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IMMUNOHISTOCHEMICAL CHARACTERISTIC OF SKIN IN PATIENTS WITH PSORIASIS AND CORRECTION OF THE REVEALED DISORDERS

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Abstract. *The purpose of the research is determination of role of inhibitors of cellular cycle in pathogenesis of psoriasis and efficacy evaluation for pathogenetic mechanisms of therapy. For the purposes of study of cytological characteristic of hyperprolipherative processes at the inhibitors of cellular cycle level in skin of diseased patients with psoriasis at steady state and progressive state a biopsy of the plaque skin was performed. On the basis of this research evaluation of pathogenetic mechanisms during psoriasis at the inhibitors of cellular cycle level was realized, proteins (p16, p19, p21, p53) expression in psoriatic patient's skin before and after therapy was evaluated.*

Key words: *psoriasis therapy, Glutaxim*

Psoriasis is multifactorial dermatosis, whose pathogenesis is based on a lot of theories [1, 3]. Despite the recent years have witnessed a great number of publications, dealing with both the study of its pathogenesis and development of new methods of treatment, psoriasis still remains an important problem of dermatology.

At present, psoriasis is characterized as a systemic autoimmune process [11, 12] with the following changes in the immune status:

- lysozyme and phagocytic activity of neutrophilic reactions is inhibited;
- the complement and content of lactoferrin in blood are increased;
- the count of T lymphocytes decreases;
- changes take place in the titre of T lymphocytes, the ratio of T and B lymphocytes and immunoregulatory index as result of a decreased count of T helpers;
- the count of “zero” lymphocytes increases.

In this situation a lower level of IgG is observed too, the number of CIC increases. All these changes are also most manifested in complicated forms of the disease, a frequently recurrent process, particularly in patients with some concomitant pathology [10, 12].

Psoriatic plaques are known to form on the basis of disorders in epidermal differentiation. The latter is a very complex and highly regulated biochemical process, characterized by morphological changes in all structural components of epithelial cells. Generally accepted is the point of view that cell renewal processes play a leading part in the morphogenesis of psoriatic plaques, whose formation is based on epidermal hyperproliferation, caused by cell cycle disorders. Several phases are observed in the process of the preparation of a cell for its division and subsequent formation of two new cells: G1, G2, S, G0. The activation of sequences of cyclin-dependent kinases (CDK) is the "motor" of the cell cycle.

Proteins p16, p19, p21 and p53 inhibit or activate different CDK complexes, which are responsible for the normal course of all phases in the cell cycle. Psoriasis is characterized by intracellular pathological processes with resultant affection of the normal cell cycle, thereby increasing CDC complexes and expression of proteins p16, p19, p21 and p53, which activate these complexes. Gene p53 is one of the central components of the system, which ensures elimination of pathological cells from the organism; p53 is known as a tumour suppressor, an activator of apoptosis.

The activation of protein p53 is caused by damage of cells via signalling pathways, which control the state of cells. Protein p53 coordinates the process of reparation or induces autocytolysis. The loss of gene p53 causes an uncontrolled accumulation of genetic damages with a resultant loss of control from the part of the organism, a pathological growth of cells and death. Gene p53 produces the protein, which consists of 393 amino acids and has the electrophoretic mobility of 53 kD (hence the name of the gene and protein). Proteins p16, p19 and p21 are members of the CDC family, i.e. representatives of negative regulators of the cell cycle, which are responsible for the normal course of the cell cycle in phase G1, take part in the system of control over the cell cycle and stop reproduction of malignant cells. A

higher activity of proteins p16, p19 and p21 causes blockage of entering phase S by cells and their premature aging.

Few studies dealt with tumour markers in psoriasis [2, 4, 8]. Tumour markers Bcl-2, Ki-67, p21 and p53 were detected. The progressive stage of psoriasis revealed a high expression of Ki-67 and Bcl-2, as well as low levels of p21 and p53. A reliable increase of p53 expression in the epidermis at the stationary stage indirectly demonstrates an intensification of apoptosis processes in the epidermal layer [8]. The appearance of expression of p21 and p53 in the suprabasal layers of the epidermis gives evidence for an early beginning of keratinization of cell membranes, which as result are not able to form the normal horny layer and may facilitate the development of parakeratosis [8].

The purpose of the present study was to reveal the role of cell cycle inhibitors in the pathogenesis of psoriasis and assess the efficacy of pathogenetic mechanisms of therapy.

Glutoxim is one of promising medicines. It represents a new class of drug preparations, thiopoetins, and has unique biological effects. Owing to them it acts on the intracellular level of thiol metabolism and plays an important part in the regulation of metabolic processes in cells and tissues. The influence of Glutoxim stimulates proliferation and differentiation of normal cells and activates processes of genetically programmed death (apoptosis) of transformed cells. The drug action is realized through an increased half-life of protein p53 and with help of influence on the cascade of phosphoprotein kinases of the Ras signalling pathway. Thus Glutoxim normalizes cell metabolism and produces the cytoprotective effect. It is for these reasons that this medicine attracted our attention, when we studied therapy of patients with psoriasis. The positive effect consists in a higher efficacy of treatment of psoriasis cases owing to the fact that Glutoxim additionally increases reactivity of the organism.

Materials and methods. The study involved 120 patients, ill with psoriasis, who were divided into two groups.

– The main group consisted of 100 patients, including:

1) 50 cases with the stationary stage of psoriasis;

2) 50 cases with the progressive stage of the disease.

– The control group was composed of patients with the same diagnosis (20 cases).

The clinical picture of the disease severity was assessed by the psoriasis area and severity index (PASI), which averaged between 15 and 20 units.

Patients with psoriasis from the main (20 cases) and control (10 cases) groups underwent histological examinations of their skin before and after the multimodality treatment. Biopsy was carried out under local anaesthesia with 0.5 % solution of Novocain. The material was fixed in 10 % buffered aqueous solution of neutral formalin and Carnoy's fluid, and underwent celloidin-paraffin double embedding. Serial 5-6 mcm slices were prepared. Hematoxylin-eosin staining after Van Gieson was used in all cases.

During morphological examinations, we calculated the infiltrate volume density per square millimetre and assessed the degree of acanthosis on the basis of the visual analogue scale:

- 1st – the mild degree of acanthosis is regarded as an insignificant thickening of the epidermis; acantholytic bands spread deep into the derma up to 2/3 of the visual field (x 210, the total visual area is 0.4 mm^2);

- 2nd – the moderate degree of acanthosis is characterized by extension of acantholytic bands and their spreading into the derma, but already to the visual field edge with the above magnification;

- 3rd – the marked degree of acanthosis manifests itself by a larger number of acantholytic bands, their spreading outside the visual field and by extreme thinning of the suprapapillary layers of the epidermis.

In order to study the immunohistochemical characteristics of hyperproliferative processes at the level of cell cycle inhibitors in the skin of patients with the stationary and progressive stages of psoriasis before and after their treatment, biopsy of the skin of psoriatic plaques was carried on. Immunohistochemical examinations were performed on 5 mcm paraffin slices, which were deparaffinized by the standard

technique. The slices were incubated with monoclonal antibodies against p53 protein (DO-7), cyclin-dependent kinase inhibitor (p19-Ink4D), p16 protein ("Novocastra", UK), p21 clone 2G12) (BD PharMingen). Avidine-biotin-peroxidase complex method (ABC method) (Novostain Universal Quick Kit [NCL-RTU-QU], Novo-Castra, UK) was used for immune staining. In all cases, nuclei were counterstained with haematoxylin.

Before treatment, PASI in the patients averaged:

- at the stationary stage of psoriasis – 15.4;
- at the progressive stage of psoriasis – 18.6.

Immunohistochemistry before treatment revealed a high expression of proteins p16, p19, p21 and p53 in 30 patients. In the cases with psoriasis, expression of proteins at the stationary stage was slightly lower than at the progressive one. The conducted studies make it possible to state that the expression of cell cycle inhibitors in psoriasis increases, this fact being taken by us into consideration during the multimodality treatment of patients from the main group.

The control group patients (20 cases) received the standard therapy, which included:

- sedative drugs;
- antihistamine drugs;
- vitamins B6 and B12 – intramuscularly every other day, No.20;
- externally – 2 % salicylic ointment onto the skin.

The main group of patients (100 cases) received, against a background of the standard therapy, Glutoxim drug in the form of intramuscular injections of 1 % solution by 1 ml, No. 10.

Results. The rate of the reverse development of the disease was assessed by the following indices: infiltration, erythema, oedema, scaling, itching, excoriations.

Against a background of their treatment, all the patients from the main group reported a significant decrease of itching, they demonstrated decreases of erythema and skin infiltration in the foci of lesion as early as on the 5th day of therapy, the infiltration and erythema almost completely regressing on the 15th day of the

multimodality therapy. After the end of the therapy the foci had only secondary pigmentation. PASI after the treatment was:

- in patients with the stationary stage of psoriasis – 2.2;
- in patients with the progressive stage – 4.3.

The immunohistochemichal indices of patients with psoriasis after their treatment were as follows:

- at the stationary stage, the expression of proteins p16, p19, p21 and p53 slowly decreased from 27-32 % to 11 %, thereby demonstrating a change in the rate of apoptosis in the skin of cases at this stage;
- at the progressive stage, there was a weak and moderate expression of proteins p16, p19, p21 and p53 from 7 % to 25 % in cells of the basal and spinous layers.

These indices indicate acceleration in cell proliferation processes (acanthosis, parakeratosis, hyperkeratosis), while a higher content of dividing cells confirms a decrease in the rate of apoptosis.

At the regressive stage, treatment with use of Glutoxim resulted in decreases of acanthosis, hypo- and hyperkeratosis (stages 1-2). A very weak expression of proteins p16 and p21, a moderate expression of protein p19 (up to 28 %) and a decreased expression of protein p53 were observed, thereby demonstrating a higher apoptic activity of keratinocytes and a decreased proliferative activity in the epidermis.

The duration of hospitalization lasted:

- for the control group of patients – 25 ± 0.4 bed days;
- for the main group of patients, whose course of treatment included Glutoxim – 20 ± 0.4 bed days.

Conclusion. Following their multimodality treatment with Glutoxim patients with psoriasis did not reveal any expression of proteins p16 and p19 in the spinous and basal layers, while the expression of proteins p21 and p53 was weak (3-5 %), these facts demonstrating a higher apoptic activity of epidermal cells and a decrease of proliferation.

The following phenomena were observed during histological studies:

- chiefly weak expression of the horny layer, in some places it had moderate hyperkeratosis;

- positive dynamics in the state of other layers of the epidermis and dermal microvasculature;

- acceleration of regression of psoriatic eruptions;

- shortening of the patients' stay at in-patient department.

Thus, the use of Glutaxim results in positive clinical and immunohistochemical dynamics and can be recommended in both in-patient and out-patient conditions.

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Резюме: Обговорюються результати вивчення імуногістохімічних характеристик гіперпроліферативних процесів на рівні інгібіторів клітинного циклу у хворих на псоріаз та оцінки патогенетичних механізмів сучасних методів терапії. Виявлено підвищення експресії білків p16, p19, p21, p53 в шкірі хворих на псоріаз. Розроблено нові імуногістохімічні тести для вибору терапії та оцінки прогнозу перебігу псоріазу. Якщо в процесі проведеної комплексної терапії експресія білків p16, p19, p21, p53 не знижується, то це свідчить про неефективність проведеної терапії, а також говорить про несприятливий перебіг псоріатичного процесу. Якщо в міжрецидивний період до появлення нових висипів на шкірі спостерігається підвищена експресія білків p16, p19, p21, p53, це свідчить про початок рецидиву і вимагає призначення противорецидивного курсу терапії.
Ключові слова: псоріаз, терапія, Глутоксим

Резюме: Обсуждаются результаты изучения иммуногистохимических характеристик гиперпролиферативных процессов на уровне ингибиторов клеточного цикла у больных псориазом и оценки патогенетических механизмов современных методов терапии. Выявлено повышение экспрессии белков p16, p19, p21, p53 в коже больных псориазом. Разработаны новые иммуногистохимические тесты для выбора терапии и оценки прогноза течения псориаза. Если в процессе проведенной комплексной терапии экспрессия белков p16, p19, p21, p53 не снижается, то это свидетельствует о неэффективности проведенной терапии, а также говорит о неблагоприятном течении псориатического процесса. Если в межрецидивный период до появления новых высыпаний на коже наблюдается повышенная экспрессия белков p16, p19, p21, p53, это свидетельствует о начале рецидива и требует назначения противорецидивного курса терапии.
Ключевые слова: псориаз, терапия, Глутаксим

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