SCIENTIFIC ARTICLE

Effect of Local Delivery of Vancomycin and Tobramycin on Bone Regeneration

Wei Han, MD, FRCS[1], Lei Zhang, MD, PhD[2], Ling-jia Yu, MD, PhD[3], Jun-qiang Wang, MD[1]

[1]Department of Traumatology, Beijing Jishuitan Hospital and [3]Department of Orthopaedics, Beijing Friendship Hospital, Capital Medical University, Beijing and [2]Department of Oral and Maxillofacial Surgery, School of Stomatology, China Medical University, Shenyang, China

Objective: A bone defect rat model was established to investigate the osteogenic effect of local delivery two antibiotics (vancomycin and tobramycin powder) on bone regeneration.

Methods: Twenty-four Sprague–Dawley (SD) male rats (6 to 8 weeks, 200 to 250 g) were used in this study. All these rats were randomly divided into four groups. Based on dose conversion between rat and human *via* body surface area, the rat dose of two antibiotics was 88μg/g and 176 μg/g for vancomycin and tobramycin, respectively. Con group (no antibiotic), Van group (vancomycin, 88 μg/g), Tob group (tobramycin 176 μg/g), and Van +Tob group (vancomycin 88μg/g combined with tobramycin 176 μg/g). A 5.0-mm full-thickness standardized mandibular bone defect was performed with a drill in each rat and different antibiotic powders were placed over the bone defect space, respectively. All these animals were sacrificed after 12 weeks post-operation. The mandible bones were harvested for further radiographic and histologic analysis. The bone volume/total volume (BV/TV) ratio, bone volume (BV), and bone fractional area (BFA) in the defect area *via* micro-computed tomography (μCT scanning) were further analyzed. Then, we performed a histological assessment *via* hematoxylin and eosin (H&E) and Masson's trichrome staining to analyze bone regeneration and also analyze the number of osteoblasts per filed.

Results: There were no postoperative deaths, signs of vancomycin-related or tobramycin-related toxicity, or signs of systemic illness in any of the four groups. All wounds healed well, and no complications or surgical site infection were observed in all rats. From the μCT scans analyses, there was less bone regeneration in the Van group than in the Con group (BV/TV: $F = 64.29$, $R^2 = 0.9602$; $P = 0.0052$; BFA: $F = 76.17$, $R^2 = 0.9662$, $P = 0.0007$; BV: $F = 194.4$, $R^2 = 0.9865$, $P = 0.0022$). However, when the tobramycin and vancomycin were combined, an increase in bone defect re-ossification was found in the Van+Tob group than in the Van group (BV/TV: $F = 64.29$, $R^2 = 0.9602$, $P = 0.0033$; BFA: $F = 76.17$, $R^2 = 0.9662$, $P = 0.0006$; BV: $F = 194.4$, $R^2 = 0.9865$, $P = 0.0033$). Routine H&E and Masson staining supported the finding of μCT scanning. Quantitative indices confirmed that both the bone regeneration and the number of osteoblasts per filed in the defect area was higher in the Van+Tob group than in the Van group (percentage of bone tissue: $F = 145.7$, $R^2 = 0.9562$, $P = 0.0008$; number of osteoblasts per file; $F = 67.3$, $R^2 = 0.9098$, $P < 0.0001$). There was no significant difference between the Con group and the Van+Tob group on the number of osteoblasts each field ($F = 145.7$, $R^2 = 0.9562$, $P > 0.9999$).

Conclusion: For bone defect, local application of vancomycin combined with tobramycin was recommended over vancomycin alone. This animal study presents data suggesting that the use of local delivery of vancomycin and tobramycin should be investigated further in clinical studies.

Key words: Bone regeneration; Local delivery; Osteogenesis; Tobramycin; Vancomycin

Address for correspondence Jun-qiang Wang, MD, Department of Traumatology, Beijing Ji shui tan Hospital, Beijing, China 100035 Tel. +86 18618460987; Email: drw_jq2020@163.com

Grant Sources: This research was funded by National Key R&D Program of China (2017YFC0110600).

Disclosure: The authors declare no conflict of interest.

Received 31 August 2020; accepted 21 March 2021

Orthopaedic Surgery 2021;13:1654–1661 • DOI: 10.1111/os.13020

Introduction

Traditional systemic intravenous prophylactic antibiotics could reduce the infection rates and has become a standard preoperative strategy. However, the surgical site infection is a common complication in orthopedic surgery that can increase the cost of treatment[1]. Furthermore, the offending organisms are becoming more resistant to antibiotics. Studies showed that more than 50% of Staphylococcus isolates from postoperative infections are insensitive to cephalosporins[2,3]. Traditional systemic intravenous prophylactic antibiotics mainly rely on the diffusion into the surgical site. Although the adequate serum antibiotics concentrations, the concentration of antibiotic scan are lower than the minimum inhibitory concentration of the target tissue. However, local application of antibiotics to the surgical sites could keep an adequate therapeutic drug level, providing an effective minimum inhibitory concentration in the surgical sites.

Local administration of antibiotics powder has been advocated as an adjuvant measure to help preventsurgical site infection. Local intrawound delivery of antibiotic powder after orthopedic surgery has been shown to reduce infection rates as much as 10-fold[1]. This prophylactic method is better than intravenous and oral antibiotics, because of the adverse effects of systemic administration of antibiotics such as nephrotoxicity[4]. Furthermore, local delivery can reduce antibiotic resistance. Thus, topical delivery antibiotic powder has become an increasing measure for reducing the postsurgical infection rate in orthopedic procedures. Recent studies have suggested that vancomycin or tobramycin is safe and could reduce postoperative infection rates[5–8].

Vancomycin is an antibiotic used to treat a number of bacterial infections, such as bone and joint infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA)[9]. Topical delivery of vancomycin in the surgical site is not correlated with vancomycin-resistant bacteria. However, topical administration of vancomycin powder can reduce the infection of gram-positive bacteria, but not the gram-negative bacterial infections[10]. There was an increased incidence of gram-negative organisms in infections after local application of vancomycin in the wound *versus* no vancomycin[11].

Tobramycin belongs to the aminoglycoside family and is derived from Streptomyces tenebrarius. Tobramycin is widely used in the treatment of moderate to severe bacterial infections due to sensitive organisms. Tobramycin transports into the cell and interferes with the initiation complex between messenger RNA and the 30S subunit, thereby inhibiting initiation of protein synthesis, consequently leading to bacterial cell death. It has a narrow spectrum and is used to treat various types of gram-negative bacteria infection. It is especially effective against species of *Pseudomonas aeruginosa*[12,13]. Vrabec *et al.*[14] evaluated the local concentration of tobramycin in an *in vivo* study and found that local tobramycin bone cement can be used to obtain short-term supratherapeutic local concentrations in the surgical site while maintaining minimum serum tobramycin levels. A recent study proved that the topical application of tobramycin combined with vancomycin powder further reduced the infection rate than vancomycin alone[15].

Another study showed that local delivery of vancomycin and tobramycin could effectively inhibit the acute prosthetic joint infection. Because they have found that all bacteria in their study resistant to tobramycin were susceptible to vancomycin and vice versa[16]. Thus, the combination of topically used of vancomycin and tobramycin powder provide a broad spectrum of antimicrobial activity.

However, studies have suggested that local delivery of vancomycin inhibits the osteogenesis process. For example, Eder *et al.*[17] used primary osteoblast cells to perform an *in vitro* study, and proved that intrawound application of high dosage vancomycin interferes with bone regeneration and increases the risk of non-union. Seavey *et al.*[18] also demonstrated that the heterotopic ossification formation could be reduced by topical application of vancomycin powder. Furthermore, Ishida *et al.*[5] performed lumbar fusion surgery on 60 Lewis rats using syngeneic iliac crest allograft mixed with clinical bone-graft substitute and two different antibiotics. They found that local application of vancomycin detriment the fusion-mass formation in a rat model. They also found that tobramycin administration could augment fusion-mass formation. Nart *et al.*[19] assessed the clinical and radiographic outcomes of the regenerative treatment of peri-implantitis using a vancomycin and tobramycin impregnated allograft after a 12-month period. They found that the use of locally delivered antibiotics together with the bone graft may reduce the undesirable effects related to the systemic administration and the risk of resistances and the application of combined antibiotics with collagen membrane yielded positive outcomes of radiographic bone fill. Thus, the combination of vancomycin and tobramycin antibiotics covers the majority of the relevant spectrum in orthopedic surgery. And the addition of tobramycin may rescue the osteogenic potential inhibited by vancomycin *in vivo*.

In this study, we would like: (i) to exam the osteogenic effects of topical delivery of vancomycin; (ii) to evaluate the combination of vancomycin and tobramycin on osteogenic effects in a bone defect model; and (iii) to discuss the safety of local application vancomycin and tobramycin powder. We first established a mandibular bone defect rat model *in vivo*. Then the vancomycin and/or tobramycin were tropical administrated in the surgical site. Twelve weeks post operation, the samples were collected. We further evaluated the osteogenic effects of topical delivery of dural antibiotics *via* radiographic and histological analysis.

Materials and Methods

Animal Care and Surgical Procedures

All animal experiments were approved by the protocol of the Institutional Animal Care and Use Committee at China Medical University (CMU). Sprague–Dawley (SD) male rats (n = 24, 6 to 8 weeks, 200 to 250g) were provided by CMU. We used clinical concentration of antibiotics in this study

(vancomycin, 1 g per person[20,21] = 14.28 μg/g; tobramycin, 2 g per person[5,22]=28.57 μg/g). The dose of reagent applied in rats was calculated based on dose conversion between rat and human *via* body surface area (BSA)[23]. Based on Formula 1, rat dose of two antibiotics was 88 μg/g and 176 μg/g for vancomycin and tobramycin, respectively.

$$Ratdose = Humandose \times \frac{humanKm}{ratKm}.$$

Formula 1: The rat dose translatedfrom human dosebased on BSA.

The bone defect model was performed as previously reported by Zhou *et al.*[24]. Briefly, all the rats were anesthetized with 2.5% isoflurane in 100% oxygen at 0.5 L/min and were operated on an animal surgery table at room temperature and 50% humidity. After hair was shaved, the skin was prepared with 75% alcohol. A 10.0-mmskin incision was made over the lateral aspect of the mandible. A 5.0-mm full-thickness standard mandibular bone defect was performed with a stainless-steel high-speed dental drill. Then, all the rats were randomly divided into four groups. There were six rats in each group of animal model: Con group (no antibiotic), Van group (vancomycin, 88 μg/g), Tob group (tobramycin, 176 μg/g), and Van+Tob group (vancomycin 88 μg/g combined with tobramycin 176 μg/g). The antibiotic powder was placed over the bone defect space, respectively. The masseter and the skin were closed. After surgery, general condition was monitored; buprenorphine (0.01 mg/kg) was administered *via* intraperitoneal injection, and soft gel food was provided for 72 hours. All the animals were sacrificed after 12 weeks postoperatively for further analysis.

Radiographic Analysis

Samples were harvested at 12 weeks, scanned using micro-computed tomography (μCT) system (InspeXio SMX-90CT Plus, SHIMADZU, Japan). μCT was used to detect the newly formed bone within the mandibular defects. Because μCT is a non-destructive three-dimensional (3D) imaging modality and the gold standard for evaluating bone microarchitecture. All the images were evaluated by two authors (L.J.Y. and W.H.) in a blinded fashion. The new bone formation was analyzed in three different ways: the bone volume (BV), bone volume/ total volume ratio (BV/TV), and bone fractional area (BFA) in the defect area were calculated by Mimics software (Materialise, Leuven, Belgium).

Histologic Analysis

After radiographic evaluation, samples were fixed in 10% Ethylene Diamine Tetraacetic Acid (EDTA) for 20 days. Then, samples were sectioned in a coronal plane at 10μm thickness. Hematoxylin and eosin (H&E) and Masson's trichrome were performed on serial sections. H&E staining is one of the principal tissue stains used in medical diagnosis. This stain shows the general layout and distribution of cells and provides a general overview of a tissue sample's structure and revealed significant new bone formation within the defects. Masson's trichrome is a three-color staining protocol used for distinguishing cells from surrounding connective tissue. Most recipes produce red keratin and muscle fibers, blue or green collagen and bone, light red or pink cytoplasm. The results were observed under a microscope and analyzed by ImageJ software.

Outcome Measurements

Bone Volume (BV)

BV is the mineralized bone with in the defect volume. It was used to evaluate the new bone formation volume in the total defect volume. The total bone defect volume was 19.625 mm^3. The maximum volume of BV was 19.6 mm^3, so BV < 1.96 mm^3 is poor, 1.97–4.9 mm^3 is fair, 5.0–9.8 mm^3 is good, and 9.9–19.6 mm^3 is excellent. The BV was compared among the four groups.

Bone Volume/ Total Volume Ratio (BV/TV)

BV/TVstands for bone volume over total volume. In other words, BV/TV is the volume of mineralized bone per unit volume of the sample. It indicates the fraction of a given volume of interest (VOI, the TV) that is occupied by mineralized bone (BV). BV/TV calculated: new bone volume (mm^3)/total bone defect volume (mm^3) × 100%. 0%–10% is considered poor, 10%–25% is fair, 25%–50% is good, and 50%–100% excellent.

Bone Fractional Area (BFA)

BFA is the percentage of new bone formation area within the total bone defect area in a 2D plane. BFA was also performed to determine the area within the defect covered by new bone formation. BFA calculated: new bone area (mm^2)/ total bone defect area (mm^2) × 100%. 0%–10% is considered poor, 10%–25% is fair, 25%–50% is good, and 50%–100% excellent.

The Number of Osteoblasts per filed

The number of osteoblasts per filedindicates the osteogenesis of the bone defect. It was used to evaluate the new bone formation volume indefect volume. It is the number of cells in a given volume of interest (VOI) of new bone area. The number of cells were compared among the four groups.

The Percentage of Bone Tissue (Bone Tissue %)

The percentage of bone tissue stands for mineralized bone area over a defect area. It indicates the bone defect re-ossification. It was used to evaluate the new bone formation area in defect area. Bone Tissue percentage calculated as new bone formation (mm^2)/volume of interest (VOI) (mm^2) × 100%. 0%–10% is considered poor, 10%–25% is fair, 25%–50% is good, and 50%–100% excellent.

Statistical Analysis

All statistical analyses were performed by Prism software 8.2 (GraphPad) for macOS. One-way analysis of variance was used to compare the differences of BV, BV/TV, BFA, number of osteoblasts per filed and the percentage of bone tissue. All the results were biologically replicated three times. All reported P values are 2-sided, and values of $P < 0.05$ were considered as statistically significant.

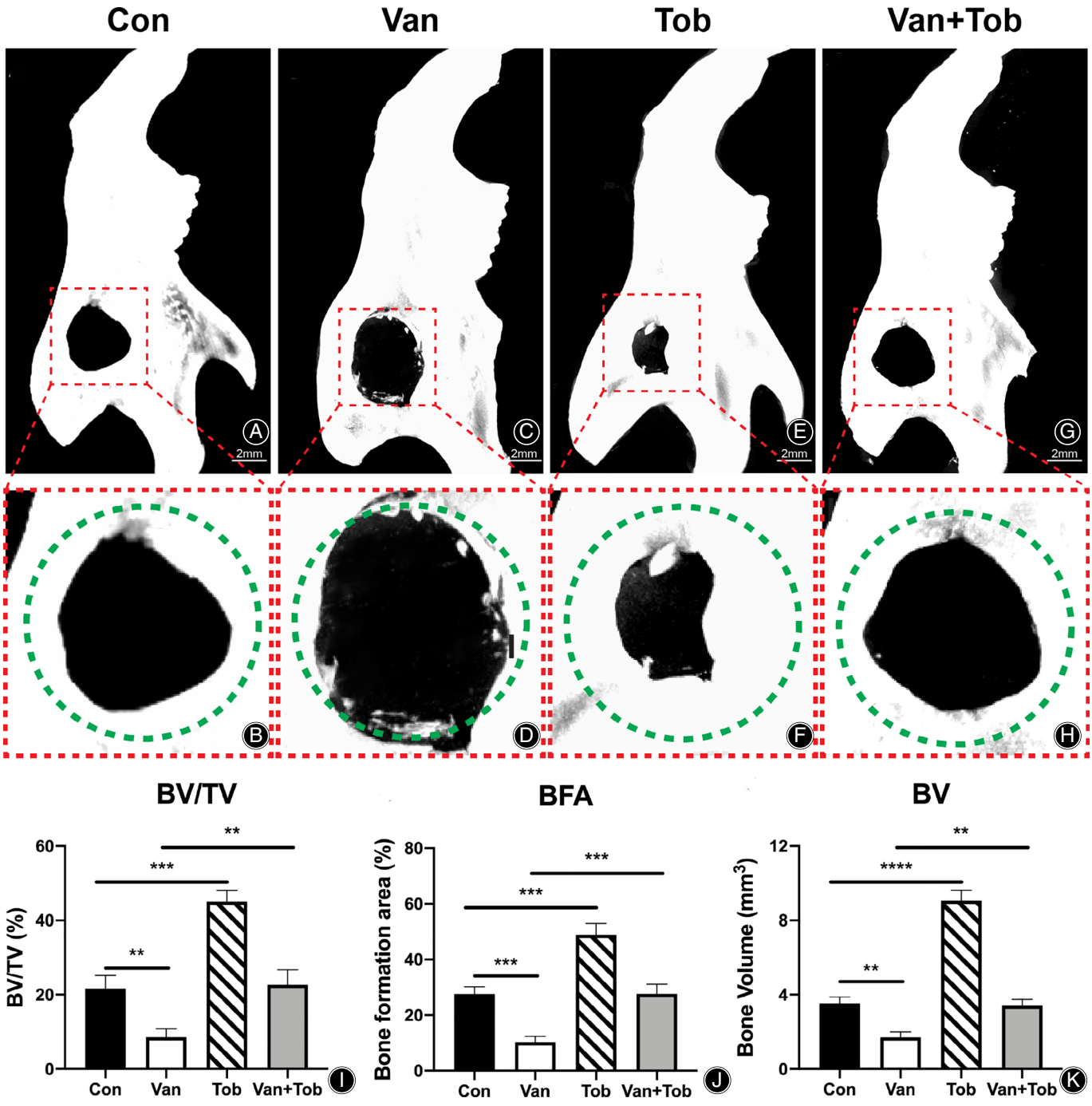

Fig. 1 Antibiotics affect bone regeneration in an animal model was evaluated by μCT scans at 12 weeks. The osteogenic effect of *in vivo* experiments from four different groups: Con group (A and B), Van group (C and D), Tob group (E and F), and Van+Tob group (G and H) (Scale bar, 2 mm). And comparisons of μCTbone defect fusion score among fourgroups: the bone volume/total volume ratio (BV/TV) (I), bone formation area (BFA) (J), and bone volume (BV) (K) in the defect area was calculated. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. (Con: control; Van: Vancomycin; Tob: tobramycin).

Results

Bone Regeneration on μCT Scans

Twelve-weeks post-operation, all wounds were healed well, and no complications were observed in all rats. After sacrifice, the new bone formation was evaluated by μCT. The representative samples of 3-dimensionalμCT scans in each group are shown in Fig. 1–H. We found there was less bone regeneration in the Van group than in the Con group (BV/TV: $F = 64.29$, $R^2 = 0.9602$, $P = 0.0052$; BFA: $F = 76.17$, $R^2 = 0.9662$, $P = 0.0007$; BV: $F = 194.4$, $R^2 = 0.9865$, $P = 0.0022$.) (Fig. 1C and D). When the tobramycin and vancomycin were combined, an increase in bone defect re-ossification was found in the Van+Tob group (Fig. 1G and H). Quantitative indices confirmed that bone formation in the defect area was higher in the Van+Tob group than in the Van group (BV/TV: $F = 64.29$, $R^2 = 0.9602$, $P = 0.0033$; BFA: $F = 76.17$, $R^2 = 0.9662$, $P = 0.0006$; BV: $F = 194.4$, $R^2 = 0.9865$, $P = 0.0033$) (Fig. 1I–K).

Bone Tissue and the Number of Osteoblasts on Histologic Analysis

Routine H&E and Masson staining (Fig. 2) supported the finding of μCT scanning. The results showed that less bone formation area was observed in the Van group (Fig. 2C and D) ($F = 145.7$, $R^2 = 0.9562$; Van *vs.* Con $P < 0.0001$; Van *vs.* Tob $P < 0.0001$; Van *vs.* Van+Tob $P = 0.0008$); The area of bone formation in the Tob group (Fig. 2E and F) was higher than that in the Con group (Fig. 2A and B) ($F = 145.7$, $R^2 = 0.9562$, $P < 0.0001$), and better bone regeneration was observed in the Van+Tob group (Fig. 2G and H) than that in the Van group (Van *vs.* Van+Tob $F = 145.7$, $R^2 = 0.9562$, $P = 0.0008$). The number of osteoblasts each field (×200) of the Tob group was higher than that of the Con group ($F = 67.3$, $R^2 = 0.9098$, $P < 0.0001$) and the number of osteoblasts per field of the Van group was the lowest among the four groups ($F = 67.3$, $R^2 = 0.9098$, Van *vs.* Con $P < 0.0001$; Van *vs.* Tob $P < 0.0001$; Van *vs.* Van+Tob $P < 0.0001$). There was no significant difference between the Con group and the

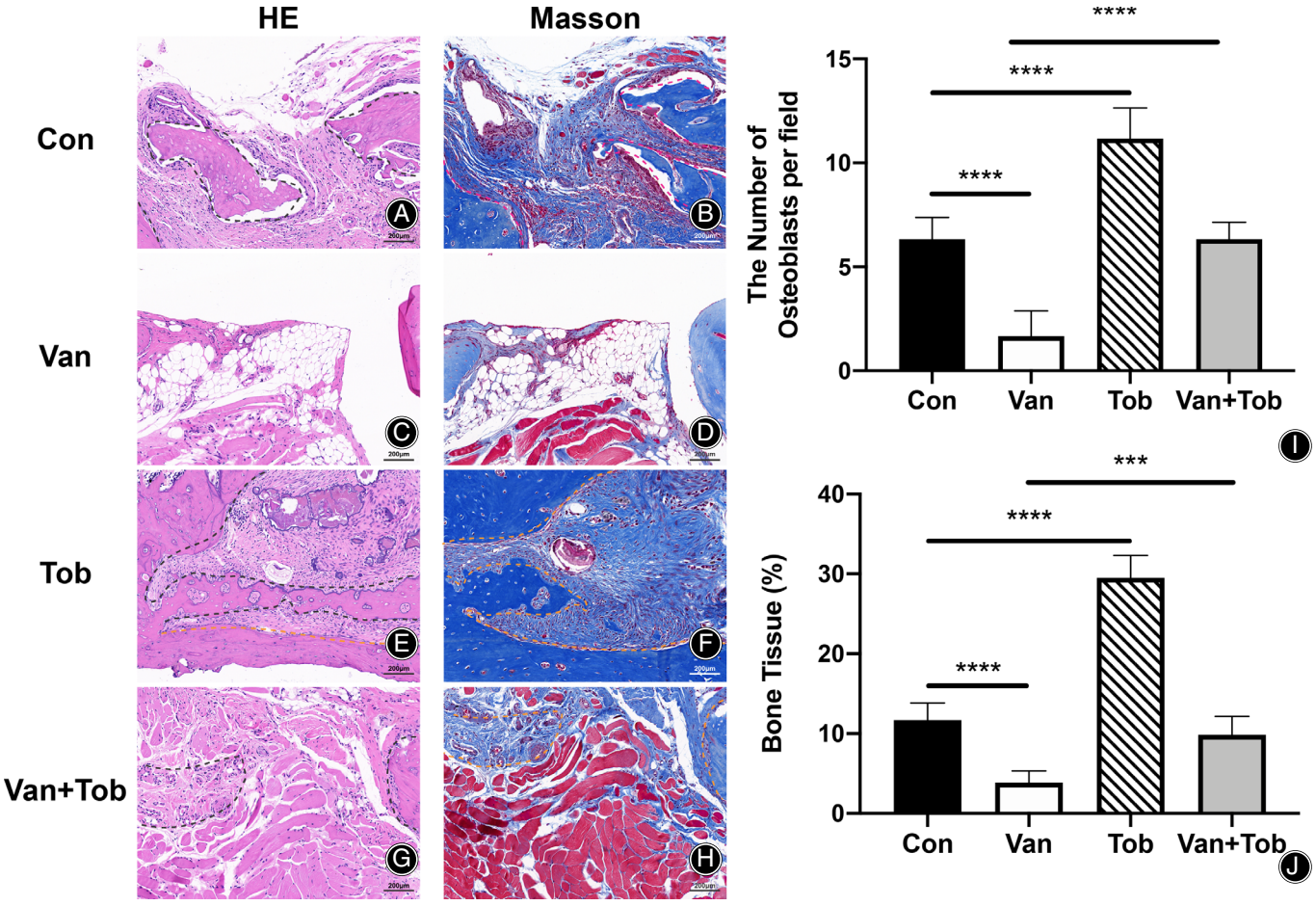

Fig. 2 Histological analysis of bone regeneration of four groups in bone defect model at 12 weeks. Representative images (Scale bar 200 μm) of H&E staining and Masson trichrome staining in the Con group (A and B), Van group (C and D), Tob group (E and F), and Van+Tob group (G and H), indicating the new bone formation inside the dashed line. The number of osteoblasts (I) and the percentage of bone tissue (J) were compared among the four groups. *** $P < 0.001$, **** $P < 0.0001$. (Con: control; Van: Vancomycin; Tob: tobramycin).

Van+Tob group ($F = 67.3$, $R^2 = 0.9098$, $P > 0.9999$) (Fig. 2I). There were no signs of surgical site infection in all the samples.

Discussion

In this study, we have demonstrated that local delivery of vancomycin may have detrimental effects on bone regeneration in the bone defect area, as evidenced by radiographic and histological analysis. However, tobramycin can promote bone regeneration. Additionally, the combination of vancomycin-tobramycin had no inhibitory effect on bone regeneration. We have proved the osteogenic effect of intraoperative administration of these two antibiotics on bone defects in an animal model.

Vancomycin Inhibits Osteogenesis

Local delivery of vancomycin and/or tobramycin therapy is an effective and safe method to reduce bacterial infections in orthopedic surgery. They have been widely used for bone defects, open fractures and bone tissue infections[19,25–27]. However, a higher concentration of vancomycin influences the pH of the cell culture medium, which reduces the formation of osteoblast pseudopodia. Furthermore, the higher concentration of vancomycin suppresses osteoblast migration, inhibits the cell proliferation and viability of osteoblast[17]. Our results proved that vancomycin can also reduce bone regeneration *in vivo*. Another *in vivo* study showed that local delivery of vancomycin powder affects the bone fusion process[5]. In contrast, another study demonstrated that bone morphogenetic protein-2 (BMP-2) combined with vancomycin did not affect osteogenesis *in vivo*[21]. However, BMP-2 addition may result in heterotopic ossification in orthopedic surgeries[28] and offset the negative effect of vancomycin on bone regeneration.

Tobramycin Promote Osteogenesis

The addition of tobramycin has a synergistic-like effect on bactericidal activity of vancomycin[22]. Several studies recommended topical administration of vancomycin combined with tobramycin powder [8,19,29] intrawound. Recently, it has been proved that tobramycin promotes fracture healing by accelerating osteogenesis differentiation of mesenchymal stem cells through activating the Wnt/β-catenin pathway[30]. Ishida *et al.*[5] also found that tobramycin appeared to augment bone fusion in a rat model, which is comparable with our findings. Another comparative study of tobramycin and vancomycin showed there were better osteogenesis scores in the tobramycin impregnated material group[31]. Additionally,

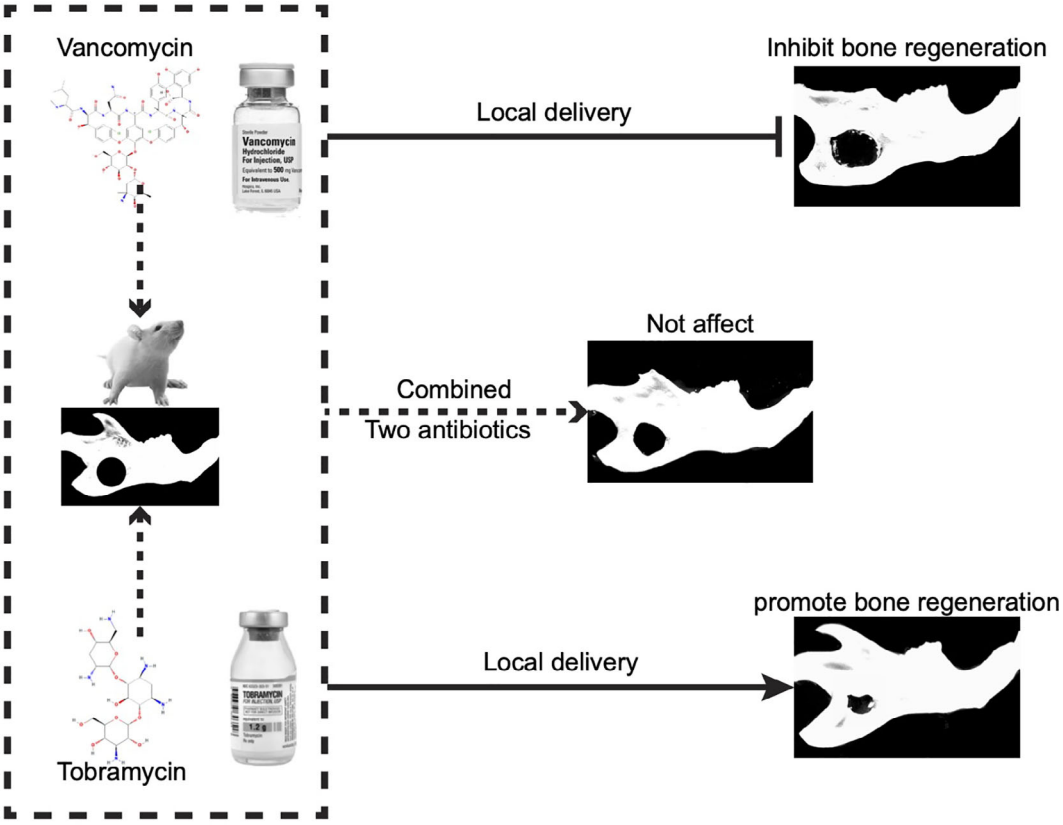

Fig. 3 The schematic diagram of this study.

a study has proved that local delivery of tobramycin hadno cytotoxic and did not affect cell number of osteoblasts[32]. Thus, tobramycin powder could promote bone formation and counteract the negative effect of vancomycin on osteogenesis (Fig. 3).

Limitations

There are several limitations in this study. First, we only explored the osteogenesis of two antibiotics (vancomycin and tobramycin) in our study, other antibiotics such as gentamicin[33], ampicillin[34], and amikacin[35] need to be further explored. Second, we only studied the clinical concentration of these antibiotics. However, we use the BSA as a factor to convert the dose of antibiotics for translation from humans to rats, which better simulate the human equivalent doses in a rat model. We need to further find out the proper concentration and ratio of vancomycin to tobramycin and recommended doses for patients. Finally, we did not address bacterial infection in our animal models. We would like to investigate this in our future *in vivo* studies.

Conclusion

In conclusion, topical delivery of vancomycin can reduce osteogenesis in the bone defect area. However, tobramycin could promote bone regeneration, and further offset the negative osteogenic effect caused by vancomycin. And we recommend the local application of vancomycin combined with tobramycin for anti-infection of bone defect surgery. The safety and efficacy local delivery of vancomycin-tobramycinin reducing the risk of both non-union and surgical site infection in orthopedic surgery needs further exam *via* prospective clinical trials.

Author Contributions

Conceptualization, Supervision, Validation: J.W.; Funding acquisition, Project Administration: W.H.; Methodology, Investigation: L.Z.; Formal Analyses and Data Curation: L.Y.

Data availability statement

All data included in this study are available upon request by contact with the corresponding author.

References

1. Sweet FA, Forsthoefel CW, Sweet AR, Dahlberg RK. Local versus systemic antibiotics for surgical infection prophylaxis in a rat model. J Bone Joint Surg Am, 2018, 100: e120.
2. Anderson DJ, Sexton DJ, Kanafani ZA, Auten G, Kaye KS. Severe surgical site infection in community hospitals: epidemiology, key procedures, and the changing prevalence of methicillin-resistant *Staphylococcus aureus*. Infect Control Hosp Epidemiol, 2007, 28: 1047–1053.
3. Styers D, Sheehan DJ, Hogan P, Sahm DF. Laboratory-based surveillance of current antimicrobial resistance patterns and trends among *Staphylococcus aureus*: 2005 status in the United States. Ann Clin Microbiol Antimicrob, 2006, 5: 2.
4. Pais GM, Liu J, Avedissian SN, *et al*. Lack of synergistic nephrotoxicity between vancomycin and piperacillin/tazobactam in a rat model and a confirmatory cellular model. J Antimicrob Chemother, 2020, 75: 1228–1236.
5. Ishida W, Perdomo-Pantoja A, Elder BD, *et al*. Effects of intraoperative Intrawound antibiotic administration on spinal fusion: a comparison of vancomycin and tobramycin in a rat model. J Bone Joint Surg Am, 2019, 101: 1741–1749.
6. Edelstein AI, Weiner JA, Cook RW, *et al*. Intra-articular vancomycin powder eliminates methicillin-resistant *S. aureus* in a rat model of a contaminated intra-articular implant. J Bone Joint Surg Am, 2017, 99: 232–238.
7. Haimoto S, Schar RT, Nishimura Y, *et al*. Reduction in surgical site infection with suprafascial intrawound application of vancomycin powder in instrumented posterior spinal fusion: a retrospective case-control study. J Neurosurg Spine, 2018, 29: 193–198.
8. Slane J, Gietman B, Squire M. Antibiotic elution from acrylic bone cement loaded with high doses of tobramycin and vancomycin. J Orthop Res, 2018, 36: 1078–1085.
9. Aljutayli A, Marsot A, Nekka F. An update on population pharmacokinetic analyses of Vancomycin, part i: in adults. Clin Pharmacokinet, 2020, 59: 671–698.
10. Li S, Rong H, Zhang X, *et al*. Meta-analysis of topical vancomycin powder for microbial profile in spinal surgical site infections. Eur Spine J, 2019, 28: 2972–2980.
11. Khanna K, Valone F 3rd, Tenorio A, *et al*. Local application of vancomycin in spine surgery does not result in increased Vancomycin-resistant Bacteria-10-year data. Spine Deform, 2019, 7: 696–701.
12. Palser S, Smith S, Nash EF, Agarwal A, Smyth AR. Treatments for preventing recurrence of infection with *Pseudomonas aeruginosa* in people with cystic fibrosis. Cochrane Database Syst Rev, 2019, 12: CD012300.
13. Marti-Carvajal AJ, Dayer M, Conterno LO, Gonzalez Garay AG, Marti-Amarista CE. A comparison of different antibiotic regimens for the treatment of infective endocarditis. Cochrane Database Syst Rev, 2020, 5: CD009880.
14. Vrabec G, Stevenson W, Elguizaoui S, Kirsch M, Pinkowski J. What is the intraarticular concentration of tobramycin using low-dose tobramycin bone cement in TKA: an in vivo analysis?. Clin Orthop Relat Res, 2016, 474: 2441–2447.
15. Lee YP, Farhan SD, Pendi A, *et al*. Does addition of tobramycin powder reduce infection rates after spine surgery?. Global Spine J, 2018, 8: 816–820.
16. Lawrie CM, Jo S, Barrack T, *et al*. Local delivery of tobramycin and vancomycin in primary total knee arthroplasty achieves minimum inhibitory concentrations for common bacteria causing acute prosthetic joint infection. Bone Joint J, 2020, 102: 163–169.
17. Eder C, Schenk S, Trifinopoulos J, *et al*. Does intrawound application of vancomycin influence bone healing in spinal surgery?. Eur Spine J, 2016, 25: 1021–1028.
18. Seavey JG, Wheatley BM, Pavey GJ, *et al*. Early local delivery of vancomycin suppresses ectopic bone formation in a rat model of trauma-induced heterotopic ossification. J Orthop Res, 2017, 35: 2397–2406.
19. Nart J, de Tapia B, Pujol A, Pascual A, Valles C. Vancomycin and tobramycin impregnated mineralized allograft for the surgical regenerative treatment of peri-implantitis: a 1-year follow-up case series. Clin Oral Investig, 2018, 22: 2199–2207.
20. Adogwa O, Elsamadicy AA, Sergesketter A, *et al*. Prophylactic use of intraoperative vancomycin powder and postoperative infection: an analysis of microbiological patterns in 1200 consecutive surgical cases. J Neurosurg Spine, 2017, 27: 328–334.
21. Mendoza MC, Sonn KA, Kannan AS, *et al*. The effect of vancomycin powder on bone healing in a rat spinal rhBMP-2 model. J Neurosurg Spine, 2016, 25: 147–153.
22. González Della Valle A, Bostrom M, Brause B, Harney C, Salvati EA. Effective bactericidal activity of tobramycin and vancomycin eluted from acrylic bone cement. Acta orthop Scand, 2001, 72: 237–240.
23. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J, 2008, 22: 659–661.
24. Zhou X, Zhang D, Wang M, Zhang D, Xu Y. Three-dimensional printed titanium scaffolds enhance osteogenic differentiation and new bone formation by cultured adipose tissue-derived stem cells through the IGF-1R/AKT/mammalian target of rapamycin complex 1 (mTORC1) pathway. Med Sci Monit, 2019, 25: 8043–8054.
25. Shekhar C. An innovative technique in local antibiotic delivery method in open infected wounds of the musculoskeletal system. Int J Low Extrem Wounds, 2019, 18: 153–160.
26. Owen MT, Keener EM, Hyde ZB, *et al*. Intraoperative topical antibiotics for infection prophylaxis in pelvic and acetabular surgery. J Orthop Trauma, 2017, 31: 589–594.
27. Tennent DJ, Shiels SM, Jennings JA, Haggard WO, Wenke JC. Local control of polymicrobial infections via a dual antibiotic delivery system. J Orthop Surg Res, 2018, 13: 53.
28. de Guzman RC, Saul JM, Ellenburg MD, *et al*. Bone regeneration with BMP-2 delivered from keratose scaffolds. Biomaterials, 2013, 34: 1644–1656.
29. Sanicola SM, Albert SF. The in vitro elution characteristics of vancomycin and tobramycin from calcium sulfate beads. J Foot Ankle Surg, 2005, 44: 121–124.

30. Rao M, Pang X, Yang J. Tobramycin promotes fracture healing by accelerating osteogenesis differentiation of MSCs through activating Wnt/beta-catenin pathway. Minerva Med, 2019, 111: 189–192.
31. Pforringer D, Harrasser N, Muhlhofer H, *et al*. Osteoinduction and -conduction through absorbable bone substitute materials based on calcium sulfate: in vivo biological behavior in a rabbit model. J Mater Sci Mater Med, 2018, 29: 17.
32. Rathbone CR, Cross JD, Brown KV, Murray CK, Wenke JC. Effect of various concentrations of antibiotics on osteogenic cell viability and activity. J Orthop Res, 2011, 29: 1070–1074.
33. Anagnostakos K, Meyer C. Antibiotic elution from hip and knee acrylic bone cement spacers: a systematic review. Biomed Res Int, 2017, 2017: 4657874.
34. Takeuchi M, Wakao N, Kamiya M, Hirasawa A, Murotani K, Takayasu M. A double-blind randomized controlled trial of the local application of vancomycin versus ampicillin powder into the operative field for thoracic and/or lumbar fusions. J Neurosurg Spine, 2018, 29: 553–559.
35. Boles LR, Awais R, Beenken KE, Smeltzer MS, Haggard WO, Jessica AJ. Local delivery of amikacin and vancomycin from chitosan sponges prevent polymicrobial implant-associated biofilm. Mil Med, 2018, 183: 459–465.