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Do steroid injections to the peripheral nerve increase perineural fibrosis? An animal experimental study

Mustafa Celiktas [1], Semih Kivanc Olguner [2], Kıvılcım Eren Erdogan [3], Remzi Caylak [4], Yusuf Kenan Daglioğlu [5]

[1] Department of Orthopaedics and Traumatology, Istinye University Faculty of Medicine Istanbul, Turkey
[2] Department of Neurosurgery, Adana City Training Research Hospital, Adana, Turkey
[3] Department of Pathology, Cukurova University School of Medicine, Adana, Turkey
[4] Department of Orthopaedics and Traumatology, Ortopedia Hospital, Adana, Turkey
[5] Department of Microbiology Kırsehir Ahievran University Faculty of Medicine Kırsehir, Turkey and President of Ahievran University Animal Experimental Research Center

ORCID ID of the author(s)

MC: 0000-0003-2084-9725
SKO: 0000-0002-5314-4636
KEE: 0000-0002-4951-8703
RC: 0000-0002-2926-4590
YKD: 0000-0002-1618-1075

Corresponding Author
Mustafa Celiktas
Department of Orthopaedics and Traumatology, Istinye University Faculty of Medicine Istanbul, Turkey
E-mail: mstfceliktas@yahoo.com

Abstract

Background/Aim: Perineural fibrosis may be seen in some surgeries after unsuccessful transforaminal injections. This animal experiment aims to investigate the effect of steroids and/or local anesthetic substances used in epidural/transforaminal injections on fibrosis.

Methods: A total of 14 male Wistar-Albino rats were separated into two groups of 7. After intraperitoneal anesthesia, right and left sciatic nerves were explored in all groups. In group 1, appropriate dose of methylprednisolone acetate and bupivacaine hydrochloride, and in group 2 only methylprednisolone acetate was administered to the explored left sciatic nerves. The right sciatic nerves were identified and explored without the application of any procedure to secure the control side of the groups. All explored areas were marked for later sampling. After 3 weeks, rats were sacrificed and samples were taken around the sciatic nerve for histopathological examination.

Results: In group 1, perineural fibrosis around the left sciatic nerve (intervention side) was seen at grade 3 in five rats and at grade 0 in two. In right sciatic nerve as the control side, grade 2 fibrosis was observed in 5 rats, and fibrosis was not observed in two rats. No statistically significant difference was defined with respect to perineural fibrosis (*P*=0.128). In group 2, perineural fibrosis was seen around the left sciatic nerve (intervention side) at grade 3 in 5 rats, at grade 4 in one rat, and not observed in one rat. In the control side of the second group, perineural fibrosis was seen at grade 3 and 1 in one each, and was not seen in five rats. The difference between intervention side and control side in the rate of perineural fibrosis seen was statistically significant (*P*=0.026).

Conclusions: The application of steroids alone to the nerve was determined to increase the risk of perineural fibrosis development. The addition of local anesthetics to the steroid in the injection may reduce the possibility of perineural fibrosis.

Keywords: Perineural fibrosis, Sciatic nerve, Methylprednisolone, Bupivacaine, Transforaminal injections

Introduction

Lumbar spinal stenosis is characterized by various degrees of lower back and leg pain due to pressure in neural and vascular tissues in the lumbar spine [1]. Spinal stenosis may be central or lateral [2], and regardless of its anatomic position, the resulting pain is explained by two mechanisms. The first of these is mechanical pressure on the nerve root with the narrowing of the bone foramen. The second is inflammatory immunological processes occurring in this region with or without mechanical pressure, which may result in radicular and neurological signs that include neural hyperemia, venous congestion and edema [3, 4] .

Treatment method of patients with radicular leg pain is determined by the severity of symptoms, comorbidities, presence of risk factors for anesthesia and surgery, and patient expectations [5, 6]. Physical treatment exercises, non-steroid anti-inflammatory drugs (NSAIDs) and activity modifications combined with epidural/foraminal steroid injections are possible options [7], from which, transforaminal injection of steroids at the closest point of the nerve root is accepted as the most definitive and effective management algorithm [8]. Transforaminal or epidural steroid injection are shown to reduce symptoms in 60-75% of patients with stenosis [9-11].

In cases where pain persists or relapses despite transforaminal injection, surgical treatments are considered, but perineural fibrosis on the affected nerve makes surgery more difficult and reduces the chance of success. Perineural fibrosis is often encountered in revision surgeries in particular.

Our hypothesis is that substances frequently used in injections may cause or increase perineural fibrosis.

Materials and methods

Experimental animals

This study was approved by the local Animal Research Ethics Committee of Çukurova University (meeting date 2017.04.14, meeting 4, decision 5). Principles of laboratory animal care (NIH publication No. 86-23, revised 1985) were followed and all animal rights have been complied with throughout the entire study.

A total of 14 young adult male Wistar-Albino rats, each weighing 200±20 g, were obtained from the breeding colony. The number of rats was determined to be the minimum suitable for statistical analysis. The rats were randomly separated into two groups for the application of Methylprednisolone acetate or the combination of Methylprednisolone acetate and Bupivacaine hydrochloride. A single appropriate dose of methylprednisolone acetate+ Bupivacaine hydrochloride was applied to the left sciatic nerve of seven rats (Group 1) and a single appropriate dose of Methylprednisolone acetate was applied to the left sciatic nerve of seven rats (Group 2). All animals were followed up in a temperature-controlled room (23±1°C), with a day/night light cycle and free access to laboratory food and tap water.

Sciatic nerve dissection

All rats were anesthetized with the intraperitoneal administration of a mixture of 10 mg/kg ketamine and 10 mg/kg xylazine. Following anesthesia both hind limbs were shaved and then prepared with 10% povidone iodine solution. A 2-cm

incision was made and using soft dissection, the right and left sciatic nerves were exposed through the gluteal muscle (Figure 1).

Figure 1: White arrow indicates the sciatic nerve exposed through the gluteal muscle
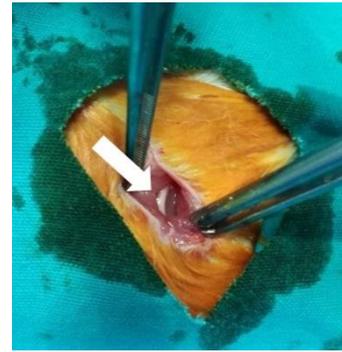
Application of drugs

The animals in group 1 received a mixture of 0.57 mg/kg Methylprednisolone acetate and 0.07 mg/kg Bupivacaine hydrochloride in same syringe, and animals in group 2 received 0.57 mg/kg Methylprednisolone acetate solution. The injections were made to the left sciatic nerve after dissection. In calculating the dose of the drug to be administered, mg/kg equivalent to one drug dose administered to adult humans was calculated. After the drug injections, a prolene marker suture (4/0) (ETHICON, San Lorenzo, USA) was placed adjacent to the nerve to create a landmark for pathological sampling. In both groups, the right sciatic nerves were identified and only marker sutures were placed adjacent to the nerve without any drug administration. In all groups, the layers on both sides were closed with vicryl (4/0) suture.

Histopathological examination

The animals were sacrificed at third week, the marked sutured areas were reopened, and tissue samples were obtained from an area of approximately 1 cm^2 surrounding the nerve. After the biopsy samples were fixed in 10% formaldehyde solution and prepared for pathological examination, 4-micron sections were stained with hematoxylin eosin. Trichrome staining was performed using the histochemical method. Pathologists performed single-blind evaluations for the samples. Perineural fibrosis around the sciatic nerve was evaluated according to the staging and histological parameters of Nahm et al. [12] by two different pathologists, on a 5-grade scale, where grade 0 defines absence of fibrosis, grade 1 defines loose or focal fibrosis, grade 2 defines loose or diffuse fibrosis (>50%), grade 3 is given in dense or focal fibrosis, and grade 4 is given in dense or diffuse fibrosis (>50%). Inflammation was evaluated based on the number of mononuclear cells and aggregation as defined by Salafia et al. [13]

Statistical analysis

Data obtained in the study was analyzed statistically using SPSS 24.0 software (IBM Corporation, Armonk, NY, USA). Comparisons between groups based on perineural fibrosis and chronic inflammation were made using the Kruskal-Wallis H Test. A value of $P<0.05$ was accepted as statistically significant.

Results

Complications or infection were not observed in the study group before the intervention or during the follow-up period of animals. In both left and right sides of all animals in

both groups, chronic inflammation was not observed according to the Salafia et al. [13] classification.

In group 1, perineural fibrosis around the left sciatic nerve (intervention side) was seen at grade 3 in 5 rats and at grade 0 in 2. In the right sciatic nerve (control side), grade 2 fibrosis was seen in 5, and perineural fibrosis was not observed in two rats. No statistically significant difference in perineural fibrosis was determined (P=0.128).

In group 2, perineural fibrosis around the left sciatic nerve (intervention side) was seen at grade 3 in 5 rats (figure 2) and grade 4 in 1, and was not seen in 1. In the right sciatic nerve (control side), perineural fibrosis was seen at grade 3 and 1 in one rat each, and was not seen in 5 (figure 3). The difference in the rate of perineural fibrosis seen was statistically significant in group 2 (P=0.026).

Figure 2: Group 2 left side (intervention side of the steroid-only group), histochemical staining with Masson's trichrome under x200 magnification. The arrow indicates fibrotic areas that have migrated into fat tissue consisting of fibroblasts and capillary vessels around nerve plexuses, consistent with grade 3 fibrosis.
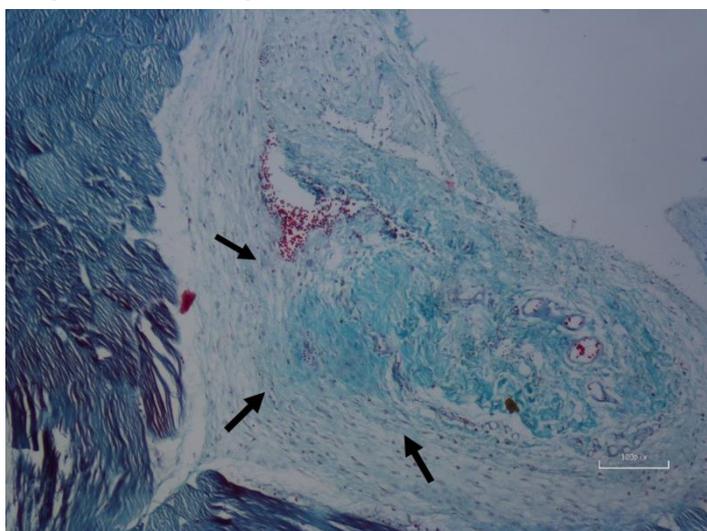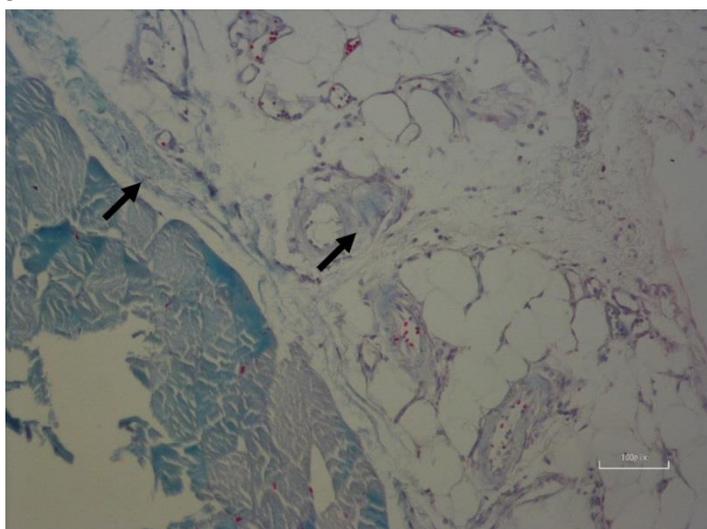

Figure 3: In Group 2 right side (control side of the steroid only group), histochemical staining of Masson's trichrome; the arrows indicate fine fibrotic band formation and small vessel proliferation detected at x200 magnification under microscope view, consistent with grade 0 fibrosis.

Mean perineural fibrosis was 2.14 (Min 0 - Max 3) on the intervention side and 1.43 (Min 0 - Max 3) on the control side in group 1 (Methylprednisolone acetate +bupivacaine hydrochloride group). Mean perineural fibrosis was 2.71 (Min 0 - Max 4) on the intervention side and 0.57 (Min 0 - Max 3) on the control side in group 2 (Methylprednisolone acetate group).

A 3-D graphic of the mean values on the intervention and control sides of both groups is shown in figure 4.

Figure 4: 3-D graphic of intervention and control mean values for both groups
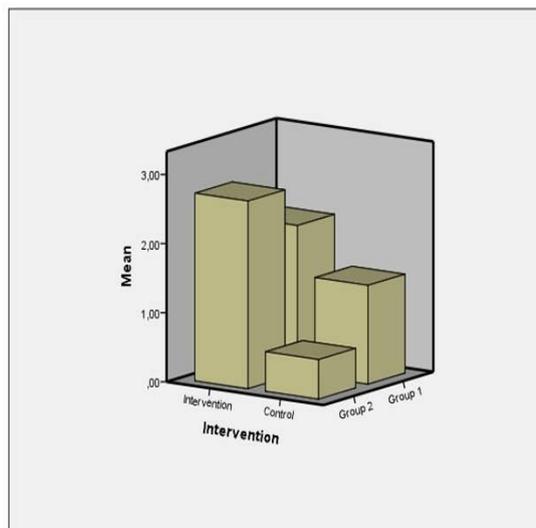
Discussion

When all conservative treatment methods of spinal stenosis are unsuccessful, transforaminal-epidural steroid injection (TFESI) and surgery are possible options [6, 14]. TFESI results in less morbidity and is a much cheaper method than surgery. When the costs are compared, the costs of nerve root injection are approximately 600 GBP, while decompression of one or two levels is 3300-4000 GBP and decompression and posterior fixation or interbody fusion is about 5800-6400 GBP [6, 15]. Therefore, injections are the first choice of procedures in spinal stenosis cases that do not respond to more conservative methods.

It has been hypothesized that continuous compression of nerve roots in spinal stenosis damages micro vessels and leads to ischemia, edema, demyelination, and C-fiber activation [16]. When there is only neurological compression, there is known to be neurological deficit rather than pain. However, progression of the inflammatory process increases nerve root sensitivity, resulting in a continuous feeling of pain, even with mild stimuli, and this demonstrates the importance of inflammatory cytokines, especially in radiculopathy cases [6]. Thus, inflammation's important role in the formation of lower back and leg pain in spinal stenosis patients is a known fact. Steroids inhibit the expression and synthesis of pro-inflammatory substances and are used in treatment to suppress the production of arachidonic acid and metabolites [2, 17], reducing the inflammatory process.

There are various assumptions regarding the repetition times of steroid injections. The general practice is to repeat an injection when a patient partially benefits. If there is no benefit, the decision to repeat the injection or to intervene surgically should be shared and decided with the patient. It is possible that surgery may not resolve all symptoms. Furthermore, by creating a scar, each operation may cause the formation of re-stenosis in the spinal canal [9, 10]. In the presence of epidural fibrosis, whether it develops with the primary disease or not, tension in the dura and nerve roots after surgery or after injection creates chronic pain. In addition to making surgery more difficult, the presence of fibrosis reduces injection efficacy in all steroid applications by preventing a sufficient dose of the drug from

reaching the site [18, 19]. The procedure applied should not have a tendency to increase fibrosis.

Percutaneous adhesiolysis is a minimally invasive method used to reduce fibrosis [20]. Park et al. [7] compared percutaneous adhesiolysis with transforaminal epidural steroid injection for the treatment of chronic radicular pain caused by lumbar foraminal spinal stenosis and reported that both methods provided significant pain relief. With the removal of epidural space barriers themselves thought to contribute to the pain, percutaneous adhesiolysis allows the delivery of pain-relieving drugs [19].

In transforaminal steroid applications, the drug administered during injection may be only steroid or may be supplemented with local anesthetic substances added to the syringe. The full inflammation suppression of steroid may require a relatively long period, but the addition of a local anesthetic substance permits the patient to be mobilized after one hour and questioned whether the pain has diminished. Thus it can be re-confirmed that the injection location was correct. Patient satisfaction (a priority) increases.

Of the local anesthetics first used, 2% lidocaine hydrochloride was one of the most common. However, side-effects associated with sympathetic nerve or motor nerve blockage (nausea, hypotension, headache, and ataxia) were seen at 1-3%, and it is known that an excessive dose may cause systemic reactions like vasovagal reaction, convulsions and respiratory depression [21, 22]. For these reasons, there has been research into the use of other substances. Some studies recommended saline injection rather than lidocaine, in addition to steroid [2]. To provide superior pain relief for chronic radiculopathy back pain secondary to foraminal stenosis, Behnam et al. [23] added hypertonic saline to epidural steroid injections (ESI) but observed no significant difference between ESI with and without hypertonic saline. In addition, some studies have stated that hypertonic solutions may be more effective in high-grade or long-lasting nerve compression, and it has been reported that hypertonics may reduce pain due to the adhesiolysis mechanism in post lumbar surgery syndrome, seen in spinal stenosis [24, 25].

Hyaluronidase addition to injections has been attempted to reduce fibrosis. Yousef et al. [26] compared the treatment outcomes of 38 patients who received either caudal injections of bupivacaine+ hypertonic saline + methylprednisolone or same combination with 1,500 units of hyaluronidase added. In this small prospective study, only the patients who received hyaluronidase continued to experience benefits at sixth and twelfth months post-treatment. In another study, it was shown that the group receiving hyaluronidase with steroid had more relief than the group who received only steroid and/or bupivacaine [27]. There is moderate evidence supporting the use of hypertonic saline and limited evidence for the use of hyaluronidase to prevent adhesion and increase the benefit seen by the patient [7]. Ng et al. [28] reported no significant difference in outcome on three month follow-up examination between the use of steroid and local anesthetic combination and the use of only local anesthetic injection. In contrast, Sahu et al. [29] used a uniform pharmacological combination of long-acting local anesthetic (0.5% Bupivacaine) and steroid

(Methylprednisolone) in patients and found a significant difference for up to six months.

It is thought that the long-term effect of local anesthetics may depend on the volume given during injection. In a study of fluid volume delivered to the epidural space during injection, injections of a greater volume were seen to provide greater pain relief. It was stated that the mechanism of this was not only that adhesiolysis was not made by the greater volume, but that by washing the epidural space, inflammatory cytokines were removed from the damaged nerves and blood flow was increased, even to ischemic nerve roots. This effect was reported to be related to the volume of the injection independent of the steroid dose, starting in short term with maintenance until the mid-term [30].

In this animal experimental study, the left leg was used as the intervention site, and the right leg as the control. This was intended to minimize individual differences in fibrosis development. In group 1, in which methylprednisolone acetate and bupivacaine hydrochloride were applied, perineural fibrosis was seen more on the intervention side than the control side, but the difference was not statistically significant. In group 2, where only methylprednisolone acetate was used, statistically significantly more perineural fibrosis was seen on the intervention side than the control side. The significant increase in fibrosis within only a three week period indicates that more fibrosis could be seen in the long term. The use of a lower volume in the group administered methylprednisolone only could have been responsible for the increase in fibrosis.

Limitations

The primary limitation of this study was the low number of animals included in the experiment population. Another limitation is that on subjects with dissection, the possibility of fibrosis caused by a drug given in injection was investigated. We think that we have overcome this handicap by exploring two of each subject's sciatica and applying medication to the left sciatica and not applying it to the right sciatica, thereby providing standardization. Thus, we evaluate only the fibrosis due to the medications given, not the open dissection and thus, overcome potential bias. Future research would be better with a third group as a combination of saline and steroid administered, to clearly understand whether the increased injection volume or the use of bupivacaine reduced the appearance of perineural fibrosis.

Conclusion

Steroid administration via injection is an effective treatment method for lumbar spine foraminal stenosis. Increasing the injection volume along with the addition of local anesthetic to the steroid may be helpful in adhesiolysis and may reduce the future development of fibrosis. Nevertheless, there is a need for further experimental studies of factors that decrease or increase fibrosis.

Acknowledgments

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