2014

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Intrawound Vancomycin Powder Eradicates Surgical Wound Contamination

An in Vivo Rabbit Study

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Background: Surgical site infection remains a complication of spine surgery despite routine use of prophylactic antibiotics. Retrospective clinical studies of intrawound vancomycin use have documented a decreased prevalence of surgical site infection after spine surgery. The purpose of the present study was to assess the efficacy of intrawound vancomycin powder in terms of eradicating a known bacterial surgical site contamination in a rabbit spine surgery model.

Methods: Twenty New Zealand White rabbits underwent lumbar partial laminectomy and wire implantation. The surgical sites were inoculated, prior to closure, by injecting 100 μL of cefazolin-sensitive and vancomycin-sensitive *Staphylococcus aureus (S. aureus)* (1×10^8 colony-forming units [CFU]/mL) into the wound. Preoperative cefazolin was administered to all rabbits, and vancomycin powder (100 mg) was placed into the wound of ten rabbits prior to closure. The rabbits were killed on postoperative day four, and tissue and wire samples were obtained for bacteriologic assessment. An independent samples t test was used to assess mean group differences, and a Fisher exact test was used to assess differences in categorical variables.

Results: The vancomycin-treated and the control rabbits were similar in weight (mean [and standard deviation], 4.1 ± 0.5 kg and 4.0 ± 0.4 kg, respectively; $p = 0.60$) and sex distribution and had similar durations of surgery (21.7 ± 7.7 minutes and 16.9 ± 6.7 minutes; $p = 0.15$). The bacterial cultures of the surgical site tissues were negative for all ten vancomycin-treated rabbits and positive for all ten control rabbits ($p < 0.0001$). Bacterial growth occurred in thirty-nine of forty samples from the control group but in zero of forty samples from the vancomycin group ($p < 0.0001$). All blood and liver samples were sterile. No rabbit had evidence of sepsis or vancomycin toxicity. Gross examination of the surgical sites showed no differences between the groups.

Conclusions: In a rabbit spine-infection model, intrawound vancomycin powder in combination with preoperative cefazolin eliminated *S. aureus* surgical site contamination. All rabbits that were managed with only prophylactic cefazolin had persistent *S. aureus* contamination.

Clinical Relevance: This animal study supports the findings in prior clinical reports that intrawound vancomycin powder helps reduce the risk of surgical site infections.

Peer Review: This article was reviewed by the Editor-in-Chief and one Deputy Editor, and it underwent blinded review by two or more outside experts. It was also reviewed by an expert in methodology and statistics. The Deputy Editor reviewed each revision of the article, and it underwent a final review by the Editor-in-Chief prior to publication. Final corrections and clarifications occurred during one or more exchanges between the author(s) and copyeditors.

Surgical site infection is a known complication of spine surgery. The prevalence of postoperative spine infection varies with the type of surgery, ranging from 1% to 3% after anterior cervical and lumbar decompression surgery to approximately 10% to 15% after fusion to treat spine trauma or neuromuscular scoliosis[1-12]. The risk of developing a surgical site infection is multifactorial. Authors of prior studies have described both patient risk factors, such as obesity, diabetes, and an immunocompromised state, and operative risk factors, such as multilevel surgery, use of

Disclosure: None of the authors received payments or services, either directly or indirectly (i.e., via his or her institution), from a third party in support of any aspect of this work. One or more of the authors, or his or her institution, has had a financial relationship, in the thirty-six months prior to submission of this work, with an entity in the biomedical arena that could be perceived to influence or have the potential to influence what is written in this work. No author has had any other relationships, or has engaged in any other activities, that could be perceived to influence or have the potential to influence what is written in this work. The complete **Disclosures of Potential Conflicts of Interest** submitted by authors are always provided with the online version of the article.

TABLE I Rabbit and Surgical Descriptive Characteristics

	Vancomycin Rabbits	Control Rabbits	P Value
Sex *(M:F)*	6:4	2:8	0.17
Weight*(kg)	4.1 ± 0.5	4.0 ± 0.4	0.60
Duration of surgery* *(min)*	21.7 ± 7.7	16.9 ± 6.7	0.15
Initial *S. aureus* concentration *(CFU)*	1×10^8	1×10^8	

*The values are given as the mean and standard deviation.

instrumentation, revision surgery, and large intraoperative blood loss[13-15].

The use of preoperative prophylactic intravenous antibiotics reduces the rate of surgical site infections and has become routine practice[16-20]. Unfortunately, surgical site infection can still develop. Local administration of antibiotics is an alternative treatment strategy to prevent surgical site infection. The advantages of locally administered antibiotics are that they are introduced directly into the surgical site, eliminate the need for diffusion into the wound that is associated with systemic antibiotics, and offer the potential to obtain a high antibiotic concentration within the surgical site. At high concentration, an antibiotic may become bactericidal even to resistant bacteria[21]. Local antibiotic administration has been studied with different delivery methods and antibiotics, with positive results[22-26].

The use of intrawound vancomycin powder in adult spine surgery has been described in several retrospective clinical series[27-31]. In these series, the authors found a significantly lower postoperative infection rate with use of intrawound vancomycin powder for diverse surgical populations, including those treated for adult degenerative disease[27,29], deformity[30], or spine trauma[28] and those treated with cervical spine surgery[31]. The purpose of the current study was to assess the efficacy of intrawound vancomycin powder with regard to reducing surgical site contamination in a rabbit spine-infection model. Our hypothesis was that rabbits managed with intrawound vancomycin powder would have less bacterial colonization and tissue infection as demonstrated by microbiological testing.

Materials and Methods

Bacterial Preparation

Staphylococcus aureus (S. aureus) was utilized for the surgical site infections because it is the most common bacterial cause of postoperative infections[3,31,32]. The *S. aureus* (American Type Culture Collection [ATCC] number 25923) isolate used for inoculation was obtained from a repository maintained at our hospital clinical microbiology laboratory. This is a methicillin-sensitive strain that has been used in prior animal studies on intrawound gentamicin application[24,25]. This bacterial strain was confirmed to be sensitive to both cefazolin and vancomycin. The identity of this isolate was confirmed on the basis of morphologic characteristics and biochemical analysis (VITEK 2; bioMérieux, Durham, North Carolina). Organisms used for this experiment were grown on 5% sheep blood agar culture (Northeast Laboratory Services, Waterville, Maine) for twenty-four hours at 37°C. Bacteria were collected with use of a sterile swab and then were suspended in sterile phosphate-buffered saline solution to the desired concentration. Colony counts and the presence of the organisms in the pure culture were verified by plating serial dilutions of the bacterial suspension on 5% sheep blood agar. In an initial phase, New Zealand White rabbits were challenged with varying concentrations of *S. aureus* to reliably create surgical site contamination. On the basis of this preliminary work and a prior report[23], a 1×10^8 colony forming units [CFU]/mL concentration of *S. aureus* was used for bacterial surgical site inoculation because it reliably produced a contaminated surgical site.

Study Design

The study protocol was approved by our Institutional Biosafety Committee and Institutional Animal Care and Use Committee. The study design was based on a prior rabbit spine-surgery infection model that reliably mimics human posterior spine surgery[33]. Twenty New Zealand White rabbits were divided into two groups of ten rabbits each. Ten control rabbits received one preoperative dose of cefazolin, and ten experimental rabbits received preoperative cefazolin as well as intrawound vancomycin powder. The preoperative 30-mg/kg cefazolin dose was based on a prior prophylactic antibiotic rabbit spine-infection study[34]. Postoperative cefazolin was not administered because a prior study had shown that a single preoperative cefazolin dose was as effective as a combination of preoperative and postoperative cefazolin in preventing surgical site infection in a rabbit-spine model[35].

Surgical Procedure

In the presurgical holding area, the rabbits were anesthetized with a ketamine and xylazine cocktail (20 mg/kg and 2.5 mg/kg, respectively, given intramuscularly) and maintained with 3% to 5% isoflurane gas. The rabbits were intubated, and the posterior aspect of the thoracic and lumbar spine was shaved. The preoperative cefazolin was administered fifteen minutes before the skin incision was made. The posterior thoracic and lumbar regions of the trunk were prepared with a Betadine (povidone-iodine) scrub, an alcohol swab, and Betadine paint, and the surgical site was sterilely draped. A 1.5-cm incision centered on approximately L3 was made, and sharp dissection through the subcutaneous tissue and fascia down to the lumbar spinous process was performed. Retractors were placed to reflect the superficial tissue and expose the spinous process, and a rongeur was used to remove the entire spinous process and surrounding musculature, creating a defect mimicking a partial laminectomy. The ligamentum flavum and the dura were not exposed. A 5-mm Kirschner wire was implanted into the transverse process to mimic posterior instrumentation. A sterile pipette was used to inoculate the wound with 100 μL of 1×10^8 CFU/mL of *S. aureus.* The experimental rabbits were treated with 100 mg of vancomycin powder placed directly within the wound prior to closure. A 100-mg vancomycin dose in a 4-kg rabbit is equivalent to a 2-g dose in an 80-kg human adult, which has been previously shown to be safe and effective in humans[27]. The deep muscle and the fascia were closed with PROLENE (polypropylene) sutures in order to contain the vancomycin powder within the surgical site, and the skin was closed with subcuticular PROLENE sutures and DERMABOND adhesive (Ethicon, Somerville, New Jersey). Postoperative analgesia included Buprenex (buprenorphine hydrochloride; 0.02 mg/kg subcutaneously) and a 25-μg/hr fentanyl patch. The animals were monitored daily for signs of pain, infection, and other complications.

TABLE II Difference in Rates of Positive Cultures Between Vancomycin-Treated and Control Rabbits on Final Bacteriologic Culture Analysis

	Fascia	Muscle	Bone	Wire	Blood	Liver
No. positive cultures/total no.						
Vancomycin-treated	0/10	0/10	0/10	0/10	0/10	0/10
Control	10/10	10/10	10/10	9/10	0/10	0/10
P value	<0.0001	<0.0001	<0.0001	<0.0001	1	1

Bacterial Evaluation

Four days postoperatively, the rabbits were resedated for blood collection and were then killed via an intravenous pentobarbital overdose. The surgical incision was prepared with a Betadine scrub, the surgical site was sterilely draped, and the skin and fascial incisions were reopened under sterile conditions in order to expose the laminectomy defect. Sterile instruments were used to harvest wound tissue, including the fascia, muscle, lamina, and transverse process, and the surgical wire and the tissues were placed into sterile test tubes. A 2 × 2-cm liver biopsy specimen was also collected under sterile conditions.

The collected specimens were divided for bacteriologic analysis. The tissues used for bacteriologic culture were individually weighed and then placed with a specified volume of sterile saline solution in a sterile tissue grinder (Fisherbrand Closed Ultra Tissue Grinder System; Thermo Fisher Scientific, Houston, Texas). Samples were ground with use of this system to achieve disruption of tissues and the release of bacterial colonies, resulting in a cloudy supernatant. A specified volume of this supernatant consisting of the ground tissue and saline solution was plated on 5% sheep blood agar and incubated for a minimum of twenty-four hours at 37°C. The retrieved implanted wire was weighed and was placed in a known volume of sterile saline solution. The wire and saline solution were sonicated (Ultrasonic FS-14; Fisher Scientific, Houston, Texas) at room temperature for ten minutes to facilitate release of bacterial colonies from the wire. A specified aliquot of this sonicated saline solution was plated on 5% sheep blood agar and incubated for a minimum of twenty-four hours at 37°C. The blood collected just prior to killing the rabbits was placed in a blood culture tube (Bac T/ALERT FA; bioMérieux), according to the manufacturer's recommendations, and was incubated at 37°C for seven days, after which it was plated on 5% sheep blood agar and then incubated at 37°C for a minimum of twenty-four hours.

After the incubation period, the colonies grown were counted and were identified on the basis of morphologic characteristics and biochemical analysis (VITEK 2; bioMérieux). Antibiotic sensitivity tests were performed on the bacteria isolated from each animal.

Statistical Analysis

An independent samples t test was used to assess mean group differences and a Fisher exact test was used to assess differences in categorical variables (SPSS; IBM, Armonk, New York). Significance was set at $p < 0.05$.

Source of Funding

No external funding was received to support the present study.

Results

The vancomycin-treated and control rabbits were similar in weight (vancomycin: 4.1 ± 0.5 kg [mean and standard deviation], control: 4.0 ± 0.4 kg; $p = 0.6$) and sex distribution (six vancomycin-treated male rabbits, two control male rabbits; $p = 0.17$), and had similar durations of surgery (vancomycin: 21.7 ± 7.7 minutes, control: 16.9 ± 6.7 minutes; $p = 0.15$) (Table I). There were no postoperative wound complications, deaths, or evidence of systemic illness. The surgical wounds of the ten vancomycin-treated rabbits did not display any evidence of local infection, and there were no apparent systemic effects from the vancomycin administration. One control rabbit had erythema and a small wound dehiscence, whereas the other nine control rabbits had subjectively unremarkable incisions. No rabbit had wound drainage.

TABLE III Results of Final Bacteriologic Culture Analysis of Control Rabbit Infections

	Bacterial Colony Count *(CFU/g)*			
Control Rabbit	Fascia	Muscle	Bone	Wire
1	6.42×10^2	*	*	4.75×10^3
2	*	*	*	2.06×10^5
3	1.79×10^4	9.92×10^4	*	2.46×10^6
4	2.71×10^4	*	8.56×10^4	1.10×10^5
5	*	*	1.48×10^4	3.0×10^4
6	1.84×10^5	*	*	6.63×10^4
7	*	*	3.45×10^4	5.0×10^3
8	7.33×10^4	*	9.74×10^4	Negative culture
9	4.08×10^4	*	3.80×10^4	8.33×10^3
10	*	*	*	2.44×10^4

*Too many bacteria to quantify.

Bacteriologic Culture

Bacteriologic cultures confirmed that all surgical sites were inoculated with 1×10^8 CFU/mL of *S. aureus*. All ten control rabbits had positive bacteriologic wound cultures on postoperative day four, whereas none of the ten vancomycin-treated rabbits had a positive culture. This difference was significant ($p < 0.0001$) (Table II). None of the forty samples (tissue or wire) from the vancomycin-treated rabbits and thirty-nine of the forty from the control rabbits had bacterial growth ($p < 0.0001$) (Table II). For the control rabbits, bacteriologic colony counts typically ranged from 10^3 to 10^5 CFU/g tissue (Table III). In some tissues, bacterial colonies were too numerous to count (Table III), which precluded a determination of recovered bacterial concentrations. All organisms grown on culture were *S. aureus*. There were no polymicrobial infections. The *S. aureus* grown on culture was sensitive to cefazolin and vancomycin. None of the blood or liver samples in either rabbit group showed bacterial growth.

Discussion

Using a rabbit spine surgical-wound-infection model, we found that intrawound vancomycin was substantially more effective at eliminating surgical site contamination than intravenous cefazolin alone. In fact, all surgical sites in the vancomycin group were sterile, whereas all control-rabbit wounds had evidence of persistent contamination on bacteriologic culture.

Systemically administered antibiotics, such as cefazolin, rely on diffusion into the surgical wound for their treatment effect against surgical site infection. The concentration of these antibiotics within the wound is expected to be low. In contrast, locally administered antibiotics achieve high concentrations at the surgical site, with safe systemic concentrations[22,36]. Locally administered vancomycin can reach levels twenty times the toxic serum levels while maintaining a safe systemic concentration, which is likely due in part to the large molecule size of vancomycin preventing systemic absorption[36]. The ability to achieve increased antibiotic concentrations with intrawound administration is important because bacteria resistant to a particular antibiotic at low concentration may be susceptible to the antibiotic at a higher concentration[21]. In addition, the spread of bacterial resistance may be less with local delivery because fewer bacteria are exposed to the antibiotic than with systemic administration. Our data suggest that the intrawound vancomycin concentration was sufficiently high to overcome a known *S. aureus* wound contamination in the experimental rabbits.

Our results for the elimination of acute surgical site bacterial contamination with use of a locally administered antibiotic are similar to those in a study by Stall et al., who reported a significantly reduced surgical site infection rate in rabbits managed with local gentamicin microspheres[12]. The two studies were similar in design and used the same *S. aureus* strain in a New Zealand White rabbit spine-infection model. However, 38% of the surgical sites in the study by Stall et al. had persistent positive bacteriologic cultures with gentamicin microspheres. The possible explanations for this discrepancy are that the microspheres acted as both an antibiotic-delivery platform and a foreign body for bacterial adhesion. In addition, local antibiotic-delivery systems were utilized to allow for a more gradual and sustained elution of the antibiotic over time. The consequence may have been that the initial local antibiotic concentration did not reach a level high enough to overwhelm the bacterial load and allowed some bacteria to survive.

Using a rat infection model, Yarboro et al. found a significantly reduced infection rate for surgical sites treated with locally injected aqueous gentamicin compared with those treated with locally administered calcium-sulfate flakes with gentamicin or treated with systemic gentamicin[24]. They proposed that this difference might have arisen because the calcium sulfate acted as a harbor for bacterial adherence. In addition, gentamicin peak concentrations may have differed between locally administered gentamicin and gentamicin-laden calcium sulfate. In a follow-up study, Cavanaugh et al. reported that the use of a combination of systemic cefazolin and local gentamicin was more effective at decreasing surgical site bacterial count than the use of systemic cefazolin or local gentamicin alone[25]. Our results support this finding, as the use of systemic cefazolin and local vancomycin was superior to the use of systemic cefazolin alone. A combination of systemic and local antibiotics may have a synergistic effect on the reduction of a surgical bacterial load.

The current standard of care for preoperative antibiotic prophylaxis for spine surgery is the use of an intravenous cephalosporin, most commonly 1 to 2 g of cefazolin (2 g for patients weighing >80 kg)[37]. We chose to use 30 mg/kg of cefazolin in the present study because this dose had been used for prior rabbit spine-infection studies and was recommended by our veterinarian (M.T.)[34,35]. Clinical reports on intrawound vancomycin powder use for spinal surgery have described the application of 1 to 2 g, and in the largest clinical series of which we are aware (911 patients) 2 g of vancomycin powder was placed subfascially without systemic toxicity[27]. On the basis of an average weight of 4 kg for the New Zealand White rabbits and surgical site area of 3.38 cm^3, application of 100 mg of intrawound vancomycin powder to the rabbit would be equivalent to 2 g of vancomycin for an 80-kg human patient.

There is concern that high concentrations of vancomycin powder applied locally to a wound may be cytotoxic to osteoblasts. Authors of prior studies have reported that a vancomycin concentration below 1000 μg/mL had little or no effect on osteoblast growth, whereas 10,000 μg/mL caused cell death[38,39]. We placed 100 mg of vancomycin into a surgical site of 3.38 cm^3, for a local vancomycin concentration of approximately 30 μg/mL. This vancomycin concentration falls well below the critical threshold for reduced cell activity and death. It is important to recognize that, in these in vitro studies, direct contact between the high-concentration vancomycin and the osteoblast cells was maintained for twenty-four to seventy-two hours. In contrast, a high concentration of local vancomycin in a surgical site is transient, and is often significantly reduced by twenty-four hours post-administration to below-cytotoxic concentration levels[27,40].

Other investigators also have used the methicillin-sensitive *S. aureus* strain that we utilized in the current study[23-25]. This bacterial strain has caused the death of untreated control rats, as reported by Yarboro et al.[24], but not of treated rats or New Zealand White rabbits[23,25]. This difference may be associated with the differing immune systems of the different species and the ability of each species to combat local and systemic infections. In the current investigation, there was no associated mortality in either treatment group. In addition, blood and liver samples were sterile, indicating that a systemic infection did not occur. As we did not include a control group that was not managed with cefazolin or vancomycin, we do not know whether untreated rabbits would have a higher bacterial inoculation or mortality rate. Also, caution should be used when extrapolating these results to infections caused by organisms other than methicillin-sensitive *S. aureus*.

We acknowledge several study limitations. First, we used a strain of methicillin-sensitive *S. aureus*. This bacterium was chosen because, in prior animal experiments, it had been shown to reliably reproduce in vivo infections. Our results may not extrapolate to other bacteria, which will be a focus of future work, but, to our knowledge, these results provide the first basic-science data that support prior clinical reports of diminished rates of surgical site infections with use of intrawound vancomycin. Second, we chose the fourth postoperative day end point to evaluate for tissue bacterial infection because the local tissue concentration of vancomycin drops steadily after the third postoperative day[27]. Thus, we are unable to offer evidence regarding the effectiveness of local vancomycin in preventing chronic, delayed infections. The fact that no bacteria were found in the vancomycin group supports the notion that the contaminant had been eliminated and that any future, deep infection would require hematogenous seeding. The primary aim of the present study was to assess whether a local vancomycin concentration could substantially eliminate a known bacterial wound contaminant, and this was shown by our results. Although our study was adequately powered to achieve our primary aim, it may have been underpowered to accurately assess the risk of complications related to high-concentration, local vancomycin. The use of a 100-mg vancomycin local dose was chosen to mimic that used in the largest clinical series of which we aware—that of Sweet et al., who used 2 g of vancomycin powder in 911 patients without reported systemic toxicity[27]. None of the vancomycin-treated rabbits had any apparent clinical vancomycin toxicity effects within the four postoperative days, but we were unable to reliably measure serum vancomycin levels in these rabbits.

In conclusion, the use of intrawound vancomycin powder eliminated *S. aureus* bacterial contamination in rabbit spine surgical wounds, whereas the use of systemic cefazolin alone did not. A high concentration of vancomycin is produced at the surgical site with no apparent systemic toxicity from this local concentration. The present study corroborates the findings in prior clinical series that demonstrated the effectiveness of intrawound vancomycin powder in spinal surgery. ■

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