Araştırma Makalesi *Research Article*

Pamukkale Tıp Dergisi
Pamukkale Medical Journal

doi:https://dx.doi.org/10.31362/patd.789163

Does vancomycin powder topically apply to the peripheral nerve cause perineural fibrosis?

Periferik sinir üzerinde topikal uygulanan vancomisin toz perinöral fibrozise neden olur mu?

Semih Kivanc Olguner, Mustafa Celiktas, Kivilcim Eren Erdogan, Kenan Daglioglu, Ismail Istemen, Ali Arslan, Vedat Acik, Ali Ihsan Okten, Yurdal Gezercan

Gönderilme tarihi:01.09.2020 Kabul tarihi:02.01.2021

Abstract

Purpose: The aim of this experimental rat study is to investigate whether vancomycin powder directly and topically applied on the sciatic nerve causes perineural fibrosis and chronic inflammation development.

Materials and Methods: Thirty young adult male Wistar-Albino rats were included in the study and divided into 3 groups of 10 rats based on the dose to be applied after intraperitoneal anesthesia. After the sciatic nerve exploration, 10 rats (group 1) were administered a single dose of vancomycin powder, 10 rats (group 2) were administered a double dose of vancomycin, and control group was created with 10 animals which were administered no medication (group 3). After 3 weeks, rats were sacrificed, tissue samples were taken around sciatic nerve and sent for histopathological examination.

Results: Grade 1 perineural fibrosis was detected in 1 animal (10%) in group 1. Grade 2 perineural fibrosis was detected in 1 animal (10%) and grade 1 perineural fibrosis was detected in 1 of 10 animals (10%) in group 2. In Group 3, grade 1 perineural fibrosis was detected in 1 experimental animal (10%). Chronic inflammation was not detected in any experimental animal. No significant difference was found between the 3 groups in terms of perineural fibrosis (p=0.753).

Conclusion: In this experimental animal study, we found that fibrosis development did not differ significantly among the groups. We believe that more objective results can be obtained as a result of experimental studies with larger participation and more detailed histopathological examinations.

Key words: Perineural fibrosis, sciatic nerve, vancomycin powder.

Olguner SK, Celiktas M, Eren Erdogan K, Daglioglu K, Istemen I, Arslan A, Acik V, Okten AI, Gezercan Y. Does vancomycin powder topically apply to the peripheral nerve cause perineural fibrosis? Pam Med J 2021;14:354-360.

Öz

Amaç: Bu deneysel sıçan çalışmasının amacı direk olarak siyatik sinir üzerine topikal uygulanan vancomisin tozun perinöral fibrozis ve kronik inflamasyon gelişimine neden olup olmadığını araştırmaktır.

Material and methods: Otuz adet erkek Wistar-Albino cins sıçan çalışmaya dahil edilerek intraperitoneal anestezi sonrası uygulanacak doz miktarına göre 10'arlı 3 gruba ayrıldı. Siyatik sinir ekplorasyonu ardından tek doz vankomisin toz verilen 10 sıçan (grup 1), çift doz vankomisin uygulanan 10 sıçan (grup 2) , 10 hayvana ise ilaç verilmeyerek kontrol grubu (grup 3) oluşturuldu. 3 hafta sonra ise sıçanlar sakrifiye edilerek siyatik sinir etrafındaki doku örnekleri alındı ve histopatolojik incelemeye gönderildi.

Bulgular: Grup 1'de 1 (%10) hayvanda evre 1 perinöral fibrosis tespit edildi. Diğer 9 (%90) deney hayvanında epidural fibrozis görülmedi. Grup 2'deki 10 deney hayvanından 1 tanesinde (%10) evre 2, 1 tanesinde ise

Semih Kivanc Olguner, MD. Department of Neurosurgery, Adana City Training Research Hospital, Adana, Turkey, e-mail: kivanc3olguner@hotmail.com (orcid.org/0000-0002-5314-4636) (Corresponding Author)

Mustafa Celiktas, Prof. Department of Spine surgery, Ortopedia Hospital, Adana, Turkey, e-mail: mstfceliktas@yahoo.com (orcid.org/0000-0003-2084-9725)

Kivilcim Eren Erdogan, MD. Department of Pathology, Cukurova University School of Medicine, Adana, Turkey, e-mail: kivilcimerenates@hotmail.com (orcid.org/0000-0002-4951-8703)

Kenan Daglioglu, Assoc. Prof. Department of Microbiology, Ahi Evran University School of Medicine, Kırşehir, Turkey, e-mail: kdagli01@gmail.com (orcid.org/0000-0003-3140-2937)

Ismail Istemen, MD. Department of Neurosurgery, Adana City Training Research Hospital, Adana, Turkey, e-mail: drismailistemen@gmail.com (orcid.org/0000-0002-2341-4818)

Ali Arslan, MD. Department of Neurosurgery, Adana City Training Research Hospital, Adana, Turkey, e-mail: aliarslan26062006@hotmail.com (orcid.org/0000-0002-7457-5283)

Vedat Acik, MD. Department of Neurosurgery, Adana City Training Research Hospital, Adana, Turkey, e-mail: vedatacik74@gmail.com (orcid.org/0000-0002-0371-5883)

Ali Ihsan Okten, Assoc. Prof. Department of Neurosurgery, Adana City Training Research Hospital, Adana, Turkey, e-mail: aihsano1@gmail.com (orcid.org/0000-0003-0292-201X)

Yurdal Gezercan, Assoc. Prof. Department of Neurosurgery, Adana City Training Research Hospital, Adana, Turkey, e-mail: gezercan@hotmail.com (orcid.org/0000-0002-4124-2036)

(%10) evre 1 perinöral fibrozis saptandı. Sekiz hayvanda (%80) ise perinöral fibrozis bulgusu saptanmadı. Grup 3 de ise 1 (%10) deney hayvanında evre 1 perinöral fibrozis saptandı. Kronik inflamasyon ise hiçbir deney hayvanında tespit edilmedi. İstatistiksel incelemeler sonucunda 3 grup arasında perinöral fibrozis açısından anlamlı farklılık saptanmadı (p=0,753).

Sonuç: Bu deneysel hayvan çalışmasında vankomisin toz uygulanan hastalarda fibrozis gelişiminin gruplar arasında anlamlı farklılık göstermediğini saptadık. Daha büyük katılımlı ve histopatolojik incelemelerin daha detaylı yapılacağı deneysel çalışmalar sonucunda daha objektif sonuçlara varılacağı kanaatindeyiz.

Anahtar kelimeler: Perinöral fibrozis, siyatik sinir, vankomisin toz.

Olguner SK, Çeliktaş M, Eren Erdoğan K, Dağlıoğlu K, İştemen İ, Arslan A, Açık V, Ökten AI, Gezercan Y. Periferik sinir üzerinde topikal uygulanan vancomisin toz perinöral fibrozise neden olur mu? Pam Tıp Derg 2021;14:354-360.

Introduction

One of the most feared complications of spine surgery is surgical site infection (SSI). It has been reported in the literature that the incidence in different series is between 0.7% and 12.0% [1-3]. SSI has been reported to increase healthcare cost, readmissions rate, and length of hospital stay [4-6]. To avoid these unfavorable situations, preventive strategies are being developed. The most common cause of wound infections in spine surgery is Staphylococcus epidermidis and Staphylococcus aureus. As the incidence of these two infections increases, the incidence of methicillin-resistant staphylococci increases, and cephalosporin and broad-spectrum antibiotics used in the treatment becomes ineffective against these resistant strains [7]. Vancomycin has been used for many years to treat infections caused by gram (+) cocci. Vancomycin, which is a tricyclic glycopeptide, shows serious toxic side effects in intravenous use, and that concerns many clinicians. Hypotension, nephrotoxicity, ototoxicity, hypersensitivity reactions, and red man syndrome are the most common side effects [4]. However, the administration of locally applied vancomycin passes to the systemic circulation at minimal doses prevents the occurrence of side effects and has been reported to be effective in the prevention of SSI [8-10]. Although systemic complications of vancomycin are well known, there are few studies on the side effects of its local use. Moreover, it has been introduced as a safe agent especially in spine surgery, it has been investigated in many studies whether it has pseudarthrosis, ototoxic and nephrotoxic effects [11, 12]. There is only one study in the literature investigating the effect of vancomycin powder on epidural fibrosis. This study was performed on rats undergoing laminectomy [5]. To the best of our knowledge, the first study conducted on peripheric nerves in the literature is our study. Based on this idea, we planned an animal experimental study considering whether vancomycin powder has an irritative effect on the peripheral nerve in histopathological terms, and investigated the presence of chronic inflammation and perineural fibrosis on rat sciatic nerve.

Materials and methods

Experimental animals

This study was approved by the local Animal Research Ethics Committee of Cukurova University and 30 young adult male Wistar-Albino rats were (bodyweight 200±20 g) obtained from the breeding colony of the animal house of University.

The rats were divided into three groups randomly corresponding to the dose and application of Vancomycin powder. Single-dose vancomycin powder was applied to 10 rats (Group 1) and double dose vancomycin powder was applied to 10 rats (Group 2). Ten rats did not take any medication and were accepted as the control group (Group 3). All animals were followed in a temperature-controlled room (23±1°C), with a day/night cycle, and had free access to laboratory food and tap water.

Sciatic nerve dissection

Rats were anesthetized by intraperitoneal administration of a mixture of 10 mg/kg ketamine and 10 mg/kg xylazine. Following anesthesia left hindlimb was shaved, prepped with 10% povidone-iodine solution. Two cm incision was made on the skin and by soft dissection, under an operation microscope, the left sciatic nerve was exposed through the gluteal muscle (Figure 1 and 2).

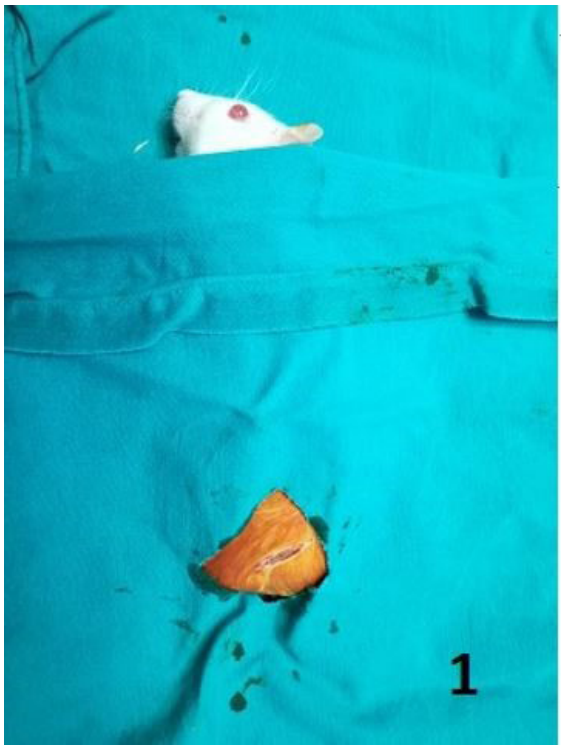

Figure 1. Following anesthesia, an incision was made on the left hindlimb and sciatic nerve exposed through gluteal muscle dissection

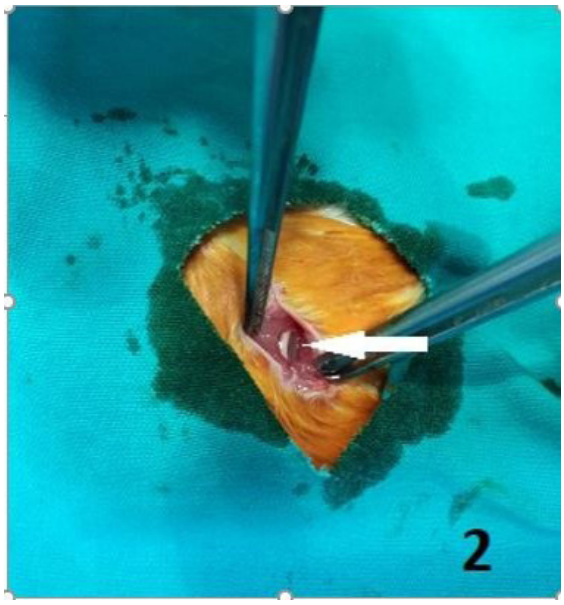

Figure 2. White arrow indicates exposed sciatic nerve through the gluteal muscle

Vancomycin powder application

Calculated as 14.3 mg/kg for a single dose of vancomycin and 28.6 mg/kg for a double dose of vancomycin, the animals were locally administrated. In calculating the dose of the drug to be administered, mg/kg required to be equivalent to 1 gr drug dose administered to adult humans were calculated. For the preparation of vancomycin powder preparations, support was received from an experienced pharmacologist. Vancomycin powder was poured onto the sciatic nerve at predetermined doses in rats in group 1 and group 2, and right after that, a marker suture prolene (4/0) (ETHICON, San Lorenzo, USA) was placed adjacent to the nerve to create a landmark for pathological sampling. In group 3, only marker sutures were placed adjacent to the nerve. In all three groups, the layers were closed with vicryl (4/0) (ETHICON, USA) suture.

Histopathological examination

Three weeks later, the animals were sacrificed, and the areas where the marker sutures were placed were reopened, and tissue samples were obtained from approximately 1 cm^2 area centering the nerve. Biopsy samples were fixated in 10% formaldehyde solution and then sampled for pathological examination. After routine fixation and follow-up procedures, 4-micron sections were stained with hematoxylin-eosin. Besides, trichrome staining was performed by the histochemical method. The cases were evaluated by pathologists with the single-blind method. In samples evaluated by two different pathologists, perineural fibrosis around the sciatic nerve was performed according to the staging and histological parameters of Nahm et al. [8]. These two parameters were the character of fibrosis (loose vs dense) and the extent of fibrosis (focal vs diffuse). Perineural fibrosis was evaluated on a 5-grade scale (grade 0 = absence of fibrosis, grade 1 = loose or focal fibrosis, grade 2 = loose or diffuse fibrosis (>50%), grade 3 = dense or focal fibrosis, and grade 4 = dense or diffuse fibrosis (>50%). Perineural inflammation was evaluated at the end of 21 days as indicated in Nahm et al.'s [8] study. Inflammation was evaluated based on the number of mononuclear cells and aggregation as defined by Salafia et al. [13]. The degree of chronic inflammation was graded as follows: grade 0 = absent, grade 1 = one focus of at least five mononuclear inflammatory cells, grade 2 = more than one focus of grade 1 or at least one focus of 5–20 mononuclear inflammatory cells, grade 3 = multiple confluent foci of grade 2, and grade 4 = diffuse and dense inflammation.

In the microscopical examination, fibrosis and inflammation were tried to observe around peripheral nerve sections.

Statistical methods

SPSS 24.0 (IBM Corporation, Armonk, New York, United States) software was used to analyze the variables. Comparison of the groups with each other based on perineural fibrosis and chronic inflammation was conducted with Kruskal-Wallis H Test, Monte Carlo Simulation, and Exact p values. Quantitative variables were shown as grouped median (Minimum-Maximum) in the tables. The statistical significance level was accepted as $p<0.05$.

Results

No infection was observed in the study group before the intervention or during the follow-up periods of the animals.

Grade 1 perineural fibrosis was detected in 1 animal (10%) in Group 1 (Figure3). Perineural fibrosis was not observed in the other 9 experimental animals (90%).

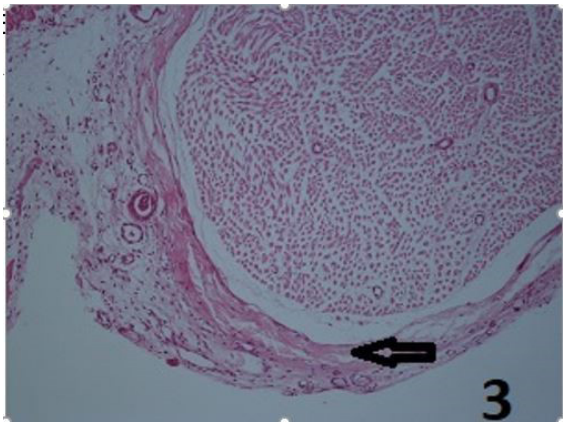

Figure 3. The histopathological evaluation revealed scattered fibroblastic activity with minimal inflammation. This finding was scored as minimal fibrosis around the nerve as graded. Following hematoxylin-eosin staining, the arrow indicates thin fibrotic band formation and small vessel proliferation detected in a rat in group 1 atx100 magnification under microscope view are consistent with grade 1 fibrosis

Grade 2 perineural fibrosis was detected in 1 animal (10%) and grade 1 perineural fibrosis was detected in 1 of 10 animals (10%) in group 2 (Figure 4). Eight animals (80%) had no signs of perineural fibrosis (Table 1).

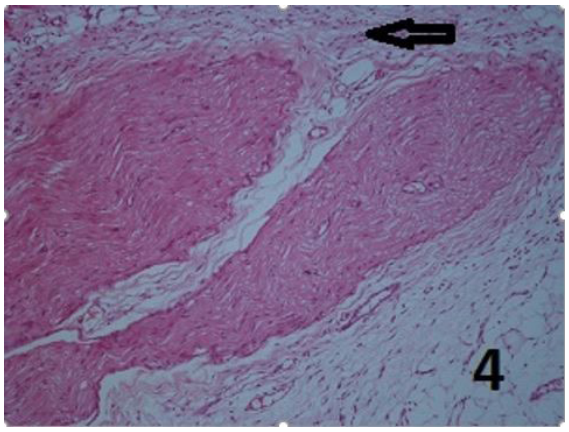

Figure 4. The histopathological examination revealed different fibrosis stages according to the grading scheme. The fibroblastic activity of more than 50% around the nerve sheath was detected in 1 rat in group 2 which was graded as 2 for perineural fibrosis. Hematoxylin-eosin sections under x40 magnification, the arrow indicates fibrosis areas that migrate into fat tissue consisting of fibroblasts and capillary vessels around nerve plexuses were consistent with grade 2 fibrosis

In Group 3, grade 1 perineural fibrosis was detected in 1 experimental animal (10%).

Chronic inflammation was not observed in any experimental animal.

No significant difference was found between the 3 groups in terms of perineural fibrosis statistically ($p=0.753$) (Table 2).

Discussion

In parallel with advances in modern medicine in recent years, access of patients to health services have become easier and surgical treatments have become more common. Spine and peripheral nerve surgery are also affected by this condition, and surgical treatments have begun to be applied especially in cases where there is no response to medical treatment. This has led to the incidence of more surgical site infections in clinical practice [1]. Vancomycin powder used to prevent postoperative surgical site infection in spine surgery has been the subject of many studies [7, 14-16]. Hypersensitivity reactions that may be caused by vancomycin used intravenously in clinical practice make many physicians reluctant to use

Table 1. The outcomes of the histopathologic evaluation of the study group

	Group 1 (n=10) n / (%)	**Group 2** (n=10) n / (%)	**Group 3** (n=10) n / (%)	**Total** (n=30) n / (%)	***p* value**
Perineural Fibrosis					
Grade 0	9 (90.0)	8 (80.0)	9 (90.0)	26 (86.7)	1
Grade 1	1 (10.0)	1 (10.0)	1 (10.0)	3 (10.0)	
Grade 2	0 (0.0)	1 (10.0)	0 (0.0)	1 (3.3)	
Chronic Inflammation					
Grade 0	10 (100.0)	10 (100.0)	10 (100.0)	30 (100.0)	-
Grade 1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

Fisher Freeman Halton Test (Monte Carlo)

*Group 1: Single dose vancomycin applied, Group 2: double dose vancomycin applied, Group 3: control group

Table 2. The statistical analysis results of the study group

Groups		**Perineural fibrosis Median (Min / Max)**	**Chronic Inflammation Median (Min / Max)**
Group 1	(n=10)	0.10 (0 / 1)	0 (0 / 0)
Group 2	(n=10)	0.22 (0 / 2)	0 (0 / 0)
Group 3	(n=10)	0.10 (0 / 1)	0 (0 / 0)
Total	(n=30)	0.14 (0 / 2)	0 (0 / 0)
P value		0.753 [a]	1 [b]

Kruskall Wallis H Test (Monte Carlo a / Exact b) / Min.: Minimum - Max.: Maximum.
Group 1: single dose vancomycin applied, Group 2: double dose vancomycin applied, Group 3: control group

this drug. Besides, the widespread intravenous use of vancomycin can lead to the formation of vancomycin-resistant strains and thus threaten public health. However, vancomycin applied locally remains in a closed area for a long time and thus causes limited participation in the systemic circulation [9, 17]. It is thought that the drug reduces the side effect profile [11]. Therefore, local and prophylactical use of vancomycin has become widespread rapidly. The studies investigating the side effects of vancomycin powder have questioned new bone formation, hypersensitivity reactions and epidural fibrosis [5, 11]. Although peripheral nerve surgery and spine surgery are generally examined under the same heading, most of the local vancomycin powder studies in the literature have been conducted in the spine surgery area, and there are no studies on peripheral nerves [6, 10, 14, 15, 18]. Therefore, we investigated the effect of local vancomycin powder directly on the sciatic nerve in our study.

In this study, as a result of vancomycin powder administration and histopathological examination in 2 different groups in different doses, grade 2 fibrosis development was observed in 1 rat in group 2 and grade 1 fibrosis development was observed in 1 rat. The nerve dissection was conducted under an operating microscope to prevent the development of epidural fibrosis from surgical trauma. No grade 2 fibrosis was detected in groups 1 and 3. Although there is no statistically significant difference in the degree of fibrosis observed between the 3 groups, we think that this result may be important. Detection of grade 1 fibrosis was detected in all 3 groups suggests that this may be due to surgical dissection. However, the presence of grade 2 epidural fibrosis in group 2, even in only one experimental animal, suggests that a dose-dependent effect may be observed. Clippinger et al. [5] applied vancomycin powder locally to rats following laminectomy and investigated the development of epidural fibrosis in their experimental study. Although no statistically significant difference was found between the control group and animals with vancomycin powder administered, it was emphasized that the result might be different in a study group with more rats.

In our study, the presence of inflammation, as well as fibrosis, was investigated, and no findings of chronic inflammation were detected in histological examination in all 3 groups. In this context, we did not find any irritant effect of vancomycin powder in our study.

The strong aspect of our study is the first study on peripheral nerves. Secondly, vancomycin powder applied to the sciatic nerve spread over a very small anatomical area. Therefore, unlike the practice in spine surgery, speculations such as the wide distribution of the drug to the surgical field and loss of its effectiveness have been prevented. Besides, since there would be no exposure to the laminectomy process, the microtrauma to be created by the surgical procedure on the dura was limited.

The weak aspect of our study is that the number of animals included in the experiment population is few. Lastly, electron microscopy examination in the histopathological examination could have contributed in terms of examining tissue qualities in a more detailed way and evaluating especially the inflammation more accurately.

In this experimental animal study, we found that fibrosis development did not differ significantly between the groups in rats administered with vancomycin powder. In this context, we think that it can be used especially in patients who undergo peripheral nerve repair to prevent adhesions and scar development in open and dirty injuries. We believe that more objective results can be obtained in further experimental studies with larger participation and more detailed histopathological examinations.

Conflict of interest: No conflict of interest was declared by the authors.

References

1. Fei Q, Li J, Lin J, et al. Risk factors for surgical site infection after spinal surgery: a meta-analysis. World Neurosurg 2016;95:507-515. http://doi.org/10.1016/j.wneu.2015.05.059
2. Meredith DS, Kepler CK, Huang RC, Brause BD, Boachie Adjei O. Postoperative infections of the lumbar spine: presentation and management. Int Orthop 2012;36:439-444. http://doi.org/10.1007/s00264-011-1427-z
3. Pull Ter Gunne AF, Cohen DB. Incidence, prevalence, and analysis of risk factors for surgical site infection following adult spinal surgery. Spine 2009;34:1422-1428. http://doi.org/10.1097/BRS.0b013e3181a03013
4. Bruniera FR, Ferreira FM, Saviolli LRM, et al. The use of vancomycin with its therapeutic and adverse effects: a review. Eur Rev Med Pharmacol Sci 2015;19:694-700.
5. Clippinger B, Ayzenberg M, Narvaez M, et al. Effect of intrasite vancomycin powder on the development of epidural fibrosis. Orthop Rev 2018;10:7777. http://doi.org/10.4081/or.2018.7777
6. Ghobrial GM, Cadotte DW, Jr Williams K, Fehlings MG, Harrop JS. Complications from the use of intrawound vancomycin in lumbar spinal surgery: a systematic review. Neurosurg Focus 2015;39:11. http://doi.org/10.3171/2015.7.FOCUS15258
7. Texakalidis P, Lu VM, Yolcu Y, et al. Impact of powdered vancomycin on preventing surgical site infections in neurosurgery: a systematic review and meta-analysis. Neurosurgery 2019;84:569-580. http://doi.org/10.1093/neuros/nyy288
8. Nahm FS, Lee PB, Choe GY, Lim YJ, Kim YC. Therapeutic effect of epidural hyaluronic acid in a rat model of foraminal stenosis. J Pain Res 2017;10:241-248. http://doi.org/10.2147/JPR.S122861

9. Chilukuri DM, Shah JC. Local delivery of vancomycin for the prophylaxis of prosthetic device-related infections. Pharm Res 2005;22:563-572. http://doi.org/10.1007/s11095-005-2497-7
10. Godil SS, Parker SL, O'Neill KR, Devin CJ, McGirt MJ. Comparative effectiveness and cost-benefit analysis of local application of vancomycin powder in posterior spinal fusion for spine trauma: clinical article. J Neurosurg Spine 2013;19:331-335. http://doi.org/10.3171/2013.6.SPINE121105
11. Sweet FA, Roh M, Sliva C. Intrawound application of vancomycin for prophylaxis in instrumented thoracolumbar fusions: efficacy, drug levels, and patient outcomes. Spine 2011;36:2084-2088. http://doi.org/10.1097/BRS.0b013e3181ff2cb1
12. Strom RG, Pacione D, Kalhorn SP, Frempong Boadu AK. Lumbar laminectomy and fusion with routine local application of vancomycin powder: decreased infection rate in instrumented and non-instrumented cases. Clin Neurol Neurosurg 2013;115:1766-1769. http://doi.org/10.1016/j.clineuro.2013.04.005
13. Salafia CM, Weigl C, Silberman L. The prevalence and distribution of acute placental inflammation in uncomplicated term pregnancies. Obstet Gynecol 1989;73:383-389.
14. Schroeder JE, Girardi FP, Sandhu H, Weinstein J, Cammisa FP, Sama A. The use of local vancomycin powder in degenerative spine surgery. Eur Spine J 2016;25:1029-1033. http://doi.org/10.1007/s00586-015-4119-3
15. Heller A, Mclff TE, Lai SM, Burton DC. Intrawound vancomycin powder decreases staphylococcal surgical site infections after posterior instrumented spinal arthrodesis. J Spinal Disord Tech 2015;28:584-589. http://doi.org/10.1097/BSD.0000000000000045
16. Theologis AA, Demirkiran G, Callahan M, Pekmezci M, Ames C, Deviren V. Local intrawound vancomycin powder decreases the risk of surgical site infections in complex adult deformity reconstruction: a cost analysis. Spine 2014;39:1875-1880. http://doi.org/10.1097/BRS.0000000000000533
17. Edin ML, Miclau T, Lester GE, Lindsey RW, Dahners LE. Effect of cefazolin and vancomycin on osteoblasts in vitro. Clin Orthop Relat Res 1996:245-251.
18. Emohare O, Ledonio CG, Hill BW, Davis RA, Polly DW, Kang MM. Cost savings analysis of intrawound vancomycin powder in posterior spinal surgery. Spine J 2014;14:2710-2715. http://doi.org/10.1016/j.spinee.2014.03.011

Acknowledgments

We certify that the manuscript has not been previously published in whole or in part or submitted elsewhere for review. We declare that the article content was composed in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval: The local Animal Research Ethics Committee (IRB: 4, Date: April 24, 2017) of Cukurova University approved this study.

Author contribution

S.K.O., M.C., K.R.E. constructed the main idea and hypothesis of the study. K.D., S.K.O., I.I., A.A. developed the theory and organized the material method section. A.I.O., Y.G. and S.K.O. analyzed the data in the results section. The discussion section of the article was written by V.A., M.C., K.E.E., S.K.O. and A.I.O., Y.G. reviewed, made the necessary corrections, and approved. Also, all authors discussed the whole study and approved its final version.