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The prevention of experimental osteomyelitis in a model of gunshot fracture in the pig

P.F. Hill and P.E. Watkins

Biomedical Sciences, CBD Porton Down,Salisbury, Wilts SP4 OJQ, UK and Royal Defence Medical College Gosport Hants PO12 2AB, UK

Abstract: Data from civilian trauma practice would support the non-operative management of low-energy transfer ballistic wounds to bone. However, there is a paucity of data to indicate whether this would be an effective treatment for similar injuries in military casualties, in whom wounds are heavily contaminated and there is a delay to receipt of medical treatment. We created a model of ballistic wounding in the proximal tibia of pigs. Fractures were contaminated with *Staphylococcus aureus*. Animals were divided into two groups; Control (n = 8), received no treatment, Treatment (n = 6), received 600 mg benzylpenicillin and 500 mg flucloxacillin by intramuscular injection for seven days, commencing six hours after injury. Animals were killed 14 days after injury. Treatment with antibiotics caused a statistically significant reduction in the incidence of osteomyelitis assessed by bacteriological examination. The results have important implications for the management of low-energy transfer wounds of bone in both civilian and military casualties.

Correspondence to: Dr P.E.Watkins
E-mail : watkinsvet@hotmail.com

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Introduction

The incidence of ballistic fractures in civilian trauma practice has increased significantly over the last 30 years, with handguns causing the majority of these injuries. They are characterised by limited bony damage and maintenance of the integrity of the surrounding bone, so that load bearing, although reduced, is still possible and operative stabilisation of the fracture is rarely necessary [17, 23]. However, these injuries place a substantial burden on health care providers [3, 14].

Recently there has been a change in management of these injuries, moving away from surgical exploration of wounds towards a non-operative policy of wound cleaning, in some cases combined with local débridement, and/or the administration of systemic antibiotics [9, 10, 16]. The incidence of wound infection is low, ranging from 0 to 4%, despite only minimal treatment [11, 17] due, probably, to a relatively low bacterial load in the wound and the rapid receipt of medical treatment [5, 8].

In most military conflicts of the last century there has been a predominance of wounding by high-energy projectiles, often bullets, in which treatment involved surgical débridement and excision [21, 22]. In more recent military conflicts there has been increasing deployment of weapons that generate multiple small fragments and aim to incapacitate, rather than kill, casualties [18, 20]. The small fragments produce multiple low-energy transfer wounds with injuries involving soft tissue and/or bone [12] and up to 90% of wounds affecting the extremities [4]. The bony injuries produced by small fragments bear some similarities to those caused by handgun injuries in civilian trauma practice, but with several differences. Military wounds are more likely to be heavily contaminated [21] and there is invariably a greater delay between wounding and the receipt of medical treatment. As a result, limb wounds caused by these munitions in recent military conflicts have been managed by surgical excision and delayed primary closure [20].

Surgeons treating casualties from the Afghanistan civil war have reported the successful non-operative management of small fragment wounds. There was often a delay of 12 hours or more to initial treatment that involved wound cleaning and systemic administration of antibiotics; however this was applied only to wounds of soft tissue [2]. By contrast, there is no data to indicate whether a similar regimen would be successful for the management of low-energy transfer wounds involving bone.

This study evaluated the efficacy of systemic antibiotics, commencing six hours after injury, in controlling infection in an animal model of a contaminated low-energy transfer wound of a long bone.

Materials and methods

Fourteen female Large White pigs were used (mean weight 45.4 kg), Animals were healthy on veterinary examination. Work was performed under the Animals (Scientific Procedures) Act, 1986.

Experimental study

Following premedication by intramuscular injection of droperidol general anaesthesia was induced by inhalation of halothane in oxygen/nitrous oxide. Animals were intubated with a cuffed endotracheal tube and anaesthesia subsequently maintained.

Animals were placed in dorsal recumbency, and the right hind leg was elevated. A ballistic injury was created at the right tibia by firing a steel fragment (mass 200 mg) at the lateral aspect of the proximal metaphysis. By use of a laser-aiming device, the aiming point was aligned 2 cm distal to the tibial tuberosity. The velocity of the fragment was measured with a chronoscope positioned between the barrel and the leg. The wound was immediately contaminated with approximately 10^7 colony-forming units of *Staphylococcus aureus* (American Type Culture Collection 29213), an organism that has been used previously in other animal models of osteomyelitis [7]. This was introduced on a strip of sterile bovine collagen passed down the entry wound into the bony defect. Animals were radiographed, and received buprenorphine prior to recovery from anaesthesia.

The energy (E) of the fragment was calculated from:

$$E = 0.5\ mv^2$$

where m = mass of fragment (kg), v = fragment velocity (ms^{-1}).

Animals were divided into two groups. Control Group (n = 8) received no antibiotics, whereas Treatment Group (n = 6) received 600 mg benzylpenicillin and 500 mg flucloxacillin by intramuscular injection every six hours for seven days, commencing six hours after injury.

Radiographic assessment was made under general anaesthesia seven and 14 days after injury. All animals were killed 14 days after injury and a post-mortem examination undertaken under sterile conditions. Both bony and soft tissues were removed for bacteriological and histological examination.

Bacteriological examination

Tissue samples and swabs taken from the wounds were inoculated onto blood agar and MacConkey agar, and were cultured aerobically and anaerobically for 24 hours. All bacterial species were identified by standard methods.

Histological examination

Tissues removed from the wound tract were fixed in buffered formaldehyde, and bony specimens were decalcified. Sections (4 µm thickness) were then cut on a rotary microtome and stained with haematoxylin and eosin.

Statistical examination

As a result of the relatively small numbers in each group the Fisher Exact Probability test was used to evaluate the effect of antibiotic treatment on the incidence of osteomyelitis. Significance was set at $p < 0.05$.

Results

A ballistic fracture of the proximal tibia was produced in all animals, and was of either the drill-hole or divot pattern (Fig. 1 & 2); the number of divot or drill-hole fractures was similar in both groups. The maximum energy transfer at wounding was 40J, confirming that all injuries were low-energy transfer wounds [19].

All animals recovered from anaesthesia without complications and were partially weight-bearing through the injured limb within 24 hours. A scab formed over the entrance to wound tracts, despite the presence of deep infection in some animals.

Fig. 1
Radiograph demonstrating drill-hole fracture of proximal tibia

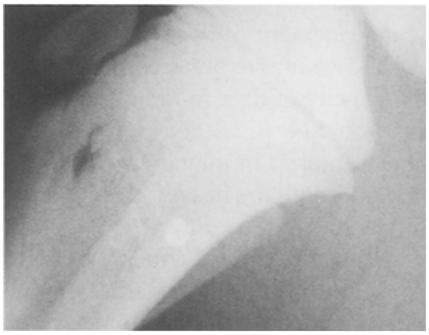

Fig. 2
Radiograph demonstrating divot fracture of proximal tibia

Control group

All animals developed osteomyelitis and infection of overlying soft tissues. Radiographs demonstrated a consistent appearance by day 14, with a central radiolucent area and surrounding sclerosis (Fig. 3). There was no evidence of a local periosteal response. In one animal an involucrum was clearly visible.

Post-mortem examination revealed purulent material in both soft tissues and in the tibia. The infecting organism was isolated from all animals.

Histological examination confirmed the diagnosis of osteomyelitis based on the presence of purulent material in bone and associated ostoenecrosis.

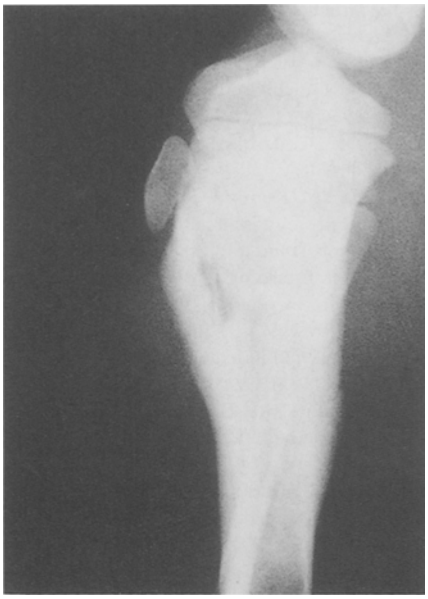

Fig. 3
Radiograph of animal in control group, 14 days after injury, demonstrating osteolytic lesion with surrounding sclerosis

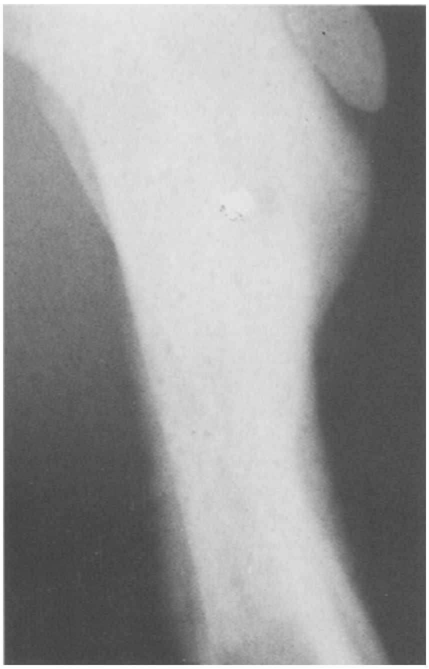

Fig. 4
Radiograph of animal in treatment group, 14 days after injury, demonstrating early periosteal new bone formation at the tibia

Treatment group

None of the animals in this group developed osteomyelitis. Radiographic examination revealed some radiolucency in the proximal tibia, but no surrounding sclerosis. A proliferative periosteal response, with new bone formation, was observed in four of six animals (Fig. 4).

At post-mortem there was a localised soft tissue abscess, superficial to the bone, in one animal only, from which the infecting organism was cultured. However, all samples of bone from all animals were sterile on culture.

Histology demonstrated a pattern of active new bone formation at and around the site of injury; however there was no evidence of either osteonecrosis or purulent material.

There was statistically significant reduction in the incidence of osteomyelitis in treatment group compared with the control group ($p = 0.00067$).

Discussion

This study has allowed the development of a model of a low-energy transfer ballistic fracture in a long bone that is directly relevant to the military casualty. Contamination of the wound immediately after injury led to the consistent development of osteomyelitis, and the administration of systemic antibiotics alone, commencing six hours after injury, could control infection in this model.

Although many models of osteomyelitis have been described in the literature [6], most have been used for the evaluation of local antibiotic therapy. In the few studies assessing systemic antibiotics, therapy was usually commenced once infection had been established, in order to evaluate the efficacy of treatments for established osteomyelitis. Limited data detail the effects of timing of antibiotic administration on the control of bacterial contamination of bone. Studies in the dog, involving an osteotomy defect contaminated with *Staphylococcus aureus*, showed that antibiotics had to be administered within 4 hours of bony injury if they were to be effective [4]. However, more recently, studies in a rabbit model of an infected fracture, demonstrated that antibiotics administered within 4 hours of contamination caused a significant reduction in the incidence of infection [24].

The delay of six hours to commencing antibiotic administration in our study would be considered exceptional by the standards of modern civilian trauma care. However, in military scenarios as a result of the logistical constraints combined with often large numbers of casualties injured in a short period of time, a delay of this time to receipt of initial therapy is not unexpected [20]. Despite this, the control of infection described here is in agreement with the findings reported from civilian trauma centres detailing the management of low-energy gunshot wounds. Rose [17], in a retrospective study, reported the successful treatment of 12 patients with localised fractures of a long bone, by local wound care and antibiotics. Woloszyn [23] described a retrospective study of 132 gunshot wounds involving bone that were treated by superficial débridement, local cleaning and administration of antibiotics, without operative fixation. The route of administration of antibiotics, either orally or intravenously, had no effect on the rate of infection.

In a prospective trial, Dickey [9] demonstrated no difference in the rate of infection amongst patients with low-energy gunshot wounds, not requiring operative treatment, regardless of whether antibiotics were administered. However, the numbers recruited to the study were too low to meet statistical requirements. More recently, Knapp [16] undertook a prospective randomised study of 222 low-energy gunshot fractures involving a long bone, in 190 patients. Treatment involved either oral or intravenous antibiotics, commenced as soon as possible after injury. Both treatment protocols were equally as effective.

The results of this experimental study, if combined with data from civilian trauma centres and the results from the non-operative management of soft tissue wounds caused by small fragments, provide support for military surgeons to reconsider their policy for management of low-energy transfer wounds. Adoption of a non-operative protocol by military surgeons, making use of systemic antibiotics alone, would aid the logistics of casualty management, since the wounding patterns are invariably multiple and would require prolonged surgical treatment, if the latter were adopted.

Despite these encouraging results, there is a need for some caution before military surgeons adopt a policy of non-operative management of small fragment wounds to bone. Successful adoption of this policy would require that clinicians be able to reliably distinguish between high-energy and low-energy transfer wounds at the time of initial assessment of the patient. A further potential complicating factor is the site of injury. Knapp [16] reported four patients with infections, (two in each group), all involving injury to the distal tibia. These findings highlight the importance of the anatomical site of injury in determining outcome, a feature that has been reported by several authors describing the management of tibial fractures [10, 15].

In conclusion, this experimental study has shown that in an animal model, directly relevant to military casualties sustaining a contaminated low-energy transfer injury to bone, osteomyelitis can be prevented by the administration of systemic antibiotics, commencing at up to six hours after injury. Despite the encouraging results there is a need for further studies to assess whether the time to commencing of antibiotic administration might be extended without prejudicing outcome. In addition, more precise, but clinically applicable methods for defining tissue damage in ballistic wounds will aid in the selection of those casualties who may be successfully managed without recourse to surgery.

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Prévention de l'ostéomyélite expérimentale à travers un exemple de fracture par balles sur le cochon. Contamination de fractures expérimentales par balles

Résumé : Les données concernant les accidents civils tendraient à soutenir le traitement non-opératoire des blessures balistiques aux os de transfert de basse énergie. Cependant, il n'y a pas suffisamment d'informations indiquant qu'il s'agirait ici d'un traitement efficace pour les blessures de ce type parmi les victimes militaires, quand les plaies sont profondément contaminées et qu'il existe des retards dans l'administration des soins médicaux. Nous avons créé un modèle de blessure balistique dans le tibia proximal du cochon. Les fractures ont été contaminées par *Staphylococcus aureus.* Les bêtes étaient réparties en deux groupes ; Contrôle (n = 8) qui n'ont reçu aucun traitement, Traitement (n = 6) qui ont reçu 600 mg de benzylpénicilline et 500 mg de flucloxacilline par piqûres intramusculaires pendant sept jours, en commençant six heures après les blessures. Les animaux ont été abattus quatorze jours après les blessures. Le traitement aux antibiotiques a causé une réduction statistiquement conséquente de l'incidence d'ostéomyélite déterminée par examen bactériologique. Les résultats ont d'importantes implications pour le traitement de blessures aux os, transfert de basse énergie autant chez les victimes civiles que militaires.

Mots-clés : Fracture – Contamination – Coup de feu – Antibiotiques