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Percutaneous absorption of a chlorhexidine digluconate solution

C. Lafforgue [a,*], L. Carret [b], F. Falson [a], M.E. Reverdy [b], J. Freney [b]

[a] *Université Claude Bernard, Lyon 1, Faculté de Pharmacie, 8 avenue Rockefeller, 69373 Lyon Cedex 08, France*
[b] *Département de Recherche en Bactériologie Médicale, UPRES-EA 1655, Faculté de Médecine Laennec, Rue Guillaume Paradin, 69372 Lyon Cedex 08, France*

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Abstract

Chlorhexidine is an antiseptic widely used to clean the skin. The present work compare the percutaneous diffusion of Hibitane® through hairless rat skin with or without stratum corneum. For the tests carried out on whole skin, the storage is more important than the diffusion; the reverse was observed for stripped skin. The results are discussed according the composition of the commercial solution and the physicochemical characteristics of chlorhexidine digluconate. © 1997 Elsevier Science B.V.

Keywords: Percutaneous absorption; Chlorhexidine digluconate; Hairless rats

Chlorhexidine is an antiseptic that has been widely used for a number of years to clean the skin and mucosa of wounds and burns. The molecule was then developed as a preservative in pharmaceutical preparations.

The various irritation tests carried out on skin and mucosa have revealed a good tolerance when recommended dosage is applied (Reverdy, 1995). Few studies have been reported about percutaneous absorption of chlorhexidine (Rieg-Falson, 1995): studies carried out on adults (Case, 1977; Hackenberger, 1992) and new-born infants (Cowem et al., 1979) have shown that serum level of chlorhexidine after absorption remains low, almost at detection level.

The purpose of our study was to examine the percutaneous absorption of chlorhexidine digluconate and to evaluate the surface retention of the molecule on whole and stripped skin, simulating intact and injured skin.

Skin absorption was studied using a static diffusion cell (type Franz). The skin selected was the abdominal skin of female hairless rats aged 8 weeks (Iffa Credo, l'Arbresle, France). The skin

* Corresponding author.

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samples were frozen at −20°C until needed. For the tests on stripped skin, the stratum corneum was removed by tearing off with adhesive strips; the operation was repeated ten times on each skin sample before placing the samples into the diffusion cells. The skin samples were thawed at room temperature, placed immediately into the diffusion cells and maintained in a water-bath for 12 h at 37°C; the receptor compartment contained a 10 ml aqueous solution of 0.9% NaCl.

At time t_0, 2 ml of a 5% chlorhexidine digluconate solution (Hibitane®) were introduced into the donor compartment and the saline solution of the receptor compartment was renewed.

At 1, 3, 6, 9, 24 and 48 h, 5 ml of the receptor medium were sampled, analyzed and replaced by 5 ml of a new saline solution. Throughout the study period, the diffusion cells were kept at 37°C.

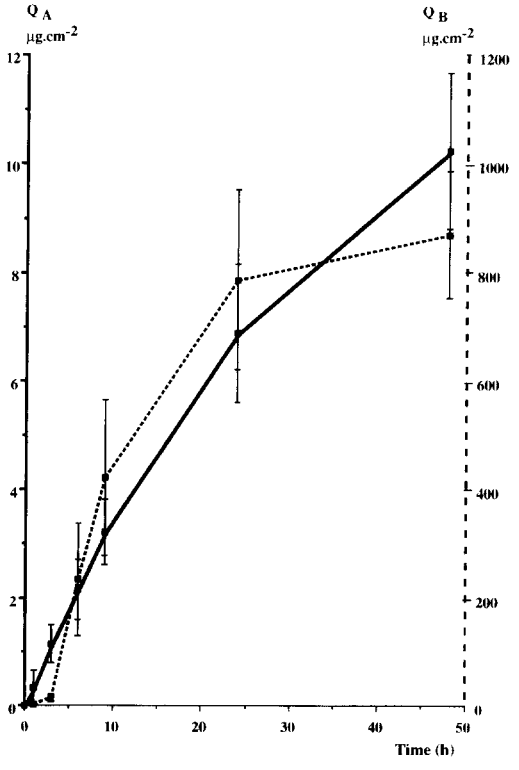

Fig. 1. Cumulated amounts of chlorhexidine digluconate ($\mu g \cdot cm^{-2}$) permeated during 48 h through whole skin (Q_A ·····) and stripped skin (Q_B — — —)

Table 1
Amounts of chlorhexidine digluconate ($mg \cdot cm^{-2}$) diffused after 48 h and stored in the cutaneous structures (average ± S.D., $n = 6$)

	Whole skin	Stripped skin
Donor ($t = 0$ h)	38.7 ± 0.32	39.49 ± 1.34
Receptor ($t = 48$ h)	0.00403 ± 0.0006	0.34241 ± 0.04633
Skin ($t = 48$ h)	0.02663 ± 0.01317	0.23865 ± 0.04648

After 48 h absorption, the skin samples were removed and immersed for 12 h in a 10 ml saline solution. Chlorhexidine released from the skin to this solution was quantified to evaluate the storage capacity of the skin.

The specimens sampled from the receptor phase were collected in propylene tubes, centrifuged for 10 min at 3000 revs./min then stored at 4°C. The HPLC tests were carried out within 24 h.

Dosage was carried out by reverse phase adsorption chromatography, using a Lichrospher 6ORP-select B (Merck) cartridge and an L-40000 UV detector. The volume of sample injected was 80 µl; the mobile phase included a mixture of acetonitrile (Lichrosorv, gradient grade, Merck) and 50 mM acetate buffer (50:50) adjusted to pH 3.15 with 96% acetic acid (RPE Merck).

Retention time of chlorhexidine digluconate was 3.44 min; detector response was linear as regard the 0–20 mg/ml concentrations; correlation coefficient was 0.998; sensitivity of the technique scored 20 ng/ml.

The permeation profiles obtained (Fig. 1) exhibit that percutaneous absorption of chlorhexidine depends of the skin state. The quantities diffused after 48 h represented 0.01% of initial quantity for intact skin, and 0.87% for stripped skin.

These results are close to those reported by Chow et al. (1978), who observed a 2–4.3% diffusion of chlorhexidine 5 days after cutaneous application of chlorhexidine dihydrochloride. For both intact and stripped skin, the steady state flux could not be calculated from the absorption profile according to Fick's first law.

The experimental curves are very similar to those obtained when irritating excipients of stra-

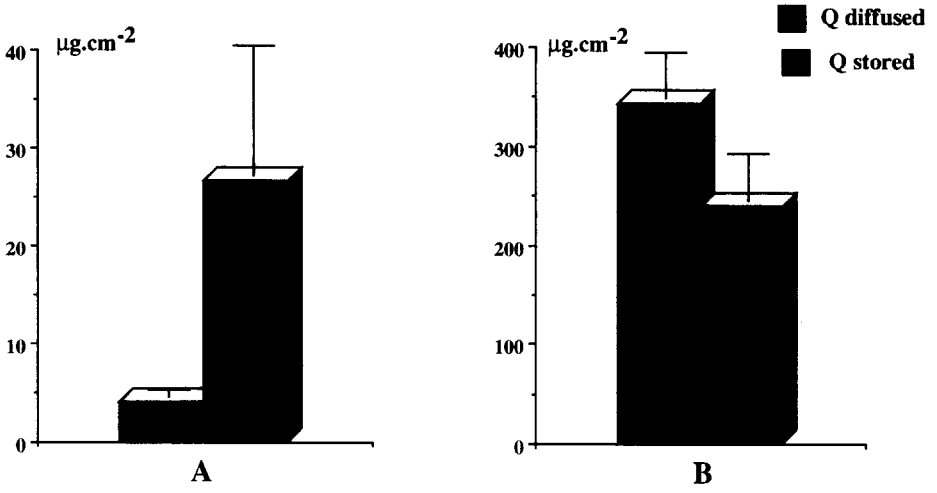

Fig. 2. Amounts of chlorhexidine digluconate ($\mu g \cdot cm^{-2}$) diffused after 48 h and stored. (A) Whole skin, (B) stripped skin.

tum corneum are applied, such as dimethylsulfoxide (DMSO) (Hotchkiss et al., 1992) and sodium lauryl sulphate (Wilhelm et al., 1991). This suggests a marked interaction between Hibitane® and skin structures. The non-ionic tensioactive molecule (octyl cresyl poloxyethylene alcohol), present in the commercial solution (Hibitane®) to obviate precipitation of the active drug would seem to have an influence on the skin lipids by enhancing diffusion.

Stripped skin revealed a profile that was close to that of intact skin during the first 24 h; after this time it deviated, indicating a slower diffusion of chlorhexidine.

The fact that a greater amount of Hibitane® diffused with stripped skin (100 times more than for whole skin) is related to the physicochemical characteristics of chlorhexidine digluconate. The chlorhexidine salt used is soluble in water, and the absence of stratum corneum enhanced the hydrophillic property of the skin structure.

As regards the tests carried out on intact skin, after 48 h there was more active drug diffused through the skin than stored (Table 1, Fig. 2). In proportion to the total amount diffused with stripped skin after 48 h, storage was lower, which suggests that release has started within the previous 24 h, as shown by a change in the diffusion profile. The presence of tensioactive in the donor compartment allowed the chlorhexidine digluconate to penetrate and remain in the intact skin, with possible formation of micelle within the lipids of the stratum corneum. When the stratum corneum was removed, hydrophilization was enhanced, which favoured the diffusion of chlorhexidine salt into the receptor phase at the expense of skin storage.

The present study has underline the importance of stratum corneum in the process of percutaneous absorption. When the skin is stripped, the amount absorbed is multiplied by approximately 100, and the amount stored in the skin by approximately 10. Thus, when such an antiseptic solution (with tensioactive molecule) is applied, according to the state of skin, storage and diffusion are enhanced or reduced.

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