Examination of the Mechanism of Oleic Acid-Induced Percutaneous Penetration Enhancement: an Ultrastructural Study

Shao Jun JIANG* and Xiao Jun ZHOU

Department of Pathology, Jinling Hospital; 305 Zhong Shan Dong Lu, Nanjing, 210002, P. R. China.

Received March 18, 2002; accepted June 11, 2002

The epidermal permeability barrier appears to be regulated primarily by the lamellar arrangement of lipid bilayers between corneocytes of the stratum corneum and presents a significant barrier to the transdermal delivery of drugs. The aim of the present study was to investigate the effects of oleic acid on the ultrastructure of stratum corneum lipids in rat skin. Wistar rats were treated topically with 10% oleic acid/propylene glycol for 2 h, the structure of stratum corneum was examined by electron microscopy using osmium tetroxide or ruthenium tetroxide postfixation, and the epidermal barrier function was evaluated in a lanthanum tracer study. Ultrastructural examination revealed that there was a marked alteration in the stratum corneum and the tracer penetrated into the intercellular spaces of the stratum corneum after application of oleic acid. These results suggest that ruthenium tetroxide postfixation is a powerful tool for the study of the stratum corneum lipid structure. Oleic acid might increase the epidermal permeability through a mechanism involving the perturbation of stratum corneum lipid bilayers and lacunae formation to enhance transdermal drug delivery.

Key words stratum corneum lipid; penetration enhancement; oleic acid; ruthenium tetroxide; lanthanum tracer study

The outermost layer of mammalian skin, the stratum corneum, by virtue of its unique architecture presents a significant barrier to the transdermal delivery of drugs. Recently, biophysical, morphological, and biochemical results have indicated that the stratum corneum forms a continuous sheath of protein-enriched corneocytes embedded in an intercellular matrix enriched in nonpolar lipids that are organized as lamellar layers. Although only 10 to 15% of the stratum corneum mass is comprised of lipids, these lipids largely dictate the overall skin permeability property. Transdermal drug delivery promises many advantages over oral or intravenous administration, and as a consequence it would be very useful to reduce the skin barrier using physical approaches and chemical enhancers, which would reversibly remove the barrier resistance of the stratum corneum and thus allow the drug to penetrate into the viable tissue and enter the systemic circulation.

There is continued interest in the development of strategies to alter the skin barrier to percutaneous absorption of compounds. Oleic acid (OA) has been studied as a skin penetration enhancer for drugs via its action primarily on stratum corneum lipid structures. Studies have shown that the modes of action of OA have generated two mechanistic scenarios, which may account for the action of this enhancer: 1) lipid fluidization; and 2) lipid phase separation. Since the stratum corneum lipids constitute the primary permeability barrier of the skin and these lipids exist in the stratum corneum intercellular spaces as a highly organized lamellar bilayers, it is reasonable to expect that OA applied to the skin would emulsify or otherwise disorganize the stratum corneum lipid lamellae and thus increase skin permeability. In the present study, we investigated the use of ruthenium tetroxide postfixation and a lanthanum tracer study to ascertain the effects of OA on the stratum corneum lipids and epidermal permeability barrier function.

MATERIALS AND METHODS

Materials OA and propylene glycol (PG) were of analytical grade and purchased from Sigma Chemical (St. Louis, MO, U.S.A.). Ruthenium tetroxide and lanthanum nitrate were from Polysciences Inc. (Warrington, PA, U.S.A.).

Experimental Protocols Male Wistar rats weighing 250—300 g were used in this study. Under general anesthesia with 4% chloral hydrate by intraperitoneal injection, the hair from the back of the animals was clipped and then shaved with an electric razor. The rats were treated on the back with 10% OA/PG-soaked cotton balls. The other group serving as a control was treated with PG-soaked cotton balls. Each was rolled gently on the skin surface for 2 h at 5-min intervals, and neither OA nor PG application produced damage to the stratum corneum.

Electron Microscopy Samples were taken from the flanks of animals for electron microscopy after either OA or PG treatment. Briefly, samples were fixed in modified Karnovsky’s fixative overnight at 4 °C, which contained 2% paraformaldehyde, 2% glutaraldehyde, 0.06% CaCl₂, in 0.1 M cacodylate buffer, pH 7.4. The tissues were then washed in 0.1 M cacodylate buffer, postfixed in either 0.25% ruthenium tetroxide (Ru₂O₄) in 0.1 M cacodylate for 45 min in the dark at room temperature or in 1% osmium tetroxide (OsO₄) in 0.1 M cacodylate buffer for 2 h at room temperature. The specimens were then rinsed in buffer, dehydrated in graded ethanol solutions, and embedded in an Epon–epoxy resin mixture. Ultrathin 60—80 nm sections were examined under a Jeol-1200 electron microscope operated at 80 kV after staining with uranyl acetate/lead citrate.

Lanthanum Tracer Studies To evaluate the extent of epidermal permeability barrier disruption after OA treatment, lanthanum nitrate was used to delineate the pathway and extent of water permeation through the epidermis. Samples were soaked in equal parts of modified Karnovsky’s fixative and 8% lanthanum nitrate solution, which contained 8% sucrose in 0.05 M Tris buffer, pH 7.6, for 1 h at room temperature. Then the samples were rinsed with fixative, fixed in modified Karnovsky’s fixative overnight at 4 °C, postfixed in 1% OsO₄ for 2 h, and processed as described above. Thin sections were stained with uranyl acetate only for electron
RESULTS AND DISCUSSION

**Ultrastructural Observations**  
Figure 1 shows that after treatment with either 10% OA/PG or PG alone using OsO4 postfixation. As shown in Fig. 1A, the corneocytes of the stratum corneum were cohesive in PG-treated epidermis. However, the stratum corneum was considerably disrupted by 2-h OA treatment. As illustrated in Fig. 1B, the corneocytes were widely separated and most of them were removed from the upper stratum corneum. This finding may reflect the simplest approach to enhance physically the percutaneous absorption of a compound across the skin by stripping off the outermost layers of the skin, the stratum corneum. A sequential increase in transepidermal water loss is observed as the stratum corneum is progressively removed, indicating that the epidermal permeability is increased.6)

To gain insight into the mechanism by which OA affects the epidermal permeability barrier, we next examined the vehicle-treated and OA-treated epidermis using RuO4 postfixation. In vehicle-treated epidermis, the intercellular lipid bi-

![Fig. 1. Electron Micrograph of PG or 10% OA-Treated Rat Skin Fixed with OsO4](image1)

After exposure to PG for 2 h, the majority of corneocytes of the stratum corneum were tightly cohesive (1A). The application of OA resulted in corneocyte separation and most were removed from the upper stratum corneum (1B). Scale bar = 1 μm.

![Fig. 2. Electron Microscopic Findings in the Stratum Corneum of Rat Skin Treated with OA Using RuO4 Fixation](image2)

Note the preservation of the intercellular lipid bilayers in PG-treated epidermis (A, arrows). After 2-h OA treatment, the expansion of lacunae (asterisks, B) was observed in the widened intercellular spaces of the stratum corneum. Scale bar = 100 nm.

![Fig. 3. Lanthanum Nitrate Was Employed as a Tracer to Evaluate the Barrier Function](image3)

The tracer is found in the intercellular spaces of the granular layers (A, arrowheads), but not in the stratum corneum in PG-treated epidermis. In contrast, after 2-h OA treatment, abundant tracer was present within the stratum corneum (B, arrowheads). Scale bar, A = 500 nm; B = 1 μm.
into the intercellular spaces of the stratum corneum (Fig. 3B). This indicates that the increased permeability to water occurs within the epidermis with OA treatment.

Lanthanum is an electron-dense trivalent cation material that can be used as a tracer for delineating extracellular spaces and intercellular junctions. It can also be used as a tracer in studying the permeability barrier. The localization of the barrier defect to the stratum corneum by the application of OA is supported by the results of lanthanum perfusion studies, which have shown that the tracer percolates through the stratum corneum interstices in OA-treated, but not control epidermis. A similar situation occurs in the inhibition of HMG CoA reductase, which results in defective barrier function that correlates with enhanced tracer permeability through the stratum corneum.

The present ultrastructural studies provide clear insight into the potential mechanism of permeability barrier alterations in the skin. Under basal conditions, the stratum corneum lipids comprise broad compact sheets, organized as a lamellar structure. In contrast, the formation of lacunae and distinct disorganization of the stratum corneum lipid bilayer structure appeared in OA-treated epidermis. It is reasonable to propose a mechanism of percutaneous penetration enhancement: the formation of lacunae could be assumed to be one type of permeable defect in the stratum corneum, which reduces the diffusional resistance to enhance water-soluble molecule transport through the stratum corneum. It is possible that with a penetration enhancer, the lacunae are enlarged and the size of the lacunar domain is increased; these spaces eventually coalesce, forming a continuous tubuloreticular network. As a result, the penetration threshold is eventually breached and forms a pathway for molecule transport.

The use of saturated and unsaturated fatty acids such as stearic acid, OA, linoleic acid, and linolenic acid for drug permeation enhancement is of interest in the area of topical and transdermal drug delivery systems research. These fatty acids have been shown to be effective penetration enhancers for a variety of drugs. Selective perturbation of the intercellular lipid bilayers in the stratum corneum appears to be the major route of enhancing the activity of fatty acids. The combined infrared spectroscopy and DSC results suggest that certain exogenously applied fatty acids can disrupt the stratum corneum lipid structure and increase motional freedom or fluidity of the lipids. It is not clear from these results, however, whether structural differences seen among the various acids are due to their relative disrupting power or simply reflect varied uptake of these compounds by the stratum corneum. Therefore further studies need to be conducted to clarify this.

In summary, we utilized the RuO4 postfixation and lanthanum perfusion methods to evaluate the effects of OA-induced skin penetration enhancement. The results presented here demonstrate that topical application of OA induces stratum corneum lipid structure disorder in vivo. OA may enhance percutaneous penetration mainly through a dual mechanism involving stratum corneum lipid bilayer perturbation and lacunae formation.

REFERENCES