ORIGINAL RESEARCH

Novel Rabbit Model for the Evaluation of Open Tibia Fractures: Effect of Delayed Surgery on Infection Rate

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ABSTRACT

Introduction: The objective of this investigation was to evaluate the effects of delayed surgery on infection rates of open tibia fractures in a rabbit model. Our hypothesis was that delaying irrigation and debridement (I&D) would increase the risk of bony and soft tissue infection when antibiotics were withheld.

Materials and methods: A drill hole was created in the rabbit tibia and inoculated with *Staphylococcus aureus*. Animals underwent I&D at controlled delayed times of 6 hours (n = 11), 12 hours (n = 9) and 24 hours (n = 10). A stainless steel pin was inserted to mimic definitive fracture fixation with a metal prosthesis. No antibiotics were administered. Animals were sacrificed at 4 weeks and evaluated for infection.

Results: The percent of soft tissue infections with I&D delays of 6, 12 and 24 hours were 18, 22 and 40%, respectively [p = 0.59; odds ratio (OR) = 3]. The percentage of animals with osteomyelitis with I&D delays of 6, 12 and 24 hours were 9, 22 and 50%, respectively (p = 0.12; OR = 10).

Discussion: In the current model, delaying I&D from 6 to 24 hours may increase the rate of postoperative osteomyelitis and further investigation is warranted.

Keywords: Infection, Open tibia fracture, Irrigation and debridement, Delayed surgery, Natural history.

INTRODUCTION

Bony and soft tissue infections are a major concern when patients sustain open fractures. Preventing infection is a major goal of the orthopaedic traumatologists and as such, efforts are made to direct the patient quickly to the operating room to undergo no less than a thorough irrigation and debridement (I&D) to remove any tissue that may affect healing. In addition, administration of antibiotics[1] and prompt soft tissue coverage[2] are important to avoid infectious complications. While immediate treatment of open fractures is common, the time from injury to operative I&D does not always correlate with a reduced rate of infections.[3-9] Hence, the benefit of emergency/urgent surgery has become a topic of intense debate.

The reason for delayed surgical intervention for open fractures is multifactorial, including time for patient transportation, time for assessment and stabilization, and delays due to operating room readiness.[5] Delays also occur secondary to management of life-threatening conditions including neurological, thoracic and abdominal trauma. It is common practice to administer antibiotics as soon as possible, preferably within 3 hours of injury.[1,10] Most studies indicate that when antibiotics are introduced quickly, there is an associated decrease in the rate of infection. This makes it reasonable to assume that early administration of antibiotics is important in the reduction of infection rates after open fractures regardless of the timing of surgical intervention.[1,4,10,11] Irrigation and debridement within shows of presentation is still considered the 'gold standard', although several studies indicate that delays of more than 6 hours do not increase infection rates.[1,3-9] There are no definitive answers on whether or not longer delays, such as 12 or 24 hours, make any difference on infection rates. Modern medicine and ethics dictate the use of all known treatments for patients with open fractures to optimize clinical outcomes. Therefore, a randomized trial controlling all the variables regarding treatment of patients with open fractures is clinically impractical.

To date, there is no animal model to demonstrate the effect of delayed surgical intervention for open fractures. However, there are animal models of fractures and osteomyelitis[12-16] which can be adapted for studying the effect of delayed surgery on infection rates of open fractures. The current study presents the results of delayed I&D of open tibia fractures when antibiotics are not administered. Our hypothesis is that a delay in I&D will result in increased risk of infection when antibiotics are not administered. We present a model for the effect of delayed surgery on the infection rate of open tibia fractures in rabbits.

MATERIALS AND METHODS

Animals: All surgical procedures were conducted within the core animal facilities of Medical University of South Carolina (MUSC, Charleston, SC; DHHS Assurance # A3428-01). All surgical interventions, pre- and postsurgical care were provided in accordance with the PHS Policy on Humane Care and use of Laboratory Animals, Guide for the Care and use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, 1996), and approved (AR#2539) by the Institutional Animal Care and use Committee of the Medical University of South

Carolina. All animals were housed (12h/12h light/dark cycle) in the facilities for laboratory animals provided by the Division of Laboratory Animal Resources under the direction of M Michael Swindle, DVM, a diplomate of ACLAM. MUSC has ongoing full accreditation from AAALAC effective November 5, 1987.

Anesthesia: Anesthesia was performed by institutional staff veterinarians. Rabbits were anesthetized using ketamine 30 mg/kg, xylazine 5 mg/kg, and atropine 1 to 3 mg/kg intramuscularly and maintained on isoflurane after intubation. The atropine was given 20 to 30 minutes prior to induction, at which time buprenorphine (0.02 mg/kg, PRN) was given pre-emptively to prevent up-regulation of nociceptive pathways and repeated every 12 hours PRN. Surgery was performed using standard aseptic techniques. A record of animal surgery was maintained in the institutional animal surgical record.

Surgical technique: Under general anesthesia (isoflurane), hair from the right leg of the rabbit (3-4 months old female New Zealand White with a mass of 3.75 ± 0.25 kg) was shaved from mid thigh to the ankle using electric clippers. The skin was then cleaned with 7.5% povidone-iodine and 70% isopropyl alcohol. An anteromedial 1.5 cm vertical incision was made over the proximal tibia just lateral to the tibial tuberosity exposing the upper tibial metaphysis. A 4 mm oblique hole was created through the anteromedial cortex just lateral to the tibial tuberosity using a drilling force directed posteriorly and distally with 4 mm trocar-shaped drill bit. A gauze sponge was packed into the medullary canal until bleeding ceased (1-5 min), at which time an inoculum of *Staphlococcus aureus*, 10^5 colony forming units (CFU) in 100 µl saline, was injected into the medullary canal. This size inoculum was chosen due to the likelihood of producing a clinically detectable infection, as previously described.[17,18] The skin was loosely closed with two interrupted sutures (4.0 Vicryl, ©Ethicon, Inc), wrapped loosely with gauze and Coban™ (2 inch, 3M™). The animals were awakened, extubated and placed in a cage in the recovery room until the debridement surgery was performed at one time point of 6 (n = 11), 12 (n = 9) or 24 (n = 10) hours after the wound creation/inoculation. At each delay time point, the animal was reanesthetized as described above. Under sterile surgical conditions, the wounds were cleaned, hematomas evacuated and debrided. Sterile saline irrigation was utilized to clean soft tissue (5 ml) and bone (5 ml in medullary canal). Then, a 3×30 mm stainless steel pin was inserted into the medullary canal. The deep fascia and subcutaneous tissue layer were closed in two layers using vicryl sutures. Prior to awakening, radiographs of the

operative leg were obtained to confirm placement of the stainless steel pin and establish a baseline for subsequent radiographs. The rabbits were then awakened, extubated and placed in a cage in the recovery room and subsequently transferred to the main housing room. After surgery, unrestricted weight bearing was allowed. All animals showing signs of pain or discomfort were given buprenorphine (0.02-0.05 mg/kg) as needed every 12 hours. Food and water was provided ad libitum.

Sacrifice: All animals were sacrificed 4 weeks postoperatively using IV overdose of pentobarbital [concentration was 390 mg/ml (1 ml/4.54 kg)].

X-rays were taken to identify signs of osteomyelitis such as periosteal reaction, bone destruction and sequestrum formation.

Under aseptic conditions (skin shaved and cleaned), the tibia was exposed through the original incision and cortical window area were swabbed for bacterial culture and typing. A soft tissue sample was then fixed in 10% buffered formalin and processed for paraffin sectioning. Then, the upper half of the tibia was harvested and separated into two segments using a rotary saw. The stainless steel pin was removed and placed in tryptic soy broth for bacterial culture. One part of the bone explant was cultured in tryptic soy broth for bacterial growth.[17] The other segment of the upper tibial bone was fixed in 10% buffered formalin, decalcified and processed for paraffin sectioning.[18] The sections were stained with hematoxylin and eosin, Masson's trichrome, and Gram stain.

The criteria for confirming a diagnosis of osteomyelitis included the presence of radiographic evidence (periosteal reaction, bone destruction and sequestrum formation), histological evidence (purulent exudate with numerous neutrophils) and *S. aureus* growth in culture medium. Similarly, soft tissue infection was confirmed by gross inspection of a palpable abscess, histological samples containing purulent exudate and *S. aureus* growth in culture medium.

Data was analyzed with PASW statistics 18 for windows (SPSS-Chicago, IL). Infection differences (dichotomous) in soft tissue and bone at 6, 12, and 24 hours was assessed through the Fisher's exact test since there were several cell sizes of <5. Odds ratios (OR) between each of the time periods was also calculated. An a *priori* power analysis was not conducted for this investigation.

RESULTS

The percent of animals that presented with soft tissue infections 4 weeks following the index surgery at controlled
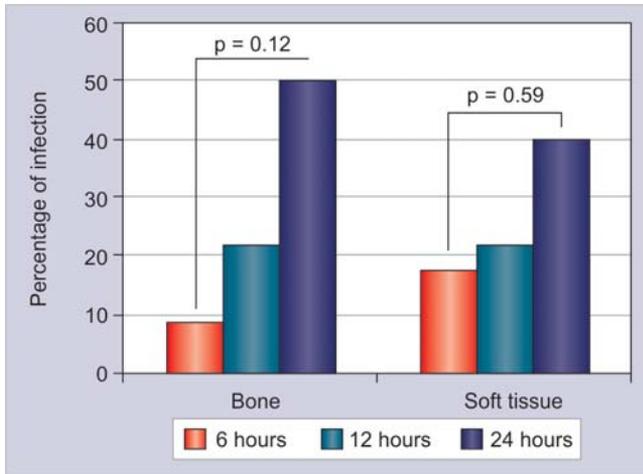
Fig. 1: Comparison of bony and soft tissue infection rates following surgical debridement at 6, 12 and 24 hours

delay times of 6, 12 and 24 hours were 18, 22 and 40%, respectively. There was an increasing trend in soft tissue infection based on delay in I & D from 6 and 24 hours, yet there were not statistically significant differences [(p = 0.59); (OR 6-12h = 1.29) OR 6-24h = 3.0), OR 12-24 h = 2.33)] as illustrated in Figure 1. The percentage of animals with osteomyelitis and controlled delay times of 6, 12 and 24 hours were 9, 22 and 50%, respectively. There was an increasing trend in osteomyelitis from 6 to 24 hours, yet there was not a statistical difference [(p = 0.12); (OR 6-12 h = 2.85); (OR 6-24 h = 10); (OR 12-24 h = 3.5)]. Figures 2A to F illustrate typical gross (Figs 2A and D), radiographic (Figs 2B and E) and histological imaging (Figs 2C and F) of representative animals with and without infection.
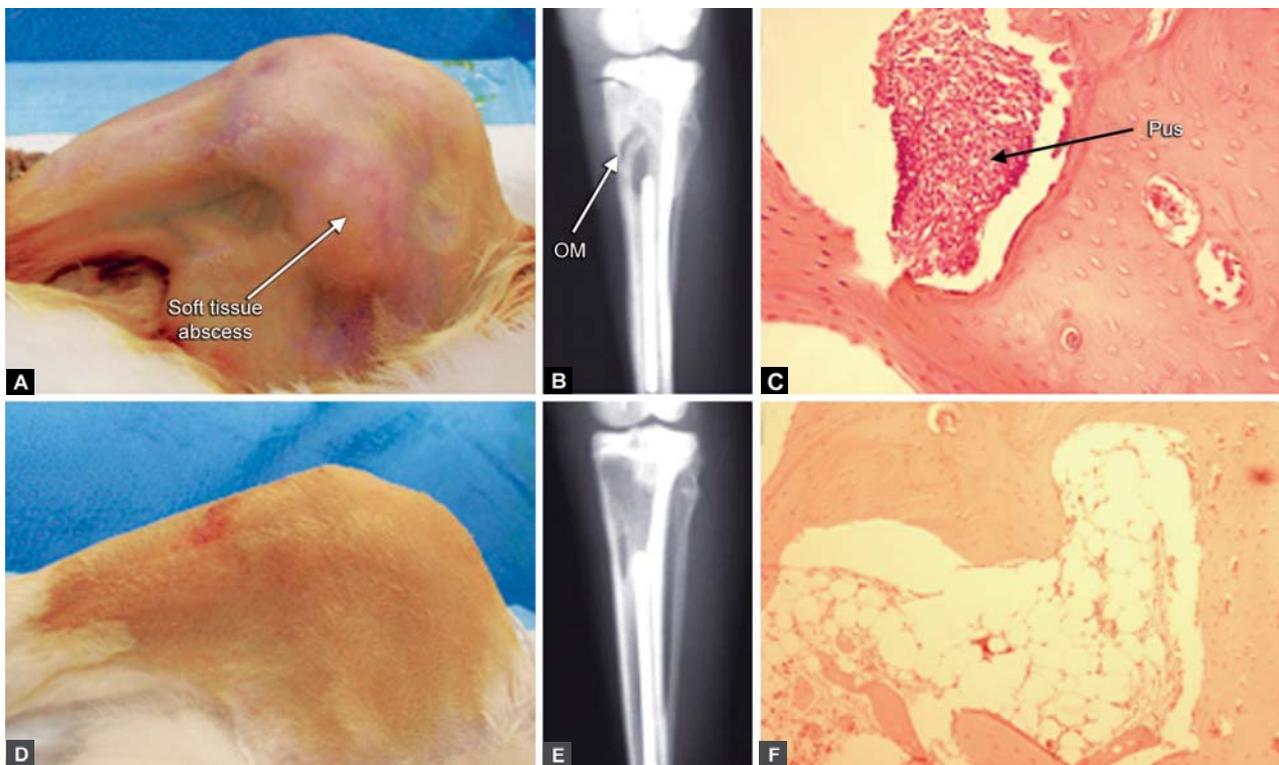
Figs 2A to F: Representative gross (A, D), radiographic (B, E) and histological (C, F) findings for rabbits with an apparent infection (A, B, C) as compared to those without an apparent infection (D, E, F)

Table 1: Effects of surgical delay on postsurgical infection rates of open fractures

Study	Time point (X hours)	Before (X hours)	After (X hours)	Difference
Patzakis 1989	12	6.8% (27/396)	7.1% (50/708)	No
Bednar 1993	6	9.1% (2/22)	3.4% (2/59)[a]	No
Kreder 1995	6	11.9% (5/42)	25% (2/8)[a]	Yes
Skaggs 2000	6	2.5% (1/40)	1.6% (1/55)	No
Harley 2002	8	8.7% (10/115)	10% (10/100)	No
Ashford 2004	6	17% (2/12)	11.1% (4/36)[b]	No
Spencer 2004	6	10.1% (7/69)	10.9% (5/46)	No
Skaggs 2005	6	3% (12/344)	2% (4/210)[a]	No
Charalambous 2005[20]	6	4.3% (8/184)	4% (8/199)	No
Al-Arabi 2007[21]	6	7.8% (12/154)	9.6% (9/94)	No

[a]6 hours < X < 24 hours; [b]6 hours < X < 37 hours

DISCUSSION

Delayed surgical intervention for open fractures is multifactorial. To date, the exact timing of surgery to best prevent infections following open fractures remains unknown. The number of relevant studies that evaluate the effect of surgical delays in postoperative infection rate are surprisingly limited.[19-21] Table 1 lists relevant studies evaluating postoperative infection rates where an identifiable time point (e.g. 6 hours) and complete statistics could be used to evaluate the effect of delayed I&D. Of these studies, most compared outcomes when surgical intervention occurred prior to, or following, a delay of 6 hours. Only one of these studies[19] reports a difference in the infection rate when operative care occurred after 6 hours. However, the statistical significance of these results are questionable considering the small sample pool. More recently, The Lower Extremity Assessment Project Study Group evaluated the relationship between time to surgical debridement and the incidence of infection after open high energy lower extremity trauma. Their results echoed those listed in Table 1 and concluded that there is no difference in infection rates based upon time from admission to surgical debridement.[22] Moreover, they concluded that there is no difference in infection rates based upon time from injury to surgical debridement when compared across 3 groups (debridement <5 h, between 5-10 h and >10 h; $p < 0.05$). Interestingly, however, it was concluded that prolonged (11-24 h) time to admission to the definitive trauma center was an independent predictor of subsequent infection. Further, prolonged transit time (>2 h) from the time of injury to admission to the definitive trauma center was an independent risk factor for developing subsequent infection.

In the current study, we adapted a rabbit tibial infection model previously reported from our laboratory[23] to assess the effect of delayed I&D on postoperative infection rates. The advantages of utilizing a rabbit model are numerous. Most importantly, we are able to circumvent the human ethical dilemma inherent in prospective studies in which the use of all known treatments (e.g. early intervention, antibiotics) is compared to methodologies which limit these treatments. Moreover, we are able to control important variables, including the following: Age, gender, weight, past medical history, comorbidities, timing of surgical procedure, type of surgical procedure, amount of bacterial inoculum, I&D delay and routine follow-up. The amount of saline used during irrigation and debridement was determined from previous experiments to determine a 50% effective dose (ED$_{50}$) of saline resulting in osteomyelitis in 50% of the animals following I&D. That volume was determined to be a total of 10 cc of saline (unpublished data). Had we decided to irrigate with a copious amount of saline, many more animals would have been needed to demonstrate a statistical difference in the infection rates. Specific practices concerning volume, delivery method and irrigation additives in the orthopaedic literature is highly variable. Most studies indicate irrigation should be adequate and ample.[24-26] Other investigators describe the amount of irrigation as arbitrary.[27,28] Whether the delivery should involve pulsatile flow is controversial. Pulsatile flow has been shown to be less effective than continuous flow, at various rates of pressure, for bacterial removal and new bone formation. Some evidence suggests that pulsatile flow forces the bacteria into the tissues, thereby increasing the consequential infection risk.[29-32]

Numerous animal models for deep soft tissue and bony infections have been described. The goat leg model described by Svoboda and Owens et al[24,25] is possibly the most rigorous model of deep infection published to date. Their model has effectively demonstrated the importance of irrigation volume, type of irrigation used and the bacteria reducing effect of early irrigation (3 h postinoculation) compared to late irrigation (12 h postinoculation). Their model does have two notable shortcomings. First, they do not utilize *Staphlococcus aureus,* which is the most prevalent bacteria found in older children and adults with osteomyelitis.[32] Instead, they use *Pseudomonas aeruginosa* due to the ease with which they are genetically engineered to be luminescent, allowing for quantitative evaluation of the bacteria remaining in the wound. Second, their endpoints are quantitative measures of bacteria remaining in the wound immediately after irrigation and debridement or 48 hours following initial inoculation as opposed to awaiting evidence of clinically relevant deep infections that result days to weeks following the initial inoculations.

Our model addresses the shortcomings of the goat leg model in that we utilize *Staphlococcus aureus*, the most common bacteria isolated in deep infections. Moreover, we follow the inoculated subjects for a total of 4 weeks. Clinical signs of infection including radiographic bony changes, histological evidence of pus and positive bacterial culture swabs were utilized to make the diagnosis of infection. Although not quantitative, these methods mimic the scenarios orthopaedic surgeons are confronted with in the emergency room, operating suite and postoperative clinics.

Notable shortcomings of this study include the qualitative nature of the clinical diagnosis. In addition, we do not utilize different types of irrigation or evaluate the effect of administering antibiotics. Witholding antibiotics in this study was used to evaluate the natural history of an experimental open fracture with specific times of

intervention by irrigation and debridement of the fracture site. Moreover, the size of the study is small.

SUMMARY

Our results demonstrate an increase in infection rates when I&D is delayed from 6 to 24 hours. Our study did not demonstrate a statistical difference, but this is due, in part, to our small sample size. Further investigation may be warranted. This study is necessary to establish animal protocols and baselines for subsequent studies in which antibiotics are administered at preselected times postbacterial inoculation. Ultimately, stratifying delay to I&D and delay to antibiotic administration will help traumatologists and general orthopaedists alike determine and guide the appropriate treatment of open fractures.

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