Injury, Int. J. Care Injured 45 (2014) 1171–1178

Contents lists available at ScienceDirect

Injury

journal homepage: www.elsevier.com/locate/injury

A pre-clinical evaluation of silver, iodine and Manuka honey based dressings in a model of traumatic extremity wounds contaminated with *Staphylococcus aureus*

Hugo C. Guthrie [a,b,*], Kevin R. Martin [a], Christopher Taylor [a], Abigail M. Spear [a], Rachel Whiting [a], Sara Macildowie [a], Jonathan C. Clasper [b], Sarah A. Watts [a]

[a] *Biomedical Sciences Department, Building 245, Defence Science and Technology Laboratory, Porton Down, Wiltshire SP4 0JQ, United Kingdom*
[b] *Academic Department of Military Surgery and Trauma, Royal Centre for Defence Medicine, Birmingham Research Park, Vincent Drive, Birmingham B15 2SQ, United Kingdom*

ARTICLE INFO

Article history:
Accepted 6 May 2014

Keywords:
Infection
Extremity
Injury
Military
S. aureus
Wounds
Dressings
Silver
Iodine
Honey

ABSTRACT

Prevention of extremity war wound infection remains a clinical challenge. *Staphylococcus aureus* is the most common pathogen in delayed infection. We hypothesised that choice of wound dressings may affect bacterial burden over 7 days reflecting the current practice of delayed primary closure of wounds within this timeframe.

A randomised controlled trial of 3 commercially available dressings (Inadine® (Johnson & Johnson, NJ, USA), Acticoat® (Smith & Nephew, Hull, UK), Activon Tulle (Advancis Medical, Nottingham, UK)) was conducted in a rabbit model of contaminated forelimb muscle injury. A positive control group treated with antibiotics was included. Groups were compared to a saline soaked gauze control. The primary outcome was a statistically significant reduction ($p < 0.05$) in tissue *S. aureus* at 7 days post-injury. Secondary outcome measurements included bacteraemias, observational data, whole blood determination, ELISA for plasma biomarkers, PCR array analysis of wound healing gene expression and muscle/lymph node histopathology.

Antibiotic, Inadine and Acticoat groups had statistically significant lower bacterial counts (mean 7.13 [95% CI 0.00–96.31] $\times 10^2$; 1.66 [0.94–2.58] $\times 10^5$; 8.86 [0.00–53.35] $\times 10^4$ cfu/g, respectively) and Activon Tulle group had significantly higher counts (2.82 [0.98–5.61] $\times 10^6$ cfu/g) than saline soaked gauze control (7.58 [1.65–17.83] $\times 10^5$ cfu/g). There were no bacteraemias or significant differences in observational data or whole blood determination. There were no significant differences in muscle/loss or pathology and lymph node cross-sectional area or morphology. There were some significant differences between treatment groups in the plasma cytokines IL-4, TNFα and MCP-1 in comparison to the control. PCR array data demonstrated more general changes in gene expression in the muscle tissue from the Activon Tulle group than the Inadine or Acticoat dressings with a limited number of genes showing significantly altered expression compared to control.

This study has demonstrated that both Acticoat® and Inadine® dressings can reduce the bacteria burden in a heavily contaminated soft tissue wound and so they may offer utility in the clinical setting particularly where surgical treatment is delayed.

Introduction

Over 400 UK military and civilian personnel have been killed as a result of hostile action in Afghanistan since 2001 [1] and many more have been injured. The majority of these casualties are the result of the improvised explosive devices used ubiquitously by insurgents in recent conflicts [2,3]. The extremities are the most commonly injured anatomical regions in war accounting for between half [2] and two thirds of all wounds [3]. Most of these

* Corresponding author at: c/o Dr Sarah Watts, Biomedical Sciences Department, Building 245, Defence Science and Technology Laboratory, Porton Down, Wiltshire SP4 0JQ, United Kingdom.
E-mail addresses: hugoguthrie@doctors.org.uk (H.C. Guthrie), sawatts1@dstl.gov.uk (S.A. Watts).

http://dx.doi.org/10.1016/j.injury.2014.05.007
0020–1383/

extremity wounds are penetrating soft tissue wounds but around a quarter involve a fracture; of which the vast majority are open fractures [2]. Studies of high-energy, complex and heavily contaminated wounds have identified high rates of infectious complications [4,5] and eventual osteomyelitis [6] which may be a result of the initial contamination or subsequent contamination during staged evacuation and treatment at medical facilities of increasing sophistication.

The microflora of war wounds evolves over time [7] and historical studies have demonstrated both seasonal [8] and geographic [9–11] variation. During the recent conflicts in Iraq and Afghanistan it is become clear that Gram-positive organisms dominate the initial contamination [12] and largely reflect bacterial carriage in the population at risk [13], Gram-negative organisms are then more prevalent in early wound colonization [14,15] or primary infection [16,17] and finally *Staphylococcus aureus* is identified in the majority of delayed infectious complications [15] which result in further surgery, delayed rehabilitation and worse functional outcome.

Frequently war wounds are anatomically complex and seen in physiologically vulnerable servicemen whose evacuation to the next level of care (where further surgical debridement can be carried out) cannot be guaranteed due to ongoing hostilities. For these reasons military surgeons cannot rely on a single surgical debridement, however expertly performed, to eradicate all initial bacterial contamination and of course prevent subsequent contamination. Therefore the evidence based use of adjuncts to surgery, such as wound dressings, is an important area of research and practice. Given the heterogenous nature of war wounds and the presence of multiple confounding variables such as poly-microbial contamination, multiple injury, haemorrhagic shock, massive blood transfusion and the effect of blast injury we believe that a clinical randomized controlled trial of war wound dressings would be extremely challenging to perform and may not answer the research question.

In order to investigate the effect of wound dressings on this most clinically significant microorganism the Defence Science and Technology Laboratory first developed an animal model of a *S. aureus* contaminated extremity war wound [18] and then conducted a 48 h randomized controlled trial of antimicrobial wound dressings [19]. This trial reproduced the common scenario where contamination persists despite current management strategies and assessed whether the 'residual' bacterial load could be reduced by dressings alone and whether there was any injurious effect associated with their use.

The aim of the current trial was to assess the antimicrobial efficacy of commercially available wound dressings which have all been used or are being considered for use by the UK military by extending the time frame to reflect the current clinical practice of delayed primary closure of clean viable war wounds at around 5–7 days after injury [20–22]. We tested the hypothesis that wounds treated with gauze dressings impregnated with iodine (Inadine®), silver (Acticoat®) or Manuka honey (Activon Tulle) will have significantly lower *S. aureus* counts after 7 days than a control group treated with saline soaked gauze dressings. In addition, we evaluated the potential harmful effects of the dressings via assessment of plasma biomarkers and tissue pathology and molecular biology.

Materials and methods

Study design and ethics

This trial was conducted under a license issued by the UK Home Office under the authority of the Animals (Scientific Procedures) Act 1986. A local ethical review board approved the work and 3R's principals were adhered to. A power calculation based on the earlier trial suggested that 6 animals in each group would be sufficient to detect a statistically significant difference in bacterial count, which was the primary outcome in this study. Secondary outcome measurements included bacteraemia, animal behaviour, weight change, temperature, whole blood determination, plasma and tissue markers of inflammation and repair and muscle and lymph node histopathology.

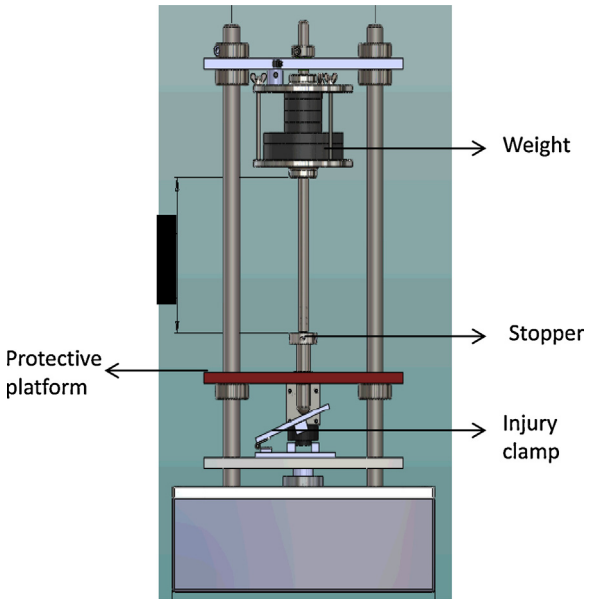

Fig. 1. Muscle injury jig. The isolated muscle is held between the jaws of the injury clamp. The weight held in place by an electronically controlled magnet on release the weight falls to the level of the stopper which pushed down on the clamp. The protection plate and design of the injury clamp ensure only the isolated muscle is injured.

Surgical, injury and microbiological technique

A detailed description of the surgical and microbiological methodology has been reported previously by this group [18].

Briefly, groups of skeletally mature female New Zealand White (NZW) rabbits (3.87 kg, 4.73–3.12 kg) were randomized to each of 5 test groups.

Under general anaesthesia the flexor carpi ulnaris (FCU) muscle belly was surgically isolated and injured using the bespoke jig (Fig. 1).

Following injury the FCU muscle belly was directly inoculated with a challenge dose of 10^6 colony forming units (CFU) of *S. aureus* (National Collection of Type Culture 4163) using a pipette.

A subcutaneous injectable non-steroidal anti-inflammatory was administered (4 mg/kg Carprofen – Rimadyl, Pfizer, Sandwich, UK) and repeat doses were administered daily for the remainder of the study. (This drug was given to reduce the post-injury pain associated with traumatic injury. Reducing the incidence of lameness with the use of an analgesic reduced the overall severity of the procedure as well as ensuring that adverse effects such as infection, if present, was more easily determined.)

A subcutaneous Implantable Programmable Temperature Transponder (IPTT, Biomedic Data Systems Inc., Delaware, USA) was inserted between the scapulae.

The rabbits remained anaesthetized and monitored for 3 h prior to application of randomized test dressing (described below) and a support bandage was applied prior to recovery from anaesthesia.