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Comparison of curcumin and methylprednisolone in the prevention of epidural fibrosis after spinal surgery: An experimental study

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Abstract

We aimed to compare the effectiveness of curcumin, which has been shown to inhibit cell migration and proliferation, with that of methylprednisolone, which has been shown to have a similar mechanism of action to that of curcumin and to delay tissue repair and wound healing, in the prevention of epidural fibrosis in an experimental laminectomy model in rats. The experiment was conducted with 40 male Wistar rats weighing 250-300 g which were divided into four groups. Laminectomy was performed from L1 to L4 in all groups. Curcumin and methylprednisolone were applied to the other two groups except the control and DMSO groups. The vertebral colon was excised en bloc and each section was examined for epidural fibrosis formation under a light microscope to evaluate histologically using the grading system. Results: The statistical analyses indicated that the treatment groups had a significant effect on the histological grades of epidural fibrosis in rats compared to the control group. Curcumin, derived from the rhizome (Rhizoma Curcumae) of turmeric (Curcuma longa) was found to show a significant effect in the prevention of epidural fibrosis formation with its potent antioxidant and anti-inflammatory properties and by inhibiting further recruitment of fibroblasts to the laminectomy area. In the present study, both histological examination and statistical analyses indicated that the administration of curcumin led to a remarkable reduction in epidural fibrosis after laminectomy.

Keywords: Curcumin, epidural, fibrosis, metilprednisolone

1. Introduction

Epidural fibrosis (EF) refers to the excessive formation of a scar tissue in the epidural space following lumbar laminectomy. EF is a common cause of persistent pain after spinal surgery and of failed back surgery syndrome. The EF incidence has been found to be 20–47% (Guler et al., 2020). Various substances have been used to prevent EF; however, the use of these substances has been limited to clinical trials (Wu et al., 2016; Wang et al., 2018). Meanwhile, it is commonly known that local administration of agents that prevent cell migration and proliferation can reduce EF formation. Curcumin (diferuloylmethane) is a fraction of Curcuma longa. Many studies have reported the antioxidant, anti-inflammatory and analgesic effects of curcumin (Ji et al., 2013). Its preventive effect on lung fibrosis has been demonstrated in experimental studies (Xu et al., 2007). Curcumin was effective in the treatment of neuropathic pain and prevented hyperalgesia by altering adrenergic and serotonergic inhibition in the brainstem (Ji et al., 2013).

Corticosteroids are frequently used for their anti-inflammatory effects. They can block COX-2 mRNA expression, resulting in suppression of prostaglandin production and also interleukins and other inflammatory mediators to reduce pain. In many studies, it has been shown that methylprednisolone, a long half-life depot steroid, has long-term contact with neural tissue and provides a wide distribution on the spinal cord surface (Rasmussen et al., 2008).

In this study, we aimed to compare the effectiveness of curcumin, which has been shown to inhibit cell migration and proliferation, with that of methylprednisolone, the mechanism of action of which is similar to that of curcumin, in preventing EF in experimental laminectomy rat model.

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2. Materials and methods

After obtaining approval from the local ethics committee for animal research, we conducted our experiment using 40 male Wistar rats weighing 250–300 g. The animals were reared in an environment with appropriate temperature and humidity levels, and they were given standard rat chow and ad libitum access to water. The rats were divided into four groups. Thirty minutes prior to the surgical procedure, prophylaxis was given via intraperitoneal administration of a single dose of cefazolin sodium (20 mg/kg) (Cefazolin, Bilim Pharmaceuticals, Istanbul, Turkey). Intramuscular ketamine hydrochloride (25 mg/kg; Ketalar, Pfizer, Istanbul, Turkey) and xylazine (5 mg/kg; Rompun, Bayer, Istanbul, Turkey) was induced for anesthesia. After the prone position fixation, the surgical site was disinfected with povidone–iodine solution (Poviod; 10% polyvinylpyrrolidone iodine complex, Saba, Turkey). A 3-cm midline skin incision was made over the spinous processes. The paravertebral muscles were stripped through blunt and sharp microdissection. L1- 4 vertebrae laminectomy was performed. The ligamantum flavum and the epidural fat tissue were removed, and the lumbar epidural space was exposed after hemostasis was achieved. None of the rats had any dural tears and injuries to a nerve root during the procedure.

The first group of rats received laminectomy only, and the layers were closed in the anatomical plane. The second group received 0.5 mg/kg curcumin (Sigma–Aldrich, Chemical Co., USA) diluted in DMSO solution at 1:10 ratio and applied topically on the epidural space with cotton pad for 5 min. After 5 min, the soaked cotton was removed from the surgical site, and the layers were closed in the anatomical plane. The third group received 1 mg/kg methylprednisolone applied topically on the epidural space with cotton for 5 min. The fourth group received topical DMSO saline solution applied to the surgical site with cotton for 5 min. Subsequently, the soaked cotton was removed from the surgical site, and the layers were closed in the anatomical plane. The rats were maintained and followed up in a room with a temperature of 28°C for post-anesthesia recovery. All surgical procedures were performed under an OpMi (Carl Zeiss, Germany) microscope with 16× magnification. None of the subjects had an infection.

After 3 weeks, the rats were sacrificed with a high dose (75–100 mg/kg) of intraperitoneal sodium thiopental (Pentothalsodium, Abbott, Italy). The vertebral column, which includes the laminectomy site, was removed cautiously.

2.1. Histopathologic examination

The vertebral colon was excised en bloc, fixed in 10 mM phosphate buffer, and decalcified in 10% formic acid for 10 days. Subsequently, samples were obtained from the laminectomy site, washed for 6 h under running water, and then subjected to routine paraffin tissue procedure. Paraffin blocks were dissected into 5-µm-thick sections and then stained with hematoxylin and eosin. Each section was examined for EF formation under a light microscope, and the EF scar tissues were graded histologically using the grading system proposed by He et al. (He et al., 1995) (Table 1).

Table 1. Histological grading of epidural

Grade 0	No epidural scar tissue
Grade 1	Only thin fibrous bands between the scar tissue and dura
Grade 2	Continuous adherence in less than 2/3 of the laminectomy defect
Grade 3	Dense epidural scar tissue, affecting more than 2/3 of the laminectomy defect

2.3. Statistical analysis

Statistical analyses were performed using SPSS for Windows Version 25.0 (IBM Corp. Released 2017, Armonk, NY: IBM Corp.). The frequency distribution of fibrosis scores was compared using the Chi-square test ($\chi 2$), and the likelihood ratio Chi-square test was performed given that values in one or more cells was smaller than 5. A p value of <0.05 was considered significant. Given that the proportion of cells with an expected value of <5 is more than 20%, the Fisher Exact test results were considered

3. Results

In the histological examination, dense epidural scar tissue formation and hemorrhage were detected in the laminectomy site in the control group. The scar tissue adhered to the dura mater, and the epidural space was covered by a fibrous tissue (Fig. 1a). A significant difference in the distribution of EF grades was observed in the different groups ($p < 0.05$). In the control, methylprednisolone, and DMSO groups, 100%, 70.0%, and 80.0%, respectively, of the EF cases are grade 3; in the curcumin group, 50.0% of the EF cases are grade 2 (Table 2). The control and curcumin groups significantly differed in terms of grade 2 EF ($p < 0.05$), wherein the incidence rate was higher in the curcumin group (50.0%) (Table 3) No significant difference was observed between the control and methylprednisolone groups ($p > 0.05$) (Table 4) as well as between the control and DMSO groups ($p > 0.05$) (Table 5) in terms of the distribution of EF grades. By contrast, a significant difference was observed between the curcumin and methylprednisolone groups in terms of the distribution of EF grades ($p <0.05$) The EF grade with a high incidence rate in the curcumin group was the grade 2 EF (50.0%) whereas that in the methylprednisolone group was grade 3 EF (70.0%) (Table 6). Similarly, a significant difference was observed between the curcumin and DMSO groups in terms of the distribution of EF grades ($p < 0.05$). The EF grade that had a high incidence rate in the curcumin group was grade 2 EF (50.0%) whereas that in the DMSO group was grade 3 EF (80.0%) (Table 7). No significant difference was found between the methylprednisolone and

DMSO groups in terms of the distribution of EF grades ($p > 0.05$) (Table 8; Fig. 2).

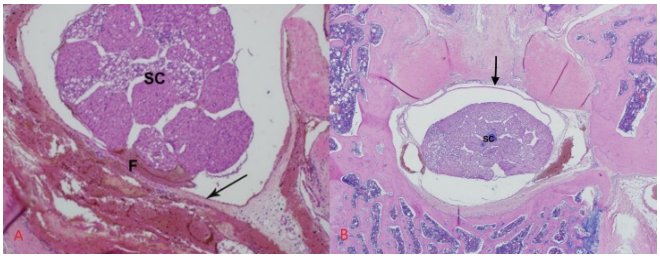

Fig. 1. A. Epidural fibrosis adhered to the dura mater. B. Curcumin shows a significant effect in the prevention of EF formation. SC: Spinal cord, F: Fibrosis

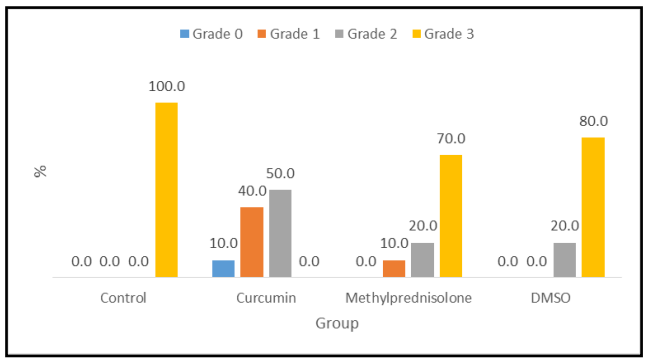

Fig. 2. The statistical analysis indicated that the treatment groups had a significant effect

Table 2. Grade stage relationship with the group

	Grade 0	Grade 1	Grade 2	Grade 3	X^2	p
Control	0. 0	0 (0.0)	0 (0.0)	10 (100.0)	25.877	0.000*
Curcumin	1 (10.0)	4 (40.0)	5 (50.0)	0 (0.0)		
Methylprednisolone	0 (0.0)	1 (10.0)	2 (20)	7 (70.0)		
DMSO	0 (0.0)	0 (0.0)	2 (20.0)	8 (80.0)		

Table 3. Relation of control, curcumin and grade stage

	Grade 0	Grade 1	Grade 2	Grade 3	X^2	p
Control	0.0	0 (0.0)	0 (0.0)	10 (100.0)	20.596	0.000*
Curcumin	1 (10.0)	4 (40.0)	5 (50.0)	0 (0.0)		

Table 4. Control, methylprednisolone and grade stage relationship

	Grade 0	Grade 1	Grade 2	Grade 3	X^2	p
Control	0.0	0 (0.0)	0 (0.0)	10 (100.0)	3.069	0.211
Methylprednisolone	0 (0.0)	1 (10.0)	2 (20)	7 (70.0)		

Table 5. Relation of control, DMSO and grade stage

	Grade 0	Grade 1	Grade 2	Grade 3	X^2	p
Control	0.0	0 (0.0)	0 (0.0)	10 (100.0)	x	0,474
DMSO	0 (0.0)	0 (0.0)	2 (20.0)	8 (80.0)		

Table 6. Curcumin, methylprednisolone and grade stage relationship

	Grade 0	Grade 1	Grade 2	Grade 3	X^2	p
Curcumin	1 (10.0)	4 (40.0)	5 (50.0)	0 (0.0)	11.085	0.006*
Methylprednisolone	0 (0.0)	1 (10.0)	2 (20)	7 (70.0)		

Table 7. Curcumin, DMSO and grade stage relationship

	Grade 0	Grade 1	Grade 2	Grade 3	X^2	p
Curcumin	1 (10.0)	4 (40.0)	5 (50.0)	0 (0.0)	14.394	0.000*
DMSO	0 (0,0)	0 (0,0)	2 (20.0)	8 (80.0)		

Table 8. Methylprednisolone, DMSO and grade stage relationship

	Grade 0	Grade 1	Grade 2	Grade 3	X^2	p
Methylprednisolone	0 (0.0)	1 (10.0)	2 (20)	7 (70.0)	1.129	0.999
DMSO	0 (0.0)	0 (0.0)	2 (20.0)	8 (80.0)		

4. Discussion

EF is defined as the excessive formation of a scar tissue in the epidural space following a lumbar laminectomy; it is a major cause of persistent pain after spinal surgery, and it often

causes hospital re-admissions. Due to fibrosis, the adhesions in the epidural region prevent the movement of a nerve in a nerve root canal (Zhang et al., 2015). Following reoperations due to epidural fibrosis, adhesions may develop again. New and varied treatment options that prevent the development of epidural fibrosis have become necessary as the current treatments are not sufficiently effective with prolonged medical treatments and re-surgical interventions.

The formation of an epidural scar tissue is an indication of wound healing. EF results from the migration of fibroblasts induced by the chemotactic agents released as a result of the degradation of erythrocytes and thrombocytes after hemorrhage. Fibrosis formation begins in the early stages of inflammation and consists of four phases, namely, fibroblast migration and proliferation, extracellular matrix deposition, angiogenesis, and finally reorganization of the scar tissue (Eming et al., 2014). The resulting fibrotic tissue causes distension or compression of a root nerve, ultimately leading to a radicular pain after a spinal surgery (Wang et al., 2018).

Fibroblast and vascular endothelial cells begin to proliferate within 24 h after tissue damage and also after a surgery, leading to granulation tissue formation, which is an indication of wound healing. Given that the volume of fluid and protein leakage from new vessels induced by angiogenesis is relatively high, the granulation tissue formed in this stage displays an edematous appearance (Martin and Nunan, 2015). Fibroblast growth factor, platelet-derived growth factor, epidermal growth factor, and transforming growth factors are well-known inducers for fibroblast migration, as well as macrophages and lymphocyte-derived cytokines. Some cytokines, such as tumor necrosis factor (TNF) and interleukin 1 (IL-1) stimulate collagen synthesis. In advanced stages of fibrosis, the cellular components of a granulation tissue diminish, leading to the formation of a rigid tissue that is denser than collagen and elastic fibers and that involves a small number of vessels (Martin and Nunan, 2015). In our study, intense fibrosis was observed in the entire control laminectomy group, and the fibrosis caused adhesions to the dura mater (Fig.1a).

Although medical agents such as analgesics, myorelaxants, steroids, and/or local anesthetic injections are commonly used in the treatment of EF to eliminate the symptoms associated with fibrosis, a standard treatment for the underlying cause of EF remains unavailable. The abovementioned medical therapies are supported by symptomatic treatment methods, such as physical therapy, bed rest, and exercise (Zhang et al., 2015). However, given that these treatments do not eliminate a scar tissue, patients' complaints are only reduced and not completely gone.

For the prevention of EF progression, the most commonly used and recommended technique that causes less tissue damage is microsurgery (Zavyalov and Pretechikov, 2016). Given that microsurgery leads to less dead space in the paraspinal region and epidural area, it is likely to result in less scar tissue. Moreover, given that the blood elements remaining in the epidural area may aggravate a scar tissue, a rapid hemostasis is needed. Another technique used to prevent EF is the preservation of the ligamentum flavum along with the epidural adipose tissue, although this technique demonstrates limited effectiveness (Dobran et al., 2017).

Literature reviews have shown that although numerous clinical and experimental studies on EF prevention have been conducted, no definitive outcomes have been obtained. Additionally, some experimental studies have used various synthetic and organic substances in combination with drugs to prevent scar tissue formation and adhesion in laminectomy defects (He et al., 1995; Tatsui et al., 2006; Tao and Fan, 2009; Wang et al., 2018).

Methylprednisolone is a type of synthetic glucocorticoid (steroid) drug. Steroids are known to exhibit anti-inflammatory, anti-allergic, and immunosuppressive activity, and they also affect the hematopoietic system. Steroids prevent the migration of cells to areas of inflammation, and they inhibit the synthesis of acute-phase reactants as well as the synthesis and release of cytokines (Liu et al., 2017). Moreover, they inhibit further recruitment of neutrophils and monocytes/macrophages in an inflammation site and reduces the number, the effect, and the proliferation of fibroblasts in a connective tissue.

A previous study reporting on the effect of long-acting steroids on EF prevention has revealed that the intraoperative irrigation of long-acting dexamethasone in the laminectomy site significantly reduced hospitalization and narcotics usage as a result of the inhibition of anti-inflammatory effects and pain mediators (Wilson et al., 2018). Consistent with these findings, our results showed that compared with the control and DMSO treatments, the administration of epidural methylprednisolone prevented EF formation. Also, the incidence rates of fibrosis grades were significantly low under this treatment.

Curcumin is the main bioactive component derived from the rhizome (Rhizoma Curcumae) of turmeric (Curcuma longa), which belongs to the ginger family Zingiberaceae. Curcumin is used in wound healing and in the treatment of arthritis owing to its antibacterial and anti-inflammatory properties; it is also used in the treatment of cancer patients, particularly in preventing the development of lymph node metastasis and the progression of tumors (Martin and Nunan, 2015; Liu et al., 2017). Additionally, curcumin has been shown to inhibit hydrogen peroxide-induced damage in human keratinocytes and fibroblasts (Liu et al., 2016; Zhao et al., 2017). This effect indicates that curcumin exhibits antioxidant activity in wound healing. Studies have evaluated the effect of curcumin on wound healing in rats, and they found that the administration of curcumin enhanced wound healing, improved cell proliferation, and demonstrated an

efficient free radical scavenging activity compared with the control and collagen treatments (Gopinath et al., 2004; Bang et al., 2018). Furthermore, curcumin significantly suppresses TNF-α-induced neuroinflammation and the expression levels of IL-6, PGE2, and COX-2. Our results showed that the rate of grade 2 EF was higher in the curcumin group than in the control group. Conversely, the rate of grade 3 EF was higher in the control group than in the curcumin group. This finding may have been caused by the various effects of curcumin. Moreover, curcumin has been found to decrease post-traumatic inflammation owing to its anti-inflammatory properties in experimental spinal cord trauma studies (Xiao et al., 2017; Bang et al., 2018).

In a study, curcumin was considered to provide therapeutic benefits by suppressing lung fibroblasts through the inhibition of the protein kinase C epsilon, leading to the absence of apoptosis in normal fibroblasts (Xu et al., 2007). The abovementioned studies strengthen the theory that fibroblasts may be inhibited by curcumin and thus may play a role in preventing EF. Our study also showed that curcumin exerted a significant effect in the prevention of EF formation by inhibiting further recruitment of fibroblasts to the laminectomy site compared with the control and methylprednisolone treatments (Fig. 1b).

Our study showed that various degrees of fibrosis occurred in the laminectomy area. Moreover, our results showed that curcumin significantly reduced fibrosis, but further studies are needed to identify which anti-inflammatory mechanisms play a role in this phenomenon. In addition, visualizing the adhesions in the epidural area through radiological examinations will render future investigations sounder.

EF is a major spinal surgery complication that causes pain. Numerous studies have reported on the surgical techniques and treatment methods used to prevent the onset of this complication. However, most of these studies have not provided definitive or clinically positive outcomes. The present study indicated that the administration of curcumin remarkably reduced the incidence of EF after laminectomy, suggesting that curcumin is an agent that can effectively reduce EF formation owing to its potent anti-inflammatory and anti-fibroblastic activity as demonstrated in the experiments.

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