# Effect of intrasite vancomycin powder on development of epidural fibrosis

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## Abstract

Placement of vancomycin powder into the surgical wound prior to closure has been shown to reduce postoperative infections in spine surgery. This study examines the effect of vancomycin powder on formation of epidural fibrosis (EF). Twenty-two rats underwent a two-level lumbar laminectomy. A control group, a low-dose and a high dose vancomycin powder (applied prior to closure) group was formed. Rats were sacrificed at 30 days and a blinded fellowship-trained pathologist evaluated the laminectomy segments for EF. 50% of the samples in the high-dose vancomycin group were EF grade 3, compared to 20% of the low-dose and 16.7% of control samples. The average fibrosis grade for the high dose, low dose and control groups were 2.4, 1.4 and 1.8, respectively. There were more grade 3 EF specimens in the high dose vancomycin group. While the average EF grade was also higher in this group, there was not a statistical difference compared with the other groups.

## Introduction

Despite advances in spine fusion techniques and surgical practices, surgical site infections (SSI) remain a significant complication of spine surgery.[1-4] The literature historically reports postoperative SSI rates between 3-40% based on the diagnosis and type/location of the procedure.[5-8] Additionally, SSI account for 22% of all healthcare-related infections, creating a significant financial burden.[9] The cost to the United States healthcare system has been found to range from $1 billion to $10 billion per year.[3,10] These costs stem from multiple reoperations, instrumentation removal, long-term antibiotic therapy and prolonged hospital stays.

To counteract SSI's, prophylactic parenteral use of a first-generation cephalosporin has been found to be safe and effective at reducing SSI, compared to placebo.[3,9] Common causes of postoperative spine infections are *Staphylococcus epidermidis*, which has the tendency to form a biofilm, and *Staphylococcus aureus*.[9] However, the incidence of infections has had a positive correlation with the increasing incidence of methicillin resistant *Staphylococcus aureus*. This has led some authors to advocate for prophylactic use of vancomycin, particularly in deformity correction where procedures are longer and hardware is critical. Parenteral vancomycin carries a risk of adverse reactions, including an anaphylactoid reaction known as red man syndrome, thrombophlebitis, nephrotoxicity, proteinuria, hepatotoxicity, ototoxicity and flushing.[1,9,10]

One solution to the adverse reactions of increased doses of parenteral vancomycin is the placement of lyophilized vancomycin powder (VP) into the surgical wound prior to closure. This method of application has now been shown to significantly reduce postoperative wound infections in animal and human studies, while avoiding systemic distribution of the drug.[4,10-13] Topical application of VP into a surgical incision can allow concentrations of antibiotic to reach up to 1,000 times the mean inhibitory concentration (MIC) needed to destroy methicillin resistant *Staphylococcus aureus*. It can also be used against bacteria moderately resistant to vancomycin at much lower systemic concentrations.[1,10] Despite increasing popularity in its use, there have been no specific animal or human studies to assess the effect of local vancomycin powder on spinal soft tissues.

Currently, the FDA does not approve vancomycin for topical application and there are no recommendations for dosing or timing of intrasite vancomycin. No studies have examined the direct effect of vancomycin-produced fibrosis at the site of a laminectomy adjacent to the dural tissue. Fibrosis and adhesion of scar tissue to adjacent dura have been shown to be relatively simple and reliable to quantify.[14] This study sought to contribute to the literature by evaluating the epidural fibrosis and local effect of direct topical application of lyophilized vancomycin to a laminectomy segment. We hypothesized that local administration of vancomycin powder directly to the epidural and intramuscular space would not demonstrate histologically significant fibrosis, adhesion, seroma formation or adverse effects on the dura.

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## Materials and Methods

The Einstein Healthcare Network Institutional Animal Care and Use Committee evaluated and approved the study protocol. Twenty-two 2-3-month-old male Sprague Dawley rats (Taconic, New York) were laminectomized at their L2-L3 spinal levels. All rats were randomized to intervention or control groups and labeled with a tattooed identification number. Three different groups were analyzed including a high dose (100 mg), low dose (50 mg), and control group (0 mg) of VP. Vancomycin powder was directly applied to the dura and dorsolaterally to the surrounding muscle, bone, and fascia after obtaining adequate exposure and for intervention groups after performing a laminectomy. Vancomycin powder was applied directly to the deepest aspect of the incision and a layer closure performed over the VP. Vancomycin dosage approximated the weight-based dose used in humans. The low dose 50 mg group correlated to the higher dose used in humans (15 to 60 mg/kg) and the high dose 100 mg

group received a doubled dose because direct application to surgical may not be uniform with clumping possible in deeper wound recesses or deep to implants. All rats received inhalational anesthetic with isoflurane during the procedure. They also received a perioperative dose of subcutaneous penicillin G benzathine/penicillin G procaine (100,000 units of penicillin G/kg) as well as Buprenex (buprenorphine 0.02 - 0.05 mg/kg IM or SC) 30 minutes prior to incision.

The rats were partially shaved and the surgical site was prepped with 10% povidone-iodide solution. A 2-3 cm incision was made in the skin and the posterior elements were identified. The paraspinal muscles were dissected from the posterior elements, and a laminectomy was performed. Controls were closed in a layered fashion with absorbable suture followed by prolene suture. Intervention groups received vancomycin powder applied directly to the dura, followed by the same closure (Figure 1). The rats were monitored in the acute postoperative period for abnormal behavior and neurological deficits. The rats were individually housed and fed a standard rat diet (Picolab Rodent Diet 20) and weighed weekly. They were allowed activity ad libitum. At 4 weeks post-operatively, all of the rats were euthanized with a lethal dose of $CO_2$. The L2-L3 vertebrae with surrounding soft tissues were excised *en bloc.* The specimens were bisected to ensure adequate fixation and fixed in 10% neutral buffered formalin (Thermo Scientific) for 5 days. The specimens were decalcified, dehydrated in alcohol, and embedded in paraffin. 5µm cross-sections were processed for hematoxylin and eosin staining as well as trichrome staining.

The histopathologic specimens with varying degrees of fibrosis were placed on slides and labeled with a randomized identification number. A fellowship-trained pathologist blinded to the process of the study assessed the slides for fibrosis. As previously described by Sae-Jung *et al*., the epidural fibrosis was graded at the dorsal aspect of the dura between the left and right margins of the laminectomy site.[14] The pathologist was familiar with the previously described technique for grading epidural fibrosis. The extent of fibrosis was rated as grade 0 (no fibrosis, dura free of fibrotic tissue), grade 1 (mild fibrosis, only thin fibrous band over dura), grade 2 (moderate fibrosis, continuous adherence less than two-thirds of laminectomy defect) and grade 3 (complete fibrosis, scar tissue adherence more than two-thirds of laminectomy defect and/or extending to nerve roots) (Figure 2). The obtained results of histopathologic grading were collected and analyzed.

## Statistical analysis

All data was analyzed using R Version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). EF grade was treated as an ordinal variable and was compared among the three groups using a Kruskal-Wallis test with increasing EF grade representing an increase in fibrosis. Fibrosis and complications were analyzed as nominal variables with an outcome of either 'yes' or 'no'. To categorize the fibrosis as a nominal value an EF grade of 0 or 1

**Table 1. Raw data, epidural fibrosis grade.**

| High Dose | Low Dose | Control |
|---|---|---|
| 3 | 1 | 2 |
| 2 | 1 | 3 |
| 3 | 1 | 2 |
| 3 | 2 | 2 |
| 1 | 3 | 1 |
| 2 | Expired | 1 |
| | | Expired |
| | | Expired |
| | | Expired |
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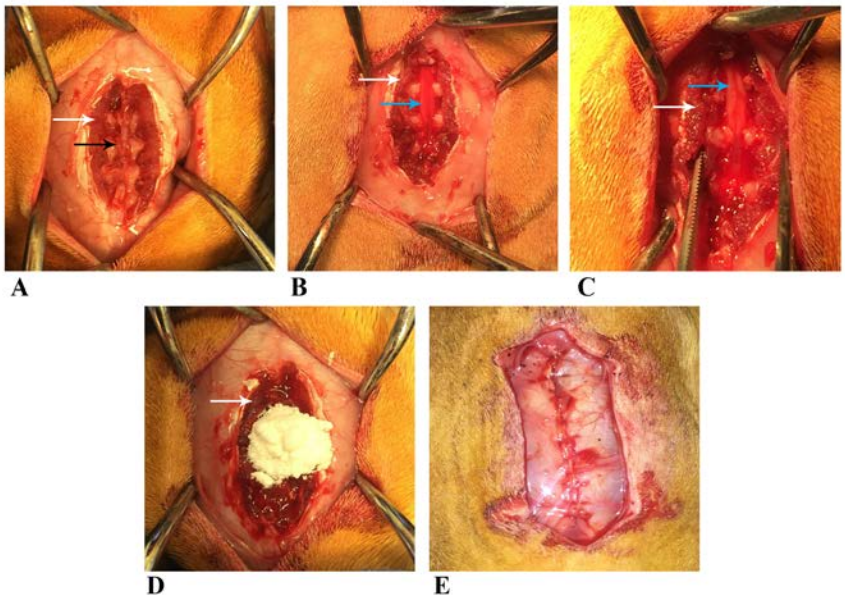


**Figure 1. A) Dorsal incision after exposure of the posterior elements of the lumbar spine. B) Dorsal incision demonstrating exposed dura after laminectomy. C) Higher magnification of the dorsal incision demonstrating exposed dura after laminectomy. D) Dorsal incision after laminectomy and addition of vancomycin powder. E) Dorsal incision after closure of fascia. (White Arrow: paraspinal muscles, black arrow: lamina, blue arrow: dura).**

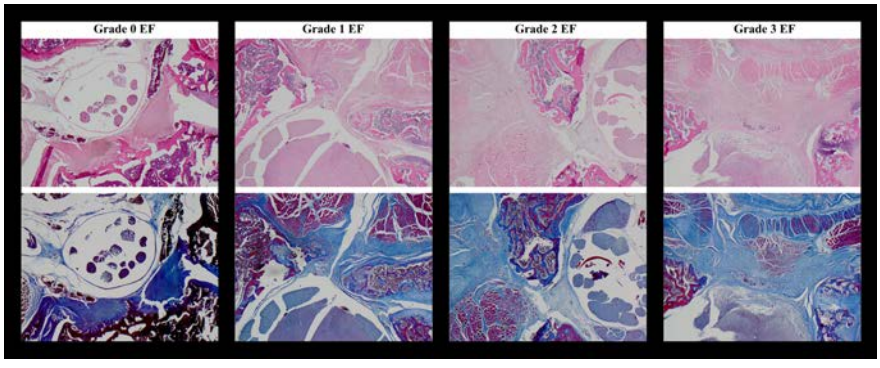


**Figure 2. Grade 0 EF (H&E stain above, Trichrome stain below). Arrow = dura. Note lack of fibrosis overlying the dura in a specimen examined immediately after dissection. Grade 1 EF (H&E stain above, Trichrome stain below). Arrow = mild amount of fibrosis overlying the dura. Grade 2 EF (H&E stain above, Trichrome stain below). Arrow = moderate amount of fibrosis overlying the dura. Grade 3 EF (H&E stain above, Trichrome stain below). Arrow = extensive amount of fibrosis overlying the dura.**

were considered 'no' while higher grades such as 2 and 3 were categorized as 'yes'. These outcomes were compared among groups using a Fisher's exact test. Given our small sample size a Fisher's exact test was chosen over a Chi-Square test to provide more precise comparisons. Statistical significance was taken at a P-value <0.05.

A retrospective power analysis was conducted for each test to determine the number of animals required to obtain statistical significance at the given power and expected effect sizes. For the Fisher's Exact Test of complications and fibrosis as a binary outcome, a large effect size of 0.8 was expected for each outcome. Using a significance level of 0.05 and power of 0.8, the number of animals needed per group was 24. For detecting differences between EF grade, a large effect size among the 3 group was also expected and set to be 0.4. With a significance level of 0.05 and a power of 0.8, 6 animals per group would be required to meet these parameters.

## Results

Raw data is demonstrated in Table 1. Three of 6 (50%) animals in the high dose vancomycin group demonstrated grade 3 EF (complete fibrosis). One of 5 (20%) of the low dose vancomycin group demonstrated grade 3 EF. One of 6 (16.7%) of control animals demonstrated grade 3 EF after undergoing laminectomy without vancomycin powder application.

Four (of 10) control animals did not have endpoint data to include in the analysis. Two expired during surgery and one expired prior to the completion of the 4-week study period without any evidence of suffering and one showed signs of distress and was euthanized prior to the endpoint. One (of 6) low-dose animals developed a foot drop and expired prior to completion of the study without evidence of distress and 1 (of 6) low-dose animals had a foot drop but showed no evidence of distress. Animals that expired prior to the primary endpoint (30 days) were not included in the histological analysis. Seventeen of 22 (77%) rats remained viable for histologic analysis at the study endpoint. There was no significant difference between the complications in any of the groups (P=0.24). There was no significant difference between EF grade when scored on the scale of 0 to 3 (P=0.32), nor was there a difference when comparing fibrosis as a binary outcome of absent (grade 0 and 1) or present (grade 2 and 3) (P=0.53).

## Discussion

The incidence, severity, and clinical implications of postoperative infections vary in spine surgery literature. Nonetheless, surgical site infections (SSI) in postsurgical spine patients place a significant burden on patients and the US medical system in the form of increased morbidity, mortality, and healthcare costs.[8,15,16] Prophylactic VP has been associated with minimal clinical adverse effects compared with parenteral administration. Intrasite VP has the benefit of producing supratherapeutic levels locally where the risk of infection is greatest, while avoiding the adverse effects of large systemic doses, including flushing, rashes, anaphylactic reactions, renal toxicity, hepatic toxicity and otological toxicity. While off-label in terms of FDA regulations, intrasite VP use been increasing in popularity and has been shown to be effective at reducing surgical site infections.[1,4,11-13,15,17,18] Though the use of intrasite VP has expanded in the past decade, little research has been done to examine its effects on local tissues.

Using a murine laminectomy model, this study applied topical vancomycin to the soft tissues of the spine prior to closure. Specifically, the VP was applied directly to the deepest aspect of the wound directly adjacent to the dura but also to surrounding structures similar to how it is spread intraoperatively in humans. To increase finding a potential effect of VP at the dura care was taken to contain nearly the entire dose directly adjacent to the dura. 3 different groups were analyzed including a high dose (100 mg), low dose (50 mg), and control group (0 mg) of VP. Dosages were designed to simulate the weight-based dose applied to humans at the low dose as well as higher dosing since direct application to wounds is not uniform and clumping of the VP may lead to a more robust local effect. While not statistically significant, our results demonstrated a trend towards a higher incidence of grade 3 EF in the high dose vancomycin group. If true, this would refute our original hypothesis that rates of fibrosis would be no different among groups. Given this data, future studies should focus on a larger sample size to ensure enough power to determine more subtle differences among groups.

The use of a murine model for preliminary analysis is appropriate due to similarities in structure to human paraspinal tissues. Furthermore, it avoids difficulties with obtaining human tissue to evaluate for scarring.[14] The pathological process, in this case fibrosis, is also more rapid, allowing an accelerated evaluation of epidural fibrosis. Additionally, the same rat model was used previously to show excellent interobserver reliability with the grading of epidural fibrosis.[14] The authors of this study also note that the histological grading of fibrosis may be applicable to axial MRI imaging in human patients.

The goal of this study was to provide histological evidence of the safety of intrasite vancomycin powder administration in spine surgery. As the first study to examine the potential effect of vancomycin powder on epidural fibrosis, we showed the murine model could be an effective tool. Our data showed no statistically significant difference among groups, suggesting no increased risk of epidural fibrosis with vancomycin administration after laminectomy. As a result of the small sample size of this study, these results should be taken in context. Moreover, given the trend towards higher grade epidural fibrosis in the high dose group, a larger study will be necessary to help determine if this is more than just chance and further delineate more subtle risks of vancomycin administration. After obtaining results, we retrospectively performed a power analysis based on our results and estimated that up to 24 animals per group would be required to determine if there was a significant difference between groups.

## Conclusions

This rat model suggests that the use of vancomycin powder does not have a significant effect on EF formed following laminectomy. However, higher concentration of vancomycin powder may increase the amount of high-grade EF. Given this data, if using vancomycin powder to help prevent SSI, we recommend avoiding direct application to the dura, applying it to the paraspinal musculature and/or lateral to the laminectomy sites and taking care to avoid large clumps where the increased concentration may lead to local effects. While vancomycin powder is used for infection prophylaxis, the optimal dose is unclear. Further studies should focus on dose-related effects of vancomycin powder and formation of EF.

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