X-ray Scattering Analysis of Human Stratum Corneum Treated by High Voltage Pulses

A. Jadoul,[1,3] V. Regnier,[1] J. Doucet,[2] D. Durand,[2] and V. Préat[1]

Received February 9, 1997; accepted June 4, 1997

KEY WORDS: X-ray scattering; human stratum corneum; electroporation, high voltage pulses.

INTRODUCTION

Stratum corneum (s.c.), the outermost layer of mammalian epidermis, acts as the main barrier for diffusion of substances through the skin. The unique morphology of s.c. has been shown to be an important determinant of its low permeability (1).

X-ray scattering analysis has provided information on the structural organization of the s.c. (2–4). Hence, this technique was applied to find a correlation between structure and barrier function alteration induced by application of penetration enhancers, liposomes or iontophoresis (5–7). Recently, the application of short high voltage pulses has been shown to promote transdermal drug delivery. The phenomenon underlying this enhancement is believed to include electroporation, i.e. the creation of small, transient "aqueous pathways" across the s.c. (8,9). Prolonged s.c. permeabilization has also been reported after application of high voltage pulses (HVP) (Vanbever, De Morre, and Préat, unpublished data). However, few studies have addressed the skin structural integrity and its recovery after electroporation (10).

The purpose of this study was to explore human s.c. integrity after *in vitro* HVP application by X-ray scattering. The scattering pattern of s.c. submitted to different electrical protocols was analysed and compared to untreated samples in order to detect potential modifications induced by HVP.

MATERIAL AND METHODS

Material

The studies were performed on human s.c. isolated by trypsin digestion (3) from fresh mammae skin, obtained from cosmetic surgery. The buffers were prepared with salts (analytical grade, UCB, Leuven, Belgium) dissolved in ultra pure water.

HVP Application

HVP application was performed at 37°C in polycarbonate cells filled with phosphate buffer pH 7.4, 0.2 M. The s.c. (1 cm^2) separated the donor and receptor compartments. The anode was introduced in the upper reservoir, facing the outer layer of the s.c. Electrodes (0.25 cm^2, Platinum pure) were connected to Easyject Plus®, a device generating exponentially decaying pulses (9). Different protocols were applied: 20X (200 V − 160 ms), 20X (200 V − 80 ms), 20X (100 V − 160 ms), 5X (200 V − 160 ms) and 60X (500 V − 1 ms). The voltage corresponds to the voltage applied to the electrodes. The resultant samples were compared to equally-long hydrated samples (control).

X-ray Scattering Experiments

The X-ray scattering experiments were carried out about 5 min after HVP using the synchrotron radiation source DCI of LURE (Orsay-France, 2). The size of the beam (wavelength 0.145 nm) was limited by a collimator with a circular aperture of 0.07 mm diameter.

Experiments were performed with the incident beam parallel to the plane of the s.c. in order to follow the scattering features originating in the lamellar structures of the intercellular lipids (SAXS, Small Angle X-ray Scattering). The chosen sample-detector distance corresponds to the range of spacing [15 nm–1.5 nm]. Patterns were also obtained in perpendicular geometry at shorter distances corresponding to the range of spacing [3 nm–0.25 nm], in order to observe the rings due to the regular packing of the lipids within the layers (WAXS, Wide Angle X-ray Scattering). Data collection was carried out for a period of 20 up to 35 min. Intensity profiles were extracted with s (scattering vector) = 2 sin θ/λ where 2θ is the scattering angle, and with d (distances) = 1/s. The profiles were normalized according to the sample volume in the beam.

RESULTS

Different HVP protocols were applied: 20X (200 V − 160 ms), 20X (200 V − 80 ms), 20X (100 V − 160 ms), 5X (200 V − 160 ms) and 60X (500 V − 1 ms). The reasons for this choice were i) to compare the effects on the s.c. of the two main electroporation protocols applied to date in transdermal drug delivery: short pulses (1–2 ms; 8) and long pulses (70–1000 ms; 9), ii) to investigate the influence of the three main electrical parameters of the pulses: the voltage, the duration and the number.

Control S.C.

SAXS

The SAXS pattern of electrically untreated s.c. (Fig. 1) was characterized by several peaks, in agreement with the literature (2,3). The strong scattering at low angle was attributed to keratin. Two peaks were visible around 6.3 and 4.7 nm and were attributed to intercellular lipid lamellar structures. According to Bouwstra et al (3), the "4.7 nm" spacing corresponds to the third order of a "13.5 nm" spacing. The experimental setting for the present study did not allow to detect properly the peak at 13.5 nm.

WAXS

The WAXS pattern of control s.c. shown in Fig. 2 is similar to those previously published (2,4). A broad band appearing at

[1] Université catholique de Louvain; Unité de Pharmacie galénique, Industrielle et Officinale, Brussels; Belgium.
[2] LURE, Université de Paris-Sud, Orsay, France.
[3] To whom correspondence should be addressed.

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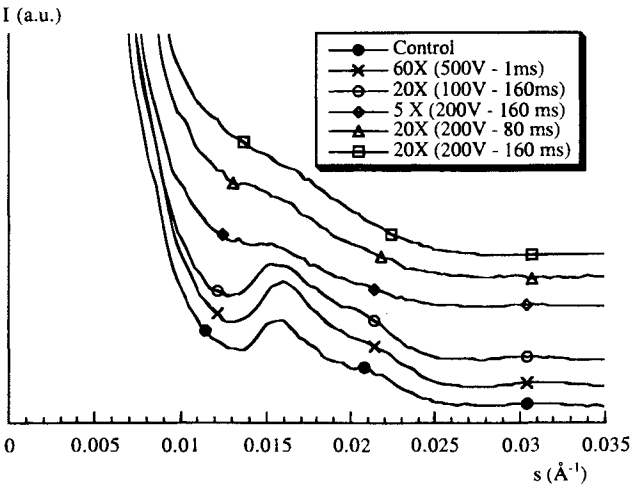

Fig. 1. Scattered intensity profiles I as a function of the scattering vector s obtained in SAXS for control samples or s.c. submitted to 60X (500 V − 1 ms), 20X (100 V − 160 ms), 20X (200 V − 80 ms), 5X (200 V − 160 ms) or 20X (200 V − 160 ms). Each curve corresponds to the mean of different scattering patterns (n = 2 or 3). The curves were vertically shifted to facilitate their viewing.

about 0.96 nm has been attributed to keratin. Another broad band located at 0.45 nm would correspond to keratin and non crystallized lipids. The presence of the two main diffraction peaks located at 0.411 nm and 0.375 nm is characteristic of an orthorhombic lateral packing of the lipids. The spacing 0.411 nm may also be due to a lateral hexagonal packing of lipids (4).

Electroporated s.c.

SAXS

The SAXS patterns of s.c. submitted to HVP are shown in fig. 1. The peaks observed at 6.3 and 4.7 nm decreased in intensity after application of 20X (200 V − 80 ms), 5X (200 V − 160 ms) and 20X (200 V − 160 ms). Furthermore, the scattering at low angle was reinforced. Only a slight intensity decrease or no decrease was detected after 20X (100 V − 160 ms) or 60X (500 V − 1 ms) respectively.

The results obtained can be compared to previous X-ray scattering work on the s.c. (5–7). Such changes in the X-ray pattern are entirely consistent with a disorganization of the intercellular lipid lamellar structure (3–7). The disorganisation was more marked as the voltage, pulse number and duration were increased.

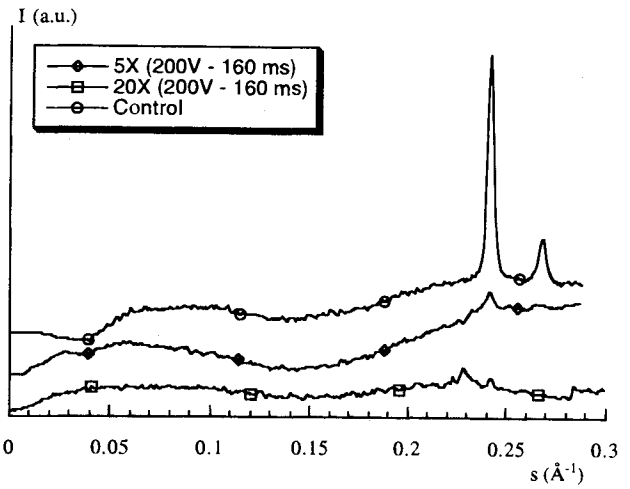

Fig. 2. Scattered intensity profiles I as a function of the scattering vector s obtained in WAXS for control samples or s.c. submitted to electroporation: 20X (200 V − 160 ms) or 5X (200 V − 160 ms). Each curve corresponds to the mean of different scattering patterns (n = 2 to 4). The curves were vertically shifted to facilitate their viewing.

WAXS

The WAXS patterns of s.c. submitted to HVP are shown in fig. 2. Application of long HVP decreased the intensity of the two lipid peaks located at 0.375 nm and 0.411 nm. The reflections were not shifted. After 5X (200 V − 160 ms) only the peak at 0.411 nm was observed. After 20X (200 V − 160 ms), this peak also disappeared. Only a slight decrease in intensity was observed after application of 60X (500 V − 1 ms): both peaks remained clearly visible (data not shown). Such decreases in intensity of the scattering peaks have previously been reported after iontophoresis performed at high current density (11) or by heating (4,12) and were interpreted as a change of the lateral packing ordering. The 0.375 nm peak disappeared for most treatments indicating that the orthorhombic packing of the lipids tends to disappear first.

DISCUSSION

The aim of this study was to detect potential changes in s.c. structure induced by HVP which could be responsible for the enhanced permeability and/or which could be considered as side effects. In contrast to short pulses, the decreases in intensity of the lipid peaks observed in SAXS and WAXS after application of long pulses suggest that both the interlamellar and intralamellar structures are affected. Further, general rather than localized disorganizations were detected after HVP application. However, since the phenomena observed in this study have been captured min after HVP treatment, they can be considered as secondary events. It is conceivable that primary defects such as the hypothetical "aqueous pathways" (8) had disappeared and/or were not detectable by X ray scattering. The general perturbation observed is in good agreement with other studies such as freeze fracture electron microscopy (FFEM) or differential scanning calorimetry (DSC) (10). Moreover, these observations can be compared to the results after iontophoresis. Indeed, a disordering of the intercellular lamellar structures was induced by the application of electrical current and depended on the intensity applied (6, 11). The WAXS pattern was also shown to be affected after high electrical current density (up to 13 mA/cm^2).

CONCLUSIONS

This X-ray scattering study investigated the s.c. structure after application of HVP. Significant changes in the X-ray patterns were detected after application of long pulses, indicating a general perturbation of the interlamellar and intralamellar lipid packing order. In contrast, few alteration could be seen after application of short HVP.

REFERENCES

1. R. O. Potts and M. L. Francoeur. *J. Invest. Dermatol.* **96**:495–499 (1991).
2. J. Garson, J. Doucet, J. L. Lévêque, and G. Tsoucaris. *J. Invest. Dermatol.* **96**:43–49 (1991).
3. J. A. Bouwstra, G. S. Gooris, J. A. van der Spek, and W. Bras. *J. Invest. Dermatol.* **97**:1005–1012 (1991).
4. J. A. Bouwstra, G. S. Gooris, M. A. de Vries, J. A. van der Spek, and W. *Int. J. Pharm.* **84**:205–216 (1992).
5. H. E. Hofland, J. A. Bouwstra, H. E. Boddé, F. Spies, and H. E. Junginger. *Br. J. Dermatol.* **132**:853–866 (1995).
6. A. Jadoul, J. Doucet, D. Durand, and Préat, V. *J. Controlled Rel.* **42**:165–173 (1996).
7. S. Chesnoy, J. Doucet, D. Durand and G. Couarraze. *Pharm. Res.* **13**:1581–1584 (1996).
8. M. R. Prausnitz, E. R. Edelman, J. A. Gimm, R. Langer, and J. C. Weaver. *Biotechnology* **13**:1205–1209 (1995).
9. R. Vanbever, N. De Morre, and V. Préat. *Pharm. Res.* **13**:1360–1366 (1996).
10. A. Jadoul, Skin perturbations induced by iontophoresis and electroporation: enhancement in drug transport and integrity of the stratum corneum, Ph D thesis, Université catholique de Louvain, Brussels, Belgium (1997).
11. W. H. M. Craane-van Hinsberg, Transdermal peptide iontophoresis: a mechanistic study of electrical skin barrier, perturbation and transport enhancement, Ph D Thesis, Leiden University, Leiden, The Netherlands, 1994.
12. J. A. Bouwstra, G. S. Gooris, W. Bras, and D. T. Downing. *J. Lip. Res.* **36**:685–695 (1995).