crush injury model. While numerous studies have investigated this association, few have utilized methods like the operating microscope to exclude iatrogenic injury. Postoperative locomotor assessment is also not routinely employed to exclude neurological injury as a potential source of allodynia. Our results indicate that there was an association between

degrees of postoperative peridural fibrosis and allodynia. We were able to exclude by way of locomotor testing that neurological injury contributed to ongoing allodynia in the laminectomy and crush injury animals.

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