Earlier Debridement and Antibiotic Administration Decrease Infection

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Timing of debridement and local antibiotic administration on infection has not been clearly defined. A contaminated critical size rat femur defect model was used to determine if earlier debridement with local antibiotics decreased infection. Defects were inoculated with Staphylococcus aureus. At 2, 6, or 24 hours following contamination, defects were irrigated and debrided then directly closed or treated with antibiotic-impregnated PMMA beads and then closed. Two weeks later, defects were examined for evidence of infection. There was a significant increase in evidence of infection between 2 and 6 hours and a further increase between 6 and 24 hours with debridement alone as well as with debridement plus local antibiotics. Treatment with antibiotics resulted in significantly less evidence of infection at 2 and 6 hours compared to debridement alone. It was concluded that early debridement in combination with local delivery of antibiotics of contaminated defects may reduce infections. (Journal of Surgical Orthopaedic Advances 19(1):18–22, 2010)

Key words: infection, irrigation, local antibiotics

Infection is one of the most common complications of wounds in extremity trauma. It can lead to extremely poor outcomes, including loss of limb and even mortality. The management of severe open fractures involves a staged approach of irrigation, serial debridements, and antimicrobial therapy to help prevent infection. Despite this, an infection rate of up to 23% has been reported in civilians with severe lower extremity injuries (1) and infection is the most common cause of delayed amputation in combat-related open tibial fractures (2).

One factor believed to influence infection rates is the time from injury to debridement and administration of antimicrobial therapy (3, 4). Unfortunately, there is little high-quality evidence for the effect of delayed treatment on infection. It is also unclear what impact local antibiotics alone have in a severely injured limb and systemic antibiotics may not achieve the required concentrations at the wound site. Randomized controlled clinical studies are difficult to perform because of the number of patients needed to control for mechanism of injury, severity of injury, patient comorbidities, and differences in surgical techniques across clinicians. Animal studies may help to elucidate the issue.

The aim of this study was to determine in a contaminated critical sized rat femur defect model the effect on the bacterial load by 1) timing of treatment (debridement alone or debridement in combination with locally delivered antibiotics) and 2) the addition of local antibiotics.

Methods

Experimental Design

A previously described contaminated critical size rat femur defect model was used (5–7). At 2, 6, or 24 hours following contamination, the defects were irrigated and debrided followed by either direct closure (No Antibiotics) or treatment with tobramycin or vancomycin-impregnated polymethylmethacrylate (PMMA) beads (Antibiotics) and closed. Each time period for the three treatment groups had a sample size of 10 rats, totaling the study to 90 rats. The rats were euthanized 2 weeks after debridement. Bacterial levels were assessed within the wound by a photon-counting camera and within the bone by traditional quantitative cultures.

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Creation and Contamination of Segmental Defect

A previously described contaminated critical size defect was created in the rat femurs (5–7). Briefly, a 6-mm segmental defect was created and stabilized under aseptic conditions in 90 rats (388.7 ± 10.68 g). Using aseptic technique, a longitudinal incision was made over the left anterolateral femur, and the entire femoral shaft was exposed using blunt dissection. A polyacetyl plate (length 25 mm, width 4 mm, and height 4 mm) was fixed to the surface of the femur using six threaded K-wires. A 6-mm mid-diaphyseal full-thickness defect was created with a small reciprocating saw blade (MicroAire 1025, MicroAire, Charlottesville, VA) under continuous irrigation with sterile saline. The defects in all animals were implanted with 30 mg of type I bovine collagen (Stryker Biotech, Hopkinton, MA) ethanol sterilized and wetted with 10^5 of colony-forming units (CFUs) of *Staphylococcus aureus* lux (Xenogen 36, Caliper Life Science, Hopkinton, MA) suspended in 0.1 mL of sterile normal saline. This isolate was transgenically modified to emit photons and was sensitive to tobramycin and vancomycin. The contaminated collagen was packed into the defect, and the wound was closed in a layered fashion. A high-resolution radiograph of each femur with stabilized defect was obtained using a Faxitron X-ray system [Faxitron X-ray Corporation, Wheeling, IL (model MX-20); image settings: time 10 s; KV 35; window level 3380/1250) at initial surgery to confirm appropriate placement of the implant and adequate creation of the defect.

Treatment

Debridement surgery was performed under aseptic conditions and the wound was irrigated with 60 mL of saline using a syringe (6). The wounds in the No Antibiotics group were reclosed. For the Antibiotics groups, two antibiotic beads were packed into the bony defect and two beads into the soft tissue space prior to closure of the wounds. The antibiotic beads were handmade to 3 mm in size using an Osteoset kit (Wright Medical Technology, Inc., Arlington, TN). The antibiotic was added to one packet of powder (40 g) and mixed with 20 mL of monomer cement. Clinically relevant doses of 2.4 g (4% weight) of tobramycin (X-GEN Pharmaceuticals, Inc., Big Flats, NY) and 2.0 g (3.33% weight) of vancomycin (Sigma Chemical Company, St. Louis, MO) were used (8, 9). The animals were allowed full activity in their cages postoperatively and were monitored daily for signs of pain and systemic infection. Following 2 weeks of recovery, the rats were euthanized by Fatal Plus.

Assessment of Bacterial Load

Photon Counts

Immediately after euthanasia, the wound site was exposed to allow bacteria quantification using a photon-counting camera [Xenogen IVIS Imaging System 100 Series using the Living Image 2.6.2. (Software Igor Pro 4.09A)]. The disarticulated extremities were placed in the Xenogen machine, a black-and-white photograph was taken first of the wound, then a luminescent image with a 2.5-second exposure time. The region of interest (ROI) was determined with elliptical ROIs positioned over the wound to include the entire femur and polyacetyl plate. This method assessed the amount of surface bacteria within the entire wound.

Quantitative Cultures

The femur with defect was harvested from the animals and used for quantitative assessment (6). The plate, K-wires, and all soft tissues were removed. Each femur was weighed, snap-frozen in liquid nitrogen, and ground to a fine powder under sterile conditions. The resulting bone powder was serially diluted in normal saline. Aliquots (100 μL) of each dilution were plated onto the surface of tryptic soy agar, and incubated at 37°C for 48 hours. The plates were examined for purity and colony morphology. Four to eight dilutions were typically required to obtain a minimum dilution level where the CFUs of bacteria were countable on the culture plate; the actual numbers of recovered CFUs of bacteria were obtained by correcting for the magnitude of the dilution used to obtain them and reported in CFUs per gram of bone tissue.

Clinical Signs of Infection

The presence of clinical signs of infection (e.g., purulent discharge) was noted when the wound was opened.

Statistics

Statistical analysis was performed with SAS V9.1 (Cary, NC). Time points and groups were compared using a Wilcoxon test with a Bonferonni adjustment made for multiple comparisons because the data were nonparametric. Statistical significance was set as $p < .05$. Comparisons between groups were made at the same time period, and comparisons made between time periods were within the same group. Values are presented as averages $\pm$ standard error of the means.

Results

Clinical Signs of Infection

Frank infection (i.e., purulence) and pin loosening were observed in 4 of the 10 wounds in the No Antibiotics group debrided at 24 hours. Pus was observed in one of the wounds in the Antibiotics group that was debrided and received antibiotics 24 hours after contamination. Clinical signs of infection were not observed in any of the other wounds.

Effect of Timing of Treatment

Quantitative Cultures

Early irrigation and debridement in the No Antibiotics groups resulted in a decrease in bacteria in the bone by an order of magnitude (Table 1). Bacteria in the bone increased significantly between the 2- and 6-hour time points ($p < .01$). There was a further increase between the 6- and 24-hour time points ($p = .22$).

There was no difference in the effectiveness of the two antibiotics ($p = .32$); therefore, the data for these two groups (tobramycin and vancomycin) were combined for each time period and treated as one group (referred to as the Antibiotics group). Administering the antibiotics at 6 hours instead of 2 hours (a 4-hour delay) resulted in almost two orders of magnitude more bacteria in the bone ($p < .01$). Delaying the antibiotics further until 24 hours resulted in an order of magnitude more bacteria than at 6 hours ($p < .01$).

Photon Counts

There was no difference in the bacteria quantity between the different time points in either the No Antibiotics or the Antibiotics groups (No Antibiotics: 2 vs. 6 hours, $p = .12$; 2 vs. 24 hours, $p = .53$; 6 vs. 24 hours, $p = 1$; Antibiotics: 2 vs. 6 hours, $p = .1$; 2 vs. 24 hours, $p = .082$; 6 vs. 24 hours, $p = .068$) (Fig. 1).

Effect of Local Antibiotics

Quantitative Cultures

The local delivery of antibiotics significantly reduced bacterial levels in bone compared to the No Antibiotics group at the 2-hour ($p < .01$) and the 6-hour ($p = .02$)

TABLE 1 Bacteria concentration (CFUs/g bone tissue) in each treatment group at the varying treatment time points

Time of Treatment	No Antibiotics	Antibiotics	P Value
2 hours	$1.98 \times 10^5 \pm 6.61 \times 10^4$ (a)	$3.00 \times 10^4 \pm 1.58 \times 10^4$ (a)	<.01
6 hours	$3.68 \times 10^6 \pm 9.23 \times 10^5$ (b)	$1.12 \times 10^6 \pm 3.41 \times 10^5$ (b)	.016
24 hours	$2.03 \times 10^7 \pm 7.56 \times 10^6$ (b)	$1.42 \times 10^7 \pm 4.13 \times 10^6$ (c)	.46

The values are expressed as the mean ± standard error of the mean. Different letters signify a difference between time periods within each treatment group ($p < .05$).

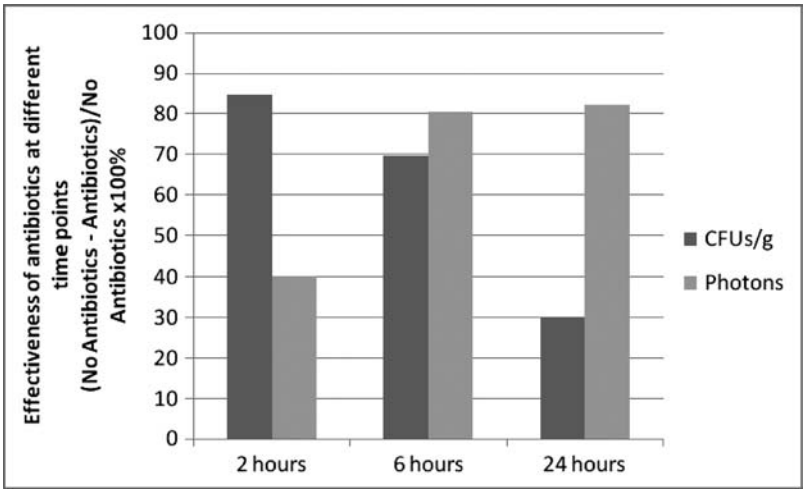

FIGURE 1 The effectiveness of antibiotics at different time points on bone and soft tissue are illustrated by the CFUs/g and photons, respectively ((No Antibiotics − Antibiotics)/No Antibiotics ×100%). Higher values indicate better effectiveness.

TABLE 2 Photon count in each treatment group at the varying treatment time points

Time of Treatment	No Antibiotics	Antibiotics	p Value
2 hours	$1.89 \times 10^4 \pm 1.51 \times 10^3$	$1.13 \times 10^4 \pm 1.05 \times 10^3$	<.01
6 hours	$5.90 \times 10^4 \pm 1.87 \times 10^4$	$1.15 \times 10^4 \pm 8.42 \times 10^2$	<.01
24 hours	$8.92 \times 10^4 \pm 3.95 \times 10^4$	$1.59 \times 10^4 \pm 1.89 \times 10^3$	.049

A. The values are expressed as the mean ± standard error of the mean. There was no difference among time periods within each treatment group.

time points. However, when antibiotics were not administered until 24 hours, the Antibiotics and No Antibiotics groups had similar amounts of bacteria in the bone ($p = .46$).

Photon Counts

Antibiotics statistically reduced the bacterial load within the wound at each time point (2 hours: $p < .01$; 6 hours: $p < .01$; 24 hours: $p = .049$) (Table 2).

Discussion

Gustilo and Anderson stated "[t]here is universal agreement that open fractures require emergency treatment including adequate debridement and irrigation of the wound." (10). Although this appears logical and is the current standard of care, there are few adequately powered, prospective, randomized, controlled trials to support the practice of early debridement and administration of antibiotics.

Several retrospective studies have demonstrated that timing of debridement does not affect the outcome. For example, there are several civilian reports that show that wounds treated after 6 hours did not have a higher rate of infection than those treated prior to 6 hours (11–15). Nor were there differences in infection rates when treatment was delayed until 8 (16) or 12 hours post injury (3). However, one investigation has reported that patients treated more than 5 hours after the injury had an infection rate 5.4 times higher than those treated earlier (17). In the military, higher infection rates resulted in combat casualties when initial debridement was delayed (18, 19). One explanation for these conflicting results could be that most patients receive early prophylactic treatment with systemic broad-spectrum antibiotics, which may confound the effect of delayed debridement. The actual timing of antibiotic administration is often not reported, but it is usually before the initial debridement. Another potential reason is that the wounds included in these studies range in severity and delay may not affect those less severe.

There is conflicting opinion on timing of administration of antibiotics to prevent infection. A retrospective study from the Falkands War reported an increase in the number of cases of soft tissue sepsis in limb injuries with a delay in antibiotic administration (19). There is also preclinical evidence that very early administration of antibiotics (1 hour post inoculation) prevents soft tissue infection in a contaminated wound tract (20). However, there are few clinical data on the effect of timing of antibiotic administration in bone infection. One retrospective study demonstrated that administration of systemic antibiotics within 3 hours of injury was an independent factor in lowering the risk of infection (3). However, a prospective study reported that the timing of antibiotics does not affect the outcome (21).

The use of systemic antibiotics per se in open fractures is universally accepted and there is evidence that it prevents wound infection in these injuries (4). It has also been demonstrated that the addition of local antibiotics to systemic antibiotics significantly reduces bone infection in severe open fractures (22).

It would be unethical to conduct a prospective, randomized clinical trial to elucidate the effect of both a delay in treatment and administration of antibiotics if this resulted in a considerably worse outcome. However, preclinical studies such as this can help. Several points can be made from this study. First, early debridement resulted in a lower level of bacterial load in bone. Second, early administration of antibiotics (at 2 hours) was more effective in reducing bacterial load in bone than delayed treatment. If the treatment is delayed for 24 hours, the antibiotics do not appreciably reduce the infection when compared with wounds that did not receive antibiotics. Third, the use of antibiotics, regardless of type, was effective in reducing the infection in both bone and soft tissue.

Knowledge gained from basic science experiments can often help explain what this particular study demonstrated and most clinicians believe. We believe that this study provides valuable information on the potential clinical effects of delaying treatment. Our two metrics CFUs/g and photon counts demonstrate a very different response to the delay of antibiotic administration. In fact, there is an inverse relationship of the effectiveness of antibiotics between the bacterial load in bone (CFUs/g) and soft tissue (photon counts) across the different time points. We believe that this may be explained by 1) the ability of *S. aureus* to form biofilms and invade osteoblasts and

2) the fact that soft tissue is easier to debride than bone. Bacterial adhesion begins quickly and by 3 hours biofilm is formed. Maturation of the biofilm can occur within 10 hours for bacteria (23). Once formed, it can provide a cohesive barrier between the bacterial cells and the external environment. This is less of a problem in soft tissue because the area that is necrotic (and presumably has the highest bacteria and biofilm) is debrided. However, in bone, biofilms and osteointegration of the bacteria markedly reduce the effectiveness of both the mechanical properties of irrigation and debridement and the ability of antibiotics to reduce the bacterial load. In fact, bacteria within a biofilm may require 1000 times higher antibiotic concentration to be effective (24). Furthermore, *S. aureus* that is internalized by the host's osteoblasts (usually within 3 hours post contamination) is protected from antibiotics (25). Many antibiotics, tobramycin and vancomycin included, cannot penetrate the osteoblastic cell membranes and are unable to obtain access to the bacteria, rendering them ineffective. This explanation is corroborated in the clinical setting. Bone that becomes infected can result in osteomyelitis, and the infected bone must be removed. Soft tissue infection is more responsive to treatment with high levels of antibiotics. Therefore, early treatment, especially in bone, can allow for better removal of bacteria through irrigation and debridement (26) and markedly improves the effectiveness of the antibiotics.

In conclusion, this study is the first to demonstrate that both early debridement and local antibiotic administration reduce infection. Although the data are from a preclinical model, they clearly suggest that the earliest introduction of antibiotics and debridement may reduce the infection rate.

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