■ TRAUMA: RESEARCH

Early antibiotics and debridement independently reduce infection in an open fracture model

J. G. Penn-Barwell,
C. K. Murray,
J. C. Wenke

From US Army Institute of Surgical Research, San Antonia, Texas, United States

Most animal studies indicate that early irrigation and debridement reduce infection after an open fracture. Unfortunately, these studies often do not involve antibiotics. Clinical studies indicate that the timing of initial debridement does not affect the rate of infection but these studies are observational and fraught with confounding variables. The purpose of this study was to control these variables using an animal model incorporating systemic antibiotics and surgical treatment.

We used a rat femur model with a defect which was contaminated with *Staphylococcus aureus* and treated with a three-day course of systemic cefazolin (5 mg/kg 12-hourly) and debridement and irrigation, both of which were initiated independently at two, six and 24 hour time points. After 14 days the bone and hardware were harvested for separate microbiological analysis.

No animal that received antibiotics and surgery two hours after injury had detectable bacteria. When antibiotics were started at two hours, a delay in surgical treatment from two to six hours significantly increased the development of infection (p = 0.047). However, delaying surgery to 24 hours increase the rate of infection, but not significantly (p = 0.054). The timing of antibiotics had a more significant effect on the proportion of positive samples than earlier surgery. Delaying antibiotics to six or 24 hours had a profoundly detrimental effect on the infection rate regardless of the timing of surgery. These findings are consistent with the concept that bacteria progress from a vulnerable planktonic form to a treatment-resistant biofilm.

■ J. G. Penn-Barwell, MRCS, Trauma and Orthopaedic Registrar, Research Fellow Royal Centre for Defence Medicine, Academic Department of Military Surgery and Trauma, Birmingham Research Park, Vincent Drive, Birmingham B15 2SQ, UK.

■ C. K. Murray, MD, Chief of Infectious Disease Service Brooke Army Medical Center, 3851 Roger Brooke Drive, Fort Sam Houston, Texas 78234, USA.

■ J. C. Wenke, PhD, Manager, Extremity Trauma & Regenerative Medicine US Army Institute of Surgical Research, 3400 Rawley E Chambers, Fort Sam Houston, San Antonio, Texas 78234, USA.

Correspondence should be sent to Surg Lt Cdr J. G. Penn-Barwell RN; e-mail: jowan@doctors.net.uk

Although open fractures are common in civilian and military practice,[1,2] there is still debate as to whether surgery is required urgently in order to prevent subsequent infection. There has been a shift away from the previous orthopaedic doctrine of operating on open fractures as an emergency.[3] This was exemplified by the change in clinical guidelines on the management of open tibial fractures from the British Orthopaedic Association (BOA) and British Association of Plastic, Reconstructive and Aesthetic Surgeons (BAPRAS). In 1997 they stated that the first orthopaedic procedure should be undertaken within six hours of injury.[4] However, in the 2009 revision of these guidelines the 'six-hour rule' had been laid aside in favour of debridement within 24 hours of injury, based on subsequent further evidence.[5] The rationale for this change was that a patient receiving early antibiotics would be better managed by senior orthopaedic and plastic surgeons than juniors, and that surgery could be delayed safely to achieve this.

The clinical evidence comes mainly from retrospective observational cohort studies, most of which suggest that, following the administration of antibiotics, surgery can be delayed by 12 to 24 hours without increasing the risk of subsequent infection.[6-8] Conversely, the animal studies that examined this question concluded that delay in surgical treatment is closely related to subsequent infection.[9]

The contradictory conclusions reached by these two different types of study are likely to be due to their respective weaknesses in design. Animal studies considered either the timing of systemic antibiotics[10] or surgery,[11] and therefore did not examine the possibility that antibiotics can make a surgical delay safe. Conversely, observational studies are susceptible to selection bias, with a tendency for clinicians to delay the treatment of cleaner, simpler fractures, with the risk of masking the independent effect of surgical delay.[3] Unfortunately, for ethical reasons, the many confounders in the clinical setting can never be controlled for in a prospective study.

In this study we used an open fracture rat model to test our hypothesis that early

Table I. The timing of treatment in the seven study groups

|	2 hour antibiotics	6 hour antibiotics	24 hour antibiotics
2 hour surgery	Group 1: 2 hour antibiotics, 2 hour surgery	Group 4: 6 hour antibiotics, 2 hour surgery	Group 7: 24 hour antibiotics, 2 hour surgery
6 hour surgery	Group 2: 2 hour antibiotics, 6 hour surgery	Group 5: 6 hour antibiotics, 6 hour surgery	-
24 hour surgery	Group 3: 2 hour antibiotics, 24 hour surgery	Group 6: 6 hour antibiotics, 24 hour surgery	-
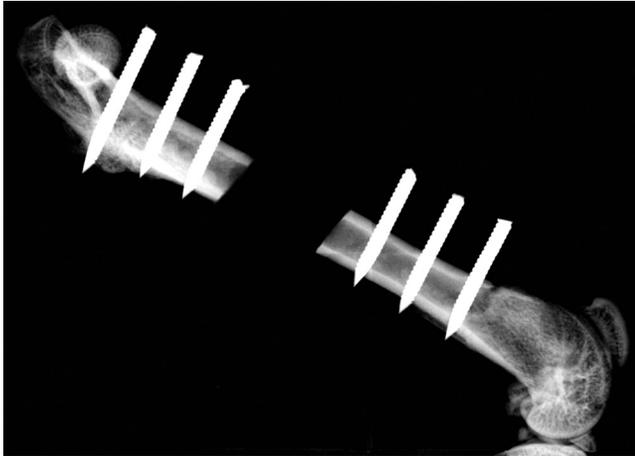
Fig. 1

Microradiograph showing a 6 mm defect in a rat femur stabilised by a radiolucent polyoxymethylene plate secured with six threaded 0.9 mm Kirschner-wires.

administration of antibiotics will reduce, but not eliminate, the detrimental effects of delaying surgical debridement.

Materials and Methods

A total of seven groups of ten rats were treated with identical operative and antibiotic regimens, initiated at two, six and 24 hours (Table I). These timings were chosen to approximate those relevant to clinical practice, with surgical debridement within two hours typical of the military setting, six hours being the previous clinical goal and 24 hours the proposed maximum operative delay that can safely be contemplated with the objective of ensuring sufficient surgical expertise. Moreover, these timings are consistent with previous preclinical studies indicating biofilm formation and maturation in bone around the six hour stage.[12]

A previously described, contaminated open fracture model was used to assess the effect of the timing of debridement and administration of antibiotics.[13] The study protocol complied with the Animal Welfare Act and principles of the Guide for the Care of Use of Laboratory Animals. Adult male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, Indiana) with a mean weight of 366 g (350 to 387) were anaesthetised with isoflurane and prepared for surgery. Their right femoral shafts were exposed and stabilised with a bespoke polyoxymethylene plate, secured to the bone with six threaded Kirschner (K)-wires. A 6 mm defect was then created in the mid-shaft by a oscillating saw, cooled with saline (Fig. 1). The defect was contaminated with 30 mg of sterile bovine collagen soaked with 1×10^5 colony-forming units (CFUs) of *Staphylococcus aureus* in 0.5 ml of saline. The (Xenogen 36) strain of *Staph. aureus* used, derived from ATCC 49525, was originally from a septic human patient (Caliper LifeSciences, Mountain View, California).

The wounds were closed in layers. At two, six or 24 hours after the initial injury, they were re-anaesthetised, their wounds opened, debrided with careful removal of all contamination and irrigated with 60 ml of sterile saline at low pressure. The wounds were once more closed in layers and post-operatively the animals were allowed full mobility, food and water.

At 14 days after the simulated injury, the animals were killed. The femur and plate were stripped of soft tissue and separated. The bone was snap-frozen in liquid nitrogen and crushed. Samples from the bone and plate were sent separately for quantitative microbiological analysis. Crushed bone samples were homogenised with 10 ml saline in an agitator and specimens from the plate were also rinsed with 10 ml of saline in an agitator. Aliquots from individual specimens were sequentially diluted and spread onto Tryptic-Soy-Agar plates. After overnight incubation at 37°C, bacterial colonies were counted and recorded; the threshold of detectability was 30 CFU/g. The recovered bacteria were examined microscopically and with a proton-count camera to confirm that they were the strain of *Staph. aureus* used to contaminate the fracture.

In addition to the operative treatment, the rats received antibiotic therapy as 5 mg/kg of cefazolin (Sigma-Aldrich, St Louis, Missouri) subcutaneously. The initial dose was at two, six or 24 hours after injury, then every 12 hours thereafter for three days. Every animal therefore received seven identical doses. This regimen was selected because it approximates to clinical practice where a 72-hour course of antibiotic therapy is recommended.[5]

The outcome measures were the absence or quantity of bacteria in the femur or attached to the hardware.

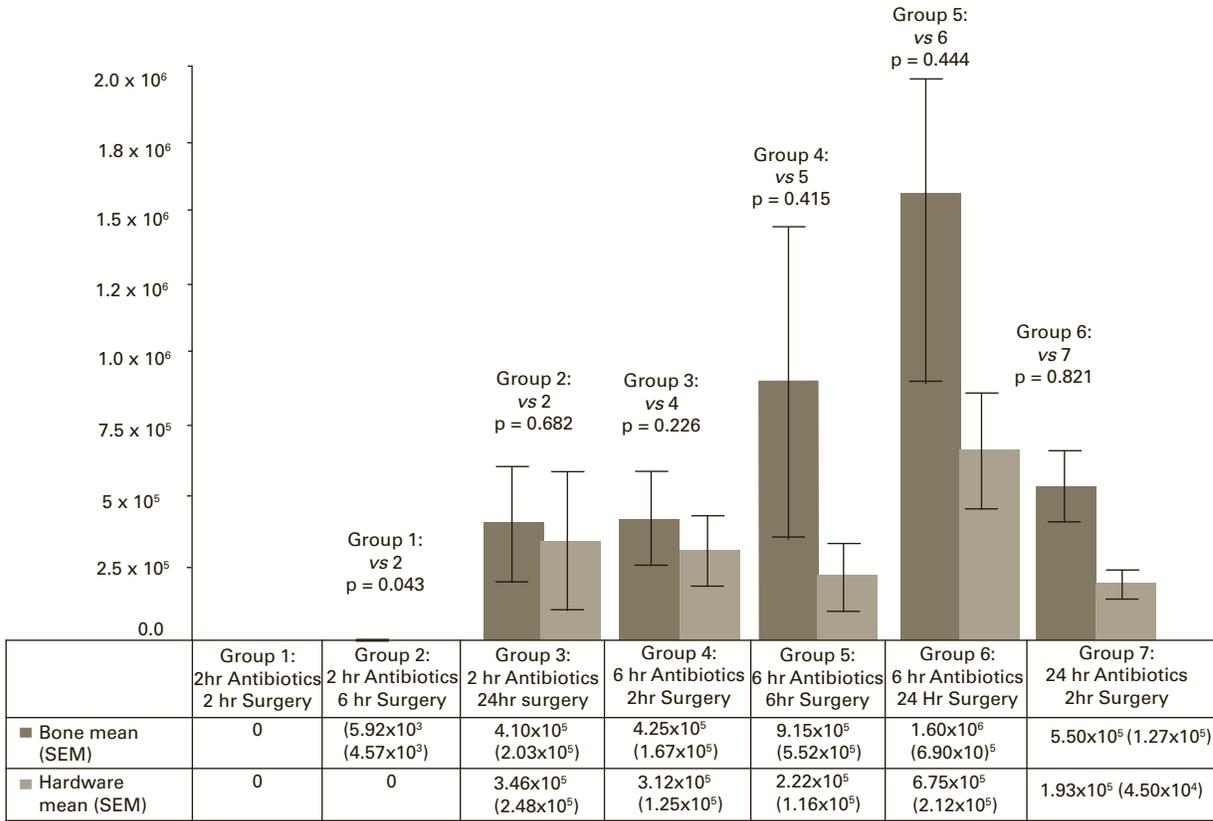|	Group 1: 2hr Antibiotics 2 hr Surgery	Group 2: 2 hr Antibiotics 6 hr Surgery	Group 3: 2 hr Antibiotics 24hr surgery	Group 4: 6 hr Antibiotics 2hr Surgery	Group 5: 6 hr Antibiotics 6hr Surgery	Group 6: 6 hr Antibiotics 24 Hr Surgery	Group 7: 24 hr Antibiotics 2hr Surgery
Bone mean (SEM)	0	(5.92×10^3) (4.57×10^3)	4.10×10^5 (2.03×10^5)	4.25×10^5 (1.67×10^5)	9.15×10^5 (5.52×10^5)	1.60×10^6 $(6.90 \times 10)^5$	$5.50 \times 10^5 (1.27 \times 10^5)$
Hardware mean (SEM)	0	0	3.46×10^5 (2.48×10^5)	3.12×10^5 (1.25×10^5)	2.22×10^5 (1.16×10^5)	6.75×10^5 (2.12×10^5)	$1.93 \times 10^5 (4.50 \times 10^4)$

Fig. 2

Bar chart showing mean bacterial quantities after varying treatment timings with statistical groupings by combined bone and hardware results. Error bars show standard error of the mean (SEM).
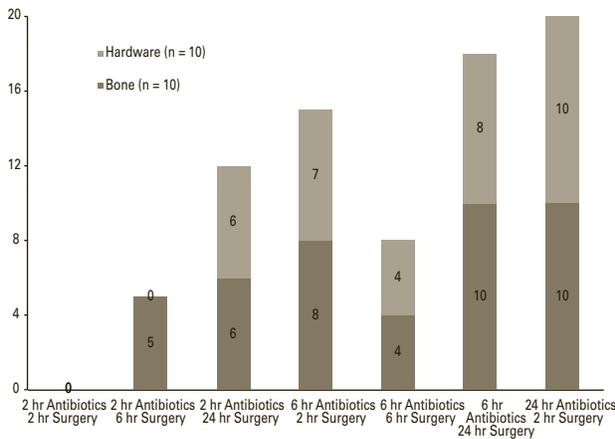

Fig. 3

Bar chart showing the proportion of samples with detectable bacteria from each treatment group.

Statistical analysis. This was by SAS software (SAS Institute Inc. Cary, North Carolina). For analysis of the quantity of bacteria, undetectable samples were regarded as zero. The log mean of the sum of bone and hardware values were compared using a Student-Newman-Keuls (SNK) analysis of variance (ANOVA). For direct comparison between study groups with regard to the presence of bacteria, Fisher's exact test was used. The threshold for significance was set at $p < 0.05$.

Results

The varying times of treatment had a marked effect on the level of bacteria (Fig. 2). No animal that received antibiotics and surgery at two hours after injury had detectable bacteria, whereas every animal in the group whose treatment was delayed for 24 hours was infected (Fig. 3). In the three groups that received antibiotics at two hours, a delay in surgery from two to six hours resulted in a significantly greater number of positive samples ($p = 0.047$). However, a further delay from six to 24 hours did not cause a significant increase in the quantity of bacteria or the number of positive samples ($p = 0.054$, Table II). This was confirmed by ANOVA of bacterial quantification across these three groups.

If antibiotic administration did not occur within six hours of injury, a delay in surgery from six to 24 hours resulted in a significant increase in the proportion of samples that were positive for bacteria ($p = 0.002$).

Table II. p-values of comparisons between treatment groups according to Fisher's exact test comparing presence of bacteria

		2 hour antibiotics			6 hour antibiotics			24 hour antibiotics
	Surgery	2hour Group 1	6 hour Group 2	24 hour Group 3	2 hour Group 4	6 hour Group 5	24 hour Group 6	2 hour Group 7
2 hour antibiotics	2 hour Group 1	-	0.047	< 0.001	< 0.001	0.003	< 0.001	< 0.001
	6 hour Group 2	0.047	-	0.054	0.004	0.501	< 0.001	< 0.001
	24 hour Group 3	< 0.001	0.054	-	0.501	0.344	0.065	0.004
6 hour antibiotics	2 hour Group 4	< 0.001	0.004	0.501	-	0.054	0.408	0.0471
	6 hour Group 5	0.003	0.501	0.344	0.054	-	0.002	< 0.001
	24 hour Group 6	< 0.001	< 0.001	0.065	0.408	0.002	-	0.487
24 hour antibiotics	2 hour Group 7	< 0.001	< 0.001	0.004	0.0471	< 0.001	0.487	-

In order to determine whether antibiotics or surgery had a more significant temporal effect on bacteria, two pairs of 'mirrored' study groups were compared; first, in groups 2 and 4 the timing of surgery and antibiotics was reversed around the two and six hour timings; and secondly, groups 3 and 7 in which treatments occurred at two and 24 hours. In both comparisons, earlier antibiotics had a significantly greater impact on the proportion of positive samples than earlier surgery. At the two and six hour treatments the difference between groups 2 and 4 the p-value was 0.004 and for the six and 24 timings, the difference between groups 3 and 7 it was 0.003 (Table II).

Discussion

In our open fracture model we found that the earlier treatment with systemic antibiotics or surgery is initiated, the greater the reduction in infection. Their effect is independent of each other and synergistic. Earlier administration of antibiotics reduces, but does not eliminate the negative impact of delayed surgery. Early antibiotics appear to have a greater impact on reducing infection than early surgery.

Although our model attempts to reproduce open fractures, it lacks several features. There was little soft-tissue damage, the defect was created surgically, there was a single operation with immediate primary closure and the type and delivery of antibiotics were standardised. It is also possible that the observations might be peculiar to the type of bacteria or antibiotic used. However, despite its limitations, it is possible with this model to control all other confounders and provide useful evidence for helping in clinical decisions. Irrigation with 60 ml of saline represents approximately 15% of the animal's mass, equivalent to 12 l in an 80 kg human patient. A staphylococcus strain was used because of its common involvement in infected open fractures.[14] We used cefazolin because cephalosporins are a recommended antibiotic option in open fractures[5] and previous experi-ence with cefazolin in similar animal models reduced the model development work required to design this study.

We believe that since this model involves an organism that is known to be sensitive to the antibiotic used, the effects observed should be similar with different combinations of bacteria and antibiotic. However, it is possible that the effects observed might be more pronounced with intravenous administration of antibiotics than oral ingestion or the sub-cutaneous route that was used in this model to avoid the morbidity from repeated venipuncture in a small mammal.

With regard to the timing of the initial surgical debridement, the earliest study, by Friedrich in 1898,[9] was based on a guinea pig model. He showed that wounds debrided within six hours had no infection, and this finding subsequently became orthopaedic doctrine as the 'six hour rule',[4] Further animal-based research endorsed these findings. Dhingra, Schauerhamer and Wangensteen[11] showed that a delay in debridement from two to four hours led to significantly greater infection rate in soft tissue wounds. More recently, Brown et al[15] used a rat model of a contaminated open fracture to show that the quantity of bacteria in subsequent infection is proportional to the initial delay until surgical debridement.

Most of the clinical evidence on the timing of initial surgery in open fractures comes from observational cohort studies, the results of which were analysed with either regression analysis or by direct comparison of infection rates between 'early' and 'delayed' cohorts. Studies using this methodology have reached divergent conclusions, with most concluding that early surgery is of little benefit. Only three studies identified a link between delayed surgery and infection. Kindsfater and Jonassen[16] examined Gustilo-Anderson grade II and III[17] open fractures of the tibia and found greater rates of infection in those debrided > 5 hours after injury compared with those debrided < 5 hours after

injury. Kreder and Armstrong[18] examined open tibial fractures in children and similarly found that surgery after six hours correlated with increased infection, but their results did not reach statistical significance. Jacob, Erpelding and Murphy[19] examined US military casualties of the 1989 invasion of Panama and found a higher rate of infection in those Gustilo-Anderson grade III fractures whose surgery was delayed until return to the US, compared with those who underwent early surgical treatment in Panama.

Conversely, others using a similar observational approach found that the risk of infection or nonunion did not increase despite delayed debridement in patients who had received early systemic antibiotics.[6,7,20-30] Also, a component of the Lower Extremity Assessment Project (LEAP) included a prospective observational study of 315 patients with Gustilo-Anderson grade III open fractures of the tibia, foot and ankle and, in a multi-variant regression analysis it was found that delay between injury and debridement was not related to the rate of infection.[31]

The opposing conclusions of the animal studies and most observational case-series can be explained by their respective methodologies. Earlier animal studies examining the timing of debridement did not involve systemic antibiotics, which could make a delay in surgery 'safer' with respect to infection. For ethical reasons all clinical studies have been observational, with the likely tendency for clinicians to prioritise the most heavily contaminated injuries for earlier debridement, thereby potentially balancing the late and early treatment arms. The groups which undergo early surgery may include more operations that are performed out of hours by less experienced, on-call staff and consequently may have received sub-optimal treatment compared with those treated on scheduled operating lists by consultant surgeons.

With regard to the timing of initiating systemic antibiotics, the first experimental work, by Altemeier, Furste and Culbertson,[32] considered the impact of the timing of antibiotic administration on the survival of guinea pigs with wounds infected with *Clostridium perfringens*. They demonstrated a significant deterioration in survival times when a regular intramuscular penicillin regime was initiated six hours after injury compared with immediately post-injury.[32] Owen-Smith and Matheson[10] showed that even with wound debridement at six hours, delaying antibiotics worsened survival in sheep with penetrating soft-tissue wounds contaminated with *C. perfringens*. Similarly, Mellor, Cooper and Bowyer[33] used a porcine penetrating injury model to show that when the start of a three-day course of benzylpenicillin was delayed from one to six hours after injury, it was ineffective in preventing infection.

Despite the early animal studies, clinical opinion remained divided on the benefit of 'prophylactic' antibiotics. Two case series of open fractures published in the 1960s did not support their use until infection was suspected.[34,35] The issue of the use of prophylactic antibiotics was settled

definitively by Patzakis, Harvey and Ilver[20] in a randomised control trial of antibiotics in all types of open fracture in 1974. They demonstrated that those not treated with antibiotics had significantly greater rates of infection compared with the group treated with a cephalosporin.[20] Unfortunately, the effect of the timing of antibiotic administration was not evaluated.

Two case series of military patients injured in separate conflicts provided a natural experiment on antibiotic timing. British servicemen in Borneo between 1963 and 1965 were issued with oral oxytetracycline to take immediately if injured.[36] Rates of wound infection were lower than in casualties with similar injuries from the 1982 Falklands conflict who did not receive antibiotics until evacuated to a medical aid post.[36,37] This difference was only observed in patients undergoing surgery within six hours and the numbers were too small to reach significance.[36,37] In the Falklands study, seven of the nine cases of wound infection did not receive antibiotics in the first six hours after wounding and there was no infection when antibiotics were administered within three hours.[37]

Two similar observational studies have provided conflicting data on the significance of antibiotic timing. Whereas Patzakis and Wilkins[38] reported that a delay in antibiotic administration greater than three hours was associated with an increased risk of wound infection, Al-Arabi et al[27] found that while a delay of > 24 hours was associated with a higher risk of infection, delays of < 24 hours were not. However, neither study was able to control for the effect of different timings of surgery.

Whereas there remains a lack of definitive clinical evidence on the timing of the administration of antibiotics, the compelling data from animal studies supports early administration and this is advised in current clinical guidelines.[5,39] With regard to the importance of early antibiotic and surgical treatment, our findings are consistent with earlier work describing the progress of bacteria from their colonising planktonic form to adherence to tissue and eventually the formation of biofilm; so-called 'race to the surface'.[40] As bacteria progress through these stages, their vulnerability to conventional treatments of debridement, irrigation and antibiotics decreases and the six-hour time point appears to be be significant.[12]

It is easier to influence the initiation of antibiotics than surgery. This study, together with the existing literature, indicates that the earlier systemic antibiotics are administered the greater their effect on infection. It is reasonable to conclude that civilian Emergency Medical Services should follow their military counterparts and regard antibiotics as a key component of pre-hospital care of the casualty with an open fracture. This study indicates that in cases where antibiotic administration has been delayed for several hours, delaying surgery for up to 24 hours is likely to result in significantly greater infection rates than emergency surgery. Even when casualties receive antibiotics soon after injury, this study also supports the position that emergency

surgical debridement can still reduce infection. However, beyond the first few hours, the advantage offered by urgent surgery appears to be negated.

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