Effect of Iontophoresis in Combination with Ionic Enhancers on the Lipid Structure of the Stratum Corneum: An X-ray Diffraction Study

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INTRODUCTION

The extracellular lipid matrix of stratum corneum constitutes the major barrier to percutaneous penetration. The penetration of drugs through the skin has been increased by using many different types of enhancers. These molecules reduce the barrier properties of the skin in different ways, some acting on the lipid bilayer while others act on the keratinized structures or cutaneous hydration. The permeation of drugs across the skin can also be enhanced by applying an external electric field (iontophoresis).

Recently, enhanced permeability by using iontophoresis in combination with penetration enhancers has been the subject of several studies but none up to now has investigated the influence of this dual system on the physico-chemical structure of the stratum corneum (1,2) and more particularly the effect produced on the stacking of the stratum corneum intercellular lipids just after the enhancer has been electro-incorporated into the stratum corneum. This work could allow us to answer to two questions: (a) is there any difference in lipids stacking if ionic enhancer is applied passively or by the use of an electrical current?, (b) is there subsequent influence of these differences on the transport rate of a model drug molecule? Here, only the first question will be considered.

By using the high X-ray flux given by a synchrotron radiation source, progress has recently been made on the understanding of the supramolecular organization of human stratum corneum lipids. According to Garson et al. (3) and Bouwstra et al. (4,5), two types of lamellar phase exist within human stratum corneum with a spacing of 45 Å for one lamellar phase and with spacings varying between 65 Å and 62 Å (4) for the other. Nevertheless, Bouwstra et al. (4,5) have found an additional lamellar system with a spacing of 134 Å. So it seems that three main different lipidic lamellar systems coexist in human stratum corneum.

During the last decade, the mechanism of action of penetration enhancers such as azone (6), terpenes (7) or sodium lauryl sulfate (8) on human stratum corneum has been analyzed by X-ray diffraction. The effect of iontophoresis on human stratum corneum has also been investigated. Jadoul et al. (9) observed a disorganization of the intercellular lipid matrix followed by a subsequent reorganization. The purpose of this study was to detect, by X-ray diffraction, possible structural modifications on stratum corneum intercellular lipids induced by electrical current alone and in combination with model ionic enhancers such as sodium lauryl sulfate and hexadecyl trimethylammonium bromide.

MATERIALS AND METHODS

Materials

Sodium lauryl sulfate and hexadecyl trimethylammonium bromide were purchased from SIGMA.

Ag/AgCl electrodes 12.5 mm in diameter and 1 mm thick were employed.

Skin and Preparation of the Skin

Skin diffusion experiments were performed on human stratum corneum obtained by trypsin treatment of heat separated epidermis. All transport studies were carried out using isotonic phosphate buffer in the receiver compartment of the diffusion cells.

Effect of Iontophoresis

Two studies were carried out, one with the cathode facing the stratum corneum and one with the anode facing the stratum corneum. A direct current of 0.17 mA/cm^2 was applied for one hour.

Effect of Ionic Enhancers

2 ml of a 20 g/l solution of either sodium lauryl sulfate alone or hexadecyl trimethylammonium bromide alone were applied to the stratum corneum for one hour.

Effect of Iontophoresis in Combination with Ionic Enhancers

Iontophoresis was performed, according to the electric charge of the ionic enhancer (cathodal iontophoresis for sodium lauryl sulfate and anodal iontophoresis for hexadecyl trimethylammonium bromide).

For each treatment, 2 ml of a 20 g/l sodium lauryl sulfate or hexadecyl trimethylammonium bromide solutions were used. For each experiment a direct current of 0.17 mA/cm^2 was applied for one hour.

Preparation of Samples

At the end of each treatments, the samples and the controls were stored in a dessicator. Before scattering experiments, they were hydrated at a water content of 10%.

Lipid Extraction

Lipids were extracted by chloroform/methanol (2:1), first, for 20 min (delipidation 1) and second, for 60 min (delipidation 2).

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X-ray Diffraction Studies

The X-ray source was the synchrotron source of the LURE center (University of Paris-Sud). The wavelength of the radiation was 1.455 Å. Small angle diffraction analysis was performed on all samples with a sample-film distance of 392.5 mm. A two-dimensional detection system with image plates was used (Molecular Dynamics scanner). Intensity profiles I(s) were extracted ($s = 2 \sin \theta/\lambda$ where 2θ is the scattering angle) and the s values can be converted into distances d through $d = 1/s$. The profiles have been normalized according to the sample volume in the beam. All diffraction analysis were performed four days after preparation of the samples.

RESULTS

The pattern of native human stratum corneum is shown in Figure 1. It displays two broad maxima located at 62.5 Å and 45 Å. Furthermore, a shoulder appears on the small-angle scattering curve at about 130 Å. These features are in agreement with those described in the literature (3,4,5).

After iontophoresis, modifications are observed on the scattering patterns (Figure 1). Anodal iontophoresis and cathodal iontophoresis patterns are superimposed. The peak at 62.5 Å becomes more intense and its position is slightly shifted towards smaller angles (its new position corresponds to 63.5 Å). The peaks at 130 Å and 45 Å are also reinforced.

The combination of iontophoresis with penetration enhancers was studied with the anionic surfactant (sodium lauryl sulfate) (cathodal treatment) and with the cationic surfactant (hexadecyl trimethylammonium bromide) (anodal treatment). Treatment with sodium lauryl sulfate alone leads to an increase in intensity of the diffraction peaks at 130 Å and 62.5 Å (Figure 2). When combined with cathodal iontophoresis, this increase is even more marked and the pattern is similar to that of cathodal iontophoresis alone but the intensity increases are more pronounced. After lipid extraction which has been performed to precise the chemical origin of the modifications, the intensity of all diffraction peaks decreases (Figure 3), and more particularly the peak at 130 Å disappears entirely. In contrast, no modification is detected for hexadecyl trimethylammonium bromide alone or for hexadecyl trimethylammonium bromide combined with anodal iontophoresis (Figure 4).

Interpretation

The intensity increase of the peaks located at 130 Å and 62.5 Å induced by iontophoresis and the fact that their width remained nearly the same indicate that the content of crystallized materials which are responsible for these peaks had increased.

We have verified by delipidation treatment that the extra crystallized materials were lipids. In other words, lipids which were in amorphous state in the native sample have been incorporated in lamellar systems between the corneocytes. The mechanism of this incorporation could be due to a fluidization of the lipids induced by the electric field. The lipids would then get more mobility and would tend to be oriented along the field thus favorizing the formation of layers. When cutting off the power, most of the previously crystallized lipids would return to the same periodic arrangement whereas newly crystallized lipids would be stacked into a system with a period of 134 Å. The scattering pattern obtained four days after application would thus correspond to the superimposition of the patterns due to the lipids previously crystallized and to the newly crystallized lipids. With such an hypothesis, we can explain the shift of the 62.5 Å peak by the superimposition of the 62.5 Å peak with the second order (67 Å) peak of the 134 Å system formed by the newly crystallized lipids, and in other hand the increase of

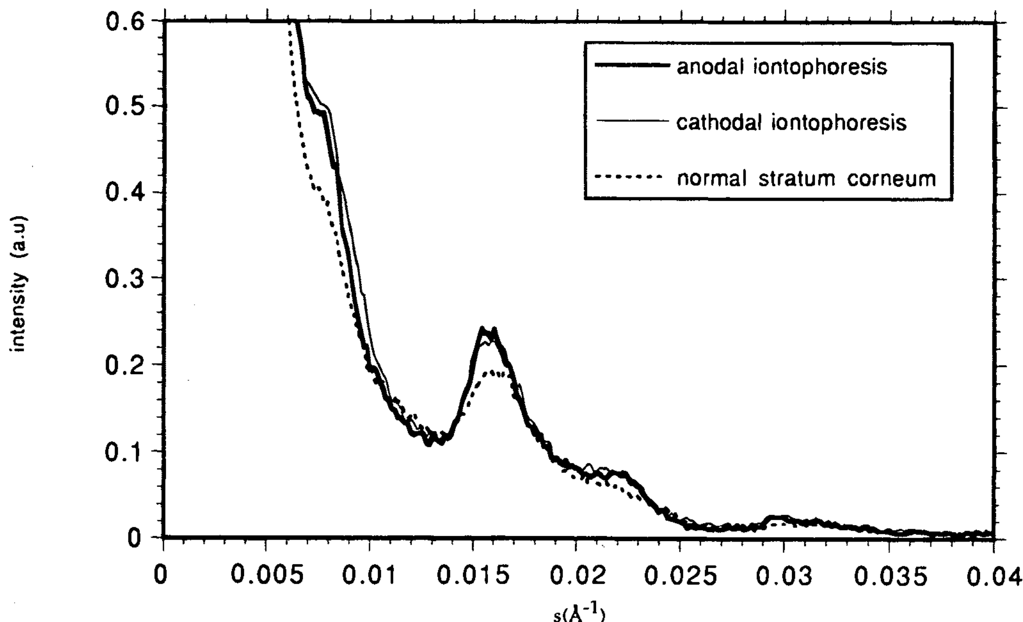

Fig. 1. Effects of iontophoresis on small angle X-ray diffraction by human skin stratum corneum.

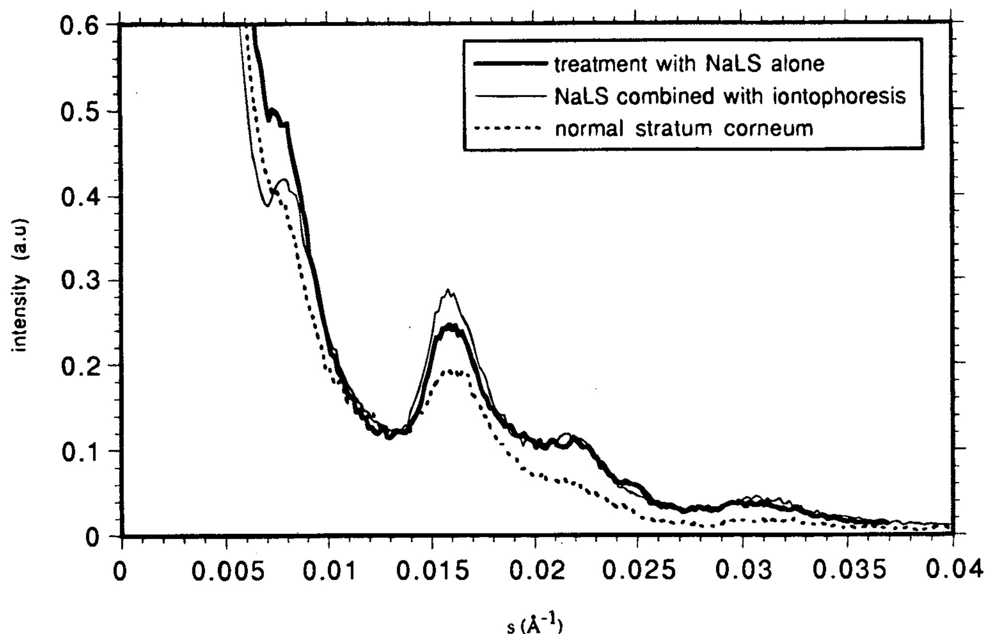

Fig. 2. Influence of sodium lauryl sulfate on small angle X-ray diffraction by human skin stratum corneum.

the 45 Å peak by the third order. The formation of a new lipid system with a period of 134 Å is not to surprising since Bouwstra (4) has observed that on heating above 90°C, a lipid melting process occurs, followed by a recrystallization into a system of 134 Å. Our observation is in favour of structural reorganization of the lipids when energy is brought to the stratum corneum. However, the fluidization should correspond to the disappearance of the lipid layer reflexions on the diffraction patterns during the electric field application. Thus, definite proof concerning the fluidization requires measurements performed during (or immediately after) the electric field application; such an analysis is in progress.

From the study with sodium lauryl sulfate it can be assumed that this enhancer promotes the fluidization process during the iontophoretic treatment. It is noteworthy that even without iontophoresis, a small fluidization effect seemed to occur. The mechanism of penetration enhancement by sodium lauryl sulfate is related (at least partly) to its effect on intercellular lipids. An opposite conclusion can be drawn for hexadecyl trimethylammonium bromide from our X-ray experiments. The

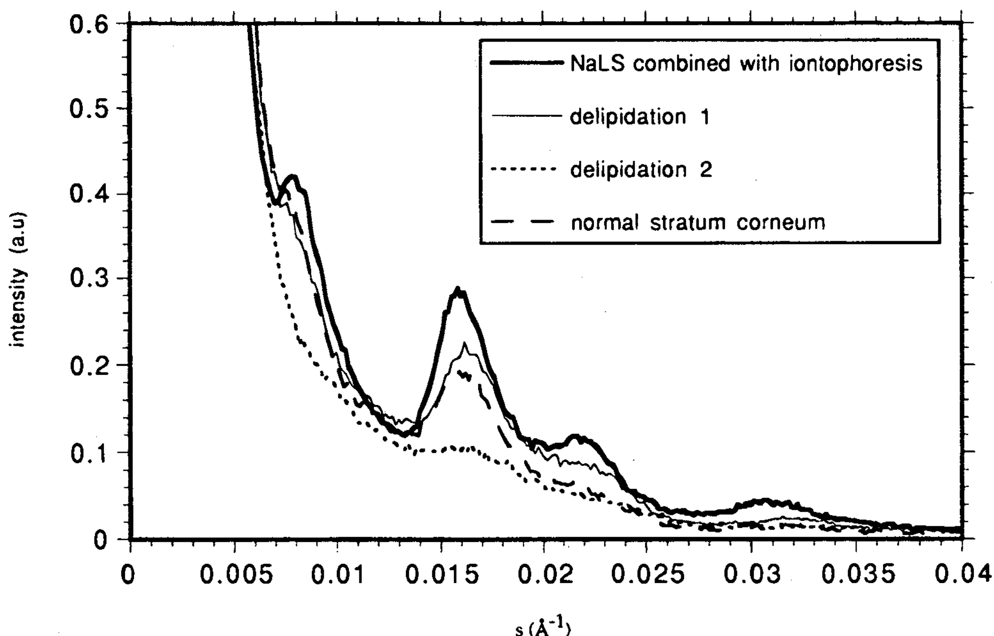

Fig. 3. Influence of delipidation on small angle X-ray diffraction by human skin stratum corneum.

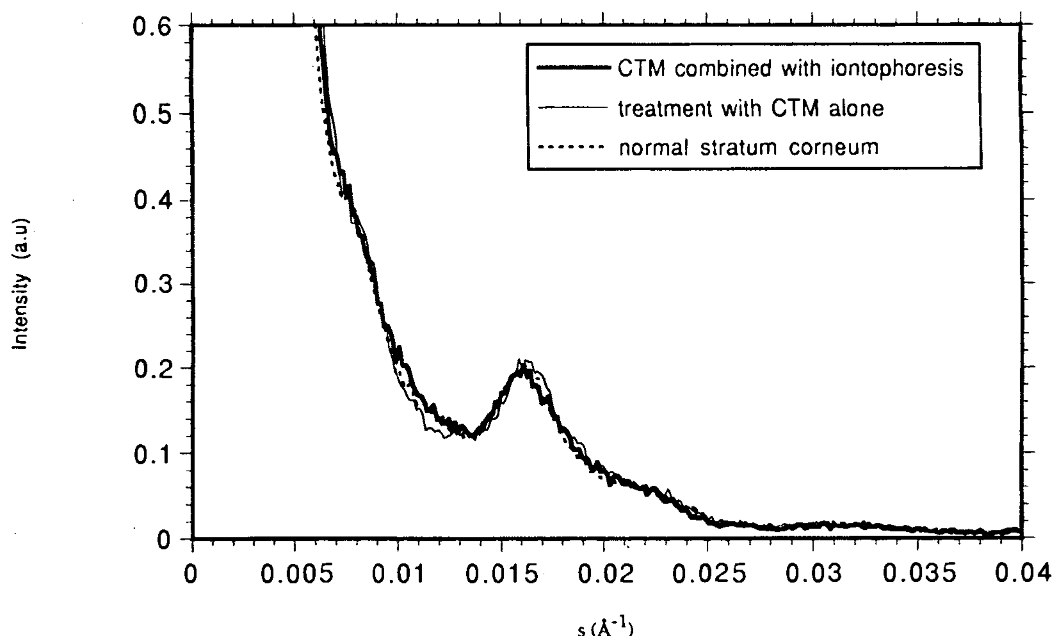

Fig. 4. Influence of hexadecyl trimethylammonium bromide on small angle X-ray diffraction by human skin stratum corneum.

mechanism of enhancement of hexadecyl trimethylammonium bromide is clearly not related to the fluidization of the lipids.

DISCUSSION

According to Jadoul et al. (9) intercorneocyte lipid stacking in human skin is greatly perturbed by an exposure to a direct current of 0.33 and 0.5 mA/cm^2 for 6 hours. More precisely, these authors observed that the scattering peaks at 45 Å and 65 Å disappeared but the phenomenon seemed to be reversible because after about one week, the pattern returned to the control one. In their study, the peak at 130 Å was not considered. One possible explanation of the difference between their results and ours is that our scattering experiments were performed 4 days after sample preparation. This delay could be long enough for the lipids which have been disorganized to reorganize into a more tight structure.

In the present study, the lipids were better organized after passive or iontophoretic treatment with sodium lauryl sulfate. However, Ribaud et al. (8) have observed by X-ray diffraction a lipid disorganization immediately after stratum corneum treatment with sodium lauryl sulfate, and Barry (10), by differential scanning calorimetry, observed a fluidization of stratum corneum lipids treated with sodium lauryl sulfate. Denda et al. (11), using Fourier transform infra-red spectroscopy in vivo, have also described a change in alkyl chain conformation of lipids just after treatment with sodium lauryl sulfate but, three days later, the values returned to their original levels. Thus it appears that our results do not contradict those of Denda et al. (11), Ribaud et al. (8) and Barry (10) since during sample storage the intercellular lipids and incorporated sodium lauryl sulfate could have reorganized themselves into a more crystallized structure.

CONCLUSION

The intercellular lipids are extensively reorganized by iontophoresis. This effect is reinforced when sodium lauryl sulfate is combined with cathodal iontophoresis. In contrast, no effect on the structure of skin lipids was detected with hexadecyl trimethylammonium bromide treatment. Thus, the experiments presented here demonstrate that X-ray diffraction is a powerful technique to characterize the mechanism of action of iontophoresis and penetration enhancers on stratum corneum lipids.

REFERENCES

1. U. T. Lashmar and J. Manger. *Int. J. Pharm.* **111**:73–82 (1994).
2. Y. Wang, L. V. Allen, JR, C. Li. Luk and Tu. Yu-Hsing. *J. Pharm. Sci.* **82** (11):1140–1144 (1993).
3. J. C. Garson, J. Doucet, J. L. Lévêque and G. Tsoucaris. *J. Invest. Dermatol.* **96**:43–49 (1991).
4. J. A. Bouwstra, G. S. Gooris, J. A. Van der Spek and W. Bras. *J. Invest. Dermatol.* **97**:1005–1012 (1991).
5. J. A. Bouwstra, G. S. Gooris, M. A. Salomons-de Vries, J. A. Van der Spek and W. Bras. *Int. J. Pharm.* **84**:205–216 (1992).
6. J. A. Bouwstra, G. S. Gooris, J. Brussee, M. A. Salomons-de Vries and W. Bras. *Int. J. Pharm.* **79**:141–148 (1992).
7. P. A. Cornwell, B. W. Barry, C. P. Stoddart and J. A. Bouwstra. *J. Pharm. Pharmacol.* **46**:938–950 (1994).
8. C. Ribaud, J. C. Garson, J. Doucet and J. L. Lévêque. *Pharm. Res.* **11**:1414–1418 (1994).
9. A. Jadoul, J. Doucet, D. Durand and V. Préat. *Proceed. Intern. Symp. Control. Rel. Bioact. Mater.* 21 (1994).
10. B. W. Barry. *J. Control. Release.* **6**:85–97 (1987).
11. M. Denda, J. Koyama, R. Namba and I. Horii. *Arch. Dermatol. Res.* **286**:41–46 (1994).