поліорганної недостатності. Автори стверджують, що позитивні результати застосування L–аргініну аспартату та тивортину за умов експериментального цирозу печінки є експериментальним обгрунтуванням доцільності тестування клінічних ефектів вказаних лікарських сполук.

Ключові слова: експериментальний цироз печінки, перекисне окислення ліпідів, морфологічні порушення, патофізіологічні механізми, L–аргініну аспартат, тивортин, патологічна морфо–функціональна дезінтеграція

Стаття надійшла 24.08.2019 р.

полиорганной недостаточности. Авторы утверждают, что положительные результаты применения L–аргинина аспартата и тивортина при экспериментальном циррозе печени является экспериментальным обоснованием целесообразности тестирования клинических эффектов указанных лекарственных препаратов.

Ключевые слова: экспериментальный цирроз печени, перекисное окисление липидов, морфологические нарушения, патофизиологические механизмы, L–аргинина аспартат, тивортин, патологическая морфо–функциональная дезинтеграция

Рецензент Костенко В.О.

DOI 10.26724/2079-8334-2020-3-73-164-168
UDC 611.32.018.73.018.1:616-053.13-097.-1].08:599.323.4

O.A. Hryhorieva, T.M. Matvieishyna, T.A. Topolenko
Zaporizhzhia State Medical University, Zaporizhzhia

DYNAMICS OF ATP-POSITIVE DENDRITIC CELLS IN RAT'S OROPHARYNGEAL SUBMUCOSA AFTER ANTENATAL ANTIGEN ADMINISTRATION

e-mail: matvieishyna.tm@zsmu.zp.ua

Purpose of the work was to establish dynamics and morphology of DCs, located in oropharyngeal submucosa the postnatal period after antenatal antigen effect on a fetus. DCs were detected on the cryostat sections of the pharynx tissue by using the Vakhshtein-Meizel method. In experimental newborns, the DCs absolute number was found to be greater than in the control and did not change during the first week, unlike in the control, where this index did not change significantly over the two weeks of life. All groups of animals have been increased DCs absolute number by third week of life, while the antigen load on the body increases. Experimental animals, regardless of the antigen administration mode, have been taken place DCs activation earlier than in control, that is, at 7th life day. Animals which underwent antenatal antigen administration during fetal period has been increased number of their processes compared to control. Although it was founded that DCs in experimental groups are stained more shade than in control group, which indicating a more active ATP accumulation.

Key words: ATP, antenatal antigen administration, dendritic cell, pharynx, local immunity.

This work is a fragment of the research project "Features of the rat's organs structure under the influence of different factors during the pre- and postnatal periods", state registration No. 0120U103118.

Dendritic cells (DCs) form a widely distributed cellular net throughout the body. DCs not only exert immune-surveillance for antigens of different origin, but also later activates naive T lymphocytes by giving rise to various immunological responses [2]. The immune complex of oral cavity and pharynx (as a part of a MALT – Mucosa Associated Immune Tissue) might represent the deserve immunological challenges continuously faced by its mucosa. DCs take a crucial part in linking innate and adaptive immunity, either as in mediating immunity or tolerance. Mucosa associated DCs, especially of oral mucosa, should be thoroughly studied in our attempt to understand formation oral immunity. Besides, it is not always possible to extrapolate oral DCs function from their counterparts in non-oral tissues [7]. Also mucus form a nonspecific physical barrier and constrains the immunogenicity of antigens by delivering tolerogenic signals [13].

It is proved that antigen-presenting cells play a central role in transferring information from the periphery of the organism to lymphoid organs. They deliver important signals which result in T cell unresponsiveness with antigen-specific tolerance induction. The initiation of effector $CD8^+$ T-cell responses needs the presentation of peptide bond derived from internalized antigen on class I major histocompatibility complex molecules by DCs in a process called cross-presentation [4].

Antigen load on body, especially on barrier mucosa, can be materialize not only bacteria and viruses but artificially by vaccination, or by antenatal antigen administration on fetus in case mother has undergone some infection during pregnancy [5]. According to Apostolopulose's opinion, a major aim in vaccine development is to induce powerful, specific T-cell responses [1]. This is achieved by targeting antigen to cell surface molecules on DCs that begins receptor mediated endocytosis for loading onto MHC molecules and stimulation of T-cell responses.

It is known, that type III interferon (IFN-λ) is important for innate immune protection at mucosal surfaces and has therapeutic benefit against influenza A virus infection (IAV). According to Hemann's opinion, IFN-λ signaling in DCs populations was critical for the development of protective IAV-specific $CD8^+$T cell responses. It is proofed that mice lacking the IFN-λ receptor had decreased $CD8^+$ T cell

responses relative to wild type and exhibited reduced survival after IAV re-challenge. Analysis of DCs revealed IFN-λ signaling directed the migration and function of $CD103^+$ DCs for development of optimal antiviral $CD8^+$ T cell responses. Thus, IFN-λ serves a critical role in bridging innate and adaptive immunity from mucosa to lymph nodes to program DCs to direct effective T cell immunity against IAV [6].

Adenosine also signaling increased IL-10 secretion while decreasing IL-12p40 secretion in human monocyte-derived DCs [9]. Actually, Antigen DCs are one of the primary targets for adenosine to suppress T and NK cell responses [3, 10].

DCs are an important component of the MALT system. The content of adenosine represents the functional activity of dendritic cells and changes throughout life, as well as with increasing antigen administration. Adenosine content dynamics in the cytoplasm of dendritic cells after antenatal antigen administration on a fetus has not been studied. Determination of adenosine accumulation features will helps to track the reactivity of dendritic cells in response to antigen administration and to reveal formation mechanisms of the oral immune system, especially for children, which mothers have undergone antigen loading during pregnancy, that will form the basis for the formation of new pediatric approaches to care for at-risk group children.

The purpose of the work was to establish dynamics and morphology of DCs, located in oropharyngeal submucosa, at the postnatal period after antenatal antigen administration on a fetus.

Materials and methods. Pharynges of the 124 white laboratory rats were taken as an object of the study. Oropharynges were taken for examination at 1, 7, 14, 21, 45 days of postnatal life. Animals were divided into four groups: I – intact animals, which were born from healthy rats without any antigen administration during pregnancy, II – animals, which were exposed to antenatal antigen administration at 18th day of prenatal development with the method of Voloshyn M.A. (2010), III – animals which were exposed to amniotic fluid antigen administration at the 18th day of prenatal development with the method of Voloshyn M. A. (2011), IV – control animals, which were exposed to antenatal intrafetal injection of saline solution on the 18^{th} day of prenatal development. Rats were born full term and absolutely healthy. It is said that all animals with any symptoms of a disease were avoided to take at experiment. Control group of animals was used for proofing that a process of operating got no effect on a fetus, but antigen leading does. Sex differences were not considered. As antigen have been used split virus inactivated Influenza vaccine Vaxigrip. DCs were detected on the cryostat sections of the pharynx tissue by using the Vakhshtein-Meizel method. Instead of more type of lymphocytes, DCs have high activity of ATPase, because of the activity of the ATP-dependent proton pump depends on the gradual decrease of pH in the endosomes and lysosomes, activation of proteases in the endocytosis of antigens. Other pharyngeal cells exhibit moderate to low activity of ATPase. Control of the reaction was carried out with histological samples rich in ATPase. Samples were embedded in glycerol-gelatin. The absolute number of DCs and its dendrites was counted in a oropharyngeal submucosa on a unit area of 15000 μm^2 using a microscope with oil immersion technique (x630). The variation statistics methods via program «STATISTICA 6.1» (StatSoft Inc., № AXXR 712D833214FAN5) was used to compare differences in number of DCs and DCs dendrites. The $p \leq 0.05$ were considered significant. Supporting and withdrawal of animals from experiment was carried out in accordance with the requirements of the European Commission Directive (86/609/EEC), Law of Ukraine № 1759-VI (15.12.2009) On the Protection of Animals from Cruelty.

Results of the study and their discussion. In newborn intact animals ATP-positive DCs are found in the submucosa of oral part of the pharynx, mainly under the basement membrane. The largest number of ATP contains in the cytoplasm, and provides membrane processes and energy metabolism. Cell bodies are of triangular elongated shape, rough contours of the plasma membrane with brown deposits in the cytoplasm. The nucleus is light, elongated, with wavy contours. The dendrites are spatially oriented mainly along the basement membrane and posses button-shaped endings. In experiment and control there is no difference between topography of DCs and their dendrites.

At the 1st day of life in the number of DCs oropharyngeal submucosa reaches 1.2±0.65 at 15000 μm^2 (Fig.1) and the number of its dendrites is 2.7±0.03 at 15000 μm^2 (fig.2). In experimental animals of the same life term dendrites number increases statistically up to 4.1±0.03 at 15000 μm^2 and 3.9±0.18 at 15000 μm^2 in the animals of the second and third groups compared to control animals. The difference in DCs number in oropharyngeal submucosa is not statistically verified between experimental and control animals (fig.1).

At the seventh day of life in experimental animals there is a tendency of a higher content of DCs number compared to control animals. The number of DCs dendrites in the experimental animals is statistically significantly higher than in control. At describing period in all group animals number of ATP-positive DCs and their dendrites has a tendency to increase compared to the previous observation period (fig. 1, 2).

At the fourteenth day of life DCs are located in the oropharyngeal submucosa beneath the basement membrane, their processes are located parallel to the basement membrane. Their number is statistically significantly increased from 7th days of life to 3.2±0.15 per 15000 μm^2. The DCs number in the experimental oropharyngeal submucosa tends to increase compared to control. In intact animals, the accumulation of ATP-positive material in the cytoplasm increases, proved by the darker staining of DCs. Statistically significantly increasing of dendrites number, compared to the previous observation period, is also revealed. However the length is reduced. Among other common findings we established are fan-shaped dendrites in experiment instead of button-shaped dendrites in control group (Fig.1, Fig.2). After antenatal antigen administration, DCs are stained more vividly compared to animals in control group. In experimental animals of both groups, DCs are characterized by greater number of dendrites than in control. DCs dendrites of experimental animals are visually thicker and more intense in color than in the animals of control group.

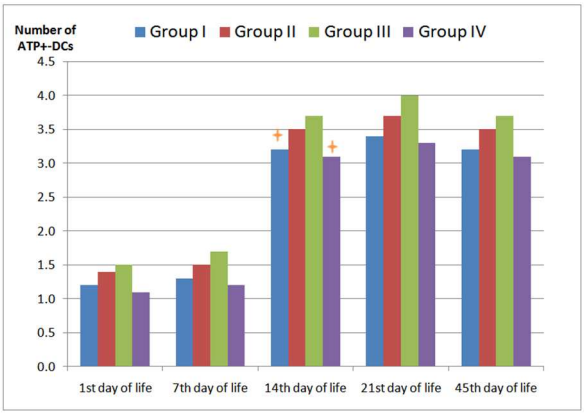

Fig.1. ATP$^+$-DCs' Dynamics in Rats' Oropharyngeal Submucosa on the Unit Area (15000 μm^2, Vakhshtein-Meizel method).

Notes: the symbol ✦ means that the result is statistically significant in relation to the previous observation period.

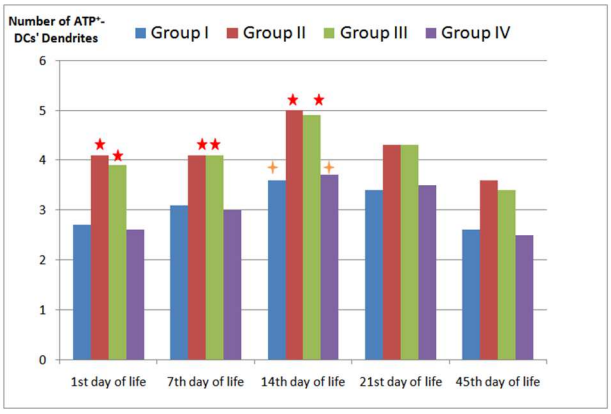

Fig.2. Dynamics of ATP$^+$-DCs' Dendrites in Rats' Oropharyngeal Submucosa on the Unit Area (15000 μm^2, Vakhshtein-Meizel method). Notes: the symbol ★ means that the result is statistically significant with respect to the control group, the symbol ✦ means that the result is statistically significant in relation to the previous observation period.

Intensive deposition of ATP-positive material contents of intracytoplasmatic inclusions of DCs persists from the twenty-first up to the forty-fifth day of life in the animals of control and intact group, the dendrites of DCs are predominantly fan-shaped. The number of them is at the same level with appropriate index of the previous observation period. In animals which underwent antenatal antigen administration during fetal period there are cells with more short predominantly fan-shaped dendrites, compared to animals of control group. At the 21 day of life there is a tendency to increase the DCs number in experimental animals, compared to control ones.

DCs number increases by 14th day of life in all groups of animals, while the antigen load on the body increases. Terms of increasing antigen load on body coincides with terms of changing type of feeding food by getting supplements (solid food) and can be explained by that. This period takes time from the 14th day up to the 21th day of postnatal life. In experimental animals, regardless of the mode of antigen administration, DCs activation takes place earlier than in animals of the control and intact groups, that is, at 7th day of life. In animals which underwent antenatal antigen effect during fetal period the DCs number does not change in comparing to animals of the control and intact groups. However, the number of their processes overgoes control and intact ones. Obtained results coordinate with our previous results [8], also statistically significantly differences between number of DCs in submucosa of nasopharynx and oropharynx was not found.

DCs initiate and modulate primary immune responses by attracting and activating naпve T cells. They are able to coordinate tolerance or immune response depending on their activation status, that is why DCs are also considered as "orchestrating" cells of the immune response [12, 15].

It is settled, that antenatal antigen administration leads to acceleration of the release of immunologically immature PNA$^+$-lymphocytes emerges from thymus to the peripheral immune organs, including pharyngeal wall as MALT-representative. According to the concept of "Lymphocyte – morphogenesis factor" PNA$^+$-lymphocytes influence on the morphogenesis of surrounding cells, changing intercellular and cellular matrix interaction. Microenvironment takes crucial point in differentiation of functional DCs [11]. Antigen presenting cells, such as DCs, are one of the primary targets for adenosine to suppress T and NK cell responses. Despite not enough understanding of molecular mechanisms of adenosine regulation of DCs it is founded that adenosine receptor stimulation strongly suppresses DCs

activation [3]. Cytokine expression profile of Adenosine-differentiated DCs is deeply altered compared to classic myeloid DCs, as well as it is characterized by a mix of proinflammatory and anti-inflammatory cytokines and up-regulated by immune suppressor and tolerogenic factors.

High activity of ATPase is explained by activation of proteases in the process of antigen endocytosis. On a one hand, adenosine is a well-studied neurotransmitter, but on the other hand as a part of ATP it also experts deep immune regulatory functions. While ATP stimulates immune responses by exact inflammasome activation, its degradation product adenosine acts rather anti-inflammatory. In this case it decreases regulation of DCs function and dampens T cell activation and cytokine secretion. DC derived adenosine can also act back onto the DCs in an autocrine manner. As a result DCs functions that are normally involved in stimulating immune responses are suppressed [14]. This likely can leads to depressed reactions of local immunity.

Conclusion

Activation and increasing of DCs number in oropharyngeal submucosa of control animals is observed at the first and fourteenth days of life. In experimental animals, regardless of the method of exposing antigen, the second wave of DCs activation takes place earlier than in control, that is a 7th day of life. Animals which were exposed to antenatal antigen administration have been increased dendrites number compared to control. In experimental groups it was founded that DCs are stained more shade than in control group, which indicating a more active ATP accumulation.

Prospects for the further research lie in the fact that it is planned to study the quantitative content of ATP in DCs cytoplasm after antenatal antigen administration.

References

1. Apostolopoulos V, Thalhammer T, Tzakos A, Stojanovska L. Targeting Antigens to Dendritic Cell Receptors for Vaccine Development. Journal of drug delivery [Internet]. 2013 [cited 19 Dec 2019]; article id 869718: 22 p. Avaliable from: https://www.hindawi.com/journals/jdd/2013/869718/

2. Castell-Rodríguez A, Piñón-Zárate G, Herrera-Enríquez M, Jarquín-Yáñez K, Medina-Solares I. Dendritic Cells: Location, Function, and Clinical Implications, Biology of Myelomonocytic Cells, Anirban Ghosh, IntechOpen [Internet]. Headquarters IntechOpen Limited**;** May 2017 [cited 19 Dec 2019]. Avaliable from: https://www.intechopen.com/books/biology-of-myelomonocytic-cells/dendritic-cells-location-function-and-clinical-implications

3. Cekic C, Kayhan M, Koyas A, Akdemir I, Savas A.C. Molecular mechanism for adenosine regulation of dendritic cells. J immunol. 2017; 198 (1): 67-8. Avaliable from: https://www.jimmunol.org/content/198/1_Supplement/67.8

4. Cohn L, Delamarre L. Dendritic cell-targeted vaccines. Front. Immunol. 2014; 5:255.

5. Grygorieva O, Apt O. Peculiarities of lymphocytes emigration from newborn thymus. Pathologia. 2017; 14 (3): 358-363.

6. Hemann EA, Green R, Turnbull JB. Interferon-λ modulates dendritic cells to facilitate T cell immunity during infection with influenza A virus. Nat Immunol. 2019; 20: 1035–1045.

7. Hovav A. Dendritic cells of the oral mucosa. Mucosal Immunol. 2014; 7: 27–37. doi:10.1038/mi.2013.42

8. Hrygorieva OA, Matvieishyna TM, Topolenko TA. Dynamics and Morphology of dendritic cells of the nasal submucosa of rats' pharynx after antenatal antigen influence. German Science Herald. 2019; 3: 6-8.

9. Kayhan M, Koyas A, Akdemir I, Savas AC, Cekic C. Adenosine Receptor Signaling Targets Both PKA and Epac Pathways to Polarize Dendritic Cells to a Suppressive Phenotype. J immunol. 2019; ji1900765.

10. Mbongue J, Nicholas D, Firek A, Langridge W. The Role of Dendritic Cells in Tissue-Specific Autoimmunity. Journal of immunology research [Internet]. 2014 [cited 24 Dec 2019]; 2014; ID 857143. Avaliable from: https://www.hindawi.com/journals/jir/2014/857143/

11. Mildner A, Jung S. Development and Function of Dendritic Cell Subsets. Immunity. 2014; 40: 642-656.

12. Osorio F, Tavernier S, Hoffmann E, Sayes Y, Martens L, Vetters, Delrue I, et al. The unfolded-protein-response sensor IRE-1α regulates the function of $CD8\alpha^+$ dendritic cells. Nat Immunol. 2014; 15: 248–257.

13. Shan M, Gentile M, Yeiser J, Walland A, Bornstein V, Chen K, He B, et al. Mucus Enhances Gut Homeostasis and Oral Tolerance by Delivering Immunoregulatory Signals. Science. 2013; 342(6157): 447-453.

14. Silva-Vilches C, Ring S, Mahnke K. ATP and Its Metabolite Adenosine as Regulators of DendriticCell Activity. Front. Immunol [Internet]. 2018 [cited 20 Dec 2019]; 9:2581. Avaliable from: https://www.frontiersin.org/articles/10.3389/fimmu.2018.02581/full

15. Solano-Gálvez SG, Tovar-Torres SM, Tron-Gómez MS, Weiser-Smeke AE, Álvarez-Hernández DA, Franyuti-Kelly GA, Tapia-Moreno M, et al. Human Dendritic Cells: Ontogeny and Their Subsets in Health and Disease. Medical Sciences. 2018; 6(4):88.

Реферати

ДИНАМІКА АТФ-ПОЗИТИВНИХ ДЕНДРИТНИХ КЛІТИН В ПІДСЛИЗОВІЙ ОСНОВІ РОТОВОЇ ЧАСТИНИ ГЛОТКИ ЩУРІВ ПІСЛЯ ВНУТРІШНЬОУТРОБНОГО ВВЕДЕННЯ АНТИГЕНА

Григор'єва О.А., Матвєйшина Т.М., Тополенко Т.А.

Метою дослідження було встановити динаміку та морфологію дендритних клітин підслизової основи ротової частини глотки щурів після внутрішньоутробного введення антигена. Дендритні

ДИНАМИКА АТФ-ПОЛОЖИТЕЛЬНЫХ ДЕНДРИТНЫХ КЛЕТОК В ПОДСЛИЗИСТОЙ ОСНОВЕ РОТОВОЙ ЧАСТИ ГЛОТКИ КРЫС ПОСЛЕ ВНУТРИУТРОБНОГО ВВЕДЕНЯ АНТИГЕНА

Григорьева Е.А., Матвейшина Т.Н., Тополенко Т.А.

Целью исследования было установить динамику и морфологию дендритных клеток подслизистой основы ротовой части глотки крыс после внутриутробного введения антигена. Дендритные клетки были выявлены на криостатных

клітини були виявлені на кріостатних зрізах тканини глотки за допомогою методу Вахштейна-Мейзеля. Встановлено, що у новонароджених експериментальних тварин абсолютна кількість дендритних клітин більша, ніж в контролі, та не змінюється протягом першого тижня, на відміну від контролю, де цей показник значно не змінюється протягом двох тижнів життя. У всіх груп тварин абсолютна кількість дендритних клітин збільшується протягом третього тижня життя, одночасно зі збільшенням антигенного навантаження на організм. В експерименті, незалежно від способу введення антигену, активація дендритних клітин відбувається раніше, ніж у контролі, тобто на 7 добу життя. У експериментальних тварин, порівняно з контролем, збільшується кількість відростків дендритних клітин, а також самі дендритні клітини забарвлені темніше, ніж у контролі, що свідчить про більш активне накопичення АТФ.

Ключові слова: АТФ, внутрішньоутробне введення антигену, дендритні клітини, глотка,

Стаття надійшла 25.08.2019 р.

срезах ткани глотки с помощью метода Вахштейна-Мейзеля. Установлено, что у новорожденных экспериментальных животных абсолютное количество дендритных клеток больше, по сравнению с контролем, на протяжении первой недели жизни и не изменяется, в отличие от контроля, где этот показатель значительно не изменяется в течение двух недель. У животных всех исследуемых групп абсолютное количество дендритных клеток увеличивается на протяжении третьей недели жизни, одновременно с увеличением антигенной нагрузки на организм. В эксперименте, активация дендритных клеток происходит раньше, чем в контроле, то есть на 7 сутки жизни. У экспериментальных животных, по сравнению с контролем, увеличивается количество отростков дендритных клеток. У экспериментальных животных увеличивается количество дендритов по сравнению с контролем, а также установлено, что сами дендритные клетки окрашены темнее, чем в контроле, что свидетельствует о более активном накоплении АТФ.

Ключевые слова: АТФ, внутриутробное введение антигена, дендритные клетки, глотка, местный иммунитет.

Рецензент Єрошенко Г.А.

DOI 10.26724/2079-8334-2020-3-73-168-175
UDC 616.12/.14–091.8:546.815/.819–022.513.2:57.084.1

I.V. Gubar[1,2], O.L. Apykhtina[1], R.F. Kaminsky, Yu.B. Chaikovsky, O.P. Yavorovsky, L.M. Sokurenko[2]
Bogomolets National Medical University, Kyiv, [1]SI "Kundiiev Institute of Occupational Health", NAMS of Ukraine, Kyiv, [2]Educational and Scientific Center "Institute of Biology and Medicine" of Taras Shevchenko National University, Kyiv

ORGANOTOXIC EFFECT OF SINGLE INTRATRACHEAL ADMINISTRATION OF LEAD NANOPARTICLES OF DIFFERENT SIZES

e-mail: ginna5@ukr.net

Cardiovasotoxic effect of a single intratracheal administration of lead nanoparticles of different sizes was studied in the experiment. Colloidal solutions of lead sulfide (PbS in sodium polyphosphate) with an average size of 26–34 nm and 50–80 nm, and lead nitrate $Pb(NO_3)_2$ in ionic form which is well soluble in water were used. Toxic effects were assessed 12 days after exposure. Morphological changes were found in the myocardium and to a greater extent in the atria. Mild disorders, such as enlarged interstitial space and dystrophic changes of individual cardiomyocytes were found after administration of $PbS_{26–34nm}$ and $Pb_{50–80nm}$ nanoparticles. Thus, with intratracheal administration of lead, the toxic effect of nanoparticles $PbS_{26–34nm}$ and $PbS_{50–80nm}$ was manifested more compared with an effect caused by the action of the ionic form of lead $Pb(NO_3)_2$. The toxic effect of lead nanoparticles was mainly evident in the atrial myocardium cardiomyocytes, while the aortic wall remained almost unaffected. The most pronounced structural changes were observed in the lungs and bronchi, which may be due to the route of the toxicant administration.

Key words: lead, nanoparticles, intratracheal intoxication, morphological changes, myocardium, aorta, lungs.

The work is a fragment of the research projects "Investigation of cardiovasotoxic action mechanisms of heavy metal nanoparticles (on the problem of biosafety of nanomaterials)", state registration No. 0119U100182; "Changes in internal organs and regulatory systems under the conditions of experimental damage and historical aspects of histology, cytology and embryology development in Ukraine", state registration No. 0116U000121 and "Study the of tissue reaction features and their modulation in lesions of various origins", state registration No. 0120U102691.

New physicochemical properties of the engineered nanoparticles make them very attractive for industrial and biomedical use. Nowadays, the manufacturing and application of nanotechnological products has reached industrial scale worldwide and has the potential for further growth and expansion. This raises concerns about the unforeseen adverse health effects on both nanoindustry workers and nanoproduct consumers [13, 8].

Zhao L [15] noted that nanoparticles (NPs) released to the work area air might contribute to the cardiopulmonary effects observed in workers. Biomarkers of lung damage, cardiovascular diseases, as well as biomarkers of oxidative stress and inflammation which were associated with the occupational exposure to the studied NPs were found in the workers of the NP factory.

It was experimentally established that the cardiotoxic effect of NPs of titanium oxide, zinc oxide, silver, carbon, silicon dioxide and iron oxide depended on both the toxicity of these compounds and the