Skin barrier defect in atopic dermatitis: increased permeability of the stratum corneum using dimethyl sulfoxide and theophylline

Takashi Yoshiike, Yosuke Aikawa, Jirot Sindhvananda*, Hajime Suto, Kumiko Nishimura, Tomoe Kawamoto and Hideoki Ogawa

Department of Dermatology, Juntendo University School of Medicine, Tokyo, Japan

(Received 12 November 1992; accepted 10 December 1992)

Key words: Penetration; Absorption; Permeability; Stratum corneum; Atopic dermatitis; Dimethyl sulfoxide; Theophylline

Abstract

The existence of a defect in the skin barrier of patients with atopic dermatitis (AD) was demonstrated and its importance in the pathogenesis of AD was emphasized. In order to evaluate the penetration properties of the stratum corneum of AD patients, the in vivo skin response to the penetration of dimethyl sulfoxide (DMSO) and in vitro response to the penetration of theophylline utilizing a diffusion chamber were studied. Both methods demonstrated an increasing level of penetration through the epidermal stratum corneum, with greatest penetration being evident with lesional skin, followed by AD non-lesional and then the normal control. However, statistical significances existed only between non-lesional and lesional skins in the case of the DMSO test, and between the normal control and non-lesional skin in the case of the diffusion chamber analysis using theophylline. Increased penetration of a non-specific nature is important in the pathogenesis of AD.

Introduction

The pathogenesis of atopic dermatitis (AD) is still very much a mystery today. There is no doubt that immunological abnormalities or ‘allergies’ play a main role in the pathogenesis. However, a considerably wide clinical spectrum, production of antibodies and sensitization to numerous antigens or haptens in the disease suggests a possible mucocutaneous barrier dysfunction in atopic diseases such as AD, bronchial asthma and allergic rhinoconjunctivitis. Furthermore, barrier dysfunction readily allows penetration of multiple antigens or haptens via mucocutaneous routes. Ogawa [1] hypothesized and emphasized the importance of barrier dysfunction in pathogenesis of AD and the ‘vicious circle’ of mucocutaneous barrier defect and allergic inflammation.

Previous morphological, pharmacophysiological and biochemical studies supported this
hypothesis. Werner et al. quantitatively demonstrated roughness of skin surface of dry skin in atopic dermatitis using computer-based scanning electron microscopic profilometry [2]. Watanabe et al. characterized the functional properties of the superficial stratum corneum in atopic xerosis [3], showing markedly higher transepidermal water loss and lower water holding capacity of stratum corneum in patients with AD. In addition, the patients had a higher transepidermal water loss following irritant exposure, indicating that the susceptibility to irritants in AD patients was closely related with breakdown of barrier function in stratum corneum [4].

Biochemical defects of stratum corneum of AD patients have been studied mainly in lipids, since they serve as a permeability barrier by forming a multi-lammellar structure in the stratum corneum ceramides, particularly ceramide 1 [5] as well as other abnormalities in lipid fractions [6]. The membrane-coating granules, the major lipid source into the extracellular space of corneocytes, was significantly larger in the dry non-lesional skin in AD, indicating a disturbance of the maturation of these granules [7].

Deteriorated barrier function of mucous membrane surfaces is another important issue. Following the ingestion of egg or milk, greater amounts of protein antigen appear in the circulation of subjects with AD [8]. Possible explanations for such a phenomenon include either increased intestinal permeability or maldigestion. Increased permeability of gut wall has been reported [9,10], but is still controversial [11].

In order to clarify enhanced penetration of multiple antigens or haptens due to skin barrier dysfunction in AD, the in vivo skin response to the penetration of dimethyl sulfoxide (DMSO) skin response and theophylline passing through the stratum corneum, respectively. They have given informed consent. Diagnosis of AD was based upon the criteria of Hanifin and Rajka [12]. The tested skin had not been treated with any ointment after bathing 24 h previously. Twenty and 15 sex- and age-matched non-atopic healthy subjects were similarly examined as controls of DMSO and theophylline studies, respectively.

For statistical evaluation, Student's t-test and paired t-test were used for the estimation of significance.

**Skin response to DMSO**

The wealing response of the skin to DMSO was quantified [13]. Briefly, 95% dimethyl sulfoxide (DMSO, Wako Chemical Co., Tokyo) was applied on lesional or non-lesional skin of forearms using a plastic block in which holes of 6 mm diameter have been drilled and which was smeared with silicone grease and firmly held with adhesive tape. The exposure time was 5 min. The fluid was then aspirated and the site dried with a cotton-tipped applicator. The weal and flare were graded separately 1 min after aspirating the DMSO as described by Frosch et al. [13], namely, 0: no weals; 1+: discrete follicular weals; 2+: mainly follicular with some merging; 3+: solid weal but only slightly elevated; 4+: solid, tense weal.

**Theophylline passing through stratum corneum**

Epidermal stratum corneum sheets were obtained from lesional or non-lesional skin of inner aspects of overarms using the cantharidin blister technique described by Kligman and Christophers [14]. Briefly, 1%
cantharidin (Sigma Chemical Co., St. Louis, MO) in acetone was applied on skin using a disk paper (6 mm in diameter) and Finn chamber. Twenty-four hours later, the turgid clear blister was cut away with iris scissors. Debridement of the loosely adherent epithelium was accomplished by firm rubbing with a cotton-tipped applicator. It was then dried and stored at room temperature until use.

The stratum corneum sheet was applied to a diffusion chamber which was prepared by modification of a Boyden chamber. The volume and diameter of upper and lower chambers were 200 μl and 4 mm, respectively. After filling to the lower chamber with n-octanol (Wako Chemical Co.), stratum corneum sheet immersed in the same solvent was placed in contact with it. The upper chamber was tightly screwed and 1 mg/ml theophylline in n-octanol was added. The receptor phases of the diffusion chambers were maintained at 25°C with stirring. Two hours later, the upper chambers were aspirated, washed with methanol once, and then the stratum corneum sheets were removed. Aliquots (100 μl) from the lower chambers were added into 1 ml of methanol and absorbance at 270 nm was measured by UV spectrophotometer. Theophylline concentrations in the lower chambers were determined by a standard curve using A270 of different contents in the same solvent.

Results

Skin response to DMSO

The mean and S.E.M. of weal grades in AD lesional, non-lesional and normal control skin were 2.87 ± 0.21, 0.74 ± 0.18 and 0.62 ± 0.23, respectively (Fig. 1.). Weal grades were significantly higher in AD lesions than in non-lesions or normal controls (P < 0.001), although there was no statistically significant difference between non-lesions and normal controls.

Theophylline passing through stratum corneum

Theophylline concentrations in lower chambers were increased in a linear fashion up to 6 h (data not shown). Absorbance at 270 nm by dissolving of stratum corneum itself in n-octanol was negligible. The mean and S.E.M. of theophylline passing rates at 2 h in AD lesional, non-lesional and normal control

![Fig. 1. Response to DMSO. Mean (column) and S.E.M. (bar) of weal grades in AD lesional (lesion +), non-lesional (lesion −) and normal control (normal) skins.](image1)

![Fig. 2. Theophylline diffusion rates of stratum corneum sheets from AD lesional (lesional +), non-lesional (lesional −) and normal control (normal) skins. Mean, column; S.E.M., bar.](image2)
skin were 12.5 ± 2.5, 9.8 ± 2.7 and 4.9 ± 1. 4 μg/ml per h, respectively (Fig. 2). It was significantly higher in AD lesional and even non-lesional skin than in normal control skin. There was no statistical difference between AD lesions and non-lesions.

Discussion

The occurrence of skin barrier dysfunction in AD was demonstrated in this study. Previous morphological, pharmacophysiological and biochemical studies [2–7] supported this finding. In order to evaluate the penetration properties of the stratum corneum of AD patients, simple in vivo and in vitro methods were employed in this study. In AD non-lesional and lesional skins, both methods demonstrated an increasing absorption or penetration through epidermal stratum corneum compared to normal controls. However, statistical significances existed between non-lesional and lesional skins by DMSO test, and between normal control and non-lesional skin by diffusion chamber analysis using theophylline. It may be explained by the different natures of these in vivo and in vitro tests. The DMSO test is a simple, quick way to assess the barrier function of the stratum corneum [13]. The intensity of the reaction was dependent mainly on thickness and integrity of the horny layer [13]. However, it may also reflect the vascular reactivity or mast cell releasibility of AD skin, since the assessment was carried out by the weal response in this test.

The theophylline passing test using a diffusion chamber reflects the penetration properties of the stratum corneum more precisely, because isolated stratum corneum sheet was analyzed in vitro. Experimental conditions were based upon the results of solubility parameters of drug and vehicle to predict flux through skin reported by Sloan et al. [15]. Although detailed analyses for diffusion coefficients was impossible due to limitation of sample sizes, a modified method in this study was found to be a rather simple and reliable technique to assess the penetration through the stratum corneum. It is of interest that penetration of theophylline was significantly increased even in non-lesional skin of AD patients, supporting the hypothesis that skin barrier defects is an important factor in the pathogenesis of AD. Even though it may be true that in normal looking skin, the injuries sustained by persistent scratching and eczematous dermatitis result in a substantial proportion of the epidermal changes [3], increased penetration of non-specific nature is still important in the pathogenesis of AD and could be an explanation for polyclonal IgE production against a variety of antigens.

References

8 Paganelli R, Atherton DJ, Levinsky RJ: Differences between normal and milk allergic subjects in their immune


