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Penetration of Chlorhexidine into Human Skin


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This study evaluated a model of skin permeation to determine the depth of delivery of chlorhexidine into full-thickness excised human skin following topical application of 2% (wt/vol) aqueous chlorhexidine digluconate. Skin permeation studies were performed on full-thickness human skin using Franz diffusion cells with exposure to chlorhexidine for 2 min, 30 min, and 24 h. The concentration of chlorhexidine extracted from skin sections was determined to a depth of 1,500 μm following serial sectioning of the skin using a microtome and analysis by high-performance liquid chromatography. Poor penetration of chlorhexidine into skin following 2-min and 30-min exposures to chlorhexidine was observed (0.157 ± 0.047 and 0.077 ± 0.015 μg/mg tissue within the top 100 μm), and levels of chlorhexidine were minimal at deeper skin depths (less than 0.002 μg/mg tissue below 300 μm). After 24 h of exposure, there was more chlorhexidine within the upper 100-μm sections (7.88 ± 1.37 μg/mg tissue); however, the levels remained low (less than 1 μg/mg tissue) at depths below 300 μm.

The aim of the current study was to use the Franz-cell skin model (6) to determine the penetration profile for CHG through excised human skin and to evaluate the skin permeation of 2% (wt/vol) aqueous CHG into the skin using this model.

MATERIALS AND METHODS

Materials. CHG, diethylamine (high-performance-liquid chromatography [HPLC] grade), dimethyl sulfoxide, phosphate-buffered saline (PBS), sodium heptane sulfonate (HPLC grade), and Tween 80 were purchased from Sigma-Aldrich (Dorset, United Kingdom). Acetic acid and methanol (both HPLC grade) were purchased from Fisher Scientific (Leicestershire, United Kingdom).

Skin samples. Full-thickness human skin samples were obtained from patients undergoing breast reduction surgery, and full ethical committee approval was obtained prior to this study (REC 2002/169). The full-thickness human skin was frozen on the day of excision and stored at −70°C until required.

Quantification of CHG. HPLC was used to measure the amounts of CHG in the skin samples obtained during the permeation studies. The analyses were performed using an Agilent 1200 series HPLC system (Agilent Technologies, United Kingdom). The samples were run at a flow rate of 1.2 ml/min at room temperature through a reverse-phase chromatography column (CPS-2 Hypersil 5-μm column; dimension, 150 by 4.6 mm [Thermo Electron Corporation, United Kingdom]), with UV detection at 254 nm. The isotropic mobile phase consisted of a methanol:water mixture (75:25) with 0.005 M sodium heptane sulfonate and 0.1% (vol/vol) diethylamine adjusted to pH 4 with glacial acetic acid. The HPLC method was validated by repeating a series of standardized CHG concentrations five times and plotting a graph of peak area versus CHG concentration. The level of detection (LOD) and level of quantification (LOQ) were calculated from the standard curve according to the following equations: LOD = (3 × standard deviation)/slope; LOQ = (10 × standard deviation)/slope.

Skin permeation studies. Skin permeation studies were performed with vertical Franz diffusion cells (Fig. 1). The receptor compartment was filled with 29 ml of PBS, maintained at 37°C by using a circulating water jacket, and agitated by stirring with a magnetic bar. Skin samples were thawed in PBS at room temperature, dried with an absorbent towel, and mounted on Franz diffusion cells with the stratum corneum (SC) uppermost, facing the donor compartment. The skin surface area exposed to the test compound was 3.14 cm² (2 cm in diameter). All entrapped air between the skin and receptor fluid was removed, and the skin was left to equilibrate for 30 min to reach the skin surface temperature of 32°C. Twenty percent (wt/vol) aqueous CHG was diluted with distilled water and
to 1,500/H9262 to the experiment by injecting a standardized quantity of CHG (128/H9262 (skin without treatment) was analyzed parallel to the test samples. Effective HPLC and the concentration of CHG (H9262 determined. Control skin of the sealed tubes at 60°C for 1 h. Following this, the samples were analyzed by placing 1 ml of HPLC mobile-phase solution in each tube, followed by incubation of each skin sample determined. Chlorhexidine was extracted from the skin by (from the surface to a depth of 600/H9262 m sections (from depths of 600/H9262 100-/H9262 m) were cut from each frozen sample in triplicate and placed on a cork disc in embedding compound (Bright Instruments, Cambs, United Kingdom). The frozen samples were sectioned horizontally with a microtome (Bright Instruments) into 20-/H9262 m sections (from the surface to a depth of 600 /H9262 μm and 30-/H9262 μm sections (from depths of 600 to 1,500 μm). Each section was placed in an Eppendorf tube and the total weight of each skin sample determined. Chlorhexidine was extracted from the skin by placing 1 ml of HPLC mobile-phase solution in each tube, followed by incubation of the sealed tubes at 60°C for 1 h. Following this, the samples were analyzed by HPLC and the concentration of CHG (μg/mg of skin) determined. Control skin (skin without treatment) was analyzed parallel to the test samples. Effective elution and recovery of CHG from the skin by this method were confirmed prior to the experiment by injecting a standardized quantity of CHG (128 μg) into 10 skin samples, extracting the CHG, and determining the recovered amount (94.4 ± 1.82%; data not shown).

RESULTS

HPLC validation. The mean retention time for CHG was 3.6 min. There were no intervening peaks from endogenous contaminating compounds within skin samples. The HPLC method gave a linear response (r² = 0.999) over the concentration range of 0.0039 μg/ml to 128 μg/ml. The level of detection and level of quantification were calculated at 0.016 μg/ml and 0.052 μg/ml, respectively.

Skin permeation studies. No CHG was detected in the receptor compartment during the 24-h exposure of excised full-thickness human skin to 2% (wt/vol) aqueous CHG.

CHG retention studies. After 2 min, 30 min, and 24 h, concentrations of chlorhexidine within the skin were highest in the surface 100-/H9262 μm sections and reduced below depths of 300 μm (Fig. 2 and 3). The concentrations of CHG within the top 100-/H9262 μm sections of skin were 0.157 (± 0.015) μg/mg tissue and 0.077 (± 0.015) μg/mg tissue after 2-min and 30-min exposures to 2% (wt/vol) CHG, respectively (Fig. 2). The concentration of CHG within deeper layers (below 300 μm) fell to less than 0.002 μg/mg tissue following both 2-min and 30-min exposures. The difference between the amounts of chlorhexidine within the top layers between 2 min and 30 min of exposure was not significant (P > 0.05) (Student’s t test, INSTAT2; Graphpad, San Diego, CA). The concentration of CHG was significantly higher within all skin sections following 24 h of exposure to CHG than with the shorter exposure times. The concentration of CHG was 7.88 (± 1.37) μg/mg tissue within the upper 100-/H9262 μm sections and less than 1 μg/mg of tissue at depths of 300 μm and below.

DISCUSSION

This study demonstrates that 2% (wt/vol) chlorhexidine, the antiseptic agent recommended within EPIC and CDC guidelines for skin antisepsis prior to central venous catheter insertion, poorly permeates into deeper layers of the skin after 2

FIG. 1. Diagram of Franz diffusion cell. The receptor compartment was filled with PBS, which was kept at 37°C by circulating water jacket. The skin was mounted between the receptor and donor compartments and clamped. The test drug was aliquoted into the donor compartment. The drug diffused through the skin was sampled by removing receptor fluid via the sampling port.

FIG. 2. Penetration profile showing the concentration and location of chlorhexidine (μg/mg tissue) in excised human skin after 2 min or 30 min of exposure to aqueous 2% (wt/vol) chlorhexidine digluconate (mean ± standard error; n = 15).

FIG. 3. Penetration profile showing the concentration and location of chlorhexidine (μg/mg tissue) in excised human skin after 2 min or 30 min (n = 15) or 24 h (n = 30) of exposure to aqueous 2% (wt/vol) CHG (mean ± standard error).
In this study, a model for studying the delivery of CHG into excised full-thickness human skin was evaluated. Skin permeation studies are commonly performed in vitro with vertical or horizontal diffusion cells using skin or artificial membranes. This study was performed using vertical diffusion cells (Franz-type diffusion cells) to evaluate the delivery of CHG through excised full-thickness human skin. Such conditions mimic the in vivo environment by maintaining the physiological receptor fluid at body temperature and the skin surface temperature at 32°C (6, 23). Skin permeation studies generally evaluate drug delivery through the skin by measuring drug diffusion into the receptor fluid through the SC or epidermis, which are the main barriers for skin permeation. However, the use of stripped skin layers, such as isolated SC or epidermal layers, for drug permeation studies may influence the results, with possible retention of the drug in the dermal layers of the skin. Full-thickness skin was used in this study to determine the location of CHG throughout the skin, rather than studying the flux of the drug through the barrier layers. Following exposure to CHG, the full-thickness human skin was sectioned to a depth of 1,500 μm by sequential sectioning with a microtome, producing a total of 60 sections per skin sample. Skin sectioning has been used in many previous studies (21); however, the SC is often removed by tape stripping prior to sectioning of the skin. In this study, the full-thickness skin samples were sectioned throughout the sample without prior removal of the surface layers. This study demonstrates that the CHG permeation through the full-thickness skin was not linear, which was expected due to the variation in structure at various layers. The top 100-μm layer of the skin, which contains SC (average of 10 to 20 μm thick) and other epidermal layers (50 to 100 μm thick), contained the largest amount of CHG following exposure to 2% (wt/vol) CHG over all time points studied. Previous research has shown that the main permeation barrier for skin absorption is the SC (3, 11, 25), which is thought to be due to its high-lipid matrix and packed layers of keratinized epithelial cells. Furthermore, this study found that below 300 μm, at the dermal layer, the level of CHG remained constantly low. Depending on the body site, dermis contains hair follicles and other skin appendages, including sebaceous glands and sudoriferous glands (sweat-producing glands), which are of interest in skin antisepsis since they may be niches for microbial colonization of the skin following skin antisepsis (7, 8). It is generally recognized that skin antisepsis does not sterilize the skin; our study confirms this and demonstrates that it may be due to poor permeation of chlorhexidine into the deeper layers of the skin.

In conclusion, this study showed poor permeation of chlorhexidine through excised full-thickness human skin after 2 min and 30 min of exposure to aqueous 2% (wt/vol) CHG. The levels of CHG were highest within the top 100-μm sections of skin and remained consistently low within the deeper layers. Furthermore, the model presented in this study is a valuable tool in determining a permeation profile for chlorhexidine through human skin in vitro. This study lays the foundation for further research within this area with a view to potentially adopting alternative strategies for enhanced skin antisepsis in clinical practice.

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