УДК 616.923.145-[214:52.14]-7

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THE ROLE OF APOPTOSIS OF EPITHELIOCYTES IN THE PROGRESSION OF SYSTEMIC INFLAMMATION IN CHRONIC OBSTRUCTIVE LUNG DISEASES ASSOCIATED WITH HYPOIODOTHYRONINEMIA IN ELDERLY AND SENILE AGE

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Abstract. The paper presents findings, concerning the intensity of the processes of apoptosis and the proliferative activity of epitheliocytes by means of carrying out an immunocytochemical research in chronic obstructive lung diseases (COLD), depending on the functional condition of the thyroid gland and the level of cortisol in patients of older age groups and their role in the formation of systemic manifestations and the mechanisms of disease progression.

Key words: chronic obstructive pulmonary diseases, apoptosis, epitheliocyte.

Intraduction. The results of recent studies indicate that there occurs a disturbance of the molecular mechanisms of regulating cellular death due to an increased activity of the genes of external modulators of apoptosis in the focus of an inflammation [4, 6, 9]. At the molecular level the process of apoptosis is a complicated cascade of reactions with the participation of protein kinases, proteases, endonucleases [1, 2, 5]. Proto-oncogenes are important intracellular effectors of apoptotic death, such as Bcl-2, Bcl-x (suppressors) and bax, bcl-xs, bak (agonists), that code the corresponding proteins which, as a matter of fact, are the regulators of scheduled cell death (SCD), determining the rheostat of the life and death of a cell [3, 7, 8]. In the pathogenesis of the formation of a chronic inflammatory reaction and the processes of bronchial remodelling an essential role belongs to the epitheliocytes of the bronchi, but the specific characteristics of the apoptosis of these cells, particularly, in case of COLD in old age and, depending on a variant of a thyroid dysfunction, haven't been practically studied by now.

The object of research. To evaluate the degree of the apoptosis of bronchial epitheliocytes and their proliferative activity and role in the progression of chronic inflammation with the formation of systemic manifectations in case of the chronic destructive disease of the lungs against a background of a thyroid hypofunction and hypocortisolemia in elderly age.

Material and methods. 18 patients with COLD were examined. The verification of the diagnosis and its formulation was carried out in accordance with the Order of Ukraine's MHP №128 of 19.03.2007. Group 1 (9 persons) was composed of patients with COLD and the biochemical signs of euthyrosis and the normosecretion of cortisol, group 2 (9 persons) – patients, suffering from COLD with functional insufficiency of the thyroid gland (TG) and hypocortisolemia. An immunocytochemical evaluation of antigens Bcl-2, Bax and PCNA (a nuclear antigen of cellular proliferation) was performed in the bronchial tissue by means of primary monoclonal antibodies to these proteins and the streptavidin-biotin system of visualization LSAB2 (DakoCytomation, Denmark), the immunocytochemical (ICC) thechnique was performed to determine internucleosomal separations of DNA – TUNEL, using the test – system TACS X L™ (R&D Systems Incorporation, USA). A percentage of PCNA – positive and TUNEL–positive nuclei of bronchial epitheliocytes was caeculated. Quntitative studies of the intensity of staining the nuclei or cytoplasm were carried out by means of obtaining digital copies («Taggerd Image File Format») of the optical image of the bronchial tissue (the microscopic objective ×100 – oil immersion) and its analysis by means of the program "Video Test – size 5.0 (Russia). The analysis was realized on the basis of probing measurements (the area of a round probe is 4 mm^2) of the staining intensity with a calculation of the index "mean optical density" (MODE). For the purpose of evaluating the intensity of apoptotic processes the number of structures identified as "apoptotic objects" (AO) – TUNEL-positive cellular nuclei or TUNEL-positive nuclear fragments were counted up. The index of apoptosis (IA) was evaluated as a ratio of the number of the TUNEL-positive to the number of the TUNEL-negative cells expressed in parts per thousand. The index of proliferation (IP) was determined as a ratio of the number of PCNA-positive to the number of the PCNA-negative cells expressed in parts per thousand. A statistical analysis of the obtained findings was implemented, using applied programs on the basis of the SPSS software, version 13,0 (StatSoft Inc., USA).

The results of the research and their discussion. The studies carried out with tissue samplings of the mucous tunic of the bronchi of patients with COLD of elderly age indicate not only the presence of the signs of an inflammation in all the smears-scrapings, but they also point out that there are available epitheliocytes with the signs of natural cell death: the appearance of nuclei with positive TUNEL staining and "apoptotic objects" that are also TUNEL-positive. An analysis of the results of the investigations has demonstrated that in case of COLD reliable differences were revealed as to the intensity of the processes of apoptosis among groups of patients with euthyrosis and a systemic deficiency with thyroid hormones and hypocortisolemia. Thus, in case of COLD with hypocortisolemia there occurred with a

considerable frequency (3,45±0,120 in parts per thonsand) TUNEL-positive nuclei (fig.1) that is regarded as early morphological changes (at the light optic level) characteristic of apoptosis.

Apoptotic corpuscls were encountered with a lesser frequency (1,23±0,017 in parts per thonsand) and they grouped in twos and fours and were of different sizes (including those considerably smaller than a nucleus) and with marked hypercromia in group 2. Immunohistochemically there occurred Bax-positive staining (fig. 2) in the cytoplasm of epitheliocytes in persons of elderly and senile age with COLD that had a micro- or macrogranular character.

The intensity of cell cytoplasm staining according to computer densitometric findings varied as far as intensity was concerned over the area of a histologic section (mean optical density – MOD) and averaged 0,51±0,011 of arbitrary units (a.u.). The highest level of apoptosis of epitheliocytes under conditions of the functional equilibrium of the thyroid gland in COLD was established in case of its macrogranular accumulation (in the form of large grains) in the cytoplasm. With such a localization of the Bax expression the share of the cells in the state of apoptosis made up 51,2±2,14 %, whereas this value equalled 17,1±1,02 % ($p<0,05$) with the Bax homogenous localization in the cytoplasm. It has been revealed that the degree of the expression of the Bax protein reliably exceeds that of the apparently healthy persons (AHP) in case of COLD with a TG normosecretion in the biopsy materials of the bronchial mucous tunic. It is statistically considerably higher than that of group 2 (6,5 % versus 2,1 %, respectively $p<0,05$). The mean values of the apoptotic index in the tissue samplings of the bronchial mucous tunic in COLD with euthyrosis also reliably exceeded the number of such values in case of COLD with hypoidothyroninemia ($p<0,05$).

In contrast to the findings presented above, an analysis of an investigation of the Bcl-2 expression in the epitheliocytes of patients afflicted with COLD indicates an essential reliable deficit of systemic antiapoptotic proteins, accounting for a high level of apoptosis in case of COLD with euthyrosis.

In particular, an ICC study of Bcl-2 has demonstrated mainly the absence of the expression of this antigen and one can visualize trace stainings (MOD made up 1,1±0,003 a.u.). The average degree of staining on Bcl-2 in the tissue samplings of the bronchial mucous tunic in COLD with euthyrosis was lower than that in AHPs 6,2 times ($p<0,01$) (fig. 3) and 2,7 times ($p<0,01$) umder the conditions of thyroid and glucororticoid deficiency.

A negative correlation of a weak degree between the degree of the Bcl-2 expression in the cytoplasm of an epitheliocyte and the presence of preapoptotic nuclei in these cells ($r=0,234$, $p=0,02$) has been established. In case of an ICC investigation of the PCNA protein, depending on a variant of the thyroid function, a positive staining was determined in 2,4 % of the nuclei of epitheliocytes on the average (fig. 4), it was never observed in the nuclei with chromatin margination or the apoptotic corpuscles.

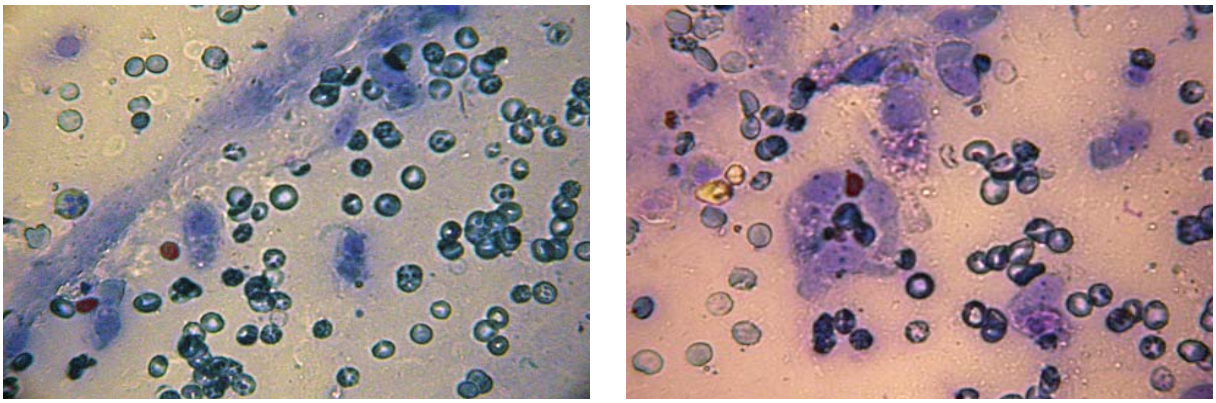

Fig. 1. TUNEL immunohistochemical technique for the verification of nuclei with internucleosomal DNA disruptions. Supplemental staining of the cellular nuclei with azure-2. Microphotographs: Ob. 100^x (oil immersion). Oc.7^x

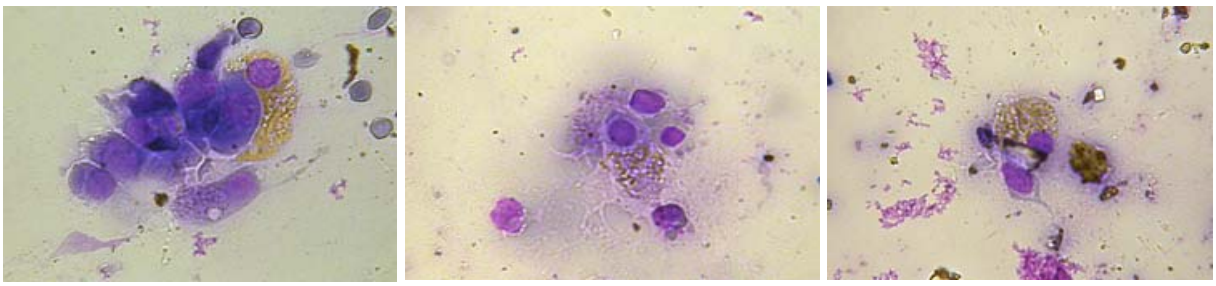

Fig. 2. Immunohistochemical technique with primary antibodies versus Bax protein and visualization of primary antibodies by means of the streptavidin-biotin method, using diaminobenzidine supplemental staining of the cellular nuclei with azure-2. Microphotographs: Ob. 100^x (oil immersion). Oc.7^x

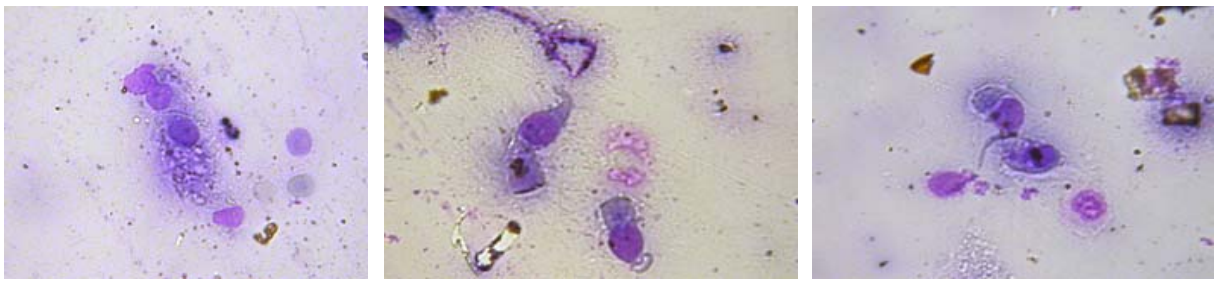

Fig. 3. Immunohistochemical technique with primary antibodies versus Bcl-2 protein and visualization of primary antibodies by means of the streptavidin-biotin method, using diaminobenzidine supplemental staining of the cellular nuclei with azure-2. Microphotographs: Ob. 100^x (oil immersion). Oc.7^x

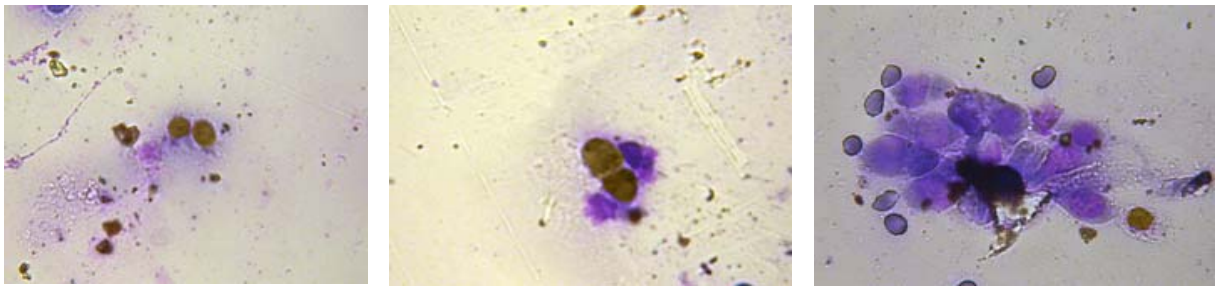

Fig. 4. Immunohistochemical technique with primary antibodies versus PCNA protein and visualization of primary antibodies by means of the streptavidin-biotin method, using diaminobenzidine supplemental staining of the cellular nuclei with azure-2. Microphotographs: Ob. 100^x (oil immersion). Oc.7^x

Conclusions

1. The dying off of epitheliocytes in COLD with euthyrosis occurs at the expense of an intensification of apoptosis TUNEL – positive nuclei encountered (with a reliable frequency) which is accompanied with a compensatory intensified proliferation of epitheliocytes (PCNA hyperexpression) and an increased production of proapoptotic BAX protein, having a macrogranular character against a background of a deficit of the synthesis of antiapoptotic factors (protein Bcl-2).

2. The intensity of apoptosis is low, programmed death of epitheliocytes takes place at the expense of necrosis and is accompanied with a low expressin of the Bax protein against a background of a hyposecretion of antiapoptic factors (Bcl-2) in case of hypoiodothyroninemia and hypocortisolemia; no positive reaction to PCNA is revealed.

Outlooks of further studies. An evaluation of the intensity of the processes of cell apoptosis in COLD in elderly age and a determination of its early manifestations stipulate a necessity of updating a regimen of pathogenetically substantiated antiinflammatory therapy.

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РОЛЬ АПОПТОЗА ЭПИТЕЛИОЦИТОВ В ПРОГРЕССИРОВАНИИ СИСТЕМНОГО ВОСПАЛЕНИЯ ПРИ ХРОНИЧЕСКИХ ОБСТРУКТИВНЫХ ЗАБОЛЕВАНИЯХ ЛЕГКИХ НА ФОНЕ ГИПОЙОДОТИРОНИНЕМИИ В ПОЖИЛОМ И СТАРЧЕСКОМ ВОЗРАСТЕ

Л.Д.Тодорико

Резюме. В работе наведены данные об интенсивности процесов апоптоза и пролиферативной активности эпителиоцитов путем проведения имуноцитохимического исследования при хронических обструктивних заболеваниях легких в зависимости от функционального состояния щитовидной железы и содержания кортизола у больных старших возрастных груп и их роль в формировании системных проявлений и механизмах прогрессирования заболевания.

Ключевые слова: хронические обструктивные заболевания легких, апоптоз, эпителиоцит.

РОЛЬ АПОПТОЗУ ЕПІТЕЛІОЦИТІВ У ПРОГРЕСУВАННІ СИСТЕМНОГО ЗАПАЛЕННЯ ПРИ ХРОНІЧНИХ ОБСТРУКТИВНИХ ЗАХВОРЮВАННЯХ ЛЕГЕНЬ НА ТЛІ ГІПОЙОДОТИРОНІНЕМІЇ У ЛІТНЬОМУ ТА СТАРЕЧОМУ ВІЦІ

Л.Д.Тодоріко

Резюме. У роботі наведені дані щодо інтенсивності процесів апоптозу та проліферативної активності епітеліоцитів, шляхом проведення імуноцитохімічного дослідження при хронічних обструктивних захворюваннях легень залежно від функціонального стану щитоподібної залози та рівня кортизолу у хворих старших вікових груп та їх роль у формуванні системних проявів та механізмах прогресування захворювання.

Ключові слова: хронічні обструктивні захворювання легень, апоптоз, епітеліоцит.

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Buk. Med. Herald. – 2009. – Vol. 13, № 3. – P.93-96

Надійшла до редакції 6.05.2009 року

Науково-практична конференція

“Нові підходи до діагностики, лікування, фізіотерапії та реабілітації захворювань внутрішніх органів”

22-23 жовтня 2009 року
м. Тернопіль

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