

Modulation of Cutaneous SP Receptors in Atopic Dermatitis after UVA Irradiation

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Atopic dermatitis is a pruritic inflammatory skin disorder, involving immunological and non-immunological factors. Substance P seems to be involved in the pathogenesis of atopic dermatitis. Substance P-containing nerve fibers are increased in the lesional skin of patients with atopic dermatitis and a reduced weal and flare reaction to intradermal injection of substance P has been observed. We investigated the distribution of substance P receptors in the involved skin of patients before and after single or repetitive UVA irradiations. Our results indicate that substance P receptors of the NK-1 subtype are expressed on blood vessels and on epidermal keratinocytes of involved skin of patients with atopic dermatitis. UVA irradiations did not modify the epidermal distribution of substance P receptors but decreased their expression intensity on blood vessels. UVA irradiations seem to decrease skin inflammation through the modulation of NK-1 receptor expression on endothelial cells. Key words: substance P; neuropeptides; photobiology; phototherapy.

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Atopic dermatitis or neurodermatitis is a pruritic inflammatory and chronically relapsing skin disorder, involving genetic, immunological, psychogenic and climatic factors (1). Some of the physiological alterations involve immunological mechanisms. Decreased delayed hypersensitivity and altered reactivity to histamine have been demonstrated. The IgE-dependent reactivity explains most of the respiratory manifestations often associated with atopic dermatitis. Non-immunological factors may also alterate skin reactivity. These factors include emotional stress, sweating, scratching and irritants (2). Current treatments for atopic dermatitis consist in a combination of local corticosteroids, emollients and phototherapy.

Neuropeptides (NP) are mediators of neurogenic inflammation involved in weal and flare reactions and play a role in immediate and delayed type hypersensitivities, suggesting their possible involvement in the pathomechanisms of atopic dermatitis (3, 4). Substance P (SP) seems to be especially implicated, since an increased number of SP immunoreactive nerve fibres (5) concomitant with a decrease of SP cutaneous levels (6) were observed in the lesional skin of atopic dermatitis. Furthermore, a skin hyporesponsiveness to intradermal injection of SP was demonstrated in atopic dermatitis (7).

SP receptor (SPR) expression in normal non-atopic skin has been described as generalized in the whole epidermis (8) or only in the upper epidermis (unpublished results) and focal in the dermis (8). The aim of the study was to investigate the distribution patterns of SPR in the involved skin of atopic dermatitis patients. As UV irradiation is a common therapy

for atopic dermatitis (1), we studied the modification of SPR expression after repetitive UVA irradiation.

PATIENTS AND METHODS

UV irradiation

An involved skin area of 6 patients with atopic dermatitis was UVA-irradiated with an intensity of 10 J/cm², five times a week, for 3 consecutive weeks (total intensity for 15 irradiations: 150 J/cm²; source: Waldmann 7001 K, 27 × 85/100 W, λ = 320–400 nm).

Skin biopsies

For each patient, three punch biopsies (4 mm) were taken: one before irradiation, the second 24 h after the first irradiation (10 J/cm²) and the third 72 h after the last irradiation. Skin biopsies were immediately frozen in liquid nitrogen and kept at –70°C until use. They were sectioned in a cryostat at 4 μm.

Substance P binding

Four-μm skin sections were incubated with 10^{–6} M of NTE-biotinyl-[Arg³]-substance P (SPB) (Peninsula Laboratories Inc., Belmont, USA) in phosphate-buffered saline (PBS) at 4°C overnight in a humidified chamber. After washing with PBS, the slides were fixed in cold acetone for 10 min at –20°C and air-dried. Sections were incubated with streptavidin-horseradish peroxidase (Dako) for 30 min at room temperature. After washing with PBS, the reaction was developed in aminoethylcarbazol (Dako) for 20 min. Sections were washed and counterstained with Mayer hematoxylin and mounted in glycerol medium (Dako).

The specificity of the reaction was checked by the absence of staining in sections incubated with PBS or unlabelled SP instead of SPB. Sections were also incubated with SPB added to a 1000-fold excess of SP or NK-1 antagonist, spantide II (ICN, France).

Slides were examined on a light microscope (Zeiss).

RESULTS

Expression of SPR in involved skin of atopic patients

As demonstrated by a strong peroxidase activity, cells of dermal blood vessels bound SPB (Fig. 2A). In the epidermis, binding of SPB was observed on the granular cell layer. The keratinocytes of the other epidermal layers were not stained in any of the samples studied. As shown in Fig. 1A, staining of moderate intensity was localized on the cell surface of keratinocytes. Cytoplasmic binding was also observed for a minority of cells.

Effects of UVA irradiation

In all patients, pruritus, erythema and infiltration were dramatically decreased after 3 weeks of UVA irradiation.

In the epidermis, no differences in the intensity or the distribution of SPB were observed after a single irradiation or after fifteen consecutive irradiations (Fig. 1B).

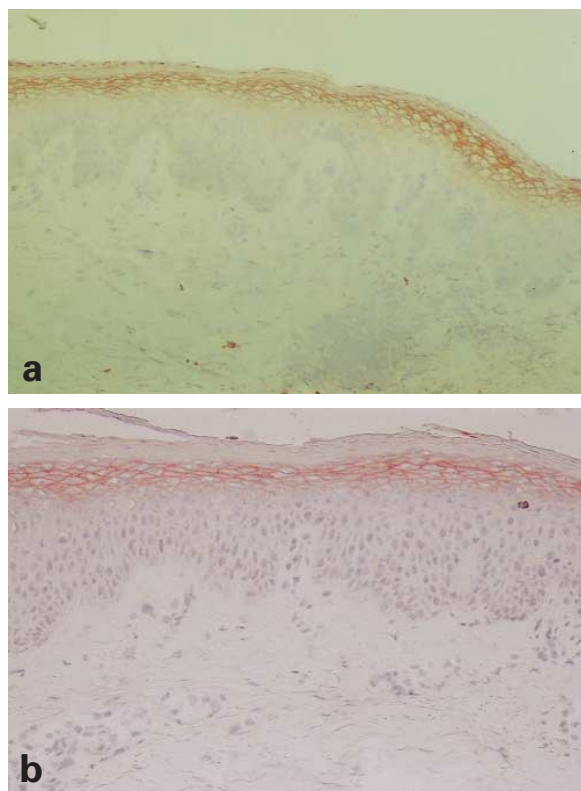


Fig. 1. Binding with biotinylated substance P in the involved skin of patients with atopic dermatitis before (A) and after UVA irradiation (150 J/cm^2) (B) (magnification: $\times 100$).

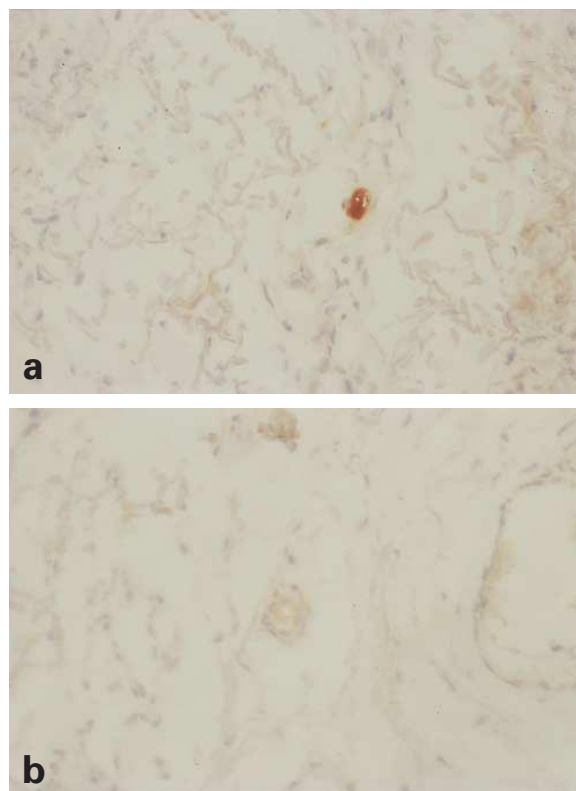


Fig. 2. Binding with biotinylated substance P on endothelial cells of patients with atopic dermatitis before (A) and after UVA irradiation (150 J/cm^2) (B) (magnification: $\times 400$).

In the dermis, the intensity of SPB binding on endothelial cells was strongly decreased after UVA irradiation of both 10 J/cm^2 and 150 J/cm^2 (Fig. 2B). The intensity of SPB binding was not proportional to the irradiation intensity.

The SPB-binding structures were not stained when skin sections were incubated without SPB, with unlabelled SP, or with SPB added to a 1000-fold excess SP receptor antagonist, assessing the specificity of the binding.

DISCUSSION

We demonstrated the presence of SPR in the skin of atopic patients. Our results indicate that SPR are expressed on blood vessels and on epidermal keratinocytes of involved skin of atopic patients. Concerning the sites of SPR expression, similar data are observed in normal skin (8). UVA irradiations did not modify the distribution of SPR but decreased the expression intensity of SPR on blood vessels in atopic dermatitis.

The moderate expression of SPR observed on granular keratinocytes of non-irradiated patients with atopic dermatitis was not modified after repetitive UVA irradiations. This localization was similar to those usually observed in the skin of normal patients (9). The level of expression seemed to be similar in atopic dermatitis and normal skin but binding *in situ* is not an adequate technique to affirm this.

Our findings indicate that SPR on endothelial cells in the

involved skin of atopic dermatitis patients are markedly decreased after single or repetitive UVA irradiations. Interestingly, the number of SP-positive nerves around blood vessels were strongly increased in the skin of atopic dermatitis patients, when compared with normal controls (5, 10, 11). We showed that similarly to non-atopic human skin, SPR of the NK-1 subtype are also expressed on endothelial cells (12, 13).

The activation of SPR leads to the expression of the adhesion molecule E-selectin by endothelial cells (14–16). E-selectin is implicated in the adhesion of leukocytes to endothelial cells and thus in the development of the inflammatory reaction. Furthermore, binding of SP to endothelial cells induces vasodilatation and vasopermeation (12). These effects may be responsible for the erythema and oedema observed in atopic dermatitis.

The observed decrease in SPB binding to blood vessels following UVA irradiations could be due to a direct effect of UVA on SPR expression, or to a chemical modification of the SP-binding sites. An occupation of SPR by release of increased endogenous SP after UVA irradiation is unlikely, as the skin inflammation was clinically improved. In contrast, exposure to the shorter wave-length erythemogenic UVB irradiation increases the SP level in rat skin nerve fibers (17).

In conclusion, our study indicates that SPR are present in the skin of atopic patients on both keratinocytes and endothelial cells, as in normal skin. The improvement after UVA irradiation observed in atopic patients may be due to an effect

of UVA irradiation on the expression of SPR on endothelial cells. A study of the effects of UVA irradiation on normal human skin would be interesting.

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