ONCOLOGY

Leukoplakia of Oral Mucosa: Pathogenesis and Possible Correction with Phytoadaptogen

O. A. Bocharova, M. M. Pozharitskaya*, T. L. Chekalina*, M. A. Lyzhenkova, R. V. Karpova, M. V. Mezentseva, and F. I. Ershov****

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Leukoplakia of the oral mucosa is characterized by impairment of the epithelial differentiation program. The use of complex phytoadaptogen in the treatment of patients with leukoplakia normalized expression of Fas-APO-1 antigen and keratin 17; increased expression of CD54 attested to activation of immune effectors. The clinical effect manifested in involution or shrinkage and loosening of the pathological focus and complete epithelialization of erosive surfaces. The phytopreparation exhibited adaptogenic effects normalizing the immune and IFN status and improving the state of adaptation systems in the body.

Key Words: *oral leukoplakia; adaptogens; adhesion molecules; immunomodulating therapy*

Prevention of oncological diseases consists in early diagnosis and adequate treatment of precancer processes. Leukoplakia is a facultative precancerous condition characterized by different degree of hornification of surface epithelium [7].

According to published data, the incidence of malignization of leukoplakia varies from 15 to 75%.

Some foreign authors studied the immunological status in leukoplakia [13,15]. However, it is now hypothesized that the main mechanism of tumor process is impairment of cell-cell adhesion, normally providing homeostasis of differentiated tissue [2].

Leukoplakia of the oral mucosa (OM) is often resistant to treatment. Therapeutic methods are effective in only 19.9% cases. The most widely used method is surgery, but relapses are very frequent.

Recently, much attention was drawn to a new group of preparations, adaptogens: Rhodiola rosea, Eleutherococcus senticosus, Aralia manchurica, *etc.* These plant preparations act as protectors and tissue differentiation regulators. Their antiblastic and antimutagenic effects are believed to be due to their immunomodulatory effect [1].

Complex phytoadaptogen Phytomix-40 (FM-40) was developed at N. N. Blokhin Russian Center of Cancer Research. The preparation was certified as a parapharmaceutical by Center of Hygienic Certification at Institute of Nutrition, Russian Academy of Medical Sciences (Hygienic Certificate No. 003323. P643.10.2001).

Phytocomposition FM-40 consists of 40 components including adaptogens. Comparative studies of *in vitro* effects of the plant extracts (phytopreparation components and their combinations) on proliferative activity of CaOv cells allowed creating an optimal composition. Experimental studies of FM-40 were carried out [3,6,8].

We studied the immunity and adhesive mechanisms in dysdifferentiation of the epithelium in the pathological focus and the possibility of correcting these disorders by using the complex phytoadaptogen.

N. N. Blokhin Russian Center of Cancer Research, Russian Academy of Medical Sciences; *Moscow State Medical Stomatological University; **N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences, Moscow

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MATERIALS AND METHODS

Fifty-four patients with OM leukoplakia, aged 21-61 years (33 men and 21 women) were treated at Department of Oral Diseases Propedeutics, Moscow State Medical Stomatological University.

Group 1 (study group) consisted of 28 patients with OM leukoplakia; combined treatment in this group included FM-40 preparation; group 2 (reference group) consisted of 26 patients with leukoplakia treated with vitamin A (retinol acetate oily solution and Aevit tablets). Control group consisted of 20 healthy subjects without dental or OM diseases.

Common clinical, stomatological, and immunological studies were carried out in all patients during treatment.

Peripheral blood immunological values were studied by the indirect immunofluorescence (IF) test.

Disorders in tissue differentiation of the oral mucosa epithelium in leukoplakia were studied by immunophenotyping (indirect IF test) by evaluating the expression of adhesion molecules (CD54, or ICAM-1) on epitheliocytes (these molecules are responsible for nonspecific cell-cell interactions and serve as receptors for immunity effectors); CD29 responsible for epitheliocyte contacts with extracellular matrix; keratin 17 differentiation protein; and CD95, or apoptosis mediating Fas APO-1 antigen.

Blood hydrocortisone level was measured by radioimmunoassay.

The interferon status was evaluated by the two-step biological method: measurements of IFN-α, IFN-γ, *etc.*, and qualitative evaluation of the preparations and IFN inductors on interferonogenesis *in vitro* [5].

Patients of the main group and controls were orally treated with FM-40 in a dose of 15 ml (with 5 ml water) 15-20 min before meals 3 times daily, the course lasting 1.5 months. A total of 3-4 courses with 2-week intervals were carried out. The dose for each subsequent course was increased by 5 ml. In order to create conditions for the maximally long contact of the involved mucosa, the patients were recommended to keep the preparation in the mouth for 10-15 min before swallowing.

The patients of the reference group were treated with group A vitamins locally and orally by the standard protocols. Retinol acetate was applied (10-15 min) onto damaged mucosa 2-3 times daily and Aevit was given orally (1 capsule twice daily for 1.5 months).

RESULTS

Examination of the patients showed squamous, verrucous, erosive ulcerative (according to A. L. Mashkilleison's classification) clinical forms of oral leukoplakia.

TABLE 1. Changes in Immunity Parameters in Patients of All Groups and Controls during Treatment (%)

Antigens	Control group			Study group			Reference group		
	before therapy	after therapy*	*p*	before therapy	after therapy*	*p*	before therapy	after therapy*	*p*
CD3 (60-75)	63.3±3.1	69.4±3.4	0.024	61.6±3.0	69.2±1.9	0.016	62.4±3.6	67.5±4.5	0.207
CD4 (35-46)	32.3±2.8	46.9±2.2	0.058	31.6±2.7	43.8±1.6	0.020	34.9±1.2	41.4±1.6	0.048
CD8 (25-30)	29.8±1.0	26.0±1.4	0.007	29.6±2.1	23.6±1.3	0.013	30.1±4.5	26.0±2.0	0.127
CD4/CD8 (1.5-1.9)	1.4±0.1	1.8±0.1	0.037	1.2±0.1	1.9±0.1	0.001	1.2±0.2	1.9±0.2	0.024
CD20 (5-15)	4.7±2.9	12.3±3.4	0.085	3.5±0.6	8.0±1.0	0.056	4.5±1.6	7.0±1.3	0.145
HLADr (7-15)	8.3±0.6	11.4±0.9	0.027	7.9±0.7	10.6±1.2	0.052	5.8±1.2	7.5±1.2	0.108
CD16 (10-20)	8.5±1.7	16.8±2.0	0.008	9.7±1.8	18.8±2.1	0.020	7.2±2.9	8.8±2.8	0.239
CD11b (15-20)	11.7±1.3	18.8±1.5	0.055	10.5±2.9	19.3±1.6	0.015	12.1±2.1	10.2±2.3	0.138
CD18 (56-64)	44.0±6.9	67.0±5.2	0.010	46.5±3.7	64.9±5.8	0.001	48.6±4.2	53.8±8.2	0.133
CD50 (70-99)	71.4±4.6	92.7±2.2	0.046	65.2±3.8	92.6±1.8	0.019	52.9±5.2	74.6±3.3	0.071
CD25 (0-5)	3.0±0.3	4.8±0.5	0.009	2.6±0.6	5.4±0.9	0.022	1.8±0.6	2.8±0.8	0.069
CD95 (10-30)	13.8±2.0	29.9±1.6	0.015	8.5±3.9	21.0±3.8	0.059	11.0±4.7	13.9±5.0	0.211

Note. Normal values are shown in parentheses. Here and in Tables 2 and 3: *compared to parameters before treatment.

TABLE 2. Changes in IFN Status of Patients during Treatment

IFN reaction of leukocytes	Percentage of patients with normal IFN level (IFN titers, IU/ml)								
	control group			study group			reference group		
	before therapy	after therapy*	*p*	before therapy	after therapy*	*p*	before therapy	after therapy*	*p*
Serum IFN (normal level <2-8 IU/ml)	73 (10.0±2.6)	96 (4.2±1.2)	0.008	62 (12.3±0.7)	92 (7.4±2.2)	0.053	57 (14.4±2.3)	55 (10.5±1.8)	0.192 —
Spontaneous IFN (normal level <2 IU/ml)	91	100		100 (<2)	100 (<2)		100 (<2)	100 (<2)	
IFN-α (normally 64-640 IU/ml)	9 (34.6±7.6)	45 (85.5±26.2)	0.053	0 (23.1±3.5)	38 (61.5±13.7)	0.012	0 (24.5±3.9)	9 (29.1±4.7)	0.326
IFN-γ (normally 32-63 IU/ml)	0 (10.9±2.3)	37 (26.2±5.2)	0.014	0 (7.9±1.7)	54 (28.3±5.1)	0.01	0 (9.0±3.7)	5 (12.5±2.4)	0.722

TABLE 3. Immunophenotyping of Surface OM Epitheliocytes in Patients (*M*±*m*)

Parameter	Control group			Study group			Reference group		
	before FM-40	after FM-40	*p*	before FM-40	after FM-40	*p*	before FM-40	after FM-40	*p*
CD29	1.7±0.4	1.4±0.6	0.650	1.8±1.1	1.3±0.6	0.376	1.4±1.2	1.9±0.7	0.668
CD54	3.9±1.4	3.5±0.8	0.141	3.4±2.7	9.0±1.7	0.046	4.2±2.0	4.6±2.3	0.137
CD95	10.1±2.2	11.4±2.3	0.792	3.8±1.3	7.9±2.1	0.020	4.6±1.3	4.8±0.8	0.820
Keratin 17	1.6±0.4	1.2±0.3	0.492	8.4±2.6	0.9±0.6	0.039	9.3±2.7	3.2±0.8	0.066

No specific disorders in immunity parameters were detected in patients with different clinical forms of the leukoplakia (Table 1).

The immune status of patients improved after the treatment: total counts of T lymphocytes and T helper inductors increased, humoral immunity values normalized, the counts of natural killers and monocytes increased. In the reference group the immunomodulating effect was observed only for T helpers.

Evaluation of the IFN status of leukoplakia patients and controls showed comprehensive picture of IFN system disorders observed in stress, viral and bacterial infections, relapsing allergic diseases, *etc.* (Table 2). No specific changes in the IFN status of patients with leukoplakia were detected. The time course of changes in IFN status under the effect of FM-40 in the study group and controls was similar and characterized by normalization of the studied parameters. The use of standard treatment for leukoplakia in the reference group did not lead to positive changes in the IFN status.

Hence, the results of immunological studies demonstrating the immunomodulating effect of FM-40, which is characteristic of plant adaptogens.

Measurements of the peripheral blood hydrocortisone level before therapy showed that the hormone level surpassed the physiological norm. This could be regarded as a homeostasis disorder in stress. Treatment with FM-40 led to normalization of this parameter. Treatment with vitamin A preparations did not lead to correction of hypercorcoidism in the reference group.

The expression of surface epitheliocytes in oral leukoplakia differs from normal. The expression of keratin 17 increased, while that of CD95 antigen decreased; on the other hand, the expression of CD54 and CD29 molecules in leukoplakia did not change in comparison with normal mucosa (Table 3).

The use of FM-40 in combined therapy of patients with leukoplakia (main group) led to normalization of the expression of all studied antigens. The expression of CD54 increased, which eventually led to the lysis

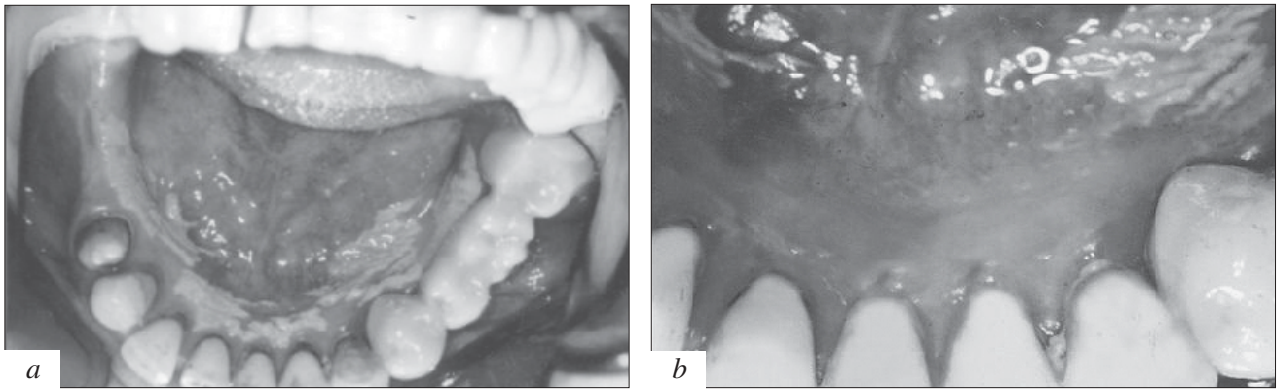

Fig. 1. Verrucous (*a*; diagnosis before treatment) and squamous (*b*; diagnosis after therapy) leukoplakia. Patient Kh., 46 years.

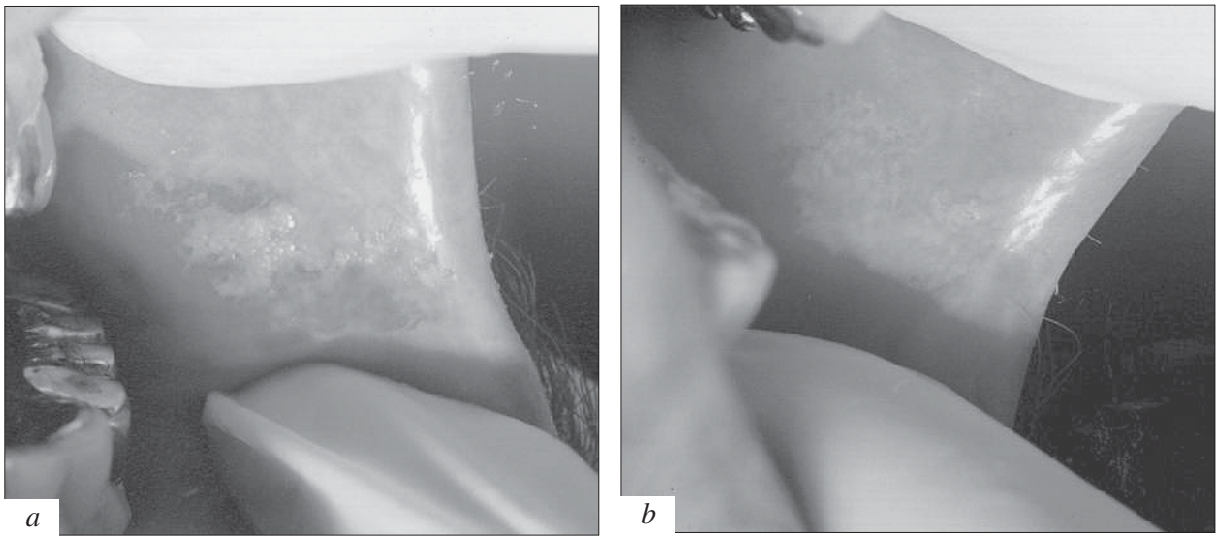

Fig. 2. Erosive ulcerative (*a*; diagnosis before therapy) and squamous (*b*; diagnosis after therapy) leukoplakia. Patient B., 35 years.

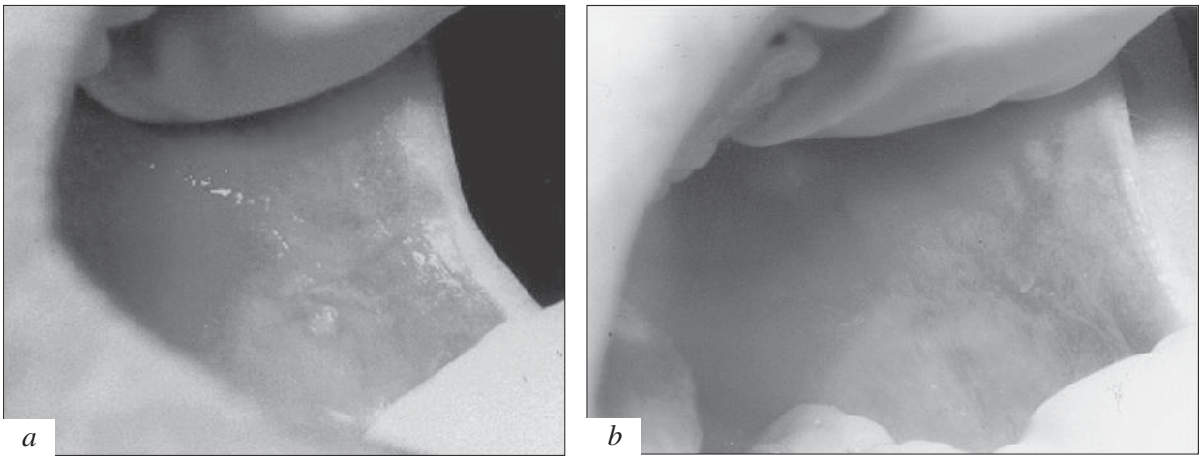

Fig. 3. Squamous leukoplakia (*a*; diagnosis before treatment) and after therapy (*b*; recovery). Patient V., 41 years.

of the pathological focus, presumably, due to activation of the immune effectors.

Treatment of patients by vitamin A preparations caused no statistically significant improvement of the parameters.

We evaluated the clinical efficiency of treatment of patients with oral leukoplakia by subjective factors (complaints, attitude to the treatment, *etc.*) and by objective signs (size, density, color of hyperkeratosis foci, time of epithelialization of OM erosions), as well as by the duration of remission.

Clinical cure was attained in 8% of patients treated with vitamin A preparations (reference group). Positive clinical shifts (epithelialization of erosions, softening of hyperkeratosis foci, sensation of comfort in the oral cavity) were observed in 27% of patients of this group. No positive shifts in the clinical picture were detected in 65% patients.

Treatment of patients with oral leukoplakia with FM-40 resulted in complete cure in 17% cases. The patients had no complaints. Involution of leukoplakia foci was observed. OM was pink, glossy, and wet. Positive changes were observed in the majority (56%) patients: the size and density of hyperkeratosis foci decreased (Fig. 1), regeneratory processes activated, complete epithelialization of erosive surfaces (Fig. 2), and even complete resolution of the process in squamous leukoplakia were noted (Fig. 3). No improvement was observed in 27% patients.

All patients receiving FM-40 (including controls) felt better, noted sleep improvement, better moods and working capacity. Moreover, the patients noted good organoleptic properties of the preparation and its deodorizing effect.

Hence, it seems that we observe perversion of the epithelium differentiation program in oral leukoplakia. This sort of "mimicry" of normal developmental biology, characteristic of the skin (intense hornification and decreased apoptosis), at this stage does not modulate the expression of ICAM-1 (CD54) receptors for immunity effectors, as well as on the expression of CD29 responsible for adhesive interactions with the extracellular matrix. Hence, the immune reactivity is not stimulated under these conditions. The pathological focus on the OM is protected from lysis by local and common (immune) mechanisms at the pretumorous stage. Attempts at detecting immunity disorders of any type in leukoplakia failed [15].

The following mechanism of therapeutic effect of FM-40 is possible. The preparation stimulates the expression of apoptosis-mediating receptors and decreases expression of protein marker of immature epitheliocytes, this indicating recovery of the parameters disordered in leukoplakia. On the other hand, expression of receptors for immunity effectors (ICAM-1) increases, which can lead to the lysis of the pathological focus, presumably, as a result of activation of immune effectors.

Our results recommend FM-40 for use in combined therapy of patients with oral leukoplakia.

Hence, oral leukoplakia is characterized by changed differentiation program of the oral mucosa epithelium in the absence of specific disorders in the immune system. FM-40 possesses immuno-, hormone-modulating, and interferonogenic activities, which characterizes it as an antistress (or adaptogenic) preparation. Presumably, the mechanism of FM-40 effect is mediated by adhesive interactions, Normalization of these processes initiates the recovery of OM epithelium differentiation program [2,4,9,12].

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