

Regional variability in stratum corneum reactivity to antiseptic formulations

PASCALE QUATRESOOZ, EMMANUELLE XHAUFLAIRE-UHODA, CLAUDINE PIÉRARD-FRANCHIMONT
AND GÉRALD E. PIÉRARD

Department of Dermatopathology, University Hospital of Liège, Liège, Belgium

Skin does not react in an identical way to the action of chemicals over all anatomic sites. Accordingly, distinct regional differences have been described in relation to irritancy. The present study assesses the regional variations of stratum corneum (SC) reactivity to 3 proprietary antiseptic solutions (povidone iodine (PVP-I), 70 mg/ml and 100 mg/ml, and chlorhexidine digluconate 50 mg/ml) using the corneoxenometry (CXM) bioassay. SC was harvested from the volar forearm, the forehead and the back in 30 young adults. Each SC sample was covered by one of the neat test product or deionized water for 2 hr at 20°C. The intrinsic staining property of each antiseptic on SC was assessed by reflectance colorimetry. For the CXM bioassay, samples were then stained by a toluidine blue-basic fuschin solution in order to show protein denaturation induced by the test products. The colorimetric index of mildness ($CIM = L^* - Chroma\ C^*$) was measured by colorimetry. Data show that PVP-I 100 mg/ml was the least reactive antiseptic to the SC. It was significantly milder than the 2 other antiseptics.

Key words: antiseptic; corneocyte; corneoxenometry; irritant contact dermatitis; regional variability.
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Accepted for publication 31 December 2006

Repeated contacts with some antiseptic formulations may lead to contact irritant dermatitis (1). Even in the absence of clinical evidence, repeated chemical aggression towards the stratum corneum (SC) may become problematic. Indeed subclinical alterations of the SC may impair the natural barrier against various xenobiotics and micro-organisms. In addition, the skin surface becomes imperceptibly harsh until it looks dry. As a result of microscopic cracks, bacteria can be harboured in higher numbers inside the SC, and these micro-organisms are to a certain extent protected from subsequent action of both cleansing and antiseptic products. Therefore, the minute damaging effects to the SC actually decrease the potential antimicrobial effect of the products with continued, prolonged use (2).

The skin response to irritants is subject to anatomic site variation (3–6). Much of the evidence that a given antiseptic product produces rough skin and irritant dermatitis often remains anecdotal. Although some clinical observations appear to have some validity, rigorous proof is confounded by a lack of suitable objective methods of evaluation (7, 8). Indeed, the interpretation of *in vivo* testing may be difficult due to large inter-individual differences. In addition, it may be dif-

ficult to ascribe the part of primary involvement of exogenous stress in the SC function in the data gathered *in vivo*. Anyway the *in vivo* cumulative irritancy test is considered to be a good predictive method for assessing the irritancy potential (9, 10). Two bioassays, namely corneoxenometry and corneoxenometry (CXM), have been shown to correlate well with the *in vivo* testing procedure (11–13).

The present study was undertaken to compare, using the CXM bioassay (13–16), the tolerance of the SC of different body sites to selected antiseptic solutions.

Materials and Methods

This study was performed in accordance with the Declaration of Helsinki and its subsequent amendments. Informed consent was obtained after the nature of the procedure had been fully explained. A total of 30 adults of both genders aged 29 ± 6 years were enrolled. Series of 4 cyanoacrylate skin surface strippings (CSSS) were harvested from each of the 3 body sites corresponding to the mid-inner forearm, the forehead and the back. For that purpose, a droplet of cyanoacrylate glue (Loctite Superglue 3; Loctite,

Brussels, Belgium) was deposited onto a sheet of clear polyethylene (Melinex O; ICI Plastic Division, Brussels, Belgium) cut at the size of a regular coverslip. This material was pressed against the surface of the skin for at least 30 s. In the presence of moisture, the cyanoacrylate polymerize and adhere to the SC. The material was gently lifted and peeled from the skin. Samples were used for CXM testing with either povidone iodine (PVP-I) present in 2 formulations (70 mg/ml Braunol[®] solution; Braun, Brussels, Belgium and 100 mg/ml iso-Betadine[®]; Meda, Brussels, Belgium), chlorhexidine digluconate (50 mg/ml Hibitane[®]; SSL Healthcare Belgium, Groot-Bijgaarden, Belgium) or deionized water. In short, each CSSS was sprayed in excess with 1 test product. After a 2-hr contact time at 20°C, samples were rinsed with tap water. Their colour was first measured following the L*C*H* system using reflectance colorimetry (Chroma Meter CR200; Minolta, Osaka, Japan). This assessment of these blank samples was performed in order to quantify the direct CSSS staining by the antiseptics. The samples were then stained for 3 min with a toluidine blue-basic fuschin solution in order to show protein denaturation in the SC. After rinsing with tap water, the samples were examined microscopically. Their colour was also measured according to the regular CXM procedure (13–16). The colorimetric index of mildness (CIM) was calculated as the difference between the L* and Chroma C* values of each CSSS test sample. The irritancy of chemicals has been previously shown to be inversely correlated with CIM (13–16).

Data gathered for each test product at each test site were expressed as medians and ranges. Between-site and between-product comparisons were made using the paired nonparametric Friedman test followed by the Dunn test. $P < 0.05$ was considered significant.

Results

Colorimetric data of the direct staining properties of the test solutions on CSSS are shown in Table 1. A discrete similar staining of the SC was present on the CSSS that had been covered by each of the PVP-I-based solutions. The differences with both water and the chlorhexidine solu-

tion were statistically significant ($P < 0.01$). For each test product, no significant intersite difference was observed.

Data yielded by the CXM bioassay are presented in Table 2. The CIM values for water were similar on the 3 test sites. Globally, PVP-I 100 mg/ml was the least reactive antiseptic to the SC at each of the test sites. The CIM difference between PVP-I 70 mg/ml and water was statistically significant ($P < 0.05$). At the 3 test sites, PVP-I 70 mg/ml and chlorhexidine 50 mg/ml were similarly more aggressive to the SC ($P < 0.001$) compared to both water and PVP-I 100 mg/ml.

For each of the 3 antiseptics, the weakest reactivity was obtained on the SC harvested from the inner forearm, followed by the forehead and the back. The difference between the forearm and back reactivities reached significance ($P < 0.01$) at each instance. For PVP-I 70 mg/ml, a significant difference ($P < 0.05$) was also reached between the forearm and the forehead. No other significant differences were yielded in the intersite comparisons.

Discussion

Due to the relatively weak irritation potential of antiseptics and to the possible interindividual differences in skin reactivity, comparative *in vivo* testing is difficult to perform. The CXM bioassay is a convenient and predictive approach to explore some chemical xenobiotics on human SC (13–16). Its use is advantageous in that it is safe, cheap and rapid. The information gained by this bioassay has been shown to be correlated with changes in skin capacitance obtained by *in vivo* testing (11–13, 17). However, CXM does not explore the effects of xenobiotics, if any, on the living epidermis and on the nature and intensity of inflammation as present in full-blown irritant dermatitis.

In the present study, the colour differences between the blank samples were so weak that they cannot explain the differences obtained at the CXM bioassay.

The CXM test performed with water explores the baseline situation, which reflects both the intrinsic properties of the SC at a given body site and the effect of previous chemical and/or physical insult to corneocytes. Among the proprietary

Table 1. Medians and ranges of the differences between the L* and Chroma C* values of blank CSSS after a 2 hr contact with antiseptics or water. The lower values indicate the higher direct staining of the samples by the test product

Test solutions	Forearm	Forehead	Back
Water	85.9 (83.2–86.1)	85.3 (83.5–86.2)	85.6 (83.7–86.3)
PVP-I			
70 mg/ml	80.2 (79.5–82.3)	79.6 (78.3–81.3)	79.8 (78.4–80.9)
100 mg/ml	82.3 (81.1–84.0)	82.2 (81.2–83.9)	82.2 (81.6–84.2)
Chlorhexidine 50 mg/ml	85.6 (82.9–85.9)	85.1 (83.4–85.7)	85.2 (83.8–86.0)

Table 2. Medians and ranges of CIM values at the CXM bioassay

Test solutions	Forearm	Forehead	Back
Water	76.4 (66.5–78.0)	75.8 (69.4–80.6)	77.7 (68.3–80.9)
PVP-I			
70 mg/ml	65.8 (47.7–79.2)	60.1 (43.5–71.5)	58.9 (41.0–64.1)
100 mg/ml	73.5 (64.7–80.6)	70.1 (63.3–73.3)	68.8 (56.2–72.4)
Chlorhexidine 50 mg/ml	63.6 (46.8–77.9)	61.3 (45.2–70.3)	59.4 (43.3–61.5)

antiseptic solutions tested, PVP-I 100 mg/ml was by far the least aggressive towards the SC. This finding is in line with previous *in vivo* studies suggesting that this PVP-I formulation exhibits a very low irritancy potential (1, 2, 18, 19). This is in contrast to the effect of iodine tincture, the irritancy of which has never been denied (1, 19). It should be noted that the 2 PVP-I formulations did not yield similar CXM data. The weaker tolerance exhibited by the PVP-I 70 mg/ml may indeed be due to the addition of iodate in this formulation. However, this remains only a working hypothesis because the present experiment does not bring information about the underlying chemical reactivity of the products with the SC.

The present study shows some regional variability in the antiseptic reactivity on the SC. The ranking from the least to the most reactive was the volar forearm, the forehead and the back. A similar ranking had been previously reported for surfactants (6). Mechanisms determining such variation in biological susceptibility of the SC are far from being understood. However, it is acknowledged that the severity of *in vivo* irritation depends not only on the nature of the chemical agent but also on individual susceptibility and on exposure conditions. In particular, anatomic site influences the proclivity to irritation (5).

Acknowledgements

We thank Mrs N. Veys for the skilful technical expertise in performing the bioassay. This work was supported by a grant from the 'Fonds d'Investissement de la Recherche Scientifique' of the University Hospital of Liège. No other sources of funding were used to assist in the preparation of this manuscript. The authors have no conflicts of interest that are directly relevant to the content of this review.

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Address:

Professor Gérald E. Piérard, MD, PhD

Department of Dermatopathology

University Hospital of Liège, CHU Sart Tilman

B-4000 Liège

Belgium

Tel: +3243662408

Fax: +3243662976

e-mail: Gerald.pierard@ulg.ac.be