Original paper

Does the corticotropin-releasing hormone system play a role in the pathogenesis of lichen planus?

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Abstract

Introduction: Lichen planus (LP) is a chronic inflammatory disease, where the psychogenic factors seem to play an important role in the pathogenesis.
Aim: To determine the expression of corticotropin-releasing hormone (CRH) receptor type 1 (CRH-R1) in LP.
Material and methods: Thirty-two LP patients and 17 age/gender-matched controls were included in the study. Detailed information about the disease and body surface area (BSA) covered by the lesions was recorded. Immunohistochemically, the expression of CRH-R1 was stained in the lesional skin of patients with LP and in the control group.
Results: The comparison of CRH-R1 expression showed a statistically significant difference between LP patients and the controls ($p < 0.05$). Additionally, we did not observe any correlation between BSA and staining intensity in LP patients.
Conclusions: Our study showed an increase in CRH-R1 expression in LP lesions. These results support the participation of the cutaneous CRH/CRH-R1 system in the pathogenesis of LP skin lesions.

Key words: corticotropin-releasing hormone, hypothalamo-hypophyseal system, lichen planus, receptors, skin.

Introduction

Lichen planus (LP) is an immune-mediated, chronic inflammatory disease that appears in 0.1% to 4% of the general population [1]. Although the exact etiology of LP is unclear, the evidence points to LP being a T-cell-mediated autoimmune inflammatory disorder [2]. CD8+ T lymphocytes are the major cell type seen in the dermal inflammatory infiltrate in LP; furthermore it is thought that these cells cause basal keratinocyte degeneration. There are differing opinions regarding the ethiopathogenesis of LP, some of them correlating stress involvement with the onset and extension of the disease [3].

Stress typically results in the release of corticotropin-releasing hormone (CRH) from the hypothalamus and regulates the hypothalamic–pituitary–adrenal (HPA) axis through activation of CRH receptor type 1 (CRH-R1) [4]. Recently in literature it has been reported that endocrine stress responses are not only under control of the central nervous system, but also occur in peripheral tissue, outside of the classical HPA axis [5]. The skin also contains the main components of a functional equivalent of the HPA axis [5, 6]. Cutaneous CRH is believed to regulate various functions of the skin that are important for local homeostasis [6, 7]. Many reports focus on the pro-inflammation role of CRH, such as CRH-induced activation of mast cells in stress-related exacerbation of cutaneous inflammatory diseases [8].

The CRH expressions were found in the inflamed tissues of patients with autoimmune and inflammatory diseases. For instance, inflammatory arthritis, psoriasis, vitiligo, alopecia areata, acne, seborrhea, androgenetic alopecia, age-associated skin xerosis, cutaneous lupus erythematosus, melanoma, and other skin cancers [9, 10]. To date in the literature, CRH expression has not been studied on the skin of LP patients.

Aim

Hereby, we have evaluated and compared the expression of the CRH/CRH-R1 system in the involved skin of LP patients and the normal skin of controls.

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Material and methods

Patients

Thirty-seven patients with clinically and histopathologically confirmed LP and seventeen healthy controls, age- and gender-matched with LP patients, were included in the study. The study protocol was approved by the local ethics committee, and the participants gave written consent. Subjects were selected based on the following criteria: age over eighteen years old, no history of other major physical or psychiatric illness, and no history of any regular systemic therapy or any systemic therapy for at least 3 months as well as any topical therapy for at least 1 month for LP prior to the study. Healthy controls with no history of skin or psychiatric disorders were also included in the study.

The first author of the study examined the subjects dermatologically, and the LP was confirmed by pathological biopsy prior to recruitment in the study. Detailed information about duration of the disease, clinical presentation, oral and nail manifestations, and body surface area (BSA) covered by the LP lesions were also recorded. The expression of CRH-R1 was stained in the lesional skin of patients with LP and in the control group.

Immunohistochemical staining

Immunohistochemically, the expression of CRH-R1 was investigated in the lichenoid lesions of the LP group and in the skin of the control group. For standard histology, the specimens were fixed in 10% formalin and processed for hematoxylin and eosin staining. For immunohistochemical staining, 4-µm sections were cut after which the slides were heated in oven for 60 min at 60°C. For dewaxing, two Xylene washes (10 min each) followed by two 96% ethyl alcohol rinses (5 min each) and followed by a wash for 3 min with tap water and for 5 min by distilled water, we used the antigen-retrieval method with exposure to microwave oven heat for 20 min at 60°C and allowed them to cool down for 5 min. Sections were immersed for 5 min in a 3% hydrogen peroxidase solution for quenching of any endogenous peroxidases and then washed in distilled water. Non-specific protein binding was blocked for 2 min. CRH-R1 rabbit polyclonal antibody (LifeSpan Biosciences (Catalog Number: LS-A6603), Inc, Seattle, USA) was incubated for 1 h with 1 : 200 dilution protein concentration in the humidified chamber. A rabbit IgG without known specificity was used at the same concentration as the specific Ab in order to control non-specific staining with anti-CRHR1 Ab. After the slides were washed with two phosphate buffered saline (PBS) (5 min each), the sections were incubated for 10 min with biotinylated secondary antibody. Tissue sections were washed with PBS for 5 min and then incubated with peroxidase-conjugated streptavidin complex for 10 min. The chromogenic substrate 3,3'-diaminobenzidine was used for the incubation of the sections for 5 min. Formalin-fixed and paraffin-embedded normal placenta samples were used as the positive control. In addition, formalin-fixed and paraffin-embedded breast samples were used as the negative control.

Sections were evaluated under a light microscope by a pathologist blinded to the groups. The staining results for the CRH-R1 expression of the epidermis, adnexal structures, and perivascular inflammatory infiltrate were evaluated. Positive staining cells were scored for intensity and percentage in the epidermis, adnexal structures, and perivascular inflammatory infiltrate. For staining intensity, the following four-point scale was used: grade 0 – no staining; grade 1 – mild staining; grade 2 – moderate staining; grade 3 – strong staining. The percentage of CRH-R1 expression was defined as the percentage of positive staining cells; grade 0 for negative, grade 1 for 1–33% positive, grade 2 for 34–66% positive, and grade 3 for 67–100% positive [11].

Statistical analysis

The Statistical Package for the Social Sciences version 16.0 (SPSS, Chicago, IL, USA) was used to perform statistical analyses. Differences in the CRH-R1 expression results between the LP patients, the control group, and the LP patients with and without oral involvement were tested with the χ^2 test. The Kruskal-Wallis test was used to compare the disease duration and BSA in the different CRH-R1 expression groups.

Results

Thirty seven patients (20 males and 17 females) were included. The mean age was 47.81 ±11.59 years (range: 19–80 years). The main clinical characteristics are presented in Table 1. All healthy biopsied volunteers were found to have microscopically and pathologically normal skin.

Table 2 represents trends of cytoplasmic CRH-R1 expression, which is statistically lower in the normal control group when compared with LP patients ($p < 0.05$). CRH-R1 expression was found to be significantly increased in the epidermis, adnexal structure, and perivascular infiltrate of LP patients ($p < 0.05$). The LP group showed grade 2 or grade 3 staining of CRH-R1 on both adnexal structures and perivascular inflammatory infiltrate. The ex-

Table 1. The main clinical characteristics of all groups

Parameter	Control	LP
Gender (F/M)	10/7	20/17
Age, mean ± SD [years]	46.53 ±13.91	47.81 ±11.59
Duration of disease, mean ± SD [month]		29.99 ±56.82
BSA, mean ± SD		21.57 ±17.98
Oral involment (Y/N)		24/13

Table 2. Immunoreactivity of CRH-R1 in controls and LP patients

Variable		Lichen planus	Control	χ^2 Test statistics	*P*-value
ISE	0	4	12	23.012	< 0.001
	+	14	5		
	++	14	0		
	+++	5	0		
ISAS	0	5	6	9.326	0.025
	+	7	7		
	++	15	3		
	+++	10	1		
ISPI	0	1	16	45.274	< 0.001
	+	16	1		
	++	19	0		
	+++	1	0		

ISE – intensity of staining of epidermis, ISAS – intensity of staining of the adnexal structure, ISPI – intensity of staining of perivascular inflammatory infiltrate. Intensity of staining: grade 0 – no staining, grade 1 (+) – mild staining, grade 2 (++) – moderate staining, grade 3 (+++) – strong staining. P < 0.05 is significant.

pression of the CRH-R1 was generally grade 0 or grade 1 on the epidermis, adnexal structures, and perivascular inflammatory infiltrate in the control group (Figures 1 A–H).

In patients with LP, there was no correlation between the BSA and expressions of CRH-R1 in the epidermis, the adnexal structure, and the perivascular infiltrate ($p > 0.05$) (Table 3). In addition, there was no relationship between the expression of CRH-R1 and age, duration of the disease, gender, and oral involvement ($p > 0.05$).

Table 3. Correlation between the BSA and expressions of CRH-R1

Variable		Mean ± SD	*P*-value[1]
ISE	0	29.50 ±11.78	0.251
	+	22.36 ±16.85	
	++	17.64 ±20.07	
	+++	24.00 ±13.86	
ISAS	0	10.80 ±6.05	0.255
	+	25.57 ±14.99	
	++	23.87 ±17.81	
	+++	20.70 ±20.83	
ISPI	0	18.00 ±0.00	0.502
	+	23.38 ±16.10	
	++	20.05 ±19.69	
	+++	25.00 ±0.00	

ISE – intensity of staining of epidermis, ISAS – intensity of staining of the adnexal structure, ISPI – intensity of staining of perivascular inflammatory infiltrate. Intensity of staining: grade 0, no staining; grade 1 (+), mild staining; grade 2 (++), moderate staining; grade 3 (+++), strong staining. [1]P-values are calculated by using mean ranks of each group.

Discussion

It is known that LP is a T-cell-mediated autoimmune disease; however, its etiology remains unknown [2]. It is thought that the disease is related to an immune response resulting from autoantigens due to the alteration of cell-mediated immunity triggered by endogenous or exogenous factors [12, 13]. The autoantigens induce the activation of CD4+ T and CD8+ T lymphocytes as well as production of cytokines such as interleukin 1 (IL-1), IL-8, IL-10, IL-12, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ) [12]. These cytokines increase the expression of intercellular adhesion molecules (ICAM-1) on Langerhans cells and macrophages, leading to the presentation of major histocompatibility complex antigens by means of keratinocytes. Finally, this altered immune response results in apoptosis of keratinocytes in the basal layer and may determine disease activity [13]. Different mechanisms that may also be involved in the ethiopathogenesis of the LP are mast cell degranulation and the activation of matrix metalloproteinases [14]. Furthermore, some authors believe that the chronicity of LP can be partly explained by a deficiency in the mechanisms of immunosuppression mediated by transforming growth factor β [13]. However, the exact mechanisms that lead to the onset and chronicity of the process have not yet been fully clarified.

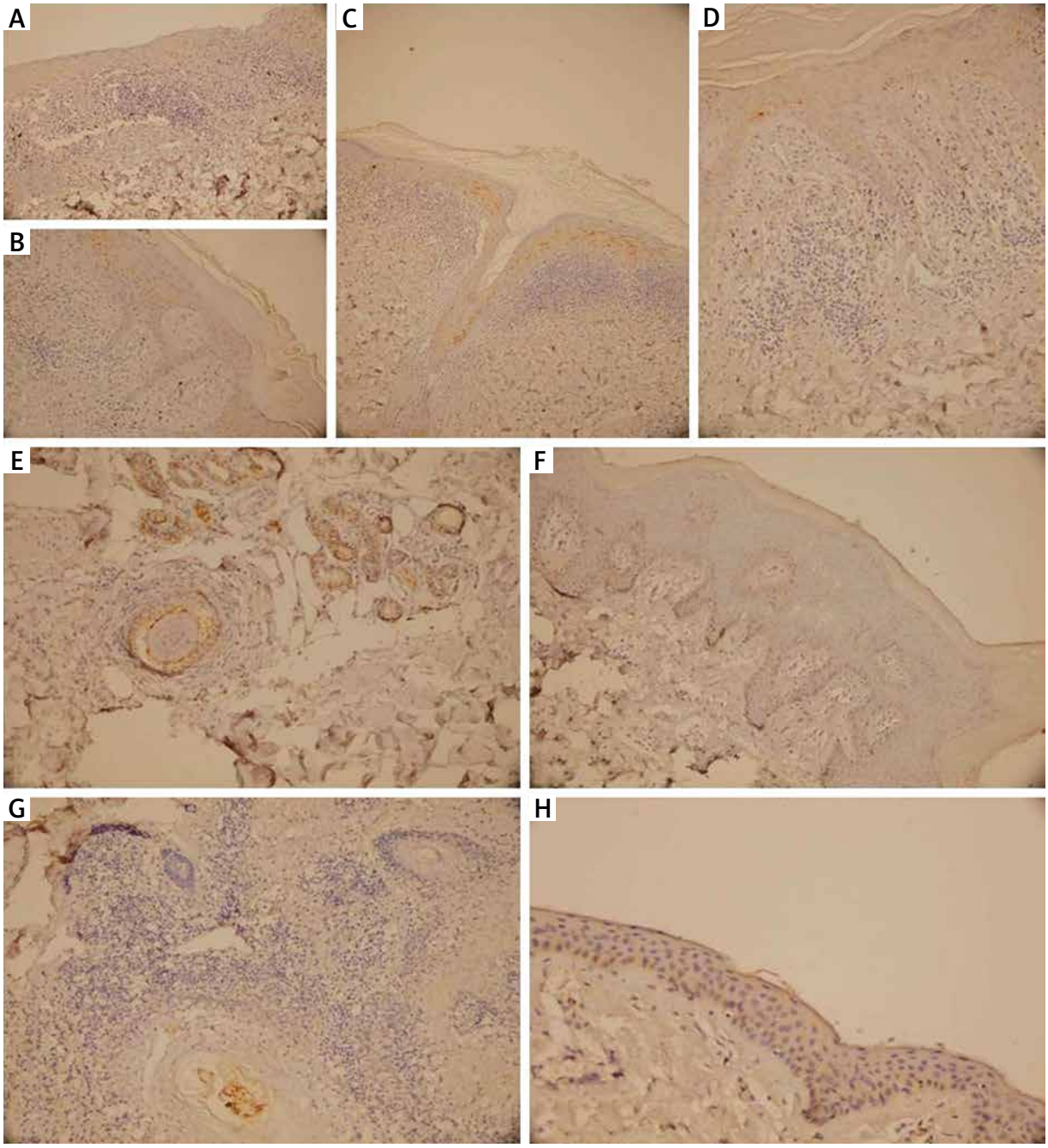

Figure 1. Sections showing immunohistochemical staining of LP patients for CRH-R1 (200×): **A –** epidermal intensity of staining (grade 1) and percentage (grade 2), intensity of staining and percentage of perivascular inflammatory infiltrate (grade 2), **B –** epidermal intensity of staining (grade 2) and percentage (grade 2), **C –** epidermal intensity of staining (grade 2) and percentage (grade 2), intensity of staining of perivascular inflammatory infiltrate (grade 2), **D –** epidermal intensity of staining (grade 3) and percentage (grade 1), **E –** intensity of staining (grade 3) and percentage (grade 3) of the adnexal structure. Sections demonstrating immunohistochemical staining of the control patients for CRH-R1 (200×), **F –** epidermal intensity of staining (grade 0) and percentage (grade 0), **G –** intensity of staining (grade 0) and percentage (grade 0) of perivascular inflammatory infiltrate and intensity of staining of the adnexal structure (grade 0), **H –** epidermal intensity of staining (grade 1) and percentage (grade 1)

The role of psychological disorders, such as depression, anxiety, and stress in the ethiopathogenesis of the LP was investigated in the patients with LP in certain studies. However, these studies produced controversial results. Some authors described more frequent occurrences or exacerbation of lesions during periods of greater emotional

tension [3, 15], while others did not find any association between LP and presence of psychological alterations [16].

The skin expresses an equivalent of the prominent HPA stress axis that may act as a cutaneous defense system, operating as a coordinator and executor of local responses to stress, in addition to its normal function in preserving homeostasis [17]. Slominski *et al.* reported that the CRH-based homeostatic response system represents an evolutionary preservation of an ancestral stress response mechanism since CRH acts as a pleiotropic cytokine in the skin [18].

In the literature, many authors have reported that CRH and CRH-Rs are expressed in the skin and have certain local functions [5, 6, 19]. The biological actions of CRH are mediated with CRH-Rs, which may be a central element [6]. CRH-R1 is the major receptor in the epidermis and dermis [18]. Recently, research has shown that the CRH peptide family has another local function in the skin through paracrine or autocrine mechanisms [18]. For example, CRH-R1 activation stimulates diverse signal transduction pathways, which leads to the regulation of differentiation, proliferation, apoptosis, and immune activities of skin cells [7, 20]. Mitsuma *et al.* reported that CRH induces the proliferation of keratinocytes via interaction with CRH receptors [21], which may indicate the possible correlation of the proliferation of keratinocytes and the degree of stress. Disorganization in differentiation and proliferation of keratinocytes might result in an abnormal barrier function of the skin [5].

The CRH also shows biological effects such as regulation of adhesion molecules and cytokines [20, 22]. Also, recently in the literature, it has been found that human mast cells were particularly rich in both CRH and the structurally related peptide urocortin [23]. Furthermore, human mast cells were able to express multiple CRH receptor isoforms, which suggests autocrine actions of CRH [24]. The ability of CRH to activate mast cells may explain its proinflammatory actions and the pathophysiology of certain skin conditions, which are precipitated or exacerbated by stress, such as atopic dermatitis, eczema, psoriasis, and urticaria [25].

Previous studies revealed the functional role of the CRH/CRH-R1 system in pathological human skin conditions [7, 11]. Cemil *et al.* reported the expression of the CRH-R1 in psoriatic skin and its correlation with PASI scores [11]. In the present study, for the first time we evaluated the protein expression of CRH-R1 in the lesions of LP patients by immunohistochemistry and found the expression of CRH-R1 to be higher in these lesions than in the normal control skin. Presumably, the peripheral functions of cutaneous CRH/CRHR1 are regulated by the way of a localized circuit, and abnormal secretion of CRH/CRH-R1 in the skin due to stress disturbs the local homeostasis. Following this pathological process, abnormal differentiation, proliferation, and activation may develop in keratinocytes and mast cells [7]. So, we believe that in LP, an increased expression of CRH-R1 may trigger and/or aggravate the disease process by interacting with keratinocytes and mast cells.

In our study, a statistically significant difference was not found between the LP patients with and without oral involvement in the expression of CRH-R1. In addition, there was no correlation between BSA and staining intensity in LP patients. Lack of serum CRH levels could be the limitation of our present study. However, following the present findings we believe that CRH-R1 may play a role in the etiology of LP and its effects independent from the severity of the disease. New studies that evaluate both serum CRH level and skin CRH expression at the same time may show the effects of the CRH on the etiology of LP.

Conclusions

We demonstrated that CRH-R1 is strongly expressed in LP skin. It may be hypothesized that a cutaneous CRH/CRH-R1 system might be aberrant in lesions of LP. The detuning of CRH/CRH-R1 regulation might contribute to the pathogenesis and development of LP. To our knowledge, our study is the first study to date to explore CRH-R1 expressions in LP. Studies with a larger patient population could be the subject of future investigations and therapies.

Conflict of interest

The authors declare no conflict of interest.

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