Propionibacterium Acnes Cause Delayed Surgical Site Infection Only In The Presence Of Implant

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Disclosures:

Introduction: It is recently reported that Propionibacterium Acnes (P. acnes) causes implant-associated infection (IAI) in orthopaedic surgeries. We previously revealed that P. acnes were frequently detected in the intraoperative specimens from the scoliosis surgery. However, no patients developed clinically apparent postoperative infection in our series. (Spine 2011) Little is known about the kinetics of P. acnes in vivo. Here we investigated whether P. acnes causes IAI under the presence or absence of titanium implant.

Methods: In the model of osteomyelitis, P.acnes (ATCC: 1×10⁸ CFU) were inoculated into the femur of the BALB/c adult male mouse with (implant group) or without (control group) 0.5×8mm titan alloy bar. Bacterial detection probe labeled with Cy5 fluorescent dye was injected intravenously, then kinetics of P. acnes in the mice was captured with the trans illumination feature of IVIS Lumina optical imaging system (Caliper) for 6 months (day1, 3, 7, 14, 28, 56, 84, and 168). We also observed the surface of implant by fluorescence microscope after Cy2 LIVE/DEAD® BacLight staining (Molecular Probes) and Scanning Electron Microscope (SEM). Histological analysis of the femurs was performed at the different time points. Anaerobic culture and PCR of purulent effusion from the femur in the 6-month mouse was performed.

Results: In the osteomyelitis model, the bacterial signal from P. acnes was clearly identified in the femur at 12 hours after the bacterial probe administration using optical imaging system (Figure1). During the first 7 days, bacterial signal from P. acnes was clearly identified in the femur in both groups. Afterward, the bacterial signal completely disappeared in the control group on 28 days. Surprisingly, in the implant group, the bacterial signal was maintained over 6 months. The signal intensities at day1, 3, 7, 14, 28, 56, 84, and 168 in the implant group were significantly higher than those in the control group (p < 0.05 each) (Figure 1). Microscopic findings showed that P. acnes survived in the biofilm around the implant, and active inflammation and abscess formation were shown over 3 months in the implant group, but not in the control group. The bacteria labeled with Cy2 LIVE/DEAD® BacLight also expressed Cy5 fluorescent dye, suggesting that the Cy5 fluorescent signal observed by optical imaging were expressed from inoculated P. acnes. SEM finding revealed that a number of P. acnes and biofilms on the surface of the implant in the implant group (Figure2). In addition, P. acnes were successfully identified from the purulent effusion from the femur in both anaerobic culture and PCR analyses (Figure 3).

Discussion: IAI is a serious complication in the field of orthopaedic surgery. According to previous reports, P. acnes have been considered to be pathogen of IAI and delayed infection. Richards and Emara (Spine, 2001) examined 489 surgically treated idiopathic scoliosis patients and identified 23 who developed delayed infections. P. acnes were positive in 12 of the 23 patients (53%) in specimens obtained at the time of instrumentation removal. Although P. acnes is generally believed to have low pathogenic potential, Bayston et al (J Biomed Mater Res A, 2007) reported that it forms biofilms, which are hard to remove and difficult to treat using antibiotics. However, little has been known about in vivo kinetics of P. acnes. In the present study, we have successfully cultured P. acnes and visualized the growth of living P. acnes by specific bacterial probe in the osteomyelitis model. To our knowledge, this is the first demonstration of delayed IAI caused by P. acnes. Surprisingly, P. acnes cause the surgical site infection only under the presence of implant, but not under the absence of implant. P. acnes could survive in the biofilm around implant for a long period. Although P. acnes were in silence in the biofilm at 6 months, they started to proliferate in anaerobic culture condition, suggesting that P. acnes in the biofilm at 6 months still have the strong ability to growth, resulting in incidence of the symptomatic late infection.

Significance: We firstly proved that P. acnes can survive in mouse osteomyelitis model for more than 6 months, and causes delayed surgical site infection. Surprisingly, the presence of implant was quite essential to bacterial survival and development of IAI.

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References:
Figure 1: Time course changes of *P. acnes* bacterial photon intensity in the mouse osteomyelitis model in the implant and the control groups. The bacterial signal completely disappeared in the control group on 28 days. Surprisingly, in the implant group, the bacterial signal was maintained over 6 months. The signal intensities at day 1, 3, 7, 14, 28, 56, 84, and 168 in the implant group were significantly higher than those in the control group. Shown are means ± standard errors of the mean (SEM).
Figure 2: The bacterial signal from *P. acnes* was clearly identified in the femur. Microscopic and SEM findings showed that *P. acnes* survived in the biofilm around the implant, and active inflammation and abscess formation were shown in the implant group.
Figure 3: The purulent effusion was collected from femoral bone marrow cavity of 6-month post-operative mice. Eight colonies were detected on the culture dish 1 week after the anaerobic culture. PCR revealed the presence of P. acnes in the specimens from the purulent effusion.

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