

Efficacy of a Tyrothricin-Containing Wound Gel in an Abrasive Wound Model for Superficial Wounds

W. Wigger-Alberti^a M. Stauss-Grabo^b K. Grigo^a S. Atiye^b R. Williams^a
H.C. Korting^c

^abioskin GmbH, Hamburg, ^bEngelhard Arzneimittel GmbH & Co. KG, Niederdorfelden, and ^cDepartment of Dermatology and Allergology, Ludwig Maximilian University, Munich, Germany

Key Words

Tyrothricin · Skin · Superficial wound · Wound healing · Abrasive wound model

Abstract

Background: Topical preparations are a common treatment for superficial acute wounds, which at the least do not interfere with healing and ideally result in enhanced wound healing irrespective of microbial colonization. **Objective:** To examine the effects of a topical antimicrobial gel and its vehicle on the wound healing of standardized, superficial abrasions. **Methods:** Thirty-three healthy volunteers were enrolled in a double-blinded, randomized, intraindividual comparison study. Three standardized, superficial abrasions were induced on their forearms. A tyrothricin 0.1% gel (Tyrosur[®] gel; Engelhard Arzneimittel GmbH & Co. KG, Niederdorfelden, Germany) and its vehicle were randomly applied to two of the test areas, and one lesion remained untreated. **Results:** A significant improvement of wound healing was seen with both tyrothricin 0.1% gel and its corresponding vehicle in the clinical assessment. The mean area under the curve (AUC) of wound healing scores was the same for both preparations and the mean reepithelization scores were comparable at all test points over the entire 12 days. A lower mean AUC representing less reepithelization was found for the untreated test fields. **Conclusion:** The use of tyrothricin 0.1% gel and its

corresponding vehicle resulted in statistically significant improved wound healing with an earlier onset of healing in particular. Based on these results obtained using an abrasive wound model, it can be concluded that the addition of tyrothricin 0.1% to the gel vehicle did not interfere with the improved wound healing seen with the vehicle alone.

Copyright © 2012 S. Karger AG, Basel

Introduction

There is a high incidence of small, superficial, acute wounds which are mainly caused by minor cuts, minor burn injuries (superficial, second degree) or accidental abrasive trauma during sport participation or other daily activities. Dirt or bacteria may have been incorporated into the fresh wounds. These wounds involve the epidermis and sometimes the superficial portion of the dermis. They are erythematous and mildly painful [1]. People generally treat such minor everyday wounds themselves, usually with dressings or wound gels and ointments with or without antibacterial ingredients which do not interfere with healing and ideally result in enhanced wound healing irrespective of microbial colonization. Topical agents

H.C.K. has deceased.

and a dressing that is minimally restrictive may be of advantage especially in wounds that are located over joints due to the limitation of movement and contracture of the skin [2]. In general, products that promote a moist wound environment produce better results in wound healing than those that promote a dry environment [3–12]. Moreover, with any open wound skin infection an increased risk of systemic infection accompanies prolonged healing. Infected wounds might also scar more severely. Therefore, the treatment with topical agents in burn and wound care should also take into consideration the risk of additional skin infection [2]. Common bacterial pathogens relevant for skin infections include Gram-positive bacteria (e.g. *Streptococcus pyogenes*, *Staphylococcus aureus*) and Gram-negative bacteria (e.g. *Pseudomonas aeruginosa*) [13], while *Candida* is a common source of – rarer – fungal infections [14]. Correspondingly, a large variety of topical antimicrobial agents such as bacitracin, polymyxin B sulfate, neomycin, povidone-iodine, silver sulfadiazine, gentamycin, nystatin and others are used in wound care [2]. Tyrothricin, an antimicrobial peptide (AMP), was identified and found its way into clinical application decades before its mode of action was uncovered [15]. AMPs are small, cationic, amphiphilic peptides with broad-spectrum microbicidal activity against both bacteria and fungi [16]. Furthermore, AMPs might help to overcome the growing problems of antibiotic resistance in the treatment of infectious skin diseases due to their special mode of action against microorganisms, namely, directly targeting and destroying their membranes [17–19]. Tyrothricin is a polypeptide antibiotic produced by *Bacillus brevis* [20] consisting of the two cyclic decapeptides gramicidin S and tyrocidine A [21]. Both peptides have broad bactericidal activity against Gram-positive bacteria; tyrothricin has been shown not to pose a risk with respect to acquired resistance of originally susceptible Gram-positive bacteria and yeasts, not even in the case of *S. aureus*, both with MSSA and MRSA strains [Korting et al., unpubl. data]. In a pilot study, a tyrothricin-containing wound gel (Tyrosur® gel; Engelhard Arzneimittel GmbH & Co. KG, Niederdorfelden, Germany) showed an improvement in the healing of noninfected wounds indicated by a higher level of reepithelization compared to the untreated test field [bioskin GmbH, unpubl. data]. The wound gel, its vehicle and a positive control (an ointment containing dexpanthenol) were applied once daily over a 12-day treatment period under semiocclusive conditions. The data supported the decision to perform the present confirmatory study with the aim of proving significantly better wound healing efficacy compared to untreated wounds. Since the

broad antimicrobial effect of tyrothricin has been established for years and for feasibility/ethical reasons, only the possible direct wound healing effect beyond antimicrobial activity was investigated here using a recently published wound model based on noninfected wound lesions only [22]. It was important to establish that tyrothricin would have no hindering effects on improved wound healing observed when using the vehicle alone.

Methods

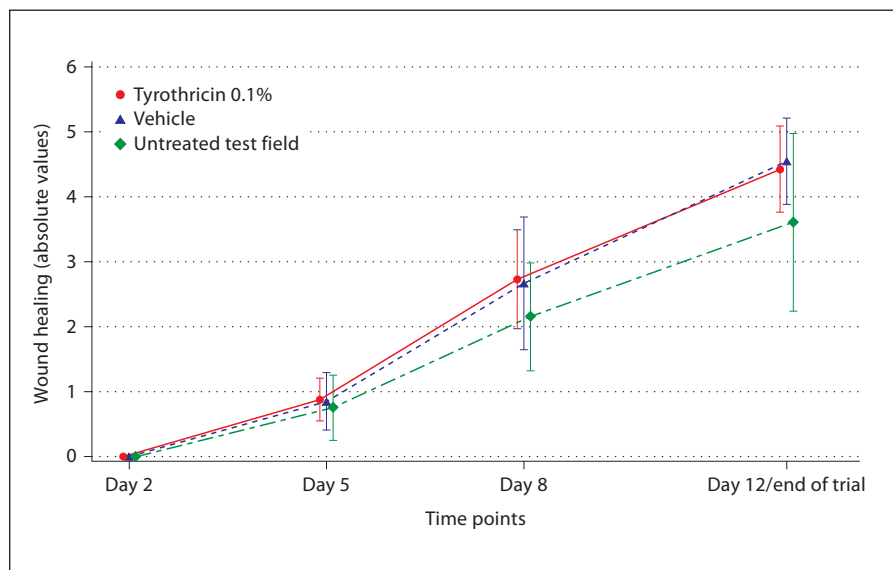
Volunteers

The study was performed at bioskin GmbH, Hamburg, Germany, from September 23 to October 8, 2010. The selection of subjects was in accordance with the requirements of the German drug law as well as the recommendations of the currently valid revision of the Helsinki Declaration and the ICH-GCP guidelines. The study documents were approved by the ethics committee of the Hamburg Medical Council. The study was registered at the competent authorities. Written informed consent was obtained from 33 male or female volunteers, aged 18 years or older with healthy skin in the test area before the start of the study. The 33 volunteers were randomized (20 women and 13 men, mean age 42.6 years, range 23–58 years). The following were the main exclusion criteria: relevant dermatological diseases such as psoriasis or lichen ruber planus; suntan, hyperpigmentation or tattoos in the test fields; dark-skinned persons; diabetes; history of wound-healing complications, or keloid and hypertrophic scarring; any clinically significant illness 4 weeks before and during the trial; known allergic reactions to components of the investigational products, and treatment with systemic or locally acting medications (e.g. antihistamines or glucocorticosteroids) within 2 weeks before the first treatment. The use of topical products other than the investigational products in the test fields was prohibited during the clinical trial. Bathing, sauna and sunbathing were not allowed. The adhesive bandages used for semiocclusion were not to be soaked through since the wound healing of the test fields might have been influenced. For the same reason exercise causing excessive sweating had to be avoided.

Test Design [22]

After enrollment 3 small superficial, abrasive wounds (10 mm in diameter) were induced, 2 on the right forearm and 1 on the left forearm of each volunteer. Treatment with tyrothricin 0.1% gel (Tyrosur gel; Engelhard Arzneimittel) and its vehicle (excipients: cetylpyridinium chloride, purified water, propylene glycol, ethanol 96%, carbomer, Trometamol) was performed topically under semiocclusive conditions. Approximately 100 µl of the formulations were applied with a gloved finger to the respective test fields on the volar forearm once daily over a 12-day study period (11 treatments, days 1–11). One test field remained untreated and served as control. All 3 wounds were covered with semiocclusive patches (Hansaplast Soft Med, hypoallergic without antiseptic silver). Wound healing was clinically assessed in all 3 test fields on days 2, 5, 8 and 12. Adverse events were recorded on each day over the entire study period.

Fig. 1. Course of mean scores (absolute values) in the clinical assessment of reepithelization of superficial wounds following treatment with Tyrosur gel and corresponding vehicle and untreated test field. Clinical assessment was performed using a 6-point scale (0 = 0% healing, 1 = 1–25% reepithelization, 2 = 26–50% reepithelization, 3 = 51–75% reepithelization, 4 = >75% but not complete reepithelization and 5 = 100% complete healing). Values are presented as means \pm SD.



Induction of Superficial Wounds

Before wounding, the forearms were disinfected with an antiseptic solution (Softasept® N; Braun, Melsungen, Germany). Two disinfected templates (robust plastic sheets) were applied to the skin. One template contained two holes (right forearm) and the other one hole (left forearm) with a diameter of 10 mm. The distance between the two holes on the right forearm was at least 1.5 cm. Epidermal abrasive wounds were induced under visual control by the same trained study nurse who repeatedly scrubbed the skin with a sterile surgical hand brush using moderate pressure until the first signs of uniform glistening and punctual bleeding were observed. At this point, the scrubbing was stopped to ensure that the lesion was superficial with only partial erosion of the papillary dermis. This procedure caused only minor pain, thus no local anesthetic was necessary [22].

Clinical Assessment of Wound Healing

After the removal of the patches and product residues the clinical assessment was performed by a trained investigator, based on the level of reepithelization represented by the following score [22]: 0 = 0% healing, 1 = 1–25% reepithelization, 2 = 26–50% reepithelization, 3 = 51–75% reepithelization, 4 = >75% but not complete reepithelization and 5 = 100% complete healing.

Statistics

Wound healing scores and their area under the time curve (AUC) were summarized. The AUC was calculated using the linear trapezoidal rule and the data assessed on days 5, 8 and 12. The hypotheses on the differences in AUCs of the wound healing scores between 0.1% tyrothricin gel and untreated as well as between the vehicle and untreated were tested by the exact Wilcoxon signed-rank test at level $\alpha = 0.025$. For evaluation of the safety extent of exposure to the investigational products, adverse events and vital signs were analyzed.

Results

The data from all 33 subjects were valid for the safety and intent-to-treat analyses while data from 32 volunteers were valid for the per protocol analysis. One subject was excluded from the per protocol analysis due to the use of a prohibited concomitant medication. Since the results of the intent-to-treat analysis were similar to those of the per protocol analysis only the results of the intent-to-treat are presented. Mean absolute clinical assessment scores are presented in figure 1. The tyrothricin 0.1% gel and the corresponding vehicle showed a comparable course of mean scores in the clinical assessment of reepithelization of superficial wounds at all test points (day 5: 0.9 and 0.8, respectively; day 8: 2.7 each; day 12: 4.4 and 4.5, respectively). The median reepithelization scores for days 5, 8 and 12 were 1.0, 3.0 and 5.0, respectively, for both investigational products. A comparable mean reepithelization score was noted for the untreated test field compared to both treated test fields on day 5 (0.8) and lower reepithelization scores were noted on days 8 and 12 (2.2 and 3.6, respectively). For the untreated field the median reepithelization scores for days 5, 8 and 12 were 1.0, 2.0 and 4.0, respectively. At the end of the 12-day treatment period complete healing was noted in more than half of the subjects in the test fields treated with tyrothricin 0.1 % gel (51.5%) and the vehicle (63.6%). In these test fields reepithelization was >75% in 39.4% (tyrothricin 0.1% gel) and 27.3% (vehicle) of the subjects and reepithelization of 51–75% was noted in 9.1% of the subjects for

each treatment. For the untreated test field complete healing was noted in fewer subjects (30.3%). Reepithelization of >50% was noted in 42.5% of the subjects (>75%: 36.4%; 51–75%: 6.1%) and reepithelization <50% in approximately 25% of the subjects. In 1 subject no healing in the untreated field was seen at the end of the trial. Mean AUCs of wound healing scores are presented in figure 2. The mean AUC of wound healing scores was the same for tyrothricin 0.1% gel and the vehicle (19.7 arbitrary units each). A lower mean AUC was calculated for the untreated test fields (15.9 arbitrary units). The treatment comparisons showed greater mean AUCs for tyrothricin 0.1% gel and the vehicle ($p = 0.0001$ and 0.0008 , respectively) compared to the untreated test fields. Furthermore, the noninferiority of tyrothricin 0.1% gel to the vehicle could be demonstrated. Altogether five nonserious adverse events were reported in 3 subjects. None of these were located in the test fields and none were considered to be related to the study medication.

Discussion

The focus of this investigation was to assess the effect of tyrothricin on noninfected wounds with respect to interference and influence on wound healing irrespective of microbial colonization. The topical use of tyrothricin in wound healing has been established over decades. It has been shown to be effective in skin infection both in vitro and in vivo [23]. In a prospective, randomized multicenter trial of 131 patients with posttraumatic and surgical cutaneous lesions a tyrothricin-containing powder demonstrated superior efficacy compared to placebo powder with respect to the radius of lesions as well as to a wound index [23]. There is an increasing interest in AMPs such as tyrothricin because these ‘nature’s antibiotics’ are promising agents for virtually new therapeutic approaches in infectious diseases, especially of the skin and for wound healing [24]. The well-known antimicrobial properties of tyrothricin also apply to the tyrothricin 0.1% gel tested in this study. Therefore, the focus of the present study was to assess the improvement of wound healing in superficial noninfected wounds by tyrothricin 0.1% gel and its vehicle in comparison to an untreated test field, independent of the antimicrobial properties. It was necessary to confirm that the composition of the vehicle contributes to improved wound healing and that the active antimicrobial ingredient tyrothricin does not interfere with this improved wound healing in noninfected superficial wounds. A recently published wound model

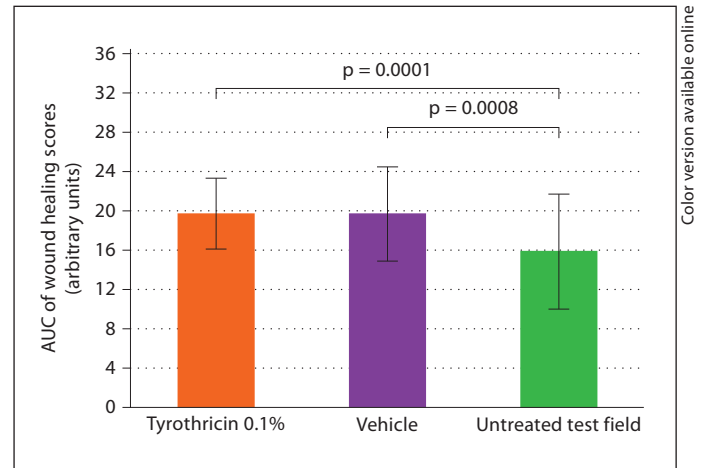


Fig. 2. AUC of wound healing scores and comparisons versus untreated test field using paired t test. Statistically significant greater mean AUCs were found for Tyrosur gel and the vehicle ($p = 0.0001$ and 0.0008 , respectively) compared to the untreated test field. Values are presented as means \pm SD.

inducing multiple uniform abrasions presents a new way to evaluate intraindividually the healing properties of commonly applied products in superficial wounds assessed by a clinical reepithelization score [22]. So far, only limited data are available on the examination of acute wounds and dressing techniques to guide clinical decisions [25, 26] and only few standardized protocols in the literature describe specific methods to examine acute wound healing [27, 28]. In another published abrasion model to examine the effects of occlusive dressings, wound healing was measured by change in wound contraction (cm^2) and change in wound color (skin color measurements of redness by chromametry), and it was demonstrated that occlusive dressings providing a moist wound environment are more effective than dry wound healing [10]. In this model, partial-thickness wounds were induced on the lower part of the leg using weighted sandpaper after the skin had been pretreated with an anesthetic ointment. Wound models remain the most effective way of objectively measuring the effects of treatment for acute superficial wounds in a clinical setting. The model used here has been published in the context of a pilot study with 10 volunteers treated with different plasters, demonstrating the benefit of moist wound healing [22]. This is the first time the model has been used in a confirmatory study with a larger number of volunteers ($n = 33$) treated with a topical wound gel. The wounds reflect the main aspects of the clinical situation of super-

ficial wounds such as partial abrasion of the epidermis, punctual bleeding and glistening. A key advantage compared to other models is that no local anesthetic is required and, therefore, subjective tolerability of products can be assessed from the first application onwards. In the present study with once daily, semioclusive application over a 12-day treatment period, tyrothricin 0.1% gel and the corresponding vehicle both showed a significant positive effect on wound healing as reflected by the same mean AUCs of wound healing scores.

A limitation of this paper is the lack of a comparison between the tyrothricin gel 0.1% and its vehicle and a reference hydroactive colloid gel.

Overall, the results showed that the totality of ingredients of the vehicle and correspondingly of the tyrothricin 0.1% gel had a positive effect on wound healing compared to untreated test fields even under semioclusive conditions. The addition of tyrothricin to the vehicle did not

interfere with the positive effects noted for the vehicle alone. Taken together with the proven antimicrobial effect of tyrothricin, tyrothricin 1% gel can be recommended for the treatment of superficial acute wounds to promote wound healing.

Disclosure Statement

The preparation of this manuscript was supported by a grant from Engelhard Arzneimittel GmbH & Co. KG, Niederdorfelden, Germany. Dr. Wigger-Alberti, R. Williams and K. Grigo performed and analyzed the trial on a contract research basis. Prof. Korting collaborated with Engelhard Arzneimittel GmbH in the development of topical drugs for skin diseases. Dr. Stauss-Grabo and S. Atiye are employed by the company that supported this investigation but do not have any personal financial interest in the investigation described in the manuscript. Data analysis and interpretation were not influenced by the company.

References

- Solem L: Classification; in Fischer SV, Helm PA (eds): *Comprehensive Rehabilitation of Burns*. Baltimore, Williams & Wilkins, 1984, pp 9–15.
- Ward RS, Saffle JR: Topical agents in burn and wound care. *Phys Ther* 1995;75:526–538.
- Kannon GA, Garret AB: Moist wound healing with occlusive dressings. *Dermatol Surg* 1995;21:583–590.
- Winter G: Formation of the scab and the rate of epithelialisation of superficial wounds in the skin of the young domestic pig. *Nature* 1962;193:293–295.
- Cho CY, Lo JS: Dressing the part. *Dermatol Clin* 1998;16:25–47.
- Eaglestein WH: Occlusive dressings. *J Dermatol Surg Oncol* 1993;19:716–720.
- Ågren MS, Karlsmark T, Hansen JB, Rygaard J: Occlusion versus air exposure on full-thickness biopsy wounds. *J Wound Care* 2001;10:301–304.
- Silverman RA, Lender J, Elmets CA: Effects of occlusive and semioclusive dressings on the return of barrier function to transepidermal water loss in standardized human wounds. *J Am Acad Dermatol* 1989;20:755–760.
- Beam JW: Management of superficial to partial-thickness wounds. *J Athl Train* 2007;42:422–424.
- Beam JW: Occlusive dressings and the healing of standardized abrasions. *J Athl Train* 2008;43:600–607.
- Thomas S: Hydrocolloid dressings in the management of acute wounds: a review of the literature. *Int Wound J* 2008;5:602–613.
- Korting HC, Schöllmann C, White R: Management of minor acute cutaneous wounds: importance of wound healing in a moist environment. *J Eur Acad Dermatol Venereol* 2011;25:130–137.
- Harkess N: Bacteriology; in Kloth LC, McCulloch JM, Feedar JA (eds): *Wound Healing: Alternatives in Management*. Philadelphia, Davis, 1990, pp 60–61.
- Hill MJ: Infections; in Hill MJ (ed): *Skin Disorders*. St Louis, Mosby Year Book, 1994, pp 89–93.
- Korting HC, Schöllmann C, Stauss-Grabo M, Schäfer-Korting M: Antimicrobial peptides and skin: a paradigm of translational medicine. *Skin Pharmacol Physiol* 2012;25:323–324.
- Schneider JJ, Unholzer A, Schaller M, Schäfer-Korting M, Korting HC: Human defensins. *J Mol Med* 2005;83:587–595.
- Wang Z, Wang G: APD: the antimicrobial peptide database. *Nucleic Acids Res* 2004;32:590–592.
- Khandelia H, Ipsen JH, Mouritsen OG: The impact of peptides on lipid membranes. *Biochem Biophys Acta* 2008;1778:1528–1536.
- Bradshaw J: Cationic antimicrobial peptides: issues for potential clinical use. *BioDrugs* 2003;17:233–240.
- Dubos RJ: Studies on a bactericidal agent extracted from a soil bacillus. *J Exp Med* 1939;70:249–256, in Tang XU, Thibault, Boyd RK: Characterisation of the tyrothricin and gramicidin fractions of the tyrothricin complex from *Bacillus brevis* using liquid chromatography and mass spectrometry. *Int J Mass Spectroscop Ion Proc* 1992;122:153–179.
- Franklin TJ, Snow GA: *Biochemistry of Antimicrobial Action*. New York, Chapman & Hall, 1988, pp 61–64.
- Wigger-Alberti W, Kuhlmann M, Ekanayake S, Wilhelm D, Buettner H, Callaghan T, Wilhelm KP: Using a novel wound model to investigate the healing properties of products for superficial wounds. *J Wound Care* 2009;18:123–131.
- Bayerl C, Völp A: Tyrothricin powder in the treatment of cutaneous lesions (in German). *Die Pharmazie* 2004;59:864–868.
- Steinstraesser L, Koehler T, Jacobsen F, Daigeler A, Goertz O, Langer S, Kesting M, Steinau H, Eriksson E, Hirsch T: Host defense peptides in wound healing. *Mol Med* 2008;14:528–537.
- Claus EE, Fusco CF, Ingram T, Ingersoll CD, Edwards JE, Melham TJ: Comparison of the effects of selected dressings on the healing of standardized abrasions. *J Athl Train* 1998;33:145–149.
- Wiechula R: The use of moist wound-healing dressings in the management of split-thickness skin graft donor sites: a systematic review. *Int J Nurs Pract* 2003;9:9–17.
- Schultz G, Mozingo D, Romanelli M, Claxton K: Wound healing and TIME: new concepts and scientific applications. *Wound Repair Regen* 2005;13:1–11.
- Keast DH, Bowering K, Evans AW, MacKean GL, Burrows C, D'Souza L: MEASURE: a proposed assessment framework for developing best practice recommendations for wound assessment. *Wound Repair Regen* 2004;12:1–17.