ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

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Evaluation of Efficacy of Collagen Material Containing Double-Release Gentamicin Sponge in Preventing Postoperative Spinal Infection: Experimental Study (Animal Experiment)

Çift Salımlı Gentamisin İçeren Kollajen Malzemenin Postoperatif Omurga Enfeksiyonunu Önlemedeki Etkinliğinin Değerlendirilmesi: Deneysel Çalışma (Hayvan Deneyi)

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ABSTRACT Objective: We studied that collagen material containing double-release gentamicin sponge for preventing of experimental postoperative spinal infection animal model. **Material and Methods:** A total of 28 adult female rats were used, in 4 groups each having 7 rats. Group 1: Only cutaneous-subcutaneous, fascia opened, muscles stripped; Group 2: Laminectomy of 2 levels+sterile normal saline; Group 3: Laminectomy of 2 levels+Staphylococcus aureus [106 colony forming unit (CFU)/10 ult]; Group 4: Laminectomy of 2 levels+S. aureus (106 CFU/10 ult)+collagen material containing double-release gentamicin sponge. With a light microscope, epidural fibrosis, inflammatory cell infiltration and fibroblast density of sections were evaluated and graded. **Results:** Group 2 had higher epidural fibrosis, fibroblast cell density and inflammatory cell density than those of Group 1. Group 3 had higher epidural fibrosis, fibroblast cell density and inflammatory cell density than those of Group 1. Group 1 had lower epidural fibrosis, fibroblast cell density and inflammatory cell density than those of Group 4. The inflammatory cell density was higher in Group 3 than that of Group 2. The epidural fibrosis and inflammatory cell density were lower in Group 4 than those of Group 2. The Group 4 had lower epidural fibrosis, fibroblast cell density and inflammatory cell density than those of Group 3. **Conclusion:** We therefore consider that the material used for this study could be used for prophylaxis to prevent epidural fibrosis and infection in spinal surgery.

Keywords: Gentamicin; collagen; spinal infection; epidural fibrosis

ÖZET Amaç: Deneysel postoperatif spinal enfeksiyon hayvan modelini önlemek için çift salımlı gentamisin sünger içeren kollajen materyalini inceledik. **Gereç ve Yöntemler:** Her birinde 7 sıçan bulunan 4 grupta toplam 28 erişkin dişi sıçan kullanıldı. Grup 1: Sadece kutanözcilt altı, fasya açık, kaslar soyulmuş; Grup 2: 2 seviyeli laminektomi+steril normal salin; Grup 3: 2 seviyeli laminektomi+Staphylococcus aureus [106 koloni oluşturan birim (colony forming unit "CFU")]/10 ult"; Grup 4: 2 seviyeli laminektomi+S. aureus (106 CFU/10 ult)+çift salımlı gentamisin sünger içeren kollajen materyal. Işık mikroskobu ile kesitlerin epidural fibrozis, inflamatuar hücre infiltrasyonu ve fibroblast yoğunluğu değerlendirildi ve derecelendirildi. **Bulgular:** Grup 2'de Grup 1'e göre daha yüksek epidural fibroz, fibroblast hücre yoğunluğu ve inflamatuar hücre yoğunluğu vardı. Grup 3'te Grup 1'e göre daha yüksek epidural fibrozis, fibroblast hücre yoğunluğu ve inflamatuar hücre yoğunluğu vardı. Grup 1, Grup 4'e göre daha düşük epidural fibrozis, fibroblast hücre yoğunluğu ve inflamatuar hücre yoğunluğuna sahipti. Grup 3'te inflamatuar hücre yoğunluğu Grup 2'ye göre daha yüksekti. Grup 4'te epidural fibrozis ve inflamatuar hücre yoğunluğu Grup 2'ye göre daha düşüktü. Grup 4, Grup 3'e göre daha düşük epidural fibrozis, fibroblast hücre yoğunluğu ve inflamatuar hücre yoğunluğuna sahipti. **Sonuç:** Bu nedenle bu çalışma için kullanılan materyalin spinal cerrahide epidural fibrozis ve enfeksiyonu önlemek için profilaksi için kullanılabileceğini düşünüyoruz.

Anahtar Kelimeler: Gentamisin; kollajen; spinal enfeksiyon; epidural fibrozis

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Although postoperative spinal infections are rare, they can result in severe and life-threatening complications in the absence of inadequate treatment.[1] Postoperative spinal infections cause severe and costly complications due to prolonged hospital stay, sometimes the need to remove the implant, reduced quality of life, repeated surgeries, occurrence of instability, and increased incidence of pseudarthrosis.[2] There are currently ongoing clinical studies and trials to prevent infections after a spinal surgery. Although there are few studies on locally administered antibiotic therapy for infection models created after lumbar spinal surgery, there are no experimental postoperative spinal infection models studied with collagen material containing double-release gentamicin sponge (Septocoll® E, Biomet GmbH Germany).[3,4]

MATERIAL AND METHODS

This research application was reviewed by the Dokuz Eylül University Faculty of Medicine Animals Animal Experiments Local Ethics Committee (date: March 18, 2011, no: 03/06/2011). "Wistar Albino" rats of the same species and similar features that were bred at laboratory conditions were used. The rats had a homogeneity of 87% and weighted around 200-220 g. Totally 28 adult female rats were used in 4 groups. The processing is defined below for each group.

In this experimental study, all animals were subjected to humane treatment in accordance with the "Guide for the Care and Use of Laboratory Animals" (www.nap.edu/catalog/5140.html). An Experimental Animals Ethics Committee approval report was received. In animal studies, care has been taken to avoid pain, suffering, and discomfort. All animals were kept at controlled temperature (21 C23 C) and 12 h light 12 h dark condition.

Group 1: Only cutaneous-subcutaneous, fascia opened, muscles stripped,

Group 2: After 2 levels laminectomy+sterile normal saline,

Group 3: After 2 levels laminectomy+*Staphylococcus aureus* [10^6 colony forming unit (CFU)/10 ult],

Group 4: After 2 levels laminectomy+*S. aureus* (10^6 CFU/10 ult)+collagen material containing double-release gentamicin sponge (Septocoll® E).

The rats were weighted before and after the experiment. The *S. aureus* American type Tissue Culture Collection (ATCC 25923) strains proved to cause osteomyelitis were used for the study.[5] *S. aureus* ATCC 25923 strains were adjusted by McFarland scale with 10^6 CFU/10 ult=10^8 CFU/mi. Because the McFarland's 0.5 value was 2x108 CFU/mL for *S. aureus*, bacterial suspension was prepared and diluted by 2 times with the initial McFarland 0.5 turbidity. 10^6 CFU/10 ult was extracted from the final concentration of bacterial suspension and inoculated on the dura and into the laminectomy site in the experiment Groups 3 and 4.

We performed this study under sterile conditions and each group of subjects at a separate session at Multidisciplinary Experimental Research Laboratory's of Dokuz Eylül University. The general anesthesia was achieved by giving rats intraperitoneal 60 mg/kg of Ketamine hydrochloride (Ketalar®, Parke-Davis, Eczacıbaşı, İstanbul, Türkiye) and 5 mg/kg of Xylazine hydrochloride (Rompun®, Bayer, İstanbul, Türkiye) prior to the surgical intervention. After anesthesia, the rats were put and fixed in the prone position, and the skin corresponding to the thoracolumbar area was shaved. The antisepsis was obtained by applying povidone iodine (POVIOD; 10% polyvinylpyrrolidone-iodine complex, Saba, Türkiye) on the skin and it is overlayed with a synthetic sterile fabric. In Group 1 (control) cutaneous-subcutaneous layer was passed through by appropriate incision at lumbar 4th and 5th spine level. The fascia was opened medially, and paravertebral muscles were bilaterally stripped by dissection. Then the fascia was sutured with Laktasorb atraumatic absorbable 4/0 [poly glycolide (90%)-co lactide (l 0%)], and the skin was sutured with Monoplene atraumatic 4/0 (polyprolen, non-absorbable, synthetic, monofilament blue). In the Group 2, additionally total laminectomy of 2 levels was performed at lumbar 4th and 5th spine level, and sterile normal saline was dropped. In the Group 3, in addition to 2 levels laminectomy, previously prepared *S. aureus* (10^6 CFU/10 ult) was inoculated on the dura and into the

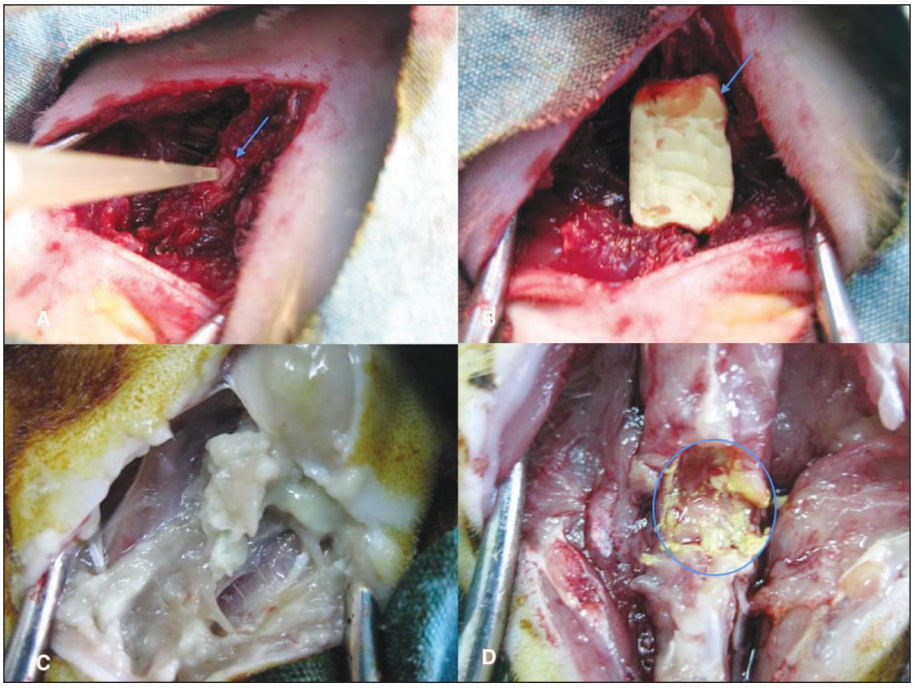

FIGURE 1: A) Inoculation of Staphylococcus aureus on the laminectomy site and dura mater (arrow); **B)** Septocoll application (arrow) after inoculation of S. aureus on the laminectomy site and dura mater; **C)** Appearance of severe infection in the postoperative wound site after sacrificing a rat in the Group 3; **D)** The laminectomy site after sacrificing a rat in the Group 4. Surgical observation revealed no abscess and infection (circle).

laminectomy site (with finn pipette 2-20 microl, thermo scientific eh44082 4500). In the Group 4, after inoculating *S. aureus* (10^6 CFU/10 ult) on the dura and into the laminectomy site, and Septocoll® E was placed in size corresponding to the distance (Figure 1A, B, C, D).

The rats were fed with compressed standard animal feed, their wound sites and activities were checked daily, and they were weighted at sacrifice. Preoperative and postoperative weight of all rats was recorded. Rats were sacrificed by high dose ether after 15 days. The vertebrae of sacrificed rats were removed in blocks and forwarded to the laboratory in formaldehyde for histopathologic examination. 2 rats in the Group 1 were excluded from the study due to contamination. 2 rats died in the Group 3 were replaced by new ones to complete the experiment.

HISTOPATHOLOGICAL CONSIDERATION

Each of the histological steps described were performed by 2 researchers blind to treatment steps of the rats. 10% formalin fixed histological specimens were holded in a phosphate buffer for 3 days, then decalcified in ethylene diamine tetraacetic acid for 6 weeks. Afterwards, the specimens were embedded in paraffin blocks. Paraffin blocks were placed in rotary microtome (RM 2255, Leica Instruments, Nußloch, Germany), and sections of 5 µm thickness were obtained with disposable metal microtome blades (Type R35, Feather Company, Osaka, Japan). All sections were stained with hematoxylin-eosin and Masson's trichrome were used for staining of all sections after deparaffinization and rehydration.

DETAILED ANALYSIS OF THE IMAGES

Computer assisted image analysis system consisting of a microscope (Olympus BX-51 is used for the analysing of the images, Japan) equipped with a high-resolution video camera (Olympus DP-71, Japan) for recording the data. Epidural fibrosis, inflammatory cell infiltration and fibroblast density in the experimental steps were evaluated and graded. For grading, the epidural fibrosis was graded as designed by He et al.[6]

Grade 0: No scar tissues on the dura,

Grade 1: Thin fibrosis bands between the scar tissue and the dura,

Grade 2: Scar tissue in less than two thirds of laminectomy defect,

Grade 3: Scar tissue greater than two thirds of laminectomy defect and scar tissue extended to the nerve roots.

To define fibroblast and inflammatory cell density in the scar tissue, cells in 3 different areas (2 edges and 1 central part of laminectomy defect) were counted and averaged.

The method used to grade fibroblast and inflammatory cell density is as follows:

Grade 0: Absence of fibroblast/inflammatory cells at x400 magnification,

Grade 1: Less than 100 fibroblast/inflammatory cells at x400 magnification,

Grade 2: 100-150 fibroblast/inflammatory cells at x400 magnification,

Grade 3: Greater than 150 fibroblast/inflammatory cells at x400 magnification.

STATISTICS

The results from counting were averaged and statistically evaluated using SPSS 15.0 (SPSS Inc. Chicago, USA). Kruskal-Wallis test was used to compare the colonization values of 4 different groups. Comparing differences in the groups were detected with Mann-Whitney U test. Values lower than $p<0.05$ were considered statistically significant.

TABLE 1: Preoperative and postooerative weights of the rats in all groups.

Group 1	Preop weight (gram)	Postop weight (gram)
1	221	228
2	217	201
3	216	205
4	210	226
5	235	248
6	214	224
7	219	238
Group 2	**Preop weight (gram)**	**Postop weight (gram)**
1	221	209
2	217	190
3	213	213
4	201	209
5	199	218
6	211	216
7	197	215
Group 3	**Preop weight (gram)**	**Postop weight (gram)**
1	196	192
2	220	233
3	225	209
4	190	193
5	206	213
6	202	207
7	200	172
Group 4	**Preop weight (gram)**	**Postop weight (gram)**
1	209	205
2	207	204
3	214	194
4	208	227
5	216	203
6	202	197
7	206	180

RESULTS

POSTOPERATIVE RESULTS

Six rats in the Group 2 and 1 rat in the Group 3 had paresis at postoperative day 1. 6 rats in the Group 3 had postoperative paraparesis in lower extremity and died at postoperative day 5. 3 rats in the group had hematuria at postoperative day 2 and died in 2 days. Died rats were changed by new rats with ethical committee approval. As part of surgical observation, no efflux and pus discharge from the wound site occurred in any of the groups including the Group 3. Ill-smelling pus came from the fascia and under the fascia of all rats in the Group 3. The preoperative and postoperative weight of all rats is presented in the Table 1.

HISTOPATHOLOGICAL RESULTS

Histopathological examination showed 2 rats in the Group 1 contaminated, thus they were excluded from the study. Surgical observation showed ill-smelling pus tissue from underneath the fascia of rats in the Group 3. Table 2 presents histopathological evaluation results according to fibrosis in the epidural space, density in the fibroblast cell and density of the cell inflammation in each group. Light microscopic examination of sections passing vertically at medulla spinalis (MS) showed a normal histopathology in the Group 1. The bone and muscular tissue surrounding the MS was normal. No fibrosis was present in the dura mater, and no acute and chronic inflammatory

TABLE 2: Evaluations of the groups about epidural fibrosis, fibroblast cell density and inflammatory cell density.

Group 1	Epidural fibrosis	Fibroblast cell density	Inflammatory cell density
1	1	0	1
2	1	1	1
3	0	0	0
4	0	0	0
5	0	0	1
Group 2	**Epidural fibrosis**	**Fibroblast cell density**	**Inflammatory cell density**
1	3	3	2
2	3	2	2
3	3	3	2
4	3	3	2
5	3	3	2
6	3	2	2
7	3	2	2
Group 3	**Epidural fibrosis**	**Fibroblast cell density**	**Inflammatory cell density**
1	3	3	3
2	3	2	3
3	3	3	3
4	3	3	3
5	3	2	3
6	3	3	3
7	3	3	3
Group 4	**Epidural fibrosis**	**Fibroblast cell density**	**Inflammatory cell density**
1	1	2	1
2	2	3	2
3	1	1	1
4	0	1	1
5	1	2	2
6	1	2	2
7	1	2	1

cell infiltration was detected in the connective tissue. The vascular structure of connective tissue was normal, and no vascular proliferation existed (Figure 2A, B). The fibrosis occurred in the dura mater and fibroblasts increased in the Group 2. Local little acute inflammatory cell infiltration was present. Vascular structure of connective tissue was normal, and no vascular proliferation was present (Figure 3A, B). Muscle and bone integrity were disrupted in subjects with induced infection of *S. aureus* after laminectomy. Significant fibrosis was present in the dura mater. There was an intense acute inflammatory cell infiltration resulting from neutrophils. Blood vessels of connective tissue had significant vascular proliferation and extravasation. There was vascular proliferation, granulation tissue areas from fibroblasts and formation of new bones. Micro-abscess foci containing necrotic bone fractures, and collagen degeneration were observed (Figure 4A, B, C, D). In the treat-

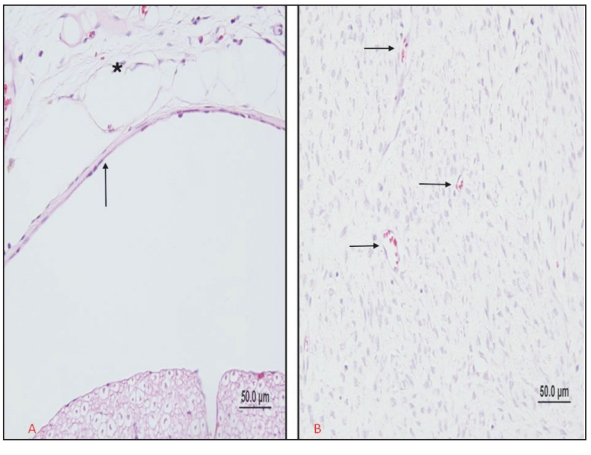

FIGURE 2: A) Normal appearance of medulla spinalis, dura mater (arrow) and connective tissue (*) in the Group 1; H&E X40; **B)** Normal appearance of connective tissue and vascular (arrrow) structures; H&E X40.

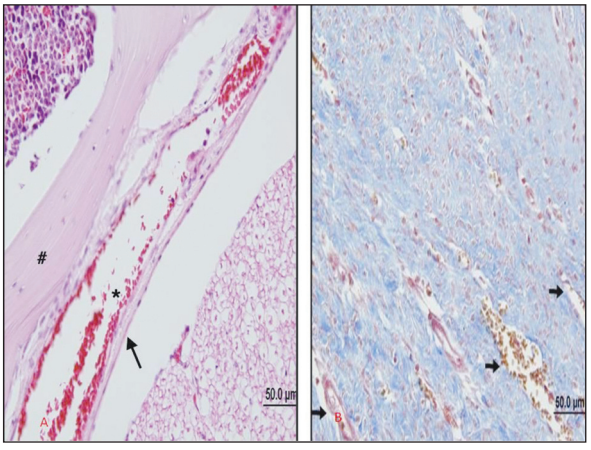

FIGURE 3: A) Thickness of the duramater in the Group 2 (arrow), vascular dilatation (*), bone tissue (#) and medulla spinalis H&E X40; **B)** Connective and vascular tissue in the Group 2 (arrow) Masson's trichrome X40.

ment group (Group 4), fibrosis and inflammatory cell infiltration were reduced on the dura mater and approximated to the control group. The granulation tissue was increased, and necrotic bone tissue was reduced in the group with induced *S. aureus* after laminectomy, but no acute inflammatory process was observed. Blood vessels of connective tissue had slight vascular proliferation (Figure 5A, B, C).

Statistically significant differences were present in epidural fibrosis, fibroblast activity and increased inflammatory cells between Groups 1, 2 and 3 and the Group 4 which was the treatment group ($p<0.05$). There were statistically significant differences in statistical comparison of all parameters of Groups 1 and 2. The Group 2 have had higher epidural fibrosis, fibroblast cell density and inflammatory cell density than those of Group 1 ($p<0.001$ for epidural fibrosis, $p<0.003$ for fibroblast cell density, $p<0.001$ for increased inflammatory cells). Significant differences were in comparison of Groups 1 and 3. The Group 3 had higher epidural fibrosis, fibroblast cell density and inflammatory cell density than those of Group 1 ($p<0.001$ for epidural fibrosis, $p<0.003$ for fibroblast cell density, $p<0.001$ for increased inflammatory cells). There were statistically significant differences in statistical comparison of all parameters of Groups 1 and 4. Group 1 have had lower characteristics than those of Group 4 ($p<0.03$ for epidural fibrosis, $p<0.05$ for fibroblast cell density, $p<0.017$ for increased inflammatory cells). A significant difference was found only in increased inflammatory cells between Groups 2 and 3. The inflammatory cell density in Group 3

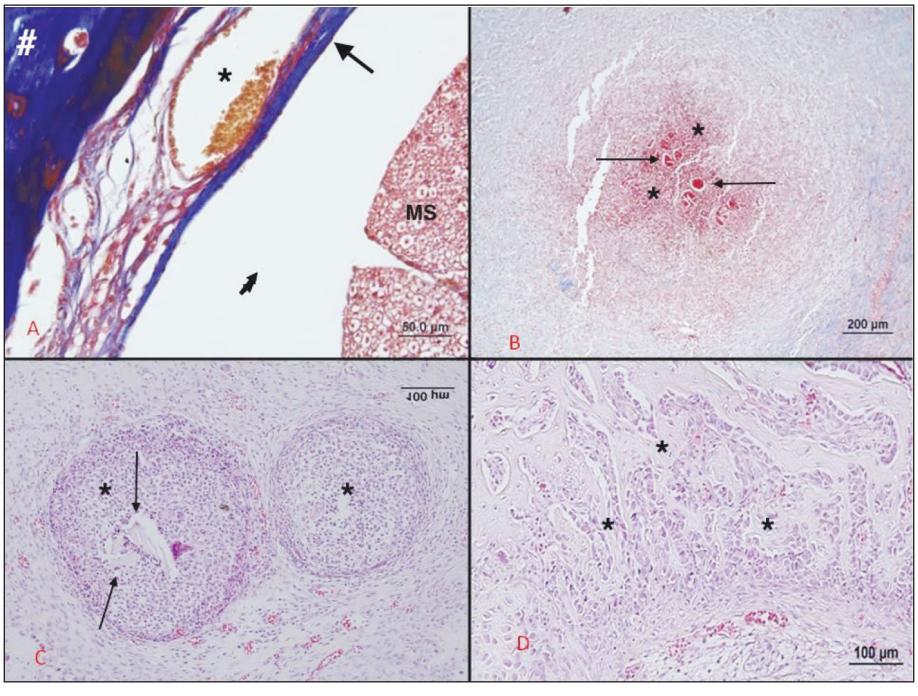

FIGURE 4: A) Epidural fibrosis in Group 3 (arrow) medulla spinalis, bone tissue (#) and vascular tissue (*), Masson's trichrome X4; **B)** Group 3: Increased inflammatory cells in the connective tissue (*) and bone spicules (arrow) Masson's trichrome XI0; **C)** Group 3: Abscess formation in the connective tissue (*) bone spicules (arrow) H&E X20; **D)** Group 3: formation of new bone (*) H&E X20.

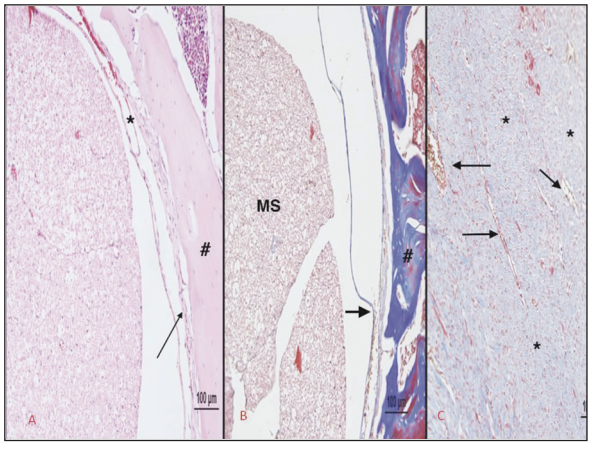

FIGURE 5: A) Group 4: reduced epidural fibrosis (arrow) medulla spinalis, dura mater (*) and bone tissue (#) H&E X40; **B)** Medulla spinalis, dura mater (arrow) and bone tissue (#) in the treatment group, Masson's trichrome X20; **C)** Connective tissue (*) and vascular structures (arrow), Masson's trichrome X40.

showed lower characteristic than that of Group 2 ($p<1$ for epidural fibrosis, $p<0.3$ for fibroblast cell density, $p<0.01$ for increased inflammatory cells). There were statistically significant differences in epidural fibrosis and inflammatory cell density between Groups 2 and 4. The epidural fibrosis and inflammatory cell density were lower in the Group 4 than those of Group 2 ($p<0.01$ for epidural fibrosis, $p<0.114$ for fibroblast cell density, $p<0.023$ for increased inflammatory cells). There were statistically significant differences in all parameters of Groups 3 and 4. The Group 4 had lower epidural fibrosis, fibroblast cell density and inflammatory cell density than those of Group 3 ($p<0.01$ for epidural fibrosis, $p<0.026$ for fibroblast cell density, $p<0.001$ for increased inflammatory cells).

DISCUSSION

Postoperative spinal infections are a substantial cause of morbidity after a spinal surgery in the science of neurosurgery, and even a cause of mortality if no measures are taken. There are ongoing studies for prophylaxis and locally effective treatments to prevent postoperative spinal infections. In addition to parenteral prophylaxis, both clinical and experimental research in the literature present examples of prophylaxis with local materials containing antibiotics. One of the disadvantages of using systemic antibiotics is that they cannot reach sufficient concentration in areas where we expect them to be effective and thus their local efficacy reduces. When using parenteral antibiotics, accession of sufficient antibiotics to the disc space is difficult due to absence of disc vascularity. In addition, systemic adverse effects of parenteral antibiotherapy are severe. Particularly, gentamicin is known to have nephrotoxic and ototoxic effects at high doses. Thus, prophylaxis is preferred using materials containing local antibiotics.[7] In the literature, local gentamicin has been used for prophylactic and treatment of bone infections. The most common application methods involve bone cement and polymethacrylate (PMMA) beads containing gentamicin in endoprosthesis surgery.[3,8,9] Another wide-spread method used for local treatment is collagen materials containing gentamicin.[7,10,11] Based on the experience of using collagen as local hemostatic in surgery, collagen materials containing gentamicin have been developed and was first used by Ascherl in 1986.[12] PMMA was the first biomaterial produced in the chemical industry. It was first used in dentistry in 1941 and tried in orthopedic surgery in 1945. Early studies gave no positive results on infection prophylaxis. A Norwegian association of arthroplasty reported favorable effects of cement treatment containing antibiotics. The bone cement treatment containing gentamicin has a risk for developing resistance to antibiotics. Following fast release of antibiotics after delivering long-term reduced concentration causes developing resistance to antibiotics.[13] In vitro studies showed that salamanders loaded with antibiotics had bacterial growth.[14] In the end, effectiveness of cement containing gentamicin on hip surgery was approved, but it was then declared that bacterial resistance needed to be investigated. Collagen containing gentamicin and PMMA beads containing gentamicin were intramedullary used by nail in fixation of tibia and femur, and only collagen containing gentamicin was shown to reach effective dose.[15] It is then reported that PMMA beads containing gentamicin needed to be removed 4-6 weeks after implantation and there was a new risk of infection for reoperation.[16] The collagen containing gentamicin was shown to be effective on sternum wound infection after cardiac surgery.[17,18] A study by Stall et al.

created a model of spinal infection with thoracolumbar implant induced by *S. aureus* on rabbits and reported protective effects of microspheres containing gentamicin.[4,19] In the study by Matsuno et al., a hyaluronic acid (HA) gel with gentamicin and vancomycin and spongostan with HA containing the same antibiotics were compared with Septocoll® and no significant differences were reported in treatment.[19] The results indicated antibacterial efficacy of absorbable materials with antibiotics containing HA gel. No significant efficacy of collagen material containing gentamicin used during prophylaxis in colorectal surgery was found.[20] Brehant et al. showed the efficacy of the collagen sponge with gentamicin in reducing the rates of the infection in the surgical site after colorectal surgery.[21] In incidence analysis of spondylodiscitis after lumbar disc surgery by Rohde et al., the group that did not receive prophylactic antibiotic therapy and the gentamicin therapy containing collagen (Sulmycin Implant, ESSEX PHARMA Gmbh, Munich, Germany) applied into intervertebral disc space, and no infection developed in the group receiving local prophylaxis.[3,22] In the same study, the infection ratio was reported to be 3.7% in the group with no prophylaxis. In the experimental rabbit study by Riegels-Nielsen, tibial bones were infected by *S. aureus* local and systemic application of Gentacoll® (collagen+gentamicin) (Schering-Plough, Copenhagen, Denmark) was compared with the control group and no infections occurred in subjects receiving Gentacoll®.[22] In a study by Lucke et al. of infection induced by *S. aureus* using Kirschner wire on rat tibia, histological and microbiological significant results were reported in preventing implant-associated infection by antibiotic covered instruments containing poly (D, L-lactide) and 10% gentamicin.[23] Mendel et al. created an osteomyelitis model induced by *S. aureus* on rat tibia, and parenteral cefazolin, gentamicin impregnated PMMA, collagen containing gentamicin (Sulmycin Implant, ESSEX PHARMA Gmbh, Munich, Germany) and addition of parenteral cefazolin therapy to the local treatment with gentamicin were compare.[24] The most effective treatment was the addition of parenteral cefazolin therapy to the collagen containing local gentamicin. In addition, it was indicated in the same study that Septocoll® material could cause encephalopathy (mad-cow disease) as it contained bovine collagen.[19]

One of the postoperative spinal surgery complications is epidural fibrosis.[18] In a variety of series, epidural fibrosis was reported at a ratio ranging 20% to 47%.[18,25,26] Epidural fibrosis is one of the steps in normal recovery in the postoperative stage caused by adherence to nerve roots. Also, intense fibrous tissue on the surface of paravertebral muscles and on fibrous layer of periost and by invasion postoperative hematoma play a critical role in forming of the epidural fibrosis. In addition, the neural canal and adhere to the dura mater and nerve roots has a potential risk for fibrosis.[9,25-27] Many methods and treatment strategy are recommended to reduce this problem. Minimal invasive techniques were reported to reduce the amount of epidural fibrosis with performing mini-incision and muscle retraction and sutitable hemostasis.[28] Powder on gloves and cotton fibers were shown to cause further reaction on the epidural site. Therefore, removal of foreign materials and toxins by lavage of the epidural area reduces the formation of fibrosis. Materials used to prevent epidural fibrosis include fat grafting, gel foam, silastik, Zenoderm (Elder Pharamaceutıcals™ Mumbai, India), Dacron, (SurgicalMesh™ Connecticut USA), Vicrylmesh (Ethicon™ Cincinnati, USA), carboxymethyl-cellulose, polyethylene oxide, Gore-tex membrane (Gore Medical Arizona, USA), and anti-adhesion barrier as well as sodium HA, steroid and anti-inflammatory therapies.[27,28] Reducing the epidural fibrosis is reported using hemostatic agents (gelatin sponge, microfibril collagen) and anti-inflammatory drugs and by mechanical barriers inserted between tissue and dura mater as well as the use of synthetic and biological materials.[25,27,29] The positive effectivity has not been reported in clinical use of these agents.[25,30] Fat graft was applied as initial treatment to prevent epidural fibrosis after lumbar discectomy. Fat graft was subcutaneously obtained from a surgical incision site and placed on the dura to cover the dura.[28] This fat grafting did not present a preventive effect for epidural fibrosis, and even to cause cauda equina syndrome.[26,28]

This study investigated histopathologic effects of collagen material containing double-release gentamicin sponge (Septocoll® E) on deep spinal infec-

tion induced on the rat model to prevent postoperative spinal infection for local treatment. Additionally, collagen's functions to prevent epidural fibrosis were also investigated. In this study, *S. aureus* ATCC 25923 strains, which is the most common pathogenic factor for postoperative spinal surgery infections, were used in a concentration that was identified to cause osteomyelitis. Septocoll® E contains 2 gentamicin salts (gentamicin sulfate and crobefate) combined at a ratio of 1:1. They have different release kinetic, therefore they show a dual action. Gentamicin sulfate is highly water soluble and thus its release is rapid. Gentamicin crobefate is yellow, less water soluble and thus its release is slower. This dual action allows antibiotic to reach high concentration fast and to last long. Septocoll® E contains equine natural Type 1 collagen fibers, is fully absorbable and not water-soluble. In collagen materials containing only gentamicin sulfate, 90% of the release of gentamicin occurs in the first 48 hours and its effectiveness diminishes at the end of day 4 whereas the release of gentamicin is maintained as long as 10 days by addition of crobefate to gentamicin sulfate.

It is natural that laminectomy performed in the Group 2 and addition of SF altered the parameters of histopathological evaluation between Groups 1 and 2. In the Group 3 with induced infection, it was an expected result that it made alterations in parameters, in inflammatory cell density in particular, compared to both the Group 1 and the Group 2. The interesting aspect of this study in terms of infection was that application of Septocoll® E significantly reduced epidural fibrosis, inflammation and fibroblast activity in the treatment group. Surgical observation presented no significant infection in the treatment group. In addition, epidural fibrosis was significantly reduced in the Group 4 where Septocoll® E was added.

CONCLUSION

We demonstrated on the experimental animal model that Septocoll® E prevented infections as well as epidural fibrosis due to collagen content. This study provides valuable data about the material used in this study that can be used in prophylaxis of the epidural fibrosis and infection. However, it is obvious that randomized clinical studies with long periods of follow-up are required in addition to experimental animal models.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Özgür Akşan;* ***Design:*** *Özgür Akşan;* ***Control/Supervision:*** *Özgür Akşan;* ***Data Collection and/or Processing:*** *Özgür Akşan;* ***Analysis and/or Interpretation:*** *Özgür Akşan;* ***Literature Review:*** *Volkan Murat Ünal, Özgür Akşan, Ali Karadağ, Seda Özbal, Kazım Tuğyan;* ***Writing the Article:*** *Özgür Akşan;* ***Critical Review:*** *Özgür Akşan, Nail Özdemir;* ***References and Fundings:*** *Özgür Akşan;* ***Materials:*** *Efsun Kolatan, Ece Sökmen Yılmaz.*

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