Effects of Prednisolone on the Cutaneous Reaction and Skin Barrier Function in Mice Treated with a Hapten

Yoshifumi Ueda, Toshiro Sone, Naoki Inagaki, and Hiroichi Nagai

*Department of Pharmacology, Gifu Pharmaceutical University; 5–6–1 Mitahoragashi, Gifu 502–8585, Japan; and
Yakult Central Institute for Microbiological Research; 1796 Yaho, Kanitachi, Tokyo 186–8650, Japan.

Received November 1, 2002; accepted January 14, 2003

Glucocorticoids are effective drugs for the treatment of allergic skin diseases. In the present study, we observed the effects of prednisolone on the cutaneous reaction and skin barrier function in mice treated with a hapten, 2,4-dinitrofluorobenzene. Repeated hapten application onto the mouse ear resulted in a potent ear swelling with an elevation of specific serum IgE. The ear swelling appeared following the second application of the hapten and peaked at 24 h after each application. Specific serum IgE was detected first after the fourth hapten application. Topical treatment with prednisolone apparently suppressed the swelling, whereas it failed to affect the serum specific IgE level. The hapten application caused an increase in transepidermal water loss, which was potentially inhibited by prednisolone, although the water content was not affected. Amounts of triglyceride and cholesterol in the ear skin increased after repeated hapten applications, whereas the relative amount of free fatty acid and ceramide diminished. Prednisolone exhibited an inhibitory effect on the changes in lipid content. Thus prednisolone apparently inhibits the alteration of skin barrier function caused by hapten application as well as the cutaneous reaction.

Key words prednisolone; skin barrier function; cutaneous reaction; hapten; transepidermal water loss; skin lipid

The skin lining the outer surface of the body is the biggest organ and plays an important role in maintaining a constant inner environment and acting as a physical barrier to prevent invasion by external organisms and substances. Breakdown of the skin barrier, therefore, facilitates the loss of water and the penetration of pathogenic materials, which may contribute to the onset and/or development of symptoms of the skin disease. Development of such diseases may result in turn in a further impairment of skin barrier.

The skin barrier is constantly maintained by reproduction of epidermal keratinocytes, which undergo differentiation and then move to the surface as interlocking layers of dead stratum corneum cells. Intercellular lipid lamellae are specifically organized between the corneocytes, and this structure is responsible for the low water permeability. It has been reported that in atopic dermatitis patients the skin barrier function is basically impaired, which may be reflected by the reduced ceramide content in the skin. Dry skin observed in atopic dermatitis patients may be the result of impaired skin barrier function.

Glucocorticoid is one of the most effective drugs for the treatment of many skin diseases including atopic dermatitis. However, the effects of glucocorticoid on the skin barrier function have rarely been investigated. In the present study, therefore, we examined the effects of prednisolone on the skin reaction and skin barrier function in a mouse model for contact hypersensitivity.

MATERIALS AND METHODS

Mice Female BALB/c mice, 8 weeks of age, obtained from Japan SLC, Inc. (Hamamatsu, Japan) were used. Experiments were undertaken following guidelines for the care and use of experimental animals made by our university and the Japanese Association for Laboratory Animal Science in 1987.

Induction and Evaluation of Cutaneous Reaction Cutaneous reaction in the mouse ear was caused by painting 2,4-dinitrofluorobenzene (DNFB) onto the ear as reported previously. In brief, 25 μl of 0.15% DNFB (Nacalai Tesque, Kyoto, Japan) acetone–olive oil (3 : 1) solution was applied to both surfaces of both ear lobes once a week for 5 weeks.

Cutaneous reaction caused by this repeated application was evaluated by measuring the thickness of ear lobes just before, and 1, 4 and 24 h after each DNFB application using a micrometer (Peacock Upright Dial Gauge, Ozaki MFG Co., Tokyo, Japan). Blood samples were obtained from orbital sinus of mice before the first DNFB application and 24 h after each application, and dinitrophenyl residue (DNP)-specific IgE levels in the sera were examined by ELISA.

Water Contents and Transepidermal Water Loss of the Mouse Ear Skin Water content and transepidermal water loss (TEWL) of the mouse ear skin were measured 24 h after the fifth DNFB application. The water content of the stratum corneum of the ear skin was measured electrically according to the method described by Tagami et al. with a skin surface hygrometer (Model Skicon-200, IBS, Inc., Hamamatsu, Japan). TEWL was measured with a tewameter (TM210, Courage+Kharazaka Electric GmbH, Germany). The instrument’s probe, which was attached to a rubber sheet with a hole 5 mm in diameter, was placed perpendicular to the mouse ear skin surface and allowed to equilibrate for 30 seconds. All measurements were carried out at 23°C and under a relative humidity of about 60%.

Measurement of Skin Lipids Mouse ear lobes were separated 24 h after the fifth DNFB application. Skin surface and stratum corneum lipids were collected from the mouse ear skin by a gentle rinse with 5 ml of hexane–methanol (2 : 3) in a glass cylinder. The solvent was transferred to a test tube and evaporated to dryness under a stream of nitrogen. Then the lipids were dissolved in 0.2 ml of chloroform–methanol (1 : 1).

The lipids were separated stepwise by chromatord-SIII (Ia-
tron Laboratories, Inc., Tokyo) coated with silica gel, and analyzed quantitatively by thin layer chromatography using a flame ionization detector (TLC-FID, Iatroscan TH-10, Iatron). Components including squalene (SQ), cholesterol ester (CE) and wax (WA) were initially developed by non-polar solvent (hexane–benzene, 1 : 1), and detected by FID excluding the origin of the chromatrod. Other components including triglyceride (TG), free fatty acids (FFA), cholesterol (CH) and phospholipid (PH) were subsequently separated by polar solvent (hexane–diethyl ether–formic acid, 70 : 30 : 1) and detected by scanning the full range of the chromatrod. Ceramide (CER) was separated by another polar solvent (chloroform–methanol–acetic acid, 190 : 9 : 1) and also detected by scanning the full range of the chromatrod. SQ, cholesterol palmitate, myristyl myristate, triolein, stearic acid, CH, phosphatidylcholine and CER types III and IV were purchased from Sigma (St. Louis, MO, U.S.A.) and used as standards.

**Prednisolone Treatment**
Prednisolone (an acetate, aqueous suspension, Shionogi & Co., Ltd., Osaka, Japan) was diluted to make 0.1% and 0.3% preparations in 70% acetone. Ten microliters of prednisolone preparations was applied onto both surfaces of both ear lobes twice a week from the day of the first DNFB application. Mice of the vehicle group (painted with acetone-olive oil without DNFB) and the DNFB group (control, DNFB-painted) were treated with 70% acetone similar to the prednisolone treatment.

**Statistics**
Data were expressed as the mean±S.E.M. Statistical significance of the difference between two experimental groups and among the three experimental groups was evaluated by Student’s t- or Welch’s t-test and by Dunnett multiple comparisons test, respectively, using InStat Program (GraphPad Software, San Diego, CA, U.S.A.). p-Values less than 0.05 were considered to be significant.

**RESULTS**

**Ear Swelling**
Changes in ear thickness upon DNFB application are indicated in Fig. 1. Weekly application onto the ear of mice resulted in a potent ear swelling, which appeared following the second application of the hapten and peaked at 24 h after each application thereafter. The peak response was gradually increased depending on the application time. Topical treatment with both 0.1% and 0.3% preparations of prednisolone significantly inhibited the swelling.

**Serum IgE Levels**
Results of serum specific IgE levels are shown in Fig. 2. Specific IgE was detected first after the fourth application of DNFB. The elevation of serum IgE levels was slightly depressed by the treatment with 0.1% prednisolone, although the change was not statistically significant.

**Water Content and TEWL of the Mouse Ear Skin**
The skin conductance of mouse ear skin was estimated. As shown in Fig. 3, no difference was observed in hydration levels between control mice and vehicle mice. Treatment with prednisolone did not affect the hydration levels significantly. Results of TEWL are shown in Fig. 4. TEWL was apparently elevated after repeated DNFB application, and the elevation was significantly depressed by the treatment with prednisolone.

**Skin Lipid Content**
The absolute amounts of major lipids are shown in Table 1. Repeated applications of DNFB increased the content of TG, CH and others (other polar lipids including monoglyceride, PH and cholesterol sulfate). The increase in TG and CH was significantly inhibited by the treatment with 0.3% prednisolone. Total lipids, a sum of all lipid fractions detected by TLC-FID, also were apparently
increased by the application of DNFB, and the increase was inhibited by 0.3% prednisolone. In contrast, at 0.1% prednisolone, SQ content increased significantly, and the inhibition of increase in total lipid level was not observed.

The relative amounts of major lipids are shown in Table 2. Proportions of TG, CH and others were increased and those of SQ, CE, WA, FFA and CER were decreased by the DNFB application. In most of these lipids, prednisolone attenuated the alteration, and its effect was significant in cases of SQ, FFA, CH and CER.

DISCUSSION

In the present study, we found that repeated hapten application causes alterations of skin lipid contents and skin barrier functions as well as cutaneous swelling in mice. Prednisolone was found to inhibit not only the cutaneous swelling but also the alterations of skin lipid contents and skin barrier functions.

Repeated application of DNFB onto the mouse ear causes the ear to swell accompanied by the rise of serum specific IgE levels. Thinning of epidermis, formation of scabs, and infiltration of abundant inflammatory cells are also induced. Repeated DNFB application induces IFN-γ mRNA expression in the ear, which may participate in the formation of a delayed type of ear swelling. In contrast, the same treatment results in the induction of IL-4 mRNA expression in the cervical lymph nodes, which may facilitate the IgE production. In this study, prednisolone clearly inhibited the ear swelling caused by DNFB application without affecting serum specific IgE levels. Therefore, topically applied prednisolone may inhibit IFN-γ induction locally, but may not affect the IL-4 expression in the cervical lymph nodes. The vascular effect of prednisolone may also participate in its inhibition of swelling.

The epidermal permeability barrier is made up of highly hydrophobic lipids, which form multiple membrane bilayers in the outer corneified cell layers. Patients with atopic dermatitis often exhibit dry skin that may correlate with a defect in the epidermal barrier structure. A decrease in the relative amount of ceramides in the stratum corneum of atopic dermatitis patients has been reported, and the absolute amount of ceramides has also been reported to be reduced in atopic dermatitis patients. In the present results, TEWL after repeated applications of DNFB rose significantly, suggesting the presence of a defect in the epidermal barrier structure. Furthermore, the relative amount of ceramide was significantly reduced in DNFB-painted mice in comparison to vehicle mice, although there was no difference in the absolute amount of ceramides between the two groups. These results indicate that repeated applications of DNFB could cause a skin lesion with some characteristics of atopic dermatitis. Treatment with prednisolone at a concentration of 0.3% apparently inhibited both the rise in TEWL and the decrease in relative amount of ceramide.

In contrast to the increase in TEWL, we found no apparent alteration in the hydration level. The water content of the stratum corneum may depend on a dynamic balance between the water supply and its loss; the relative humidity of the environment may also affect it. To elucidate the relationship between the water content and TEWL, additional experiments must be made.

We evaluated 8 fractions of skin lipids in the present study and found that some of them increased and some decreased after repeated applications of DNFB. The total lipid level was markedly increased, accompanied by a significant increase in absolute amount of triglyceride. The relative amount of triglyceride was also increased. Triglyceride is synthesized in the sebaceous gland and the synthesis is known to increase in thickened skin such as hyperkeratosis. One of the gross pathological changes in the ear skin of DNFB-painted mice is thickening of the epidermis. The in-
creased synthesis of triglyceride in DNFB-painted mice may be due to lipase suppression and may coincide well with the decreased proportion of free fatty acid. On the other hand, a wide variety of hydrolytic enzyme activities related to sterol esterification have been demonstrated in the stratum corneum, and these activities were reduced in psoriasis, ichthyosis and atopic dermatitis patients.\textsuperscript{18,19} In the present results, the absolute amount of cholesterol ester did not change in spite of a significant increase in cholesterol content in DNFB-painted mice. It can be suggested, therefore, that the esterification is relatively reduced. Although the proportion of ceramide was lowered in DNFB-painted mice, there was no difference in the absolute amount of ceramide. Present data suggest that the defect in the epidermal barrier may correlate not only with the decreased relative amount of ceramide but also with alterations of the other lipids. Most of the alterations observed in lipid contents were attenuated by the treatment with prednisolone at a concentration of 0.3%, and the effects of this drug may contribute to the inhibition of disturbed skin barrier function. In contrast, the results of 0.1% prednisolone showed a similar tendency to those of the 0.3% preparation except for squalene content and total lipid amount. The differences remain to be examined.

REFERENCES