Efficacy and safety of intra-articular-only meropenem after one-stage revision for treating *Escherichia coli*-induced periprosthetic joint infection in a rat model

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Aims

The optimum type of antibiotics and their administration route for treating Gram-negative (GN) periprosthetic joint infection (PJI) remain controversial. This study aimed to determine the GN bacterial species and antibacterial resistance rates related to clinical GN-PJI, and to determine the efficacy and safety of intra-articular (IA) antibiotic injection after one-stage revision in a GN pathogen-induced PJI rat model of total knee arthroplasty.

Methods

A total of 36 consecutive PJI patients who had been infected with GN bacteria between February 2015 and December 2021 were retrospectively recruited in order to analyze the GN bacterial species involvement and antibacterial resistance rates. Antibiotic susceptibility assays of the GN bacterial species were performed to screen for the most sensitive antibiotic, which was then used to treat the most common GN pathogen-induced PJI rat model. The rats were randomized either to a PJI control group or to three meropenem groups (intraperitoneal (IP), IA, and IP + IA groups). After two weeks of treatment, infection control level, the side effects, and the volume of antibiotic use were evaluated.

Results

Escherichia coli was the most common pathogen in GN-PJI, and meropenem was the most sensitive antibiotic. Serum inflammatory markers, weightbearing activity, and Rissing score were significantly improved by meropenem, especially in the IA and IP + IA groups ($p < 0.05$). Meropenem in the IA group eradicated *E. coli* from soft-tissue, bone, and prosthetic surfaces, with the same effect as in the IP + IA group. Radiological results revealed that IA and IP + IA meropenem were effective at relieving bone damage. Haematoxylin and eosin staining also showed that IA and IP + IA meropenem improved synovial inflammation and bone destruction. No pathological changes in the main organs or abnormal serum markers were observed in any of the meropenem-treated rats. The IA group required the lowest amount of meropenem, followed by the IP and IP + IA groups.

Conclusion

IA-only meropenem with a two-week treatment course was effective and safe for PJI control following one-stage revision in a rat model, with less meropenem use.

Bone & Joint Research

Article focus

- We tested whether the use of intra-articular (IA)-only meropenem after one-stage revision proved to be safer and better at controlling periprosthetic joint infection (PJI) than systemic intraperitoneal (IP) meropenem administration, and achieved a similar level of infection control to IA + IP meropenem administration.
- We performed an in vivo evaluation of IA meropenem treatment compared with IP meropenem treatment and combined treatment following one-stage revision in a PJI rat model of total knee arthroplasty.

Key messages

- IA-only meropenem performed better in eliminating *Escherichia coli* infection than systemic meropenem administration for infections, on both the implant and in joint tissues, after one-stage revision over a two-week course in a PJI rat model.
- IA-only meropenem reduced the amount of antibiotic required.

Strengths and limitations

- We demonstrated that IA-only meropenem injection is effective and safe for the eradication of *E. coli*-induced PJI after one-stage revision, and performs better than systemic meropenem in a rat model, which allowed for a reduction in selection bias and observation bias compared with existing retrospective clinical studies and case reports.
- The current study is a rat-based study, which could not exactly mimic the PJI process in humans, and the rat knee only represents the therapeutic effect in PJI involving non-resistant Gram-negative bacteria. For patients with multi-drug-resistant bacterial infection, the presence of a sinus tract, or immune deficiency, it may be necessary to extend the duration of IA-only treatment or use IA plus systemic antibiotic treatment.
- Our study compared the efficacy of different meropenem administration approaches without an oral step-down therapy, which may have affected the ability of the systemic meropenem to eliminate the infection.

Introduction

Gram-negative (GN) periprosthetic joint infection (PJI) is a refractory complication of arthroplasty, and its proportion in PJIs has grown from 3% to 6% in historical case series[1–3] to 15% to 36% more recently.[4] Moreover, there is a growing burden of GN-PJI because of the huge annual increases in the numbers of primary and revision arthroplasties.[5] Therefore, there is an urgent need to formulate a safe and effective anti-infection treatment.

One-stage revision has been frequently recommended in recent years due to its fewer operations, faster recovery, and lower morbidity.[6,7] However, the infection control rate varies greatly, ranging from 45.5%[8] to 95%.[9] Treatment failure is mainly attributed to bacterial biofilms, which protect pathogens from the immune system, antibiotics, and even mechanical debridement.[10,11] A study reported that the minimum biofilm eradication concentrations (MBECs) of antibiotics are nearly 10^2 to 10^3 times greater than the minimum inhibitory concentrations (MICs) for the planktonic bacteria.[12] However, in joints and infected tissues, intravenous (IV) antibiotics can only reach two to three times the MIC,[13] which is not sufficient to eradicate biofilm bacteria. Moreover, IV antibiotics would cause systemic toxicity before reaching the MBEC at the joint site.[12] Thus, local delivery of antibiotics is recommended to improve the outcomes of one-stage revision. Clinical researchers have proposed that intra-articular (IA) infusion not only provides a high antibiotic concentration in the joint cavity, but also ensures a low systemic toxicity.[14,15]

As a carbapenem, meropenem has the advantages of β-lactamase stability and remarkable antibacterial activity against GN bacteria, and it is regarded as the last line of defence against multidrug-resistant GN bacterial strains.[16] IA carbapenem has been used to control GN-PJI after one-stage revision.[17,18] Although the infection control rate has shown promise, the effects of IA-only carbapenem remain unclear because IV antibiotics have been used simultaneously. Moreover, concerns about potentially poor infection control prevent us from discontinuing IV antibiotics. In addition, retrospective studies and experience-sharing do not provide high-grade evidence. Therefore, we used an *E. coli*-induced PJI rat model to determine the efficacy and safety of IA-only meropenem after one-stage revision, thus providing an experimental basis for rational antibiotic administration after one-stage revision.

Methods

Patients

All consecutive PJI patients in our centre who were infected with GN bacteria between February 2015 and December 2021 were retrospectively included. A total of 36 patients were included. Data on sex, age, BMI, involved joints, presence of a sinus tract, duration of infection, prior transfusion, comorbidities, CRP ESR, prior antibiotic use, American Society of Anesthesiologists (ASA) grade,[19] Musculoskeletal Infection Society (MSIS)[20] category, and surgery-related factors were recorded (Supplementary Table i). The GN bacteria in the GN-PJI patients were analyzed in terms of species, production of extended-spectrum beta-lactamases, co-infections, and antibiotic resistance rates.

Bacterial preparation

E. coli strain ATCC25922 was streaked onto plates containing Luria-Bertani (LB) broth plus agar (1.5%) (Becton Dickinson, USA), and incubated overnight at 37°C for 24 hours. Colonies were cultured overnight in LB broth at 37°C with shaking at 220 rpm. The overnight culture was diluted (1:50) and subcultured for 2.5 hours at 37°C. The mid-logarithmic-phase bacteria were washed and centrifuged (2,057 g, 5 mins) three times and resuspended in sterile phosphate-buffered saline (PBS) to generate concentrations for the PJI rat model described below. The number of colony-forming units (CFUs) was determined by absorbance at 600 nm and verified after overnight culture on LB plates.

Animals and main reagents

Male specific-pathogen-free Sprague-Dawley rats (weighing 286 g ± 10 g) were housed at a humidity of 55% ± 5%, a temperature of 25°C ± 2°C, and a 12/12-hour light/dark cycle. The protocol for the animal experiments was approved by the Institutional Animal Care and Use Committee, and all

procedures were performed following the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care. An ARRIVE checklist is included in the Supplementary Material.

E.coli ATCC25922 was obtained from our centre. Clinical-grade meropenem was obtained from Sumitomo Dainippon Pharma (Japan). The implant was purchased from Suzhou Baiortho Medical Instruments (China). Rat serum alpha-1 acid glycoprotein, interleukin (IL)-6, and tumour necrosis factor (TNF)-α enzyme-linked immunosorbent assay (ELISA) kits and serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine (Cr) kits were purchased from Cusabio (China).

Antibiotic susceptibility of planktonic and biofilm *E. coli*

Each of the four antibiotic susceptibility assays were performed in triplicate. The antibiotics tested were meropenem, imipenem, amikacin, and tobramycin.

Minimum inhibitory concentration (MIC) values were determined by the microtiter method as previously described.[21] Briefly, *E. coli* ATCC25922 was subcultured on tryptic soy agar (Becton Dickinson, Germany) at 37°C for 24 hours. Next, the *E. coli* was inoculated into each well of a 96-well microtiter plate containing twofold dilutions of each antibiotic for incubation at 37°C and 5% CO_2 for 24 hours. MIC values were defined as the lowest antibiotic concentration at which there was no visible *E. coli* growth. Minimum bactericidal concentration (MBC) values were determined using the flash microbicide method as previously described.[22] Briefly, after the 24 hours of incubation at the end of the MIC assay, 10 µl of the solution in each well were mixed with 190 µl tryptic soy broth in a new 96-well plate for static incubation at 37°C and 5% CO_2 for 24 hours. MBC values were defined as the lowest antibiotic concentration at which there was no visible *E. coli* growth. Minimum biofilm inhibitory concentration (MBIC) values were determined as previously described.[23] Briefly, 10^6 CFU/ml *E. coli* ATCC25922 was inoculated into the wells of a microtiter plate, covered with a titanium sheet for bacterial adhesion, and incubated at 37°C for 48 hours. The titanium sheet was rinsed twice and then submerged in a microtiter plate containing twofold dilutions of each antibiotic for static incubation at 37°C and 5% CO_2 for at least 20 hours. MBIC values were defined as the lowest antibiotic concentration at which there was no visible *E. coli* growth. Minimum biofilm eradication concentration (MBEC) values were determined as previously described.[23] Briefly, the lid from the aforementioned MBIC assay was rinsed two times in a plate with wells containing 200 µl saline, and then placed in another plate containing 200 µl tryptic soy broth for static incubation at 37°C and 5% CO_2 for 24 hours. The MBEC values were defined as the lowest antibiotic concentration at which no *E. coli* growth was observed by the naked eye.

Establishment and treatment of PJI rat model

An *E. coli*-induced PJI rat model was established based on Li et al's[24] protocol with slight modifications. Briefly, the joint capsule of the right hind knee was opened through a medial parapatellar arthrotomy after euthanasia using inhalation of 2.5% isoflurane. The femoral canal was reamed using sequentially larger needles until a prosthesis (diameter, 1.2 mm; length, 5 mm) could be manually implanted using a screwdriver, with the 1 mm screw cap protruding into the joint. After reduction of the patella, the capsule was sutured and the joint cavity was injected with 50 µl of 1.0×10^6 CFU *E. coli* ATCC25922.

Two weeks after the PJI model was established, one-stage revision was conducted. The infected prosthesis was removed and the infected soft-tissues and bones were cleaned from the knee joint. A new revision prosthesis from the same manufacturer (diameter, 1.4 mm; length, 8 mm) was implanted. Finally, the surgical incision was closed.

One day after the one-stage revision, the rats were randomly divided into the following four groups (11 rats/group): PJI group, IA group (44 mg/kg meropenem every 24 hours), intraperitoneal (IP) group (88 mg/kg meropenem every eight hours, which represents a therapeutic dose), or IP + IA group. The meropenem administration began on the first day after one-stage revision.[25] Mean and total doses of meropenem were recorded in each group in the first and second weeks after one-stage revision.

Two weeks after meropenem administration was started, the rats were euthanized for prosthesis retrieval, blood collection, and tissue harvesting in accordance with the Institutional Animal Care and Use Committee-approved protocol (Supplementary Figure a).

Systemic and local response analyses

The body weight and body temperature of all rats were measured and recorded before surgery, and once a week after one-stage revision (days 0, 14, 21, and 28). Blood samples were collected from the left ventricle immediately after euthanasia, centrifuged (2,057 g, 10 mins) to obtain serum, and used for ELISAs to assess serum AST, ALT, and Cr levels before surgery and at two weeks after one-stage revision. The rats' weight-bearing activity was evaluated using ink blot analysis (the front paws were covered with blue ink, and the hind paws with red ink), and was graded for each rat as full (3 points), partial (2 points), toe-touch (1 point), or non-weightbearing (0 points).[26] Anteroposterior (AP) and lateral radiograph images of the right hind limbs were taken two weeks after one-stage revision. Based on the AP radiograph images, the maximal femoral width was calculated perpendicular to the anatomical axis of the distal portion of the femur. Radiological score (indicating bone damage) was assessed by an observer (SW) who was blinded to the treatment groups, based on a previously described method.[27] The X-ray machine (MX-20; Faxitron X-Ray Corp, USA) was set at an exposure time of 6,000 ms and a voltage of 50 kVp. The Rissing scale score was used to evaluate the degree of local histopathological changes in the right knee joint.[28]

Scanning electron microscopy

For observation using a JEOL-6610 scanning electron microscope (JEOL, Japan), all prostheses were fixed for three hours with 2.5% (w/v) glutaraldehyde in 0.15 M sodium cacodylate buffer, rinsed with 0.15 M sodium cacodylate buffer, fixed for one hour with 1% (v/v) osmium tetroxide in sodium cacodylate buffer, dehydrated with ethanol, incubated with hexamethyldisilazane, dried in a desiccator overnight, and sputter-coated with gold palladium.

Table I. Analysis of drug resistance of Gram-negative organisms.

Antibiotics	Gram-negative organisms			*E. coli*		
Penicillins	**S, n**	**R, n**	**RR, n (%)**	**S, n**	**R, n**	**RR, n (%)**
Ampicillin	2	18	18/20 (90)	1	11	11/12 (91.7)
Piperacillin	14	17	17/31 (54.8)	2	8	8/10 (80)
Monoamide						
Aztreonam	20	12	12/32 (37.5)	8	5	5/13 (38.5)
Cephalosporin						
Cefazolin	6	20	20/26 (76.9)	2	10	10/12 (83.3)
Cefuroxime axetil	8	16	16/24 (66.7)	3	7	7/10 (70)
Ceftazidime	24	12	12/36 (33.3)	7	5	5/12 (41.7)
Cefepime	25	11	11/36 (30.6)	8	4	4/12 (33.3)
Quinolones						
Ciprofloxacin	24	12	12/36 (33.3)	7	6	6/13 (46.2)
Levofloxacin	24	12	12/36 (33.3)	6	7	7/13 (53.8)
Sulfonamides						
Cotrimoxazole	20	14	14/34 (41.2)	5	7	7/12 (58.3)
Aminoglycosides						
Tobramycin	29	6	6/35 (17.1)	10	3	3/13 (23.1)
Gentamycin	28	9	9/37 (24.3)	7	5	5/12 (41.7)
Amikacin	26	3	3/29 (10.3)	11	1	1/12 (8.3)
Carbapenems						
Imipenem	37	2	2/39 (5.1)	13	0	0/13 (0)
Meropenem	37	2	2/39 (5.1)	13	0	0/13 (0)

E. coli, *Escherichia coli*; R, resistance; RR, resistance rate; S, sensitivity.

Table II. Antimicrobial susceptibility of *Escherichia coli* ATCC25922 strain.

Variable, mg/l	Meropenem	Imipenem	Amikacin	Tobramycin
MIC	0.0625	2	16	4
MBC	0.0625	2	16	8
MBIC	0.25	2	16	8
MBEC	4	128	2,048	1,024

MBC, minimum bactericidal concentration; MBEC, minimum biofilm eradication concentration; MBIC, minimum biofilm inhibition concentration; MIC, minimum inhibition concentration.

Ex vivo bacterial burden

After euthanasia, the surgical skin incision was reopened under sterile conditions. The prosthesis was carefully removed using a screwdriver and placed in 5 ml sterile PBS with 0.3% Tween 20 for ultrasonic oscillation, which was intended to release bacteria from the biofilm. The bone and soft-tissues were also harvested, each placed in 10 ml PBS, and homogenized using a sterile tissue grinder. Next, 100 µl of the supernatants of the prosthesis, bone, and soft-tissue were inoculated onto LB plates and cultured for 24 hours at 37°C, and the bacterial colonies were quantified (CFU/ml) using the plate count method.

Peripheral blood was obtained via heart puncture and placed into blood culture bottles. Pairs of bottles (2.5 ml each; aerobic and anaerobic) were loaded into a BacT/ALERT 3D blood culture system (bioMérieux, France), and monitored over five to seven days of incubation.

Micro-CT

High-resolution micro-CT using a SkyScan 1172 Scanner was used to analyze the level of bone remodelling around the prosthesis. The data were subsequently reconstructed (NRecon v1.6), analyzed (CTAN, v1.9), and visualized as a 3D model (CTVol, v2.0). The micro-CT scanner was set at a 360° scan with 18 µm step size and a voltage of 50 kVp. The coronal view of the 1.0 cm distal femur was selected for 3D histomorphometric analysis. Around the prosthesis, a 2 mm region was identified as the region of interest, and the bone mineral density (BMD) was measured.

Histological analyses

Histological analyses were carried out to assess the bone remodelling, inflammatory response in the capsule and intestinal tract, and liver and kidney tissue impairment. The

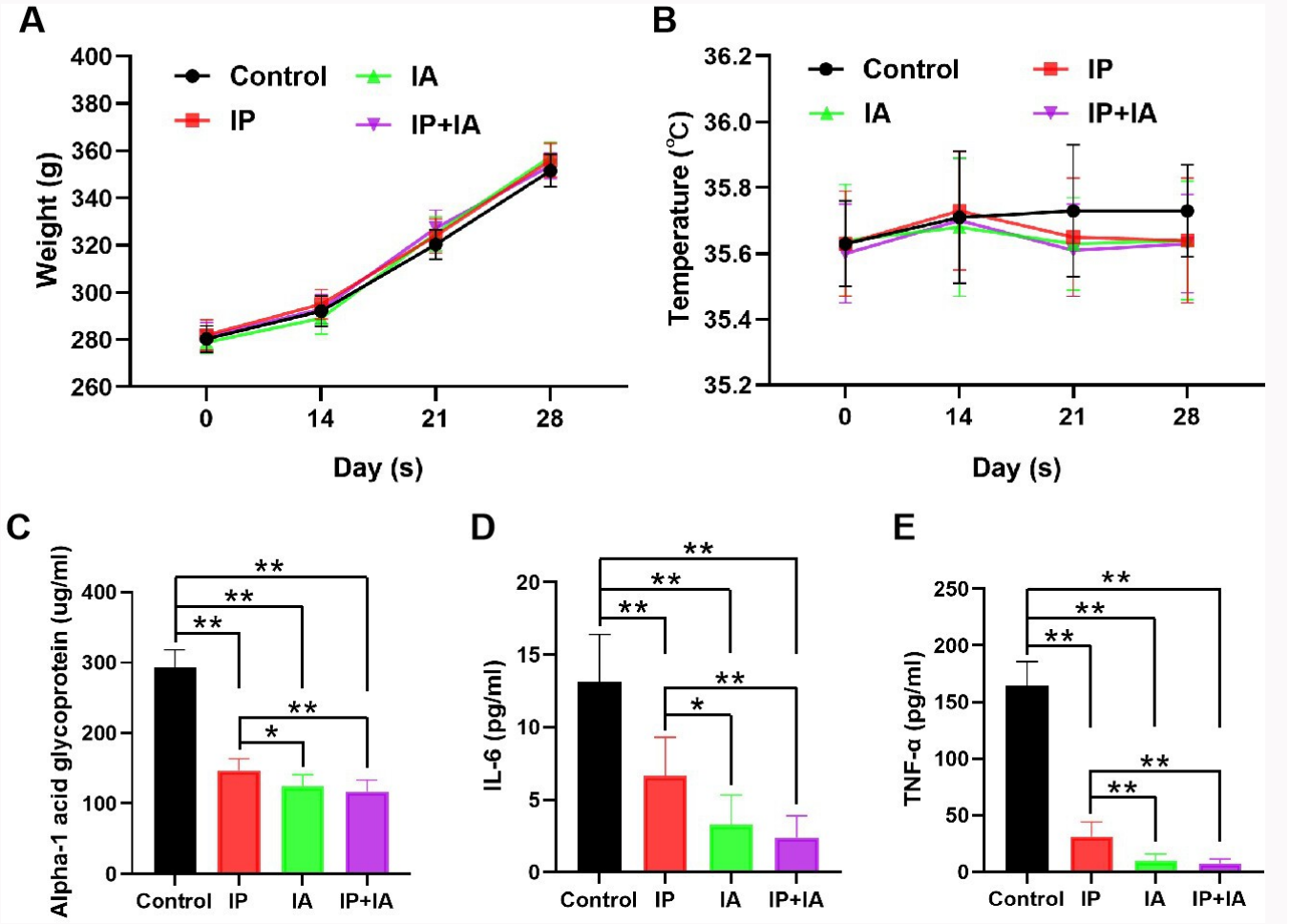

Fig. 1

Changes in general bodily condition in periprosthetic joint infection rats after one-stage revision. a) and b) Changes in body weight and temperature during the whole experiment (days 0, 14, 21, and 28). c) to e) Changes in serum alpha-1 acid glycoprotein, interleukin (IL)-6, and tumour necrosis factor (TNF)-α levels at two weeks after one-stage revision. N = 8 per group. *p < 0.05, **p < 0.01; one-way analysis of variance with Tukey's multiple comparison test or Kruskal-Wallis test with Dunn's multiple comparison test. IA, intra-articular; IP, intraperitoneal.

harvested knee joints of rats were fixed in 4% paraformaldehyde for 24 hours, decalcified for three weeks, embedded in paraffin, and sectioned (4 μm). Fields of interest were selected for observation. Sagittal cross-sections of the femur, tibia, and joint capsule, and gastrointestinal tract and liver tissues, underwent haematoxylin and eosin (H&E) staining for histological observation.

Statistical analysis

The quantitative data were analyzed using GraphPad Prism v8 (GraphPad Software, USA), and are presented as mean and SD. Normal distribution of data was tested with Shapiro-Wilk test. One-way analysis of variance (ANOVA) with Tukey's multiple comparison test, or Kruskal Wallis test with Dunn's multiple comparison test, was used for more than two groups. Significance was evaluated using the non-parametric Mann-Whitney U test for the comparison of colony counts between different treatment conditions, and using Fisher's exact test for the comparison of the percentages of cultures that had any bacterial growth (i.e. were positive). A p-value < 0.05 indicated a significant difference. Each experiment consisted of at least three replicates.

Results

Bacterial species and antibiotic susceptibility in GN-PJI patients

Regarding the 39 GN bacterial species in the 36 GN-PJI patients, the GN-PJIs were mainly caused by *E. coli* (13/39, 33.3%), *Klebsiella pneumoniae* (7/39, 17.9%), and *Enterobacter cloacae* (6/39, 15.4%). Seven of the 39 GN bacteria (3 *E. coli*, 2 *Klebsiella pneumoniae*, and 2 *Proteus mirabilis*) produced extended-spectrum beta-lactamases. There were nine PJI co-infections (six Gram-positive + GN and three GN + GN). Among the GN bacteria, the antibiotic resistance rates were all > 30% except for aminoglycosides and carbapenems, and the penicillin and first- and second-generation cephalosporin resistance rates were > 40%. Of note, the third-generation cephalosporin and gentamycin resistance rates of *E. coli* were > 40%. The GN bacteria showed high sensitivity to carbapenems (especially meropenem and imipenem), amikacin, and tobramycin (Table I).

Antibiotic susceptibility of planktonic and biofilm *E. coli*

To further clarify the sensitivity of *E. coli* to commonly used clinical antibiotics, antibiotic susceptibility assays were performed using both planktonic and biofilm *E. coli*

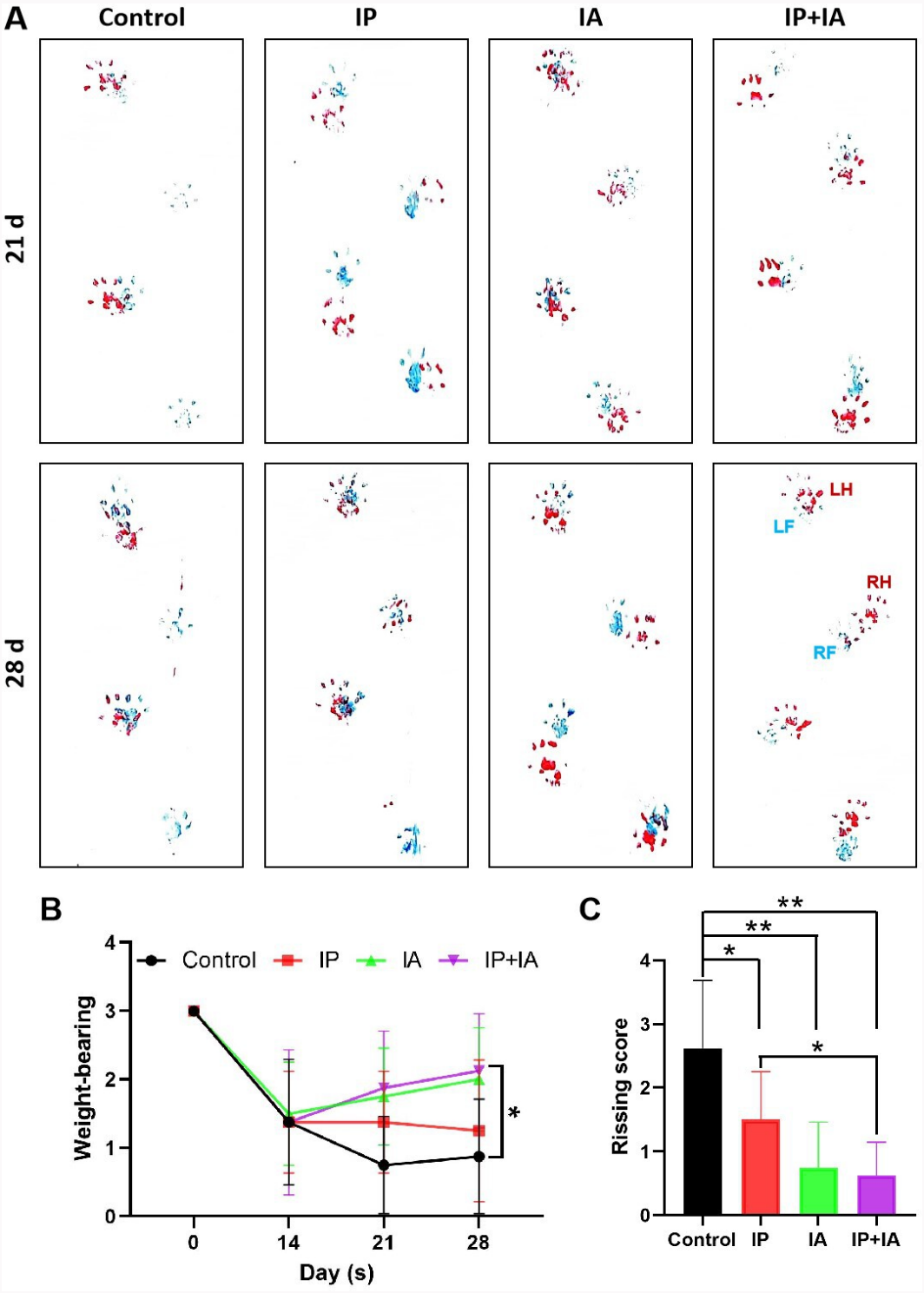

Fig. 2

Changes in weightbearing activity and Rissing scale score in periprosthetic joint infection rats after one-stage revision. a) Representative images of ink blot analysis. b) Weightbearing activity grade. c) Rissing scale scores indicating soft-tissue and bone damage. N = 8 per group. *p < 0.05, **p < 0.01, one-way analysis of variance with Tukey's multiple comparison test or Kruskal-Wallis test with Dunn's multiple comparison test. IA, intra-articular; IP, intraperitoneal; LF, left front (blue); LH, left hind (red); RF, right front (blue); RH, right hind (red).

ATCC25922. Regarding the planktonic state, the MIC and MBC were determined for meropenem (0.0625 and 0.0625 mg/l), imipenem (2 and 2 mg/l), amikacin (16 and 16 mg/l), and tobramycin (4 and 8 mg/l). Regarding the biofilm state, the MBIC and MBEC were determined for meropenem (0.25 and 4 mg/l), imipenem (2 and 128 mg/l), amikacin (16 and 2,048 mg/l), and tobramycin (8 and 1,024 mg/l) (Table II and Supplementary Figure b).

Systemic and local inflammatory responses in rats

To assess the anti-infection efficacy of meropenem after one-stage revision, the systemic and local responses were analyzed. Regarding the systemic responses, there were

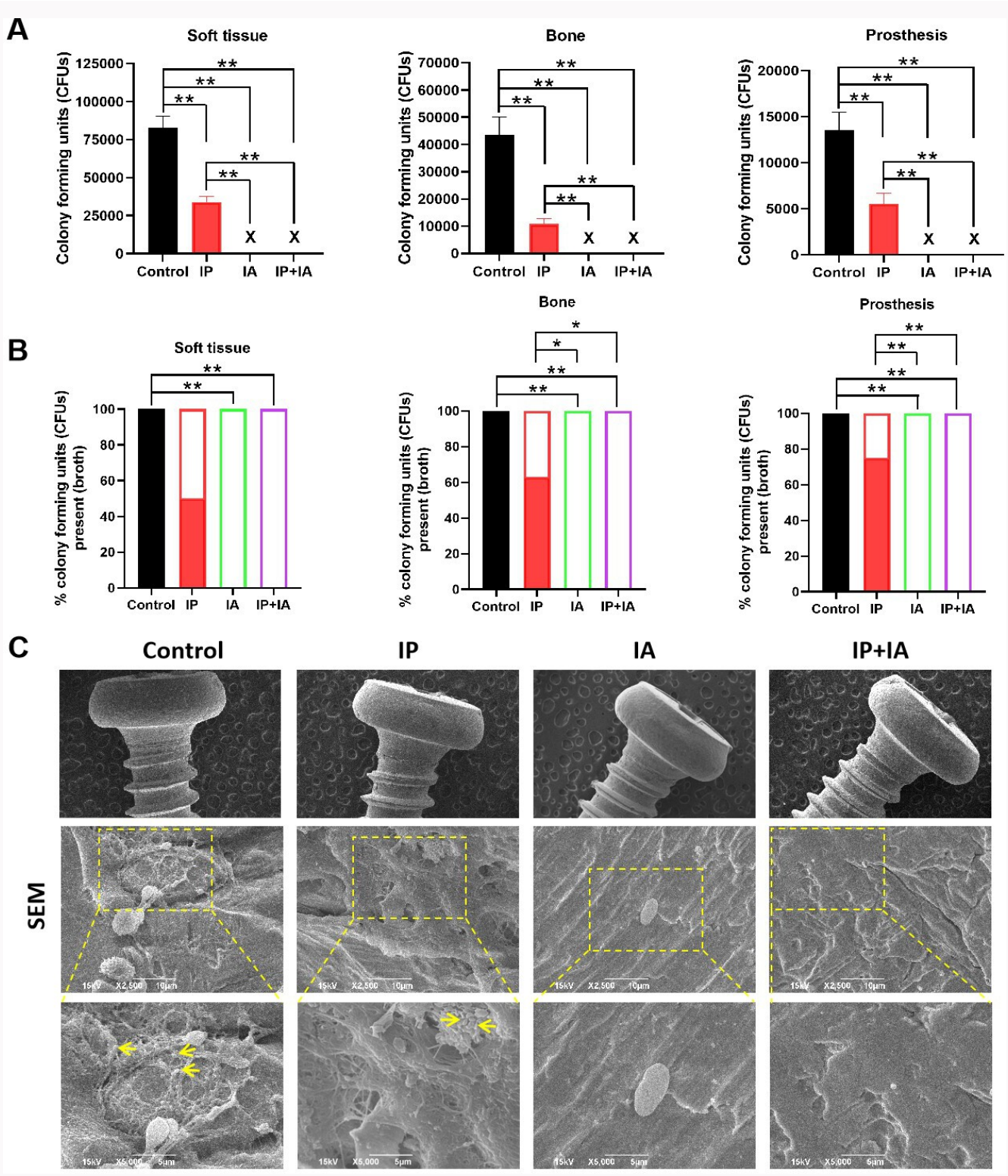

Fig. 3

Escherichia coli (*E. coli*) load in the soft-tissue, bone, and prostheses from periprosthetic joint infection (PJI) rats two weeks after one-stage revision. a) Colony-forming units (CFU)/ml of *E. coli* isolated from the soft-tissue, bone, and prostheses. b) Percentages of cultures showing growth of *E. coli* isolated from the soft-tissue, bone, and prostheses. N = 8 per group. *p < 0.05, **p < 0.01; non-parametric Mann-Whitney U test for the comparison of colony counts between different treatment conditions, and Fisher's exact test for the comparison of the percentages of cultures that had any bacterial growth. c) Representative scanning electron microscopy (SEM) images showing biofilm formation on the prostheses in three meropenem groups (intra-articular (IA), intraperitoneal (IP), and IP + IA) and PJI control group (all treated with *E. coli* to induce PJI). N = 3 per group. The arrows point to *E. coli* cells.

no significant differences among the four groups in body weight or body temperature during the whole experiment (Figures 1a and 1b). However, serum inflammatory markers were significantly reduced in the IP, IA, and/or IP + IA groups compared to the PJI control group. Of note, alpha-1 acid glycoprotein, IL-6, and TNF-α levels were prominently

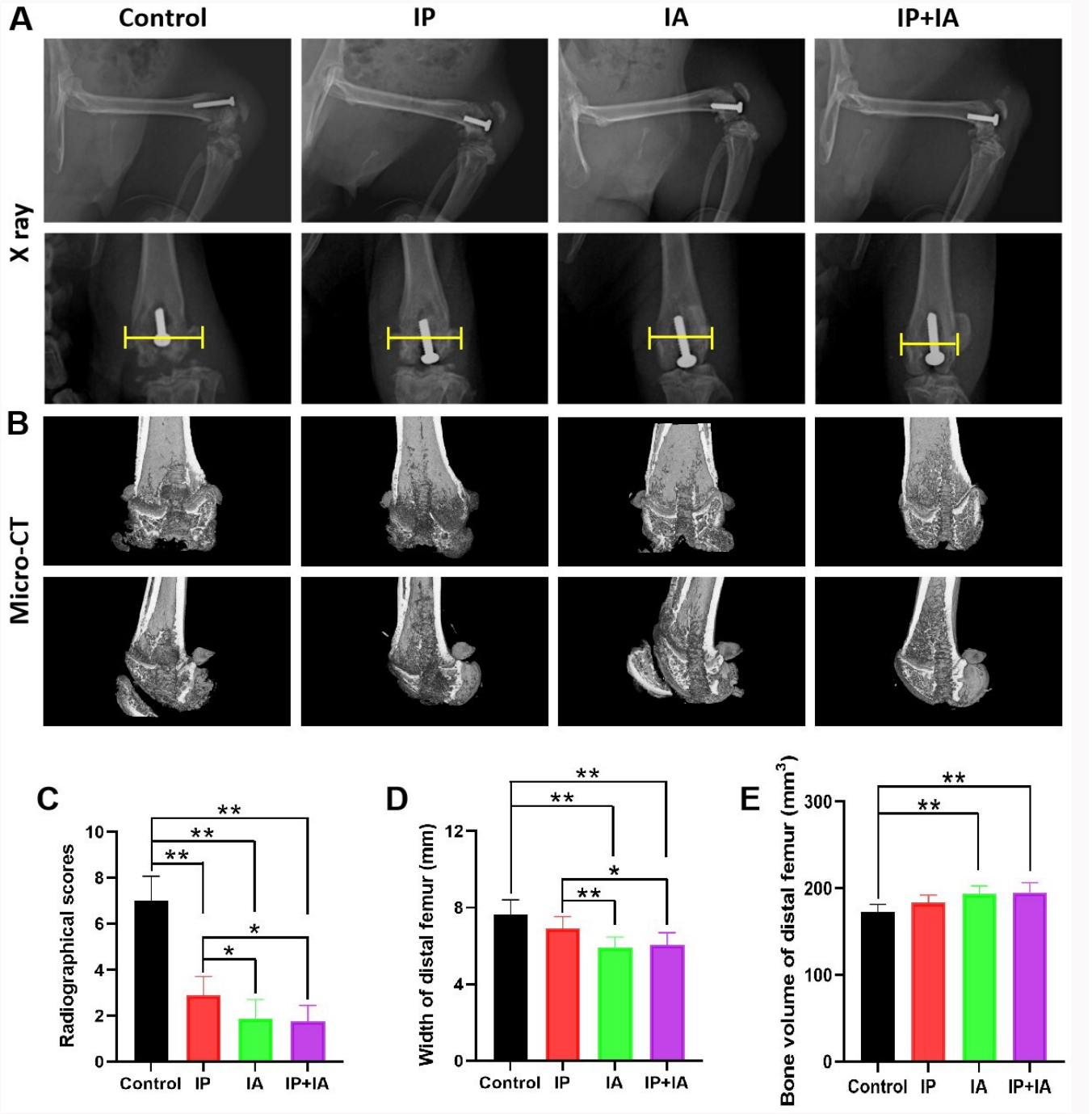

Fig. 4

Radiological evaluation of the knee joint in periprosthetic joint infection rats at two weeks after one-stage revision. a) Representative anteroposterior radiograph images. b) 3D CT scans and distal femur reconstruction. c) Radiological scores indicating bone damage based on radiograph images. d) Quantitative analysis of distal femur width. e) Quantitative analysis of distal femur bone volume. N = 8 per group. *p < 0.05, **p < 0.01; one-way analysis of variance with Tukey's multiple comparison test, or Kruskal-Wallis test with Dunn's multiple comparison test. IA, intra-articular; IP, intraperitoneal.

decreased in the IA and IP + IA groups compared to the IP group (Figures 1c to 1e). Regarding the local responses, weightbearing was markedly decreased in the PJI control and IP groups, while it was significantly improved in the IP + IA group compared to the PJI control group (Figures 2a and 2b; p = 0.037, one-way ANOVA). Moreover, the Rissing scale score was significantly reduced in the three meropenem groups compared to the PJI control group, especially in the IA and IP + IA groups (Figure 2c; p = 0.005 and 0.002, both Kruskal-Wallis test).

Bacterial load on prosthesis surface and surrounding tissues in rats

To confirm the anti-infection effect of meropenem after one-stage revision, CFU/ml measurement and scanning electron microscopy (SEM) were used. The CFU/ml values for the tissue, bone, and prostheses were significantly lower in

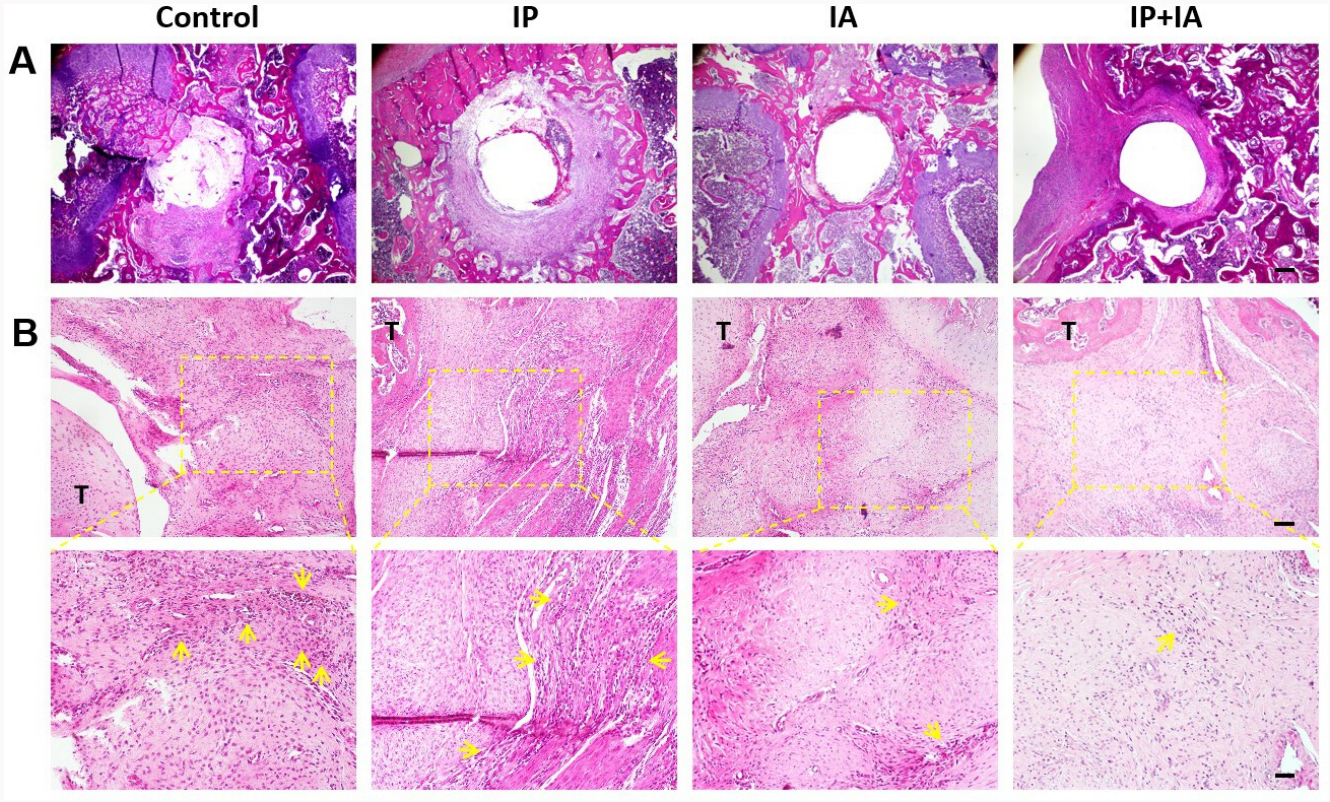

Fig. 5
Histopathological assessment of haematoxylin and eosin-stained knee joints from periprosthetic joint infection rats two weeks after one-stage revision. a) Degree of periprosthesis bone destruction in femur sagittal cross-sections. Scale bar, 200 μm. b) Knee joint (tibia and joint capsule) sagittal cross-sections. Scale bar, 100 μm (top panels) or 50 μm (bottom panels). The arrows point to inflammatory cells. T, tibia.

the three meropenem groups than the PJI control group, with the IA and IP + IA groups reducing the bacterial load (Figure 3a). Moreover, the percentages of culture-positive soft-tissue, bone, and prosthesis samples were markedly higher in the IP group (soft-tissue: 50% (4/8), bone: 62.5% (5/8), prostheses: 75% (6/8)) than in the IA and IP + IA groups (0% for all) (Figure 3b and Supplementary Table ii). There were no culture-positive blood samples in any group (Supplementary Table ii). SEM showed that a small amount of *E. coli* was observed on the prostheses in the IP group, while the IP + IA group had no *E. coli* growth (i.e. the *E. coli* on the prosthesis surface were completely eradicated) (Figure 3c).

Bone microstructure in rats

To assess the changes in the distal femur microstructure, radiograph, micro-CT, and H&E staining were used. The periosteal reaction and osteolysis around the prosthesis were severe in the PJI control and IP groups, but alleviated in the IA group and even more in the IP + IA group (Figures 4a and 4b). The radiological score (indicating bone damage based on radiograph images) was visibly reduced in the three meropenem groups, especially in the IA and IP + IA groups (Figure 4c). The distal femur widths were not markedly increased in the three meropenem groups, with no significant difference between the IA and IP + IA groups (Figure 4d). The distal femur bone volume was significantly higher in the IA and IP + IA groups than in the PJI control group, with no significant difference among the meropenem-treated groups (Figure 4e). Severe bone damage and synovial inflammation around the prosthesis, accompanied by abscess formation, were observed in the PJI control group. The three meropenem groups exhibited reduced inflammation, most prominently in the IA and IP + IA groups (Figures 5a and 5b).

Safety evaluation in rats

The safety of meropenem in major organs was assessed. No obvious pathological changes (such as degeneration or necrosis) were observed in the livers and kidneys of any of the rats (Figure 6a). The serum ALT, AST, and Cr levels in the four groups were all within the normal ranges, and there were no significant differences among them (Figures 6b to 6d). There was no leucocyte infiltration, fibrosis, high vascular density, or colon wall thickening in the H&E-stained intestinal sections in any group (Figure 6a).

Antibiotic dosage analysis in rats

The mean and total doses of meropenem that were applied by injection to the rats (both in the first and second weeks after one-stage revision) were lowest in the IA group followed by the IP and IP + IA groups. The total dose of meropenem in the IA group was twice and three times that in the IP and IP + IA groups, respectively (Supplementary Figure c).

Discussion

Although the advantages of IA injection for PJI are understood, there is concern that IA-only injection may lead to the failure of anti-infection therapy, so combination therapy is used instead (including IV antibacterials, which have been

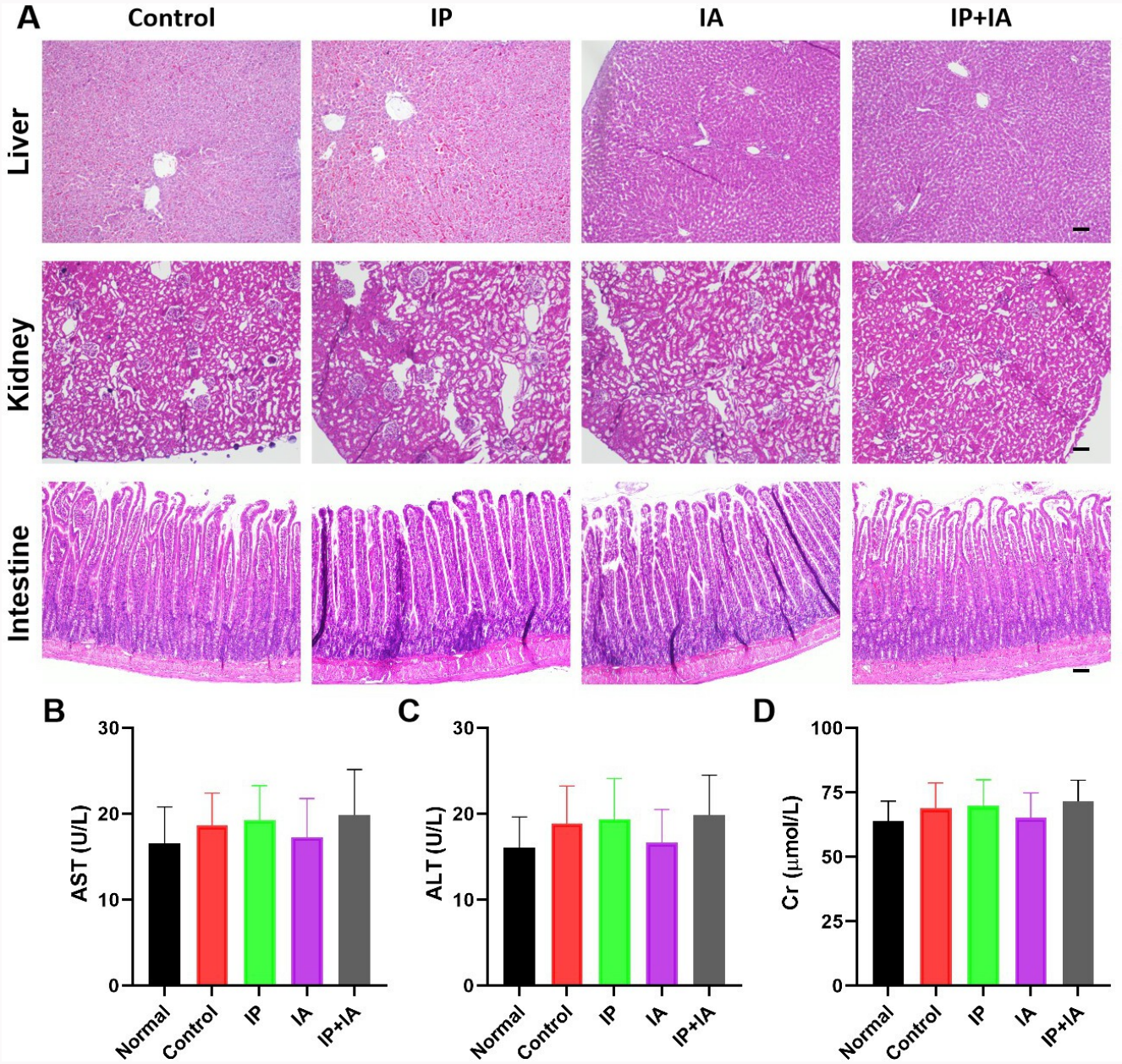

Fig. 6

Photographs of haematoxylin and eosin (H&E)-stained liver, kidney, and intestine sections, and liver and kidney biochemical indicators in periprosthetic joint infection rats. a) H&E-stained liver, kidney, and intestine sections. b) to d) Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and Cr levels. "Normal" indicates normal serum biochemical values before surgery. N = 8 per group. Scale bar, 100 μm (top panels) or 50 μm (middle and bottom panels). IA, intra-articular; IP, intraperitoneal.

used for decades).[17] However, prolonged systemic administration of antibiotics, especially vancomycin, can cause adverse events, such as kidney toxicity.[29] Fortunately, patients with knee and hip PJI treated with one-stage revision combined with IA-only antibiotics had satisfactory infection control rates (94.4 to 95%), indicating that IA-only antibiotic treatment is feasible.[14,30,31] However, these retrospective and empirical reports with small sample sizes required verification. To our knowledge, our study is the first to report on the GN bacterial species and antibiotic susceptibility related to clinical GN-PJI cases, which could be used to guide empirical treatment before culture results are obtained, and used a simplified rat model of IA-only meropenem treatment for GN-PJI after one-stage revision, which provides supplemental evidence concerning the effectiveness and safety of IA-only meropenem.

The selection of pathogen-sensitive antibiotics is the key to infection eradication after surgery. Although fluoroquinolones and cephalosporins are the first-line antibiotics for GN bacterial infections (which typically involve pneumonia, urinary tract infection, and gastroenteritis), the associated resistance rates are continually increasing.[32,33] We found a resistance rate of 30.6% to 76.9% for cephalosporins, 33.3% for quinolones, 54.8% to 90% for penicillins, and 41.2% for sulfonamides, which makes anti-infection treatment very challenging. Of note, the clinical GN-PJI strains were most sensitive to carbapenems (resistance rate, 5.1%), which achieve a bactericidal effect by inhibiting bacterial

peptidoglycan synthesis.[16] Our in vitro results on *E. coli* ATCC25922 revealed that, in a planktonic state, the MIC of meropenem was 32 times lower than the value for imipenem, 256 times lower than the value for amikacin, and 64 times lower than the value for tobramycin. In a biofilm state, the MBIC and MBEC of meropenem were eight and 32 times lower than the values for imipenem, 64 and 512 times lower than the values for amikacin, and 32 and 256 times lower than the values for tobramycin, respectively. Therefore, we believe that meropenem can be considered as the first-choice antibiotic for GN-PJI.

It is essential to ensure a high local concentration of antibiotics, as a small amount of non-visible biofilms remains in the surgical area after mechanical and chemical debridement.[34,35] However, PJI impairs the blood supply to the bone and soft-tissue around the joint, strongly affecting the concentration of antibiotics that can reach the joint through IV infusion, thus reducing anti-infection efficacy.[13] The results from our IP group support this theory, showing that local and systemic inflammation was not controlled by IP-only infusion, and many pathogenic bacteria remained in the joint. In contrast, as the infection occurs in the joint cavity, confined to a closed space, the loss of IA antibiotics is limited.[17,18] Roy et al[13] reported that IA injection not only provided a high concentration of antibiotics that could be sustained for weeks or months, but also achieved therapeutic serum levels (greater than the concentration required to prevent the development of resistance). We found that IA-only meropenem was able to eradicate the pathogenic bacteria in the joint cavity, thereby suppressing the local and systemic inflammatory responses and aiding the maintenance of the bone microstructure around the prosthesis. Although IP + IA meropenem also achieved excellent efficacy, it is difficult to ignore the side effects of long-term IV antibiotic use. Moreover, the culture-negative blood samples suggest that IV antibiotics may not be necessary. Additionally, all the patients were elderly, with inevitable organ tissue degeneration, so long-term high-dose IV antibiotics should be used cautiously.

Of note, Wei et al[25] conducted a similar study to ours, and suggested that IP + IA vancomycin was the ideal treatment for methicillin-resistant *Staphylococcus aureus* (MRSA)-induced PJI after one-stage revision, as IA vancomycin failed to eradicate MRSA while IP + IA vancomycin did not. We believe that inconsistencies in the conclusions between our study and theirs may be related to differences in the method and scope of debridement (which is a prerequisite for successful anti-infection treatment) and the pathogenic bacteria involved. Our study strictly followed our clinical procedures for one-stage revision; more radical debridement may have been performed in our study,[18,36,37] as the CFUs cultured from the three sample types were lower in our study than in Wei et al.[25] Furthermore, MRSA is highly virulent and has a strong ability to produce biofilms, which makes anti-infection treatment challenging. Despite the differences in conclusions, our findings at least indicate that IA meropenem is the best choice for *E. coli*-induced PJI after one-stage revision.

In addition to the anti-infection effect, the safety of IA meropenem was a focus of this study. Previous research reported systemic side effects of meropenem, mainly including thrombocytopenia, hepatobiliary events, and gastrointestinal events.[16] However, there were no abnormal elevations of serum markers related to liver or kidney function in the IA, IP, or IP + IA groups, and the H&E-stained tissue sections of the main organs did not show degeneration or necrosis. Notably, among the three meropenem groups, the total and mean doses of meropenem (in both the first and second weeks after one-stage revision) were smallest in the IA group. In alignment with the "antibiotic de-escalation" strategy, which involves reducing or stopping antibiotics early, we suggest that IA-only meropenem after one-stage revision is the preferred treatment for GN-PJI.

This study has several limitations. First, although measuring the meropenem concentration in the joint fluid can help to optimize the dosage required, the volume of the rat joint cavity is small and filled with a lot of scar tissue, so it is difficult to obtain joint fluid. Therefore, a large animal model should be established to monitor the antibiotic concentration in synovial fluid. Second, our results may only represent the therapeutic effect in PJI involving non-resistant GN bacteria. For patients with multidrug-resistant bacterial infection, the presence of a sinus tract, or immune deficiency, it may be necessary to extend the duration of IA-only treatment or use IA + IV treatment. Third, the dosage and frequency of meropenem should be further studied in order to optimize the existing empirical anti-infection treatment regimens.

To sum up, we identified that *E. coli* was the most common pathogen in GN-PJI, and meropenem was the most sensitive antibiotic. Two-week IA-only meropenem after one-stage revision can completely eradicate the residual GN bacteria in the joint cavity, effectively inhibit the local and systemic inflammatory responses, protect the host bone microstructure, and cause no side effects. Moreover, IA-only meropenem can reduce the amount of meropenem required. Taken together, our results show that IA-only meropenem can increase the success rate of one-stage revision, which provides a reference for the adjustment of clinical medication regimens.

Supplementary material

Tables showing patient characteristics and the proportions of positive cultures by specimen type and treatment group. Figures illustrating the treatment regimens in the rat study, susceptibility of planktonic and biofilm *Escherichia coli* ATCC25922 to four antibiotics, and the doses of meropenem in the first or second weeks after one-stage revision. An ARRIVE checklist is also included to show that the ARRIVE guidelines were adhered to in this study.

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