

ORIGINAL ARTICLE

Locally Used Antibiotics for Spinal Infection Prophylaxis and Their Effects on Epidural Fibrosis: an Experimental Laminectomy Study in Rats Using Rifamycin and Gentamycin

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Abstract— The study aims to assess the effects of antibiotics (ABs), which are typically used in spinal infection prophylaxis, on the formation of epidural fibrosis (EF). Specifically, we investigated the effect of rifamycin and gentamycin on EF formation in laminectomized rats. Thirty-two rats were randomly and equally divided into four groups as follows: laminectomy and physiological saline (0.9% NaCl) solution (control); laminectomy and rifamycin; laminectomy and gentamicin; and laminectomy and a mixture of rifamycin and gentamicin. Laminectomy was performed on L1 and L2 vertebrae in all rats. One month after spinal surgery, spinal tissue samples surrounding the laminectomy were cut with a microtome and stained with hematoxylin-eosin and Masson's trichrome. The histopathological analysis included examining the extent of EF, fibroblast cell density, and cartilage and bone regeneration. Statistical analysis was performed using the IBM SPSS Statistics 22 program (SPSS IBM, Turkey). A value of $p < 0.05$ was considered statistically significant. EF value differences between the AB treatment groups and the control group were statistically significant ($p = 0.030$). Specifically, binary comparisons indicated that the EF value was significantly higher in the rifamycin group than that in the control group ($p = 0.003$; $p < 0.05$). Our study suggests that locally applied ABs, especially rifamycin, should be diluted before administration to the epidural space.

KEY WORDS: epidural fibrosis; failed back surgery syndrome; gentamicin; inflammation; irrigation solution; prophylaxis; rifamycin; spinal infections.

INTRODUCTION

Postoperative epidural fibrosis (EF) is an extradural adhesion that develops after spinal surgery (SS) and is the result of the natural healing process and is normally seen to some extent. If this process progresses, fibrotic tissue increases and takes the place of the normal epidural fat tissue causing stretching and compression of the roots and leading to failed back surgery syndrome (FBSS). As the most common unwanted complication of SS, FBSS is observed in 10–40% of cases with SS [1–5], and currently, there is no effective treatment of this syndrome [1–3, 6, 7]. Additionally, parallel with the increase in SS, the risk of spinal

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infection (SI), which is one of the devastating complications of this intervention, is increasing, and this pathology can also result in FBSS or even death [4, 7–11]. It has been reported that the most common pathogen causing SI is *Staphylococcus aureus* [9, 11–14]. Rifamycin and gentamycin are the preferred antibiotics (ABs) in SI prophylaxis because of their proven effectiveness against *S. aureus* and cost-efficiency [12, 13, 15–23].

Furthermore, if these ABs are administered systemically, the side effects are excessive, which is the advantage of local AB administration [24, 25]. Prophylaxis against SI is the best method to prevent these problems. However, thus far, no study has compared the effects of these ABs on EF, which are typically applied locally to the area of laminectomy as dilutions (using the physiological saline solution as diluent) to provide SI prophylaxis. In this study, we aimed to investigate the effect of undiluted rifamycin and gentamycin on the formation of EF in a rat model of laminectomy.

MATERIALS AND METHODS

Experimental Design and Animal Care

The experimental procedures in this study were approved by the Local Ethics Committee of the Animal Experiments of the University of Health Sciences. The animals, Sprague-Dawley rats, were given *ad libitum* access to a standard rodent diet and water and were housed at a constant temperature (22 °C) on a 12:12-h light:dark cycle.

Surgical Procedure

Thirty-two (the number of animals was determined by power analysis) adult male Sprague-Dawley rats weighing 350 ± 50 g were randomly assigned to four study groups of equal size ($n = 8$): control group 1, laminectomy and physiologic saline solution; group 2, laminectomy and rifamycin; group 3, laminectomy and gentamicin; and group 4, laminectomy and rifamycin and gentamicin. Prior to surgery, rats were anesthetized by intraperitoneal administration of ketamine hydrochloride (60 mg/kg, Ketalar, Pfizer, Istanbul, Turkey) and xylazine hydrochloride (10 mg/kg, Rompun 2%, Bayer, Istanbul, Turkey). The rats were placed on the operating table in the prone position, the dorsal hair was shaved, and the surgical area was disinfected with 10% povidone-iodine (PVP-I) solution and covered with sterile towels. A midline incision was made between the 12th dorsal and third lumbar vertebrae. After paravertebral dissection of the muscles, a full laminectomy was performed on the lumbar two vertebrae. After laminectomy was performed on the rats in group 1, a

0.5×0.5 -cm surgical patty soaked with physiological saline (0.9% NaCl) was placed on the laminectomized area for 10 min. In group 2, a 0.5×0.5 -cm surgical patty soaked with 0.1 ml of undiluted rifamycin (maximal dose) from the ampoule containing 250 mg/3 ml rifamycin SV (Sanofi Aventis, Istanbul, Turkey) was placed on the laminectomized area and allowed to remain in contact with the epidural space for 10 min. In group 3, a 0.5×0.5 -cm surgical patty soaked with 0.1 ml of undiluted gentamicin (maximal dose) from the ampoule containing 80 mg/2 ml gentamicin (IE Ulagay, Istanbul, Turkey) was placed on the laminectomy area for a 10-min contact period with the epidural space. In group 4, the 0.5×0.5 -cm surgical patty was soaked with 0.05 ml of rifamycin and 0.05 ml of gentamicin (half-maximal dose of each AB) and placed on the laminectomy area for a 10-min contact period with the epidural space. The skin was closed using a non-absorbable surgical suture (3-0 Silk Braided Surgical Suture, Trabzon, Turkey), and the procedure was completed by applying PVP-I on the incision.

Histopathological Analysis

Four weeks after the surgery, each of the spinal blocks that were transferred into buffered formalin (10%) was coded and kept separately in the fixation and decalcification solution (BiocalC, Code: RRDC3/G, Specifications: EDTA < 1%, potassium sodium tartrate < 1%, sodium tartrate < 1%, hydrochloric acid < 1%; Biostain, UK) for 36 h and three horizontal strips of 2–3-mm thickness were sampled to include the area of laminectomy from each block. Cassettes placed in the Tissue Tracking Device (Leica ASP 300S, Wetzlar, Germany) were paraffin-blocked using the Tissue Embedding Device (Shandon Histocentre 3, MN, USA) after tissue tracing. Two slides were prepared in 4- μ m sections from each of the blocks with a microtome. After deparaffinization, one of the slides was manually stained with hematoxylin-eosin (Shandon Harris hematoxylin-eosin Y-UK) and the other with Masson trichrome (Bio-Optica kit, Milan-Italia). All histopathological evaluation was done

Table 1. Grading of Epidural Fibrosis Tissue

Grade	Definition
0	The dura mater was free of scar tissue.
I	Only thin fibrous bands between scar tissue and dura mater were observed.
II	Continuous adherence was observed but was less than two thirds of laminectomy defect.
III	Scar tissue adherence was large, more than two thirds of laminectomy defect, and/or extended to the nerve roots.

Table 2. Grading of Fibroblast Cell Density

Grade	Average number of fibroblasts
I	Less than 100 fibroblast cells per $\times 400$ field (+)
II	100–150 fibroblast cells per $\times 400$ field (++)
III	More than 150 fibroblast cells per $\times 400$ field (+++)

blindly by the same pathologist. Microphotographs were taken with an adaptive digital camera using an Olympus CX41 RF trinocular light microscope (Tokyo, Japan). The grade of EF extension was determined according to the criteria established by He et al. (Table 1) [26]. Fibroblast cell density grades are presented in Table 2.

Statistical Analysis

The experimental data were statistically analyzed using IBM SPSS Statistics 22 (SPSS IBM, Turkey). When the study data were evaluated, the suitability of parameters to the normal distribution was assessed by the Shapiro-Wilks test. The Kruskal-Wallis test was used for comparing the parameters that did not show a normal distribution in comparisons of quantitative data; the Mann-Whitney *U* test was used to identify the group that caused the difference among multiple groups. Fisher-Freeman-Halton test was used for comparing qualitative data. Differences were considered to be significant at $p < 0.05$. Statistical evaluation was done blindly by an experienced statistician.

RESULTS

Clinical Observation

None of the rats developed an infection, cerebrospinal fluid accumulation, redness, or hematoma in the wound area and the sutured area of the peripheral tissues.

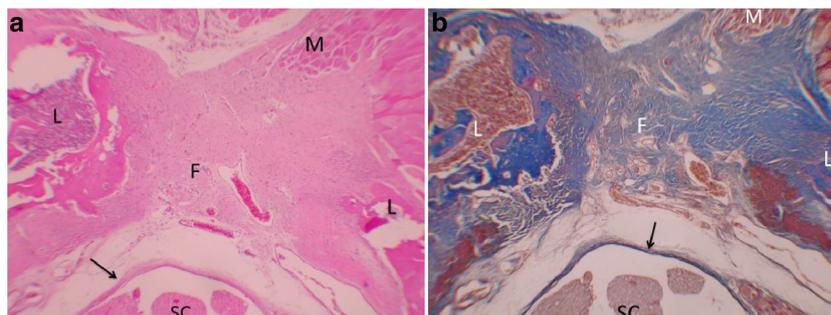


Fig. 1. Histology of spinal sections from control group rats not treated with ABs. Grade I fibrosis at the laminectomy area revealed by **a** hematoxylin and eosin (H&E) ($\times 40$) and **b** Masson trichrome ($\times 40$). L, lamina; F, fibrosis; SC, spinal cord; arrow, dura; M, Muscle.

Furthermore, no paraplegia or paraparesis was observed in any animal. However, during the 1-month observation period following the surgical procedure, four rats in total, two rats each from the gentamicin group and the rifamycin and gentamicin mixture group, died. Therefore, the histopathological analysis was performed on 28 rats.

Histopathological Evaluation

In the control group rats without AB treatment, grade 1 (62.5%) and grade 2 (37.5%) EF were observed but not grade 3 EF (Fig. 1). However, in the rifamycin group, grade 2 and grade 3 EF were found to be equally distributed among the animals (50%), whereas grade 1 EF was not observed (Fig. 2). The gentamicin group had grade 1 (33.3%) and grade 2 EF (16.7%) in 50% of the rats and grade 3 EF in the other 50% (Fig. 3). In the group subjected to the combination treatment of rifamycin and gentamicin, most animals had grade 2 (66.7%) EF, and grade 1 and grade 3 EF were also observed but at a lower rate (16.7%) (Fig. 4). There was a statistically significant increase in EF values in the AB-treated groups as compared to that in the control without ABs ($p = 0.030$, $p < 0.05$) (Table 3). The binary comparisons indicated that the EF values of the rifamycin group were statistically significantly higher than that of the control group ($p = 0.003$; $p < 0.05$) (Table 3). However, there was no statistically significant difference in EF values between the other groups ($p > 0.05$). Furthermore, there was no statistically significant difference between the groups regarding fibroblast density values, rates of cartilage regeneration, and rates of bone regeneration ($p > 0.05$) (Table 3).

DISCUSSION

SI is also one of the causes of FBSS, and despite the prophylaxis with systemic AB, after SS, SI occurs at a rate

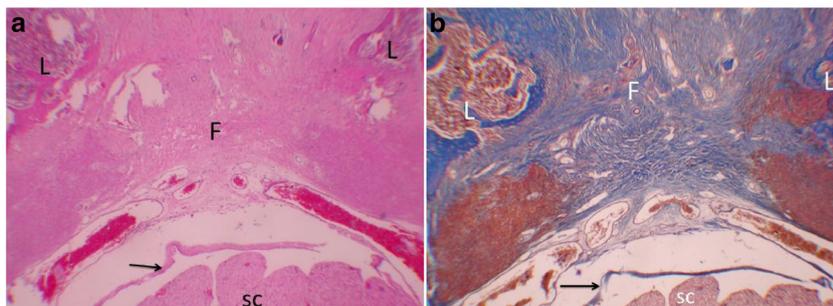


Fig. 2. Histology of spinal sections from laminectomized rats treated with rifamycin. Grade III fibrosis at the laminectomy area revealed by **a** hematoxylin and eosin (H&E) ($\times 40$) and **b** Masson trichrome ($\times 40$). L, lamina; F, fibrosis; SC, spinal cord; arrow, dura.

of 0.7–16%, and if instrumentation is applied, the risk increases to 20% [27–29]. Because hematoma, seroma, and ischemic tissues in surgical wounds prevent the affected regions from receiving the systemic dose of ABs, local administration of ABs achieves a high drug concentration in these regions and limits the systemic toxicity [30, 31]. Although it is assumed that bacteria are iatrogenically inoculated at the time of surgery, which should be eliminated by systemic AB prophylaxis, some authors claim that in cases with increased disc degeneration, parenterally administered ABs cannot penetrate to the disc space and, thus, cannot protect against SI [7, 32]. Many studies have already shown that preoperative local AB administration significantly reduced the postoperative (PO) infection rate as compared to that of preoperative systemic AB administration [10, 23, 28].

Because of its wound healing properties, rifamycin has been used in the irrigation of open and closed wounds since 1963 [33, 34]. According to the literature, the local administration of rifamycin to wounds is rarely associated with side effects [35], but its effect on EF is still not fully known. To the best of our knowledge, there is no study comparing the effects of ABs on EF that are typically applied locally to the laminectomy area for SI prophylaxis.

In our study, we determined that administration of the maximal rifamycin dose to the epidural area resulted in a statistically significant increase in EF in the first PO month as compared to that of the control group (Table 3).

Rifamycin is a semisynthetic macrolide obtained by oxidizing rifamycin B, which is produced by the gram-positive bacterium *Streptomyces mediterranei*. This AB has a high efficacy in treating *Mycobacterium tuberculosis*, *Mycobacterium leprae*, and *Staphylococcus aureus* infections. Rifamycin acts in these bacteria by inhibiting the initiation of RNA synthesis due to binding to the β -subunit of the RNA polymerase [36]. In addition to its antibacterial effect, rifamycin exerts anti-inflammatory and immunomodulatory effects that are exploited in rheumatoid arthritis therapy [37]. Rifamycin inhibits the synthesis of proinflammatory cytokines (IL-6, TNF α) and chemokines (chemokine RANTES) in monocytes, macrophages, and CD4⁺ T cells [38]. In our study, we monitored the effect of rifamycin on EF formation during the first post-SS month. However, wound healing is a long process, and the inflammation phase during the initial stage lasts for more than 2 weeks, followed by proliferation and remodeling as the last stages. The fibrillar connective tissue accumulates around the lesion and develops into a scar tissue that can

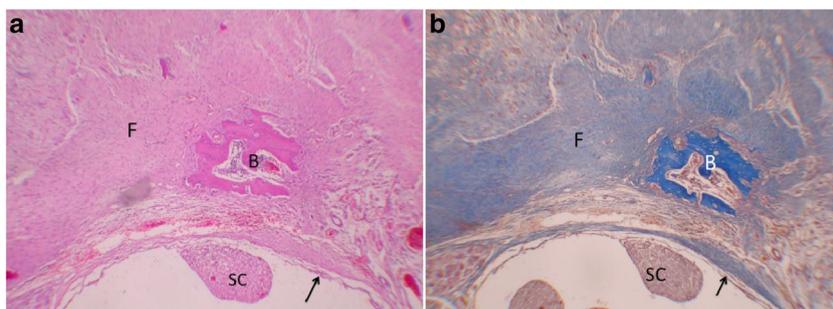


Fig. 3. Histology of spinal sections from laminectomized rats treated with gentamycin. Grade II fibrosis at the laminectomy area revealed by **a** hematoxylin and eosin (H&E) ($\times 40$) and **b** Masson trichrome ($\times 40$). F, fibrosis; B, bone regeneration; SC, spinal cord; arrow, dura.

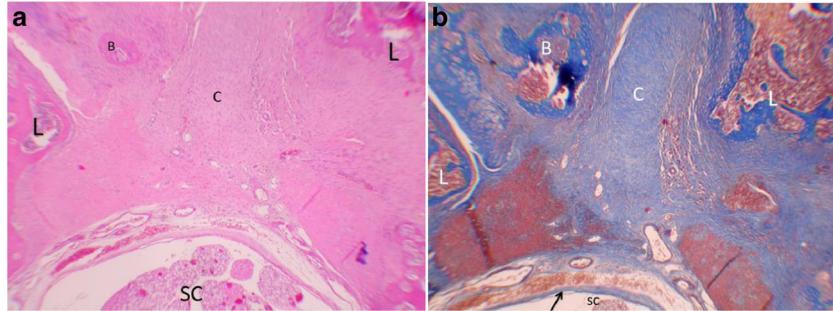


Fig. 4. Histology of spinal sections from laminectomized rats treated with rifamycin and gentamycin. Grade III fibrosis at the laminectomy area revealed by **a** hematoxylin and eosin (H&E) ($\times 40$) and **b** Masson trichrome ($\times 40$). B, bone regeneration; C, chondroid regeneration; L, lamina; SC, spinal cord.

take months or even years to progress [5, 26, 39]. In addition, considering that Caruso et al. showed that the anti-inflammatory effect of rifamycin started after 5 months post-SS, an observation period of 90 days or more will be required to assess the impact of rifamycin on FBSS [37]. Therefore, it will be critical for assessing the potential effect of AB treatment on FBSS formation to extend the period for observing the effects of select ABs on the EF etiology in FBSS beyond the first-month post-SS.

Many antiseptic agents used in wound care have been shown to prolong inflammation and delay collagen synthesis by toxic effects on fibroblasts [34, 40]. In SS, acceleration of wound healing in the skin and the epidural space is important, and the agents used in these areas should not be toxic to fibroblasts. In our study, we did not observe a statistically significant difference in fibroblast density values between the groups, indicating the absence of adverse effects on these cells by the locally used ABs ($p > 0.05$) (Table 3).

The first study on the origin of epidural scar tissue was performed on discs excised from dogs by Key and Ford in 1948, suggesting that EF originated from surgically injured annulus fibrosis [41]. Three decades after this study, LaRocca and Macnab found that the surgical lesion behind the spinal canal facilitates the formation of the epidural scar by proliferating fibroblasts in the deep layers of the sacrospinalis muscles; the authors called this formation the laminectomy membrane [42]. However, the three-dimensional adhesion mechanism developed by Songer and Ghosh Spencer found broader acceptance [5, 43]. These authors suggested that the scar tissue around the dura mater originated not only from the sacrospinalis located posterior but also from the fibrous ring and the posterior longitudinal ligament located further away, suggesting that the fibrous tissue hyperplasia around the ventrolateral nerve root was caused by epidural adhesions [43]. In our histopathological analysis, we did not detect any EF in the ventrolateral portion of

Table 3. Results of Histopathological Analysis. Epidural fibrosis Grades, Densities of Fibroblasts, Cartilage, and Bone Regenerations

		Total	Rifamycin	Gentamycin	Rif and genta	Control	<i>p</i>
Epidural fibrosis	+	8 (28.6%)	–	2 (33.3%)	1 (16.7%)	5 (62.5%)	
	++	12 (42.9%)	4 (50%)	1 (16.7%)	4 (66.7%)	3 (37.5%)	
	+++	8 (28.6%)	4 (50%)	3 (50%)	1 (16.7%)	–	
	Median (Min-Max)	2 (1–3)	2.5 (2–3)	2.5 (1–3)	2 (1.75–2.25)	1 (1–2)	0.030 ^a
Fibroblast density	+	12 (42.9%)	3 (37.5%)	3 (50%)	1 (16.7%)	5 (62.5%)	
	++	8 (28.6%)	4 (50)	1 (16.7%)	2 (33.3%)	1 (12.5 %)	
	+++	8 (28.6%)	1 (12.5%)	2 (33.3%)	3 (50%)	2 (25%)	
	Median (Min-Max)	2 (1–3)	2 (1–2)	1.5 (1–3)	2.5 (1.75–3)	1 (1–2.75)	0.445 ^a
Cartilage regeneration	(–)	16 (57.1%)	5 (62.5%)	5 (83.3%)	1 (16.7%)	5 (62.5%)	0.154 ^b
	(+)	12 (42.9%)	3 (37.5 %)	1 (16.7%)	5 (83.3%)	3 (37.5%)	
Bone regeneration	(–)	15 (53.6%)	5 (62.5%)	4 (66.7%)	1 (16.7%)	5 (62.5%)	0.300 ^b
	(+)	13 (46.4%)	3 (37.5%)	2 (33.3%)	5 (83.3%)	3 (37.5%)	

^a Kruskal-Wallis test

^b Fisher-Freeman-Halton test

* $p < 0.05$

the nerve roots. This finding may also suggest that locally applied AB does not cause FBSS after 4 weeks.

Moreover, we found that the amount of EF formed during co-administration of ABs at half-maximal doses was lower than that at maximal doses (Table 3), suggesting that the EF formation rate was proportional to the dose of ABs.

Gentamycin, an aminoglycoside antibiotic, is widely used in irrigation solutions in surgery because it has a broad spectrum of activity against most gram-positive and gram-negative bacteria but its high systemic absorption is associated with ototoxicity and nephrotoxicity [21, 44]. Four rats died in our study during the 1-month observation period after SS, two rats in the group receiving a mixture of gentamicin and rifamycin and two rats in the gentamicin group but none in the control group or rifamycin-treated group (Table 3). The result demonstrated that gentamicin can be toxic even at the half-maximal dose.

Our study examined the early effects on EF after SS caused by the administration of undiluted ABs, which are currently applied in clinical practice as dilutions (using the physiological saline solution as diluent) for prophylaxis against SI in SS. It will be necessary to determine whether the increase in EF, which occurred during the early period after SS by applying undiluted rifamycin, will continue and lead to FBSS. Therefore, there is a need for larger animal studies comparing EF formation when using ABs at the maximal dose or ABs at different dilutions with physiological saline during post-SS periods that are longer than 1 month.

CONCLUSIONS

In our study on the post-SS period, we found that local administration of the maximal rifamycin dose into the epidural space for SI prophylaxis led to an increase in EF during the first month after the surgery. This result was statistically significant with the maximal dose of the AB whereas the half-maximal dose did not cause a statistically significant difference in EF values. However, the 1-month post-SS observation period implemented in this study may not be enough to predict the long-term effect of this AB agent on FBSS. Hence, maximal rifamycin dose should not be used in the epidural space until the results of animal studies on the long-term effect of this AB on FBSS are available.

FUNDING INFORMATION

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COMPLIANCE WITH ETHICAL STANDARDS

Ethical Approval. “All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.”

Conflict of Interest. The authors declare that they have no conflict of interest.

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