Status of Free-Radical Oxidation and Proliferation Processes in Patients with Atopic Dermatitis and Lichen Planus

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We studied the status of proliferation processes (by Ki-67 expression) and biogenesis of free radicals (by chemiluminescence method) in skin biopsy specimens from patients with atopic dermatitis and lichen planus. The index of Ki-67-positive nuclei significantly ($p<0.05$) increased to 28.40±2.00% in patients with dermatitis and to 32.6±1.9% in patients with lichen planus *vs.* 8.65±1.31% in the reference sample. Decompensated accumulation of free-radical oxidation products and inhibition of detoxification systems in skin biopsy specimens indicate the development of local oxidative stress. After therapy consisting of two 10-day courses of thymodepressin injections (0.1%, 1.0 ml) over 30 days, normalization of epidermis proliferation was observed. Labeling index in atopic dermatitis and lichen planus significantly decreased ($p<0.05$) to 17.00±1.87 and 10.9±1.1%, respectively. The role of free radicals in the development of hyperregeneratory processes during dermatoses is discussed.

Key Words: *free-radical oxidation; proliferation; lichen planus; apotic dermatitis*

The notions about alterative and toxic influence of free radical oxidation (FRO) products were recently supplemented by ample data on their involvement into regulation of physiological functions [5]. Free radicals were reported to play an important role in the regulation of cell proliferation [8]. Disorders in free radical biogenesis are accompanied by disturbances in cell proliferation [10].

In our previous studies, morphometric proofs of hyperregeneratory reaction in atopic dermatitis (AD) and lichen planus (LP) against the background of systemic activation of FRO were obtained [6]. Apart from hyperregeneratory reaction of the epidermis, immune dysfunction plays an important role in the pathogenesis of both diseases. Thymodepressin, a new-generation immunocorrector exhibiting immunosuppressive effects, is indicated in psoriasis; it is known that hyperregeneratory process is an important pathogenetic component in this condition [4].

We found no published data on local manifestations of FRO and the state of cell division in LP and AD. Here we studied the peculiarities of local free-radical status and proliferation processes in skin biopsy specimens from patients with hyperregeneratory dermatoses (AD and LP).

MATERIALS AND METHODS

We examined 18 patients with AD and 16 patients with LP. Skin biopsy specimens were taken from affected skin sites before and after the therapy. Specimens of normal skin taken from 24 patients during surgery for midline hernia and hernias of the left and right iliac areas served as the control. Patients with AD and LP received intramuscular injections of thymodepressin (0.1%, 1.0 ml, two 10-day courses with a 10-day inter-

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val). The specimens were fixed in 10% neutral formalin in phosphate buffered saline. Histovax-embedded sections (7 μ) were mounted on slides coated with poly-L-lysine (Sigma). Expression of Ki-67 antigen was analyzed using Polymer Detection Sistem Novolink immunohistochemical technology (Novocastro). The sections were poststained with Lily—Mayer hematoxylin. Index of Ki-67 expression was expressed in percent of total number of nuclei in the regeneratory zone of the epidermis per 1000 examined nuclei.

Free radical biogenesis in skin biopsy specimens was evaluated by the method of chemiluminescence [1,2]. Chemiluminescence was recorded on an LS 50B luminescent spectrometer (PERKIN ELMER). The intensity of generation of free radicals (Ssp), including superoxide anion radicals (Sluc), hydroxyl radicals (Slum) and peroxide radicals (Sind-1) was analyzed; the concentration of lipid peroxides (h), peroxidation resistance of the substrate (H), and activity of antioxidant antiradical defense (Sind-2) were determined. The intensity of chemiluminescence (in mV) was normalized per 1 mg wet tissue and expressed in relative units. The data were processed statistically using Student's *t* test.

RESULTS

Analysis of proliferative processes in the skin attests to hyperregeneratory reaction in both dermatoses. The index of Ki-67-positive nuclei significantly ($p<0.05$) increased to 28.40±2.00% in patients with dermatitis and to 32.6±1.9% in patients with lichen planus *vs.* 8.65±1.31% in the control (undamaged donor skin). Apart from the increase in the index of Ki-67-positive nuclei we observed expansion of the generative zone. In unchanged skin, Ki-67-positive nuclei were located in the basal layer and in the lower part of the prickle-cell layer. In AD and LP, labeled nuclei were identified in the basal layers and in the higher parts of the prickle-cell layer. Solitary labeled nuclei were seen in the granular layer and the lower part of the prickle-cell layer.

Chemiluminescent analysis revealed increased activity of free radical processes in skin biopsy specimens from patients with dermatoses: Ssp production in AP and LP surpassed the control level by 1.64 and 1.78 times, respectively (Table 1), production of Sluc increased by 1.34 and 1.77 times and Slum by 1.71 and 1.84 times, respectively. We also observed intensification of LPO processes: the concentration of lipid hydroperoxides (h) in AP and LP surpassed the control level by 2.11 and 2.32 times and the rate of Sind-1 accumulation increased by 1.58 and 1.90 times, respectively. Impaired processing of free radicals was determined by inhibition of detoxification systems: parameter Sind-2 (inversely proportional to activity of the antioxidant antiradical system) in AD and LP increased by 2.12 and 2.21 times, respectively. Peroxidation resistance decreased judging from the increase in parameter H by 2.10 and 2.48 times in AD and LP, respectively. The observed changes in the free-radical status attest to decompensated accumulation of FRO against the background of suppressed antioxidant antiradical defense, *i.e.* to the formation of local oxidative stress in patients with dermatosis exacerbation.

The course therapy led to considerable improvement of the clinical picture, which manifested in normalization of sleep and alleviation of erythema and itch. Clinical improvement was associated with partial (AD) and even complete (LP) normalization of proliferation processes. Labeling index in atopic dermatitis and lichen planus significantly decreased ($p<0.05$) to

TABLE 1. Parameters of Chemiluminescence (rel. units) in Skin Biopsy Specimens from Patients with AD and LP during Therapy with Thymodepressin (*M*±*m*)

Parameter	Control	AD		LP	
		before treatment	after treatment	before treatment	after treatment
Ssp	0.083±0.005	0.136±0.009*	0.109±0.005*+	0.148±0.009*	0.107±0.007*+
Sind-1	0.195±0.007	0.309±0.010*	0.253±0.006*+	0.371±0.010*	0.290±0.008*+
h	0.071±0.003	0.150±0.008*	0.120±0.005*+	0.165±0.009*	0.111±0.005*+
Sluc	0.067±0.003	0.090±0.006*	0.082±0.004*	0.119±0.008*	0.080±0.004*+
Slum	0.096±0.005	0.164±0.011*	0.126±0.005*+	0.177±0.010*	0.145±0.006*+
Sind-2	0.135±0.011	0.286±0.017*	0.204±0.008*+	0.303±0.015*	0.237±0.010*+
H	0.104±0.005	0.217±0.011*	0.161±0.005*+	0.258±0.010*	0.189±0.005*+

Note. $p<0.05$ compared to: *control, +parameter before treatment.

17.00±1.87 and 10.90±1.11%, respectively, compared to the corresponding values before treatment. Normalization of proliferation processes was accompanied by changes in biogenesis of free radicals. All studied parameters of the free-radical status of skin biopsy specimens (except for Sluc in AD) still significantly differed from the control values, but markedly decreased compared to the corresponding parameters before treatment (Table 1).

The mechanism underlying the development of oxidative stress in our experiments includes several components. The level of free radicals in the inflammation foci can be modulated by proinflammatory cytokines TNF-α, IL-1, IL-2, IL-8 [5,7]; their content increases in hyperregeneratory dermatoses [3]. Previous studies demonstrated normalization of the content of proinflammatory cytokines under the effects of thymodepressin [4]. The hyperregeneratory reaction is triggered by various factors, including free-radicals [10].

There are various pathways of realization of the mitogenic signal under conditions of oxidative stress. Free radicals regulate activity of MAP-kinases by controlling cell division [11,12] and can directly activate NF-κB [9].

Activation of FRO associated with enhanced hyperregeneratory processes in patients with AD and LP confirms this assumption. Stimulation of cell division under conditions of oxidative stress is aimed at the maintenance of tissue homeostasis and epithelial layer integrity. Under conditions of oxidative stress, this adaptive reaction becomes a pathogenetic factor of dermatosis. Acceleration of cell cycle under conditions of the excess of reactive oxygen species leads to disturbances in differentiation processes. This is confirmed by expansion of the regeneration zone in AD and LP observed in our study. Partial (AD) or complete (LP) normalization of proliferation processes was associated with a decrease in the intensity of local oxidative stress, which also attested to the involvement of free radicals into the development of the hyperregeneratory process.

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