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Research paper

In vivo investigation of the efficiency of a nanoparticle-emulsion containing polihexanide on the human skin

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ABSTRACT

Skin antisepsis is a key element for the prevention of surgical site infections, as well as for infections after injection and punctures. Recent investigations have shown that about 25% of the resident bacterial flora of the human skin resides within the hair follicle. These findings strongly suggest that the skin appendages play the role of a bacterial reservoir. The bacteria within the hair follicles therefore may be the cause of endogenous germ repopulation after skin antisepsis, highlighting the need for new antiseptic formulations that can sufficiently penetrate into the hair follicles. Various experiments have found that nano-sized particles as well as oil-in-water emulsions are efficient carriers for substances into the hair follicles.

In the present study, we investigated the in vivo antiseptic potential of the particle-associated and aqueous polihexanide on the human skin by monitoring bacterial growth after antisepsis over a period of 2.5 h. The experiments suggest that the use of a particle-bound antiseptic can achieve a better and longer lasting antisepsis of the human skin than in non-particulate form.

1. Introduction

Preoperative skin antisepsis is a key element within the multi-barrier strategy for the prevention of surgical site infections (SSI) [1–3] as well as for safe injection [4] and puncture [5]. Alcohol based antiseptics are the agents of choice for skin antisepsis [6,7]. By addition of remanent antiseptic agents to alcohols the efficacy can be prolonged [8]. Another possibility to reduce the risk of SSI is to fix the residual skin flora by so-called sealing [9]. A hopeful perspective could be the antisepsis of hair follicles [10]. Lange-Asschenfeldt et al. [11] examined the distribution of bacteria in the epidermal layers of hair follicles of the human skin. Using the methods of differential stripping, cyanoacrylate biopsies and follicular mapping results stated that whereas the portion of hair follicle per square cm on the human forearm is approximately 0.09%, about 25% of the human skin bacteria are hair follicle related. Further investigations regarding the distribution of the bacterial flora on the human skin demonstrate that the hair follicle acts as a bacterial reservoir, shielding bacteria from exogenous factors such as antisepsis [11,12]. These findings depict the hair follicle as a bacterial reservoir that is likely to be an important source for the endogenous repopulation of the bacterial flora after skin antisepsis. As a consequence, the hair follicle structure should be one of the main focuses for the development of modern antiseptics.

The hair follicle structure has been the object of an increasing research interest in recent years [13,14]. Current studies have shown that the hair follicles play an important role in skin penetration [15–17]. Other investigations have shown that hair follicles are an efficient long-time reservoir for topically applied substances. Otberg et al. [18] found the storage capacity of the hair follicles to be comparable to the storage capacity of the stratum corneum on several parts of the body. Lademann et al. investigated the storage capacity of the stratum corneum in comparison to the storage capacity of the hair follicle infundibulum. The results showed a 10 times longer storage within the hair follicles [15].

In particular, nanoparticles represent an increasingly interesting physical approach to topical drug application and delivery to the hair follicle structure [19–21]. Lademann et al. [16] used differential stripping and laser scanning microscopy to investigate the in vitro penetration and storage behavior of dye-containing nanoparticles into the hair follicles of pig ear skin. The obtained measurements were compared to the penetration and storage achieved by using the same amount of dye in the non-particulate form. Nanoparticles penetrated significantly deeper into the hair

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follicles than the dye in the non-particulate form. Also, the dye without particle-carrier could only be detected for up to 4 days, while dye-containing nanoparticles could be visualized within the hair follicles for a time span of up to 10 days. Results also showed that the superiority of the dye-containing nanoparticles was linked to the appliance of a massage during the topical application of the substances.

Previous studies examining the penetration of nanoparticles into hair follicle structures suggested that the rigid hair shaft may function as a geared pump, moving applied nanoparticles down the hair follicles and thus ensuring a deeper penetration into the skin [15]. In particular, liposomes and oil-in-water emulsions represent an effective drug carrier system [22,23], possibly within the follicular pathway. Toll et al. [24] investigated the penetration efficacy of fluorescent dye-labeled microspheres of different size (0.75–6 μm) into hair follicles. Small particles of approximately 750 nm in diameter penetrated most efficiently into the hair follicle structure. Jung et al. [25] compared the penetration and storage behavior of dye-containing liposomes of 100 nm in diameter using laser scanning microscopy and found them to be efficient carriers for drug/substance delivery into the hair follicle.

The aim of the present study was to assess the antiseptic capacity of a particle-associated antiseptic in comparison with the same antiseptic in non-particulate form. The antiseptic under investigation was polyhexamethylene biguanide hydrochloride (polihexanide, PHMB), a polymeric cationic antimicrobial agent with fast acting broad-spectrum biocide acting against a wide range of both Gram positive and Gram negative bacteria, which is characterized by low systemic toxicity. Therefore, polihexanide is an agent of choice in a series of commercially available antiseptic solutions [26]. Polihexanide is highly water-soluble and has been reported to support wound healing. Müller et al. [23] demonstrated that the combination of lipid particles and polihexanide decreases the cytotoxicity of PHMB while preserving its antimicrobial efficacy. As the antiseptic examined is highly water-soluble, the carrier system chosen for this experiment was a phospholipid oil-in-water (o/w) emulsion. In a previous study, Ulmer et al. [27] demonstrated that both the o/w-emulsion and the same emulsion with antiseptic molecules attached to the oil droplets used in this study, penetrated efficiently into the hair follicles.

2. Material and methods

2.1. Study design

The assessment of the antiseptic efficacy of polihexanide in non-particulate (PHMB) and in particulate form in Lipofundin (PHMB + L) was studied on the flexor forearm of 12 volunteers. Twelve defined 5 × 5 cm testing areas were marked on both flexor forearms (6 on each) of each volunteer prior to the experiment, using a permanent marker. Seventy percent of isopropyl alcohol (70% Iso) was applied as a positive control, whereas for negative control (C), the corresponding skin area remained untreated.

The different treatments of the testing areas and the bacteriological sampling at time points 30 min, 90 min, and 150 min were performed as illustrated in Fig. 1.

2.2. Volunteers

The investigation was carried out on 12 healthy male Caucasian volunteers aged from 20 to 50 years with normal BMI. The experiment had been approved by the Ethics Board of the Charité (EA1/080/09). The volunteers were not allowed to wash their forearms for at least 12 h prior to the experiment. The distribution of the testing areas on the forearms was determined for all 12 experiments by means of a randomized procedure prior to the experiment, in order to reduce the bias caused by the high heterogeneity of the follicle density and distribution and the differences in bacterial population. The program used was the general-purpose computer algebra system Maple® (Version 13, Waterloo Maple (Maplesoft), Waterloo, Canada). The randomization process resulted in an individual topical application map for each volunteer.

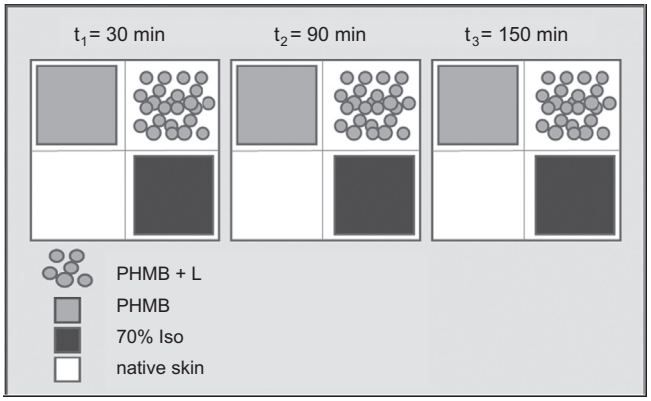

Fig. 1. Study design. Illustration of the testing areas corresponding to the sampling times.

A double-blind procedure was used to avoid bias during the investigations and placebo effects. The volunteers were oblivious as to the substance applied onto each area. Furthermore, the labels on the agar plates were removed prior to the bacteriological count by an independent assistant and replaced by a code unknown to the person performing the experiment. After completion of the investigations, the results were decoded using a key, and all agar plates were assessed.

2.3. Formulations

2.3.1. Antiseptics

1. *PHMB*: 0.05% PHMB aqueous solution prepared by combining 10 ml 0.1% PHMB and 10 ml sterile water was evaluated.
2. *PHMB + L*: The particles selected for the present study were obtained from the commercially available and commonly used product Lipofundin® MCT 20% (B. Braun, Melsungen, Germany). Lipofundin® MCT 20% as an oil-in-water (o/w) emulsion contains 200 g/L of 1:1 (w/w) mixture of medium chain triglycerides (MCT) and long chain triglycerides (LCT; soybean oil), glycerol (2.5%), sodium oleate (0.03%), and D,L-a-tocopherol, which were stabilized with egg lecithin [23]. The adherence of polihexanide to the oil droplets was achieved by mixing 25 mL Lipofundin MCT 20% with 25 mL 0.1% polihexanide. This resulted in 0.05% polihexanide in Lipofundin MCT solution with 0.6% egg lecithin. The carrier particles selected for this investigation displayed an average size of 295.1 ± 8.2 nm (*z*-average) and a polydispersity index (PI) of 0.081 ± 0.08. (B. Braun, photon correlation spectroscopy (PSC)).
3. *The 70% Iso (propan-2-ol (isopropyl alcohol))*: The 70% Iso was used as a positive quality control. The 99.9% product (MERCK®, Darmstadt, Germany) was diluted with distilled water so as to obtain 70% propan-2-ol (70% Iso).

2.4. Application protocol

100 μl of each of PHMB, PHMB + L and 70% Iso were applied onto 3 of the 12 testing areas of every volunteer according to the predetermined randomized order. The solutions were administrated