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Development of a contaminated ischemic porcine wound model and the evaluation of bromelain based enzymatic debridement

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ARTICLE INFO

Article history:
Accepted 27 July 2017
Available online xxx

Keywords:
Animal model
Necrotic wounds
Eschar
Porcine

ABSTRACT

Objectives: There are no well accepted animal models of chronic wounds, limiting advances in understanding and treatment of chronic ulcers. We developed a porcine wound model which combines multiple factors involved in chronic wounds to create a contaminated necrotic eschar and evaluated the debriding efficacy of a novel bromelain based enzymatic debriding agent (EscharEx).

Methods: Contaminated ischemic wounds were created on the flanks of domestic pigs by 'sandwiching' the skin between 2 'O' rings (1 placed on the surface of the skin and the other underneath the skin) for 24 h prior to dermatomal excision of the necrotic eschar and its contamination with *Staphylococcus aureus* and *Candida albicans*. After confirming the development of infected eschars, additional animals were used to compare the effects of daily application of topical EscharEx or its hydrating vehicle on eschar debridement as a control.

Results: In all cases, application of the 'O' rings resulted in full thickness necrotic ecshars with invasive infections, which did not reepithelialize and sloughed off spontaneously within 14–21 days. All wounds reepithelialized within 28–42 days forming contracted scars. All EscharEx treated eschars were completely debrided within 7–9 days, while no debridement was evident in eschars treated with the control gel.

Conclusions: Our model simulates the initial phase of chronic wounds characterized by a contaminated necrotic eschar allowing evaluation of wound debriding agents, and that a bromelain-based debriding agent completely debrides the contaminated necrotic eschars within one week in this model.

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http://dx.doi.org/10.1016/j.burns.2017.07.022
0305-4179/

1. Introduction

Chronic wounds, such as venous, arterial and diabetic ulcers, affect an estimated 7 million patients in the U.S. alone each year, with costs exceeding $25 billion annually [1]. While multiple topical therapies have been evaluated, few have shown significant benefit in healing chronic wounds. However, a cornerstone of wound therapy is wound debridement [2], whereby necrotic, and often contaminated tissue is removed by means of autolytic, enzymatic, or surgical debridement.

Unlike acute wounds that heal in an orderly and timely manner, chronic wounds heal slowly or not at all. While the etiology of many chronic wounds is multifactorial, ischemia, infection, and acute injury are often contributing factors [3]. In order to better understand the underlying pathobiology and mechanisms contributing to chronic wounds, animal models are greatly needed [4]. These models can also be used to evaluate potential therapeutic agents prior to advancing to human trials. Unfortunately, there are few (if any) well-accepted and validated large animal models of chronic wounds. Methods to simulate chronic wounds have included induction of diabetes, administration of corticosteroids, or local radiation [5–8]. Local application of pressure [9–12] as well as the creation of ischemic flaps [13] or models of arterial devitalization [14–16] have also been used. However, none of these models fully replicate the multitude of factors that contribute to chronic wounds.

The aims of the current study were twofold. First, to develop a porcine wound model that combines ischemia, acute injury and bacterial and fungal contamination, reproducing a contaminated wound eschar typical of many chronic wounds. Second, to compare the debridement efficacy of ischemic contaminated wounds treated with a bromelain based enzymatic agent versus its control vehicle.

2. Materials and methods

2.1. Animals and preparation

Our study was approved by the Institutional Animal Use and Care Committee. We used six female Yorkshire pigs (20–25 kg) following national guidelines [17]. The animals were fasted overnight and sedated with acepromazine 0.1 mg/kg, atropine 0.02 mg/kg, ketamine 20 mg/kg, and xylazine 2.2 mg/kg by intramuscular injection. The pigs were endotracheally intubated and maintained under a surgical plane of anesthesia with isoflurane 1.0–5.0%. The hair was removed with electric clippers and the skin was scrubbed with antibacterial soap and water and dried.

2.2. Model development

In four animals we developed an ischemic, acutely injured and contaminated wound model that was designed to mimic the most common features of chronic wounds. Ischemia of the skin was produced with a pair of stainless steel "O" rings that compressed the skin's blood supply until full thickness necrosis developed (Fig. 1A). A full thickness incision was made through the skin over the paravertebral area of the animal. The incision was 1–2 cm longer than the diameter of the 5 cm "O" rings. Using blunt dissection, two subcutaneous pockets were created, one on either side of the incision to allow insertion of the bottom ring with attached screws (Fig. 1B). Small holes were punched through the skin over the screws

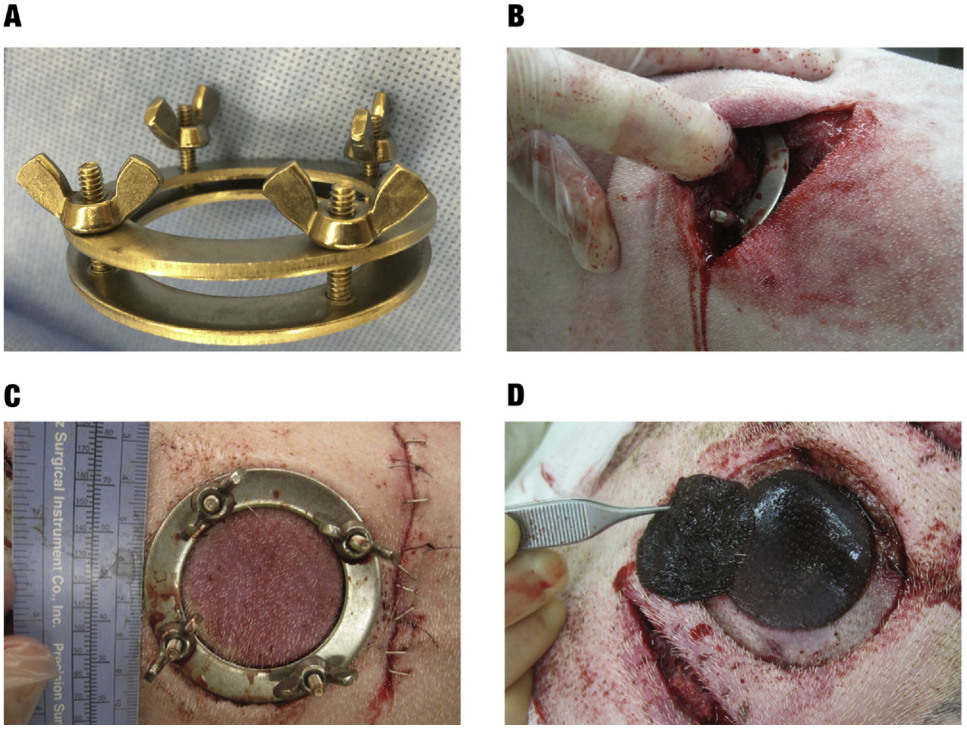

Fig. 1 – Creation of ischemic wounds. "O" ring setup and components (A), subcutaneous insertion of one "O" ring with attached screws (B), final placement of "O" ring system (C), and appearance of the necrotic eschar 48 h later (D).

Please cite this article in press as: A.J. Singer, et al., Development of a contaminated ischemic porcine wound model and the evaluation of bromelain based enzymatic debridement, Burns (2018), http://dx.doi.org/10.1016/j.burns.2017.07.022