BURNS 42 (2016) 556–563

Available online at www.sciencedirect.com

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

Measurements in wound healing with observations on the effects of topical agents on full thickness dermal incised wounds

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ARTICLE INFO

Article history:
Accepted 19 September 2015

Keywords:
Wound healing
Topical agents
Epithelial growth factor
Porcine model
Computer image analysis

ABSTRACT

Introduction: A multitude of topical wound treatments are used today. Although it is well established that the micro-environment of healing wounds can be altered to improve healing, it is difficult to measure the subtle differences in outcome where therapies are compared.

Method: We compared wound healing properties between four different topical agents in surgically incised wounds in a pig model. The four topical agents, 5% Povidone-Iodine cream, 1% Silver-Sulphadiazine, 2% Mupirocin, and 1% Silver-Sulphadiazine plus 1 mg/100 g recombinant-human epithelial growth factor (EGF) were randomly assigned to four test animals each. Test agents were compared to each other and to untreated controls. We investigated existing and new methodologies of measurement of wound healing: clinical and histological visual scoring systems, immuno-histochemistry, and computerized image analysis of the wounds on days 3, 7, and 28.

Results: All agents were found to have improved healing rates with better cellular architecture. Healing was faster, histological appearance resembled normal architecture sooner, clinical appearance improved, mitotic activity was stimulated and more collagen was deposited in comparison to the wounds with no agents. EGF-treated wounds showed an increased rate of epithelisation, but the rate of healing did not correlate well with evaluation of cosmetic outcome.

Conclusion: Topical agents improve all aspects of wound healing. The addition of a human recombinant EGF to Silver-Sulphadiazine increases epithelial growth and amounts of collagen in the regenerating wounds at day 7.

1. Introduction

Inert topical agents in the form of creams, lotions and ointments are often applied to burn wounds to create an environment conducive to wound healing. These agents, although not pharmacologically active on the intact skin, have activity when applied to wounds where the stratum corneum barrier to penetration is absent. Although effective against organisms causing burn wound infection, they are also

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http://dx.doi.org/10.1016/j.burns.2015.09.014
0305-4179/

potentially cytotoxic to regenerating epithelial cells. It is therefore prudent that the selection of an antibacterial agent is not only based on its antibacterial effect, but also on its wound modulation and cytotoxic effects on immature and non-adherent keratinocytes [1].

Central to in vivo and in vitro studies of wounds and wound healing is the correct choice of an experimental animal model and need for accurate methods to evaluate the effect of topical agents on wound healing [2–4].

There are many methods used for the analysis of wounds and the healing process. Parameters used for measuring outcome should be unambiguous and measurements should be accurate and reproducible. Studies in pig models have been used extensively to investigate the mechanisms of wound healing. Clinical evaluation is usually subjective and not quantitative, resulting in unacceptable levels of inter- and intra-observer variation. Similarly lacking are clear histological correlates of what would be considered good healing characteristics of a wound. The need for objective measurements becomes more urgent to evaluate and compare treatment options in view of the large numbers of topical agents currently in use.

Previous measurement systems were developed, ranging from visual scoring systems to measurement of biological and chemical wound constituents. The structural and ultrastructural elements of healing wounds remain difficult to measure, although immuno-histochemistry and scanning electron microscopy allows some quantification and simple morphometric measurements [5].

With current advances in computer technology, image analysis offers dynamic functional imaging, linking multiple data sources to provide composite quantitative systems.

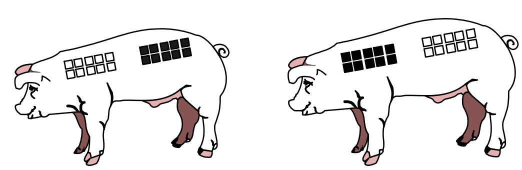
The aim of this porcine study was to explore two aspects: comparing healing of wounds treated with four different topical agents, and the use and value of computerized image analysis along with existing scoring systems to evaluate wound healing.

2. Materials and methods

The experimentation was performed in an established animal research facility and the study was approved by the Animal Research Review Committee at the University of Cape Town [Project Number 97/024]. Sixteen female pigs of between 15 and 20 kg were used. The animals were stabled in clean individual pens throughout the period of experimentation and received regular feeds and water ad libitum. The pigs were sedated before induction of anaesthesia. Inhalation anaesthesia with halothane was used during all procedures. Post-operative analgesia was provided on a scale that would be appropriate for humans, subjected to the same procedure. Intravenous buprenorphene hydrochloride (Temgesic) was administered at a dose of 0.004 mg/kg 12 hourly for the first 48 h post operative and by intramuscular injection thereafter. The dorsum of the pig was shaved and no washing or prepping with anti-septic solution was done prior to surgery to prevent any carry over effect from the agent to influence the outcome.

After induction, a random assignment was made to one of the treatment agents by an independent assistant and a study number was marked on the ear with a marker pen. A plastic template was used to mark 16 paravertebral wounds of 2 cm × 2 cm on the dorsum of the animal for treatment of the test agent(s). They were positioned either towards the head or the tail of the animal. Sixteen additional paravertebral wounds, using the same method, were then marked in a similar way towards the other end of the animal. These wounds were left untreated and served as controls. Animal allocation and wound orientation is depicted in Fig. 1. To exclude the effect that regional placement of wounding might have on healing properties, two of the four animals assigned to a specific treatment had the test wounds towards the tail and the control wounds towards the head. This order was reversed for the other 2 animals. Full thickness wound were created by using a scalpel blade to excise, in a square fashion, the skin of each area down to the fascial plane. The level of excision included the panniculus carnosus thereby eliminating the effect of the latter on wound healing/contracture. All wounds created were clinically at the same depth.

The four topical agents, randomly assigned to 16 test animals, were 5% Povidone-Iodine cream, 1% Silver-Sulphadiazine, 2% Mupirocin, and 1% Silver-Sulphadiazine plus 1 mg/100 g recombinant-human epithelial growth factor (Hebermin–Heber Biotech, S.A., Havana, Cuba). The test agents (approximately 10 ml per site and filling the whole wound), were then applied to the freshly created wounds and replenished daily without disturbing the healing process. No occlusive dressings were used, while the control wounds were left untreated.



Wound Allocation A
Control Forward
Treatment Backward

Wound Allocation B
Treatment Forward
Control Backward

Pig Number	Wound treatment	Wound Allocation	Harvesting Day
1	Povidone Iodine	A	7
2	Povidone Iodine	B	28
3	Silver Sulphadiazine	A	7
4	Mupirocin	B	28
5	Povidone Iodine	A	28
6	Mupirocin	B	7
7	Hebermin	A	7
8	Silver Sulphadiazine	B	7
9	Povidone Iodine	B	7
10	Silver Sulphadiazine	A	28
11	Hebermin	B	28
12	Mupirocin	A	7
13	Mupirocin	A	28
14	Hebermin	B	7
15	Silver Sulphadiazine	B	28
16	Hebermin	A	28

Fig. 1 – Animal wound allocation table.