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Kinetics of Physiological Skin Flora in a Suction Blister Wound Model on Healthy Subjects after Treatment with Water-Filtered Infrared-A Radiation

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Key Words
Water-filtered infrared-A radiation · Suction blister · Wound colonization · Energy supply · Oxygen supply

Abstract
The effect of water-filtered infrared-A radiation (wIRA) on normal skin flora was investigated by generating experimental wounds on the forearms of volunteers utilizing the suction blister technique. Over 7 days, recolonization was monitored parallel to wound healing. Four groups of treatment were compared: no therapy (A), dexpanthenol cream once daily (B), 20 min wIRA irradiation at 30 cm distance (C), and wIRA irradiation for 30 min once daily together with dexpanthenol cream once daily (D). All treatments strongly inhibited the recolonization of the wounds. Whereas dexpanthenol completely suppressed recolonization over the test period, recolonization after wIRA without (C) and in combination with dexpanthenol (D) was suppressed, but started on day 5 with considerably higher amounts after the combination treatment (D). Whereas the consequence without treatment (A) was an increasing amount of physiological skin flora including coagulase-negative staphylococci, all treatments (B–D) led to a reduction in physiological skin flora, including coagulase-negative staphylococci. In healthy volunteers, wIRA alone and in combination with dexpanthenol strongly inhibited bacterial recolonization with physiological skin flora after artificial wound setting using a suction-blister wound model. This could support the beneficial effects of wIRA in the promotion of wound healing.

Introduction

Water-filtered infrared-A radiation (wIRA) is a new development in thermotherapy and was introduced in 1989 for chronic wounds [1] and in 2000 for acute wounds [2]. wIRA, as a specific form of heat radiation allowing high tissue penetration with low thermal stress to the skin surface, has proven to be clinically efficient as a supportive treatment tool in wound medicine [3–5]. Altogether, the radiation with wIRA evokes an increase in local temperature [6–9], oxygen partial pressure [6] and perfusion [8, 9]. These features are of substantial benefit especially for chronic wounds [3, 4, 10], as a result of chronic energy and oxygen deficiency in the wound milieu combined with hypothermic conditions [11–16]. As a consequence, pain and the required dose of pain medication as well as inflammation and hypersecretion are reduced [5, 17]. Additionally, wIRA has positive immunomodulatory/anti-infective effects, improving wound

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healing and shortening the time until complete wound closure and therewith hospitalization. Another possible wIRA effect is a mild photodynamic therapy (endogenous PDT-like effect), since irradiation with VIS and wIRA presumably acts with endogenous protoporphyrin IX or protoporphyrin IX of bacteria and, therefore, could additionally support wound healing by improving cell regeneration and evoking antibacterial effects [18].

Typical irradiances in thermotherapy rank between 80 and 160 mW/cm^2, corresponding to approximately 60–120 mW/cm^2 wIRA [3]. Meanwhile, wIRA complements other therapeutic procedures, such as compression therapy of chronic venous insufficiency [19].

In the light of the proven efficacy of wIRA therapy, the question of the influence to the microbial burden is of substantial interest, because positive effects could stimulate wound healing by reducing the wound flora in analogy to antimicrobial effects by other physically based treatments like electrostimulation [20]. To clarify this question, a study investigating bacterial growth kinetics in swabs of artificial wounds over 7 days was performed.

Materials and Methods

Volunteers

In a prospective, randomized, controlled study over 7 days, 13 healthy volunteers (aged 23–47 years, 7 females and 6 males) were investigated at the Charité-Universitätsmedizin Berlin, Germany. The study had been approved by the Ethics Committee of the Charité-Universitätsmedizin Berlin and was conducted in accordance with standard ethic rules stated in the Declaration of Helsinki principles. Prior to the experiments, the volunteers had given their informed consent.

Induction of Standardized Artificial Wounds

Experimental superficial wounds (5 mm diameter) as an acute wound model were generated by the suction blister technique [21], removing the roof of the blister with a scalpel and sterile forceps.

Study Protocol

On both forearms of all volunteers, four areas at equal distances to each other were subjected and randomized to one of four different treatment modi. During a test period of 7 days, the volunteers had to avoid any external contact, including water as well as any systemic treatment. To avoid skin contamination, the wounds were covered with a sterile non-occlusive dressing.

Experimental Skin Injury

On day 1, on each of the four marked skin areas (A–D), two suction blisters 5 mm in diameter were artificially generated at a distance of 6 cm from each other using a negative pressure of –200 mB, as described by Lademann et al. [21].

Skin injury was performed in an air-conditioned room with constant air dryness at a constant temperature of 21°C. Before the experiments started, the volunteers had relaxed in the treatment room for 30 min. After suctioning, the roofs of the blisters were aseptically removed using a scalpel and sterile forceps.

Table 1. Test scheme

Action	Day						
	1	2	3	4	5	6	7
Setting of suction blister	x						
Microbiological sampling	x		x		x		x
Treatment	x	x	x	x	x	x	x

Bacteriological investigations were performed on day 1 directly after wound setting, and on days 3, 5 and 7, whilst the wound treatment was performed on days 1–7 (table 1).

Four treatment modi were applied: (A) no therapy; (B) ointment with 2 mg/cm^2 dexpanthenol (Bepanthen®; Bayer, Leverkusen Germany); (C) irradiation with wIRA (Hydrosun Medizintechnik, Müllheim, Germany) (30 min, wIRA 210 mW/cm^2), and (D) ointment with 2 mg/cm^2 dexpanthenol (Bepanthen) and irradiation with wIRA (30 min, wIRA 210 mW/cm^2).

Irradiation Source

Hydrosun® (Hydrosun Medizintechnik) radiator type 501, 10-mm water cuvette, orange filter OG590, water-filtered spectrum: 590–1,400 nm with a dominant amount of wIRA.

Microbiology

For quantitative microbiology, sterile swabs (Dacron, Brescia, Italy) with 5 ml of clear Amies transport medium MW170 (anorganic phosphate buffer without charcoal; Transswab, Medical Wire & Equipment, Corsham, UK) were premoistened with sterile normotonic saline. After a 3-min skin antisepsis with 70% ethanol, consecutive suction samples were taken from the artificial wounds after aseptically removing the blister roof with a scalpel by dipping the tip of the swab into the wound ground over 30 s. For transport, the swabs were placed into the transport medium and sent to the microbiological laboratory. In the laboratory, the swabs were transferred into a sterile vial containing 1 ml of sterile 0.01 M phosphate-buffered saline and vortexed for 15 s. 100 μl of the obtained suspension and another 100 μl of a 1/10 dilution with phosphate-buffered saline were plated onto Columbia blood agar (containing 5% sheep's blood; Oxoid, Basingstoke, UK). Both plates were aerobically incubated at 36°C for 48 h. Colony-forming units (CFU) were counted visually per plate for both the suspension and the dilution, and the results calculated as number of CFU per wound (swab). Cultured bacteria were identified after subculturing on selective agar (MacConkey agar for Gram-negative rods, mannitol agar for staphylococci and esculin agar for streptococci; all media provided by Oxoid) by visual aspects of cultural morphology, Gram staining and biochemical differentiation with the microstrip ATB System (bioMérieux, Nürtingen, Germany). Cultured colonies suspected for staphylococci were identified using the clumping factor test (Staphaurex; Remel, Dartford, UK), the aerobic acidification of mannitol-salt-agar (Oxoid), the DNAse test (DNAse agar; bioMérieux) and the microstrip ATB System (bioMérieux).

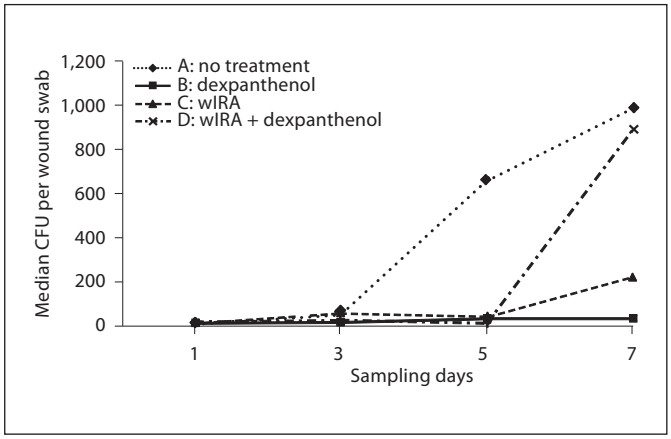

Fig. 1. Total bacteria (median CFU/wound sample) cultured from artificial suction blister wounds on the forearms of healthy individuals on days 1, 3, 5 and 7 after different treatment modi.

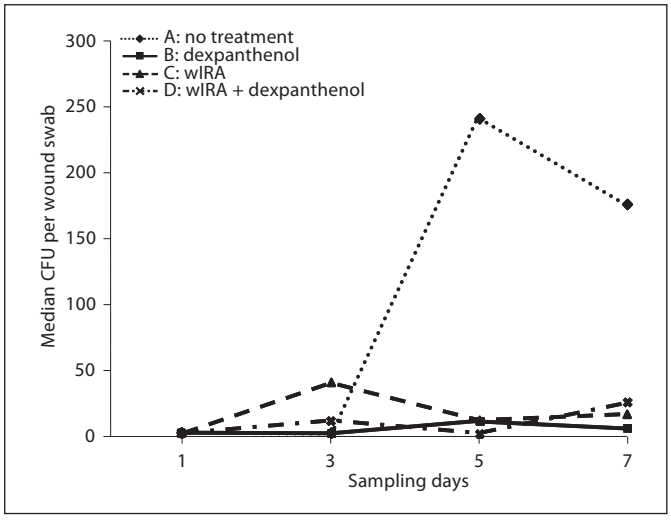

Fig. 2. Coagulase-negative staphylococci (median CFU/wound sample) cultured from artificial suction blister wounds on the forearms of healthy individuals on days 1, 3, 5 and 7 after different treatment modi.

Results

The effects of the different treatment modes on skin flora during healing between days 1 and 7 are shown in figure 1 (median total flora, CFU/wound) and figure 2 (median staphylococcal flora, CFU/wound). The total flora was composed of typical species of physiological skin flora: coagulase-negative staphylococci, aerobe bacilli, micrococci, coryneiforms and non-hemolytic streptococci. Only one sample of 1 volunteer revealed growth of potential pathogen (volunteer 1 with *Staphylococcus aureus*, without treatment at day 7, 10 CFU/wound swab, oxacillin-susceptible; data not shown).

From day 1 to day 3, the total bacterial load of the wounds was minimal and remained unchanged in all four groups. On the control wound without any treatment (A), a prominent increase in colonization developed from day 3 to day 7. In contrast, all treatments showed inhibited or at least delayed bacterial growth. Dexpanthenol treatment showed no increasing skin flora over the whole test period. The wIRA treatment resulted in strongly inhibited and delayed growth at least until day 5 with an initial and weak increase measured at day 7. The combination of wIRA with dexpanthenol showed inhibited bacterial growth until day 5 with then increasing levels reaching nearly the peak level of the untreated group (A) at day 7 (delayed growth, same peak levels). The effect resembles the untreated group, however with a delay of 2 days.

When only coagulase-negative staphylococci as the dominating part of the skin flora were analyzed, treatments B–D led to nearly complete inhibition of the bacterial recrudescence in contrast to their influence on total flora. It was only in control group A, without treatment, that the staphylococci started increasing growth at day 3, as expected.

Consequently, wIRA with and without dexpanthenol (D, C) strongly inhibited the growth of colonization flora after artificial skin injury on healthy forearm skin, showing increasing levels not before day 5 (stronger inhibition by wIRA alone than by wIRA + dexpanthenol). This increase was caused by germs, other than staphylococci and comprised aerobe spores, C*orynebacteria* spp., *Kocuria* spp. and viridans streptococci (data not shown). Dexpanthenol alone completely inhibited the detection of bacterial recolonization (all species) over 7 days.

Discussion

The study was carried out to analyze the effects of wIRA on bacterial wound contamination in the early healing phase, following artificial skin injury using a blister suction model. As controls, no treatment (A), treatment with dexpanthenol alone (B), and wIRA + dexpanthenol (D) were compared. The rationale behind this study is the hypothesis that wIRA treatment could directly or indirectly interfere with bacteria, thus reducing and/or modifying the spectrum of wound flora so that colonization in the early wound healing phase is inhibited. Con-

sequently, this efficacy could reduce the risk for critical colonization and infection in the wound. This hypothetic effect would flank the beneficial role in wound healing by wIRA treatment, providing energy to the wound with consecutive support of wound healing [3, 4, 7].

Over the first 3 days after skin injury, almost no growth could be recognized, regardless of which treatment regime had been applied. This was expected because after adequate skin antisepsis, skin flora is substantially reduced for up to 6 h and normally remains low because of sterile dressing [22, 23]. Without treatment, the first prominent increase in growth can be measured at day 3 after skin injury (modus A; fig. 1, 2). In the treatment groups, this physiological skin recolonization, which certainly starts by diffusion of neighbored untreated intact skin and possibly inevitable contamination during the manipulations (i.e. dressing), is suppressed, which may be attributed to the specific treatments B–D.

The most prominent depression of the recolonization after skin injury was shown by dexpanthenol treatment alone followed by wIRA alone (C), followed by wIRA with dexpanthenol (D). The bacterial growth did not increase before day 5, which was caused by germs other than staphylococci (aerobe spore-forming bacilli, C*orynebacteria* spp., *Kocuria* spp., non-hemolyzing streptococci and *Candida* spp.; data not shown).

The total depression of recolonization by dexpanthenol is due to the complete covering of the wound with the ointment during the whole test period [24]. This reflects a physical barrier effect by hindering contamination flora to enter the wound space, as dexpanthenol is declared by the manufacturer as being a non-antimicrobially active substance [25]. The combination of wIRA with dexpanthenol was also effective in inhibiting bacterial contamination after skin injury, but less effective than wIRA alone. This was shown by the same retarding effect, but more pronounced was the reducing effect with wIRA alone. This is of importance, because dexpanthenol plus wIRA was most effective in a similar study [5] testing the influence of wIRA on wound healing (wound closure and epithelialization measured by laser scanning microscopy). In this study, wound healing was excellent for all four treatments. The treatment modi showed only minor differences, with slight advantages for the combination of wIRA and dexpanthenol cream and of dexpanthenol cream alone with regard to the relative change in the wound size and subjective sensation of the wound area [26]. However, laser scanning microscopy revealed differences especially on days 5–7: the most effective formation of the stratum corneum was seen in wounds treated with wIRA and dexpanthenol cream, secondly wIRA alone, thirdly dexpanthenol cream alone and the least effective were untreated wounds. This result was explained by a suggested amelioration of penetration of dexpanthenol in the skin by the wIRA irradiation [5]. As a conclusion, also in this model, treating wounds with wIRA supports healing, which, combined with dexpanthenol ointment, could provide a new treatment option. Since the ointment represents a purely physical barrier, the antimicrobial effect of the combination (D) can be explained as being related only to the wIRA. The base of this antimicrobial efficacy has not yet been determined and is potentially related to the prolongation of the development of the physiological crusty barrier (see below) together with pushing the natural defense mechanisms into the deeper tissue (triggered by reinforced oxygenization and microcirculation). On the other hand, it cannot be fully excluded that dexpanthenol provides an additional antimicrobial effect by interfering with the bacterial CoA pathway as demonstrated by Kumar et al. [27] against *Mycobacterium tuberculosis* and *Escherichia coli.*

During physiological wound healing, natural wound secretions cover the fresh wound and develop a functional biodressing protecting the wound from physical, chemical and biological stress including microbial invasiveness. Soon after contact of the wound fluid with ambient air, desiccation occurs and hardens the outer wound film to form a crusty barrier. Besides wound protection, this barrier delays wound healing. The very low bacterial load in the test wounds in our experiments during the first 3 days is a result of the antimicrobial properties of the wound fluid in synergy with the barrier exerted by the crust. This crust breaks after 3 days forming several fissures allowing penetration of contamination flora into the wound bed. This breakup is prevented by fatty ointments like dexpanthenol on account of its mollifying effect, resulting in sustained stability and closure of the film barrier, thus preventing microbial penetration from both sides with consecutive growth as seen in group B (nearly no bacterial growth during the complete study). Nearly the same effect can be seen after wIRA irradiation with suppressed growth until day 5. This can be explained by two facts: first of all, an additional desiccating effect inhibiting bacterial penetration and growth in the moment of fissuring of the crust, and secondly by indirect effects as a result of supported tissue oxygenization improving the wound healing process, which coincides with a stronger elimination of invading microbes. Direct antimicrobial effects by wIRA could not be deduced and were not found in vitro elsewhere [unpubl. data] and additionally could hardly be explained by conventional irradiation physics. This means

that wIRA alone seems to be able to influence wound contamination in the first days after injury and in the absence of heavy skin and environmental bioburden at the time of injury and directly thereafter. These results may be discussed as a first demonstration of wIRA in the role of an infection-preventive treatment at the initial wound phase of acute wounds lasting up to 5 days.

In conclusion, we were able to demonstrate that wIRA irradiation with conventional dosage can inhibit spontaneous wound colonization of fresh acute wounds, thus protecting the wounds from bacterial infectious risks. In healthy individuals, this protection lasts at least over the early healing phase for the first 5 days after injury. wIRA in combination with dexpanthenol seems to be an interesting option in the treatment of contaminated acute and chronic wounds. Our results contribute to the beneficial therapeutical effects in the same suction blister model showing potential additional wIRA effects inhibiting and delaying bacterial recolonization in acute wounds [21].

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