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Reactive and destructive changes of Peyer's patches in rats with experimental burn disease under infusion of detoxification solutions

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The pathogenesis of burn immune dysfunction and burn enteropathy needs further clarification given that the cellular lesions of lymphoid tissue associated with mucous membranes are the least studied. The purpose of the study was to establish reactive and destructive changes in Peyer's patches of rats after burn injury of the skin with the use of intravenous infusion of isotonic sodium chloride solution and combined colloid-hyperosmolar solutions. White male rats weighing 160-200 g at 6 months of age were divided into 4 groups (18 animals in each group): I, II, III - rats with burn skin injury (grade II-III burn with an area of 23% of body surface area and the development of moderate-severity shock state) which was administered a separate intravenous infusion once a day for the first 7 days of the experiment with isotonic sodium chloride, lactoprotein with sorbitol and HAES-LX-5%, in each case at a dose of 10 ml/kg; IV - intact animals. The material was collected from rats under deep thiopental intraperitoneal anesthesia after 1, 3, 7, 14, 21 and 30 days after burn injury. Biopsies from Peyer's patches for histological and electron microscopic examination were processed using conventional methods. Investigation of histological preparations stained with hematoxylin-eosin was performed on an Olympus BX51 microscope. Ultrathin sections were contrasted on copper support meshes with uranyl acetate and lead citrate according to Reynolds and studied using a PEM-125K electron microscope. Electron and light microscopy data indicate that intravenous infusion of colloidal-hyperosmolar solutions (lactoprotein with sorbitol and HAES-LX-5%) promotes suppression of inflammatory response, inhibits necrosis, and optimizes lymphoid apoptosis at Peyer's patches of rats with experimental burn disease caused by burn injury to the skin of 21-23% of the body surface. Apoptotic lymphocytes and their apoptotic bodies are effectively phagocytosed by macrophages and are digestible in heterophagolysosomes. The apoptotic altered dendritic cells in Peyer's patches are characterized by osmiophilic cytoplasm and a nucleus with high electron density amorphous nucleoplasm. In the cytoplasm are located mitochondria with enliterated matrix and destroyed cristae, irregularly expanded tubules of variable configuration of a granular endoplasmic reticulum with electronically transparent content and numerous derivatives of their vacuole transformation, which are sharply darkened. The fusion of vacuoles leads to the formation of large electron-luminous cavities filled with various residues of compacted degraded cellular structures. Vacuolization promotes site segmentation of condensed cytoplasm of apoptotic dendritic cells and formation of apoptotic blebs, which are subject to entrapment and subsequent sequential degradation with the participation of neighboring macrophages. The structural changes of the organelles of the protein-synthesizing apparatus found in Peyer's plaque cells in rats with experimental burn disease can be regarded as a manifestation caused by functional overload of the granular endoplasmic reticulum (ER-stress). The consequence of optimal development of ER-stress and subsequent unfolded protein response is the apoptotic degradation of the corresponding cell, the course of which is modified by the use of colloid-hyperosmolar solutions.

Keywords: Peyer's patches, structural changes, burn disease, detoxification solutions.

Introduction

It is generally accepted [16] that severe thermal skin burns cause the development of burn disease, the main factor of which is endogenous intoxication. That is why infusion of detoxification solutions is considered to be a mandatory component of the treatment of burn disease, which makes it possible to correct its course or, even, to prevent the development of some of its stages and complications [5, 24].

Considering the stage of burn disease and the different orientation of the individual parts of its pathogenesis, infusion therapy raises not only the question of restoration of water-electrolyte balance and detoxification of the organism, but also a number of tasks for the protection of intact and repair of damaged cells, normalization and stabilization of vital (including immune) functions of the body. It stimulates the development of new infusion drugs and studies the differences of their effect on different organs, tissues and cells [4, 6, 7, 9, 11, 12, 17, 20, 21].

The benefits of widespread introduction of colloidal solutions have, until recently, seemed convincing and valid [4, 10, 18, 23]. However, in recent times the discussion regarding the feasibility and benefits of using colloidal and crystalloid drugs in infusion therapy has become more acute [13, 14, 22, 29].

One of the components of burn disease is immune system dysfunction [16] and burn enteropathy [7, 9]. However, the pathogenesis of burn immune dysfunction and burn enteropathy needs further clarification given that the cellular damage of the lymphoid tissue associated with the mucous membranes (quantitatively the largest part of the immune system) is the least studied. In turn, it does not allow adequately developing and effectively improving the means of correcting the immune status of burns and the treatment of burn enteropathy. This actualizes the study of the structural changes of Peyer's patches in burn disease, which by modern definition [15] are immune sensors of the small intestine, which provide the induction of immune tolerance to commensal bacteria and dietary antigens, as well as provide protection against pathogenic intestinal content [3, 8, 19].

The aim of this study is to establish reactive and destructive changes in Peyer's patches (clusters of lymph nodes of the ileum) of rats at the stages of development of burn disease caused by experimental thermal burn injury of the skin under the conditions of intravenous infusion of isotonic tonic solution and combined colloid-hyperosmolar solutions (lactoprotein with sorbitol and HAES-LX-5%).

Materials and methods

Reactive and destructive changes in Peyer's patches in rats with experimental burn disease under infusion of detoxification solutions (isotonic sodium chloride solution and colloid-hyperosmolar solutions) were studied in 72 white male rats weighing 200-200 g in age 6 month.

Animal retention, as well as experimentation with

thermal burn injury of the skin, infusion of detoxification solutions and other related manipulations were carried out in full compliance with the requirements of the "General Ethical Principles of Animal Experiments", approved by the First National Congress on Bioethics (Kyiv, 2001), in compliance with all the recommendations of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes", and the provisions of the Methodological Recommendations for the "Preclinical study of medicines".

The experimental animals, the material of which was subjected to histological and electron microscopic examination, were divided into 4 groups (18 animals in each group): I, II, III - rats with burn injury to the skin, which were subjected to a separate infusion of isotonic sodium chloride solution, lactoprotein with sorbitol and HAES-LX-5%, in each case at a dose of 10 ml/kg; IV - intact animals (control group).

Simulation of burn disease was performed by applying to the side surfaces of the trunk of animals four copper plates (two plates on each side, the surface area of each plate was 13.86 cm²), which was previously kept for 6 minutes in water at a constant temperature of 100°C. The severity of injury in burn injury was assessed by the severity index of the injury, which takes into account the parameters of the area and depth of burns. The results of the calculation showed that the total area of skin burn in rats was 21-23% of the body surface, the exposure was 10 s, which is sufficient for the formation of burns II-III degree and the development of a shock state of moderate severity.

Intravenous infusion of detoxification solutions at a dose of 10 ml/kg was performed for 5 minutes in a caudal vena cava after its catheterization in aseptic conditions through the femoral vein. The first infusion was performed 1 hour after burn, subsequent injections were performed once a day for the first 7 days of the experiment.

Material extraction from rats was performed under conditions of deep thiopental intraperitoneal anesthesia after 1, 3, 7, 14, 21 and 30 days after the experimental burn injury of the skin.

The material for histological and electron microscopic examination (biopsies from Peyer's patches) was processed using conventional methods. Investigation of histological preparations stained with hematoxylin-eosin was performed on an Olympus BX51 microscope. Ultrathin sections were contrasted on copper support meshes with uranyl acetate and lead citrate according to Reynolds and studied using a PEM-125K electron microscope.

Results

In rats with burn disease that underwent intravenous infusion of isotonic sodium chloride in Peyer's patches cells, destructive changes outweigh the reactive ones. A common manifestation of destructive changes is necrosis and apoptosis of functionally different cells, which occur

on the background of expressive changes in blood vessels of the hemo- and lymphomicrocirculatory bed. Crucial in this process is the change in cell death types of lymphocytes and dendritic cells - the main immunocompetent cells of Peyer's patches. It should be noted that under normal conditions, only single dendritic cells and individual lymphocytes are asynchronously killed by apoptosis; it is characterized by all classical structural manifestations and is terminated by a macrophage reaction that provides phagocytosis of apoptotic bodies and cells.

Under conditions of burn disease, apoptosis and the necrotic changes of lymphocyte and dendritic cell groups occur, to which necrosis of initially apoptotic altered dendritic cells and lymphocytes is involved. Under these conditions, the macrophages of Peyer's patches shift to a state of phagocytic over-voltage, which often ends in their necrotic death.

In phagocytic macrophages, the concentration of dilated and electron-dense contents of tubules of granular endoplasmic reticulum is associated with an increase in the number of lysosomes in the cytoplasm. Lysosomes coalesce with phagosomes and ultimately form different sizes of heterophagolysosomes with varying degrees of structure. The cytoplasm of these macrophages looks swollen, sometimes vacuolated; some mitochondria contain an enlightened matrix, partially destroyed cristae, and a locally defective inner mitochondrial membrane (Fig. 1). The expression of the combination of necrotic and apoptotic changes in lymphocytes and dendritic cells of Peyer's patches is the presence in the focal necrotic cell detritus of apoptotic cells and apoptotic bodies (Fig. 2).

In Peyer's patches of rats with burn disease under conditions of infusion of isotonic sodium chloride solution in dendritic cells, vacuolation of the cytoplasm was observed, which was combined with an increase in cyto- and nucleoplasmic osmiophilia. Such dendritic cells are characterized by destruction of the mitochondria (most often in the form of destruction of the inner membrane and cristae, the remains of which are well contoured against the background of the enlightened mitochondrial matrix). Fragmentation of the tubules of the granular endoplasmic reticulum occurs, which leads to disruption of the ordering of the tubules and their transformation into a vacuole-like formation. The formation of different vacuoles in the cytoplasm of dendritic cells indicates the depletion of the reparative capacity of the cell and the beginning of its death.

Vacuoles originating from tubules of the granular endoplasmic reticulum have an irregular (sometimes wavy) shape, often turning into cavities with uneven edges, but single ribosomes are attached to their surrounding membranes. Transformation of the tubules of the granular endoplasmic reticulum in the vacuole is a transitional stage to the ultimate disintegration of these organelles and is identified, most often, in hyperchromic shrunken dendritic cells. A large number of light vacuoles (Fig. 3, 4) compressing the cytoplasm to the level of transitions

Fig. 1. Macrophage phagocytosed (and partially digested in phagolysosomes) apoptotic body and its fragments in a Peyer's patches 14 days after experimental burn injury under conditions of Isotonic Sodium Chloride infusion. 1 - large phagolysosome. Electronic micrograph. x20000.

Fig. 2. Focal ultrastructural changes of cells in the Peyer's patches of the rat with experimental burn injury under the conditions of infusion of Isotonic Sodium Chloride Solution. The combination of apoptotic and necrotic signs of cell death. The arrow indicates the lumen of the blood capillary. 1 - dendritic cell necrosis; 2 - nucleus of apoptotic lymphocyte; 3 - erythrocyte. Electronic micrograph. x4000.

Fig. 3. Blebbing in the cytoplasm of apoptotic dendritic cells of Peyer's patches of rat 21 days after the experimental burn injury under the conditions of infusion of Isotonic Sodium Chloride Solution. 1 - nucleus of apoptotic dendritic cell; 2 - a bubble (surrounded by small vesicles and vacuoles) in the cytoplasm of an apoptotic dendritic cell; 3 - neutrophil leukocyte cytoplasm; 4 - lymphocytes. Electronic micrograph. x7000.

Fig. 4. Intense blebbing in the cytoplasm of the apoptotic dendritic cell of a Peyer's patches of rat 30 days after the experimental burn injury under the conditions of infusion of Isotonic Sodium Chloride Solution. Dystrophic cytopathological changes of cells, swelling and destruction of cristae mitochondria. 1 - shrunken cytoplasm of apoptotic dendritic cell; 2 - a bubble (surrounded by small vesicles and vacuoles) in the cytoplasm of an apoptotic dendritic cell; ← damaged mitochondria. Electronic micrograph. x10000.

Fig. 5. Phagocytosis by macrophage of apoptotic bodies in a Peyer's patches of the rat 3 days after the experimental burn injury under infusion of Lactoprotein with Sorbitol. 1 - macrophage nucleus. Electronic micrograph. x15000.

Fig. 6. Blebbing in the process of supercondensed dendritic cell in a Peyer's patches of the rat 14 days after the experimental burn injury under infusion of HAES-LX-5%. 1 - cytoplasm of a "dark" dendritic cell of normal structure; ← blebbing. Electronic micrograph. x15000.

between transparent vacuoles, creates a common enlightened honeycomb background, contrasting with a homogeneous dark osmiophilic nucleus framed by a narrow electron-light band around the nucleus. The described destructive changes are a classic morphological manifestation of apoptotic "bubble formation" or "blebbing". Subsequently, the long, narrow, branched branches of apoptotic dendritic cells are fragmented into apoptotic blebs, and the separated nucleated dendritic cell section is transformed into a larger apoptotic body.

Our findings indicate that intravenous infusion of colloidal-hyperosmolar solutions (lactoprotein with sorbitol and HAES-LX-5%) promotes suppression of inflammatory response, inhibits necrosis, and optimizes apoptosis of lymphocytes and dendritic cells in Peyer's patches of rats with experimental burn disease. Apoptotic lymphocytes and their apoptotic little bodies (Fig. 5) are effectively phagocytosed by macrophages and are digestible in heterophagolysosomes.

Dendritic cells with osmiophilic cytoplasm in Peyer's patches are characterized by a nucleus with high electron density amorphous nucleoplasm. In the cytoplasm are located mitochondria with enlightened matrix and destroyed cristae, irregularly expanded tubules of variable configuration of a granular endoplasmic reticulum with electronically transparent content and numerous derivatives of their vacuole transformation, which are sharply darkened. The fusion of vacuoles leads to the formation of large electron-luminous cavities filled with various residues of compacted degraded cellular structures (Fig. 6). Vacuolization promotes segmental segmentation of condensed cytoplasm of apoptotic dendritic cells, fragments of which are subject to entrapment and subsequent sequential degradation with the participation of neighboring macrophages.

Discussion

We have established significant reactive and destructive transformations of Peyer's patches lymphocytes and dendritic cells in rats with experimental burn disease under the conditions of intravenous infusion of various detoxification solutions.

According to modern concepts [15], dendritic cells are the major antigen-presenting cells in the immune system (and, in particular, in Peyer's patches). The presentation of antigens to lymphocytes is a finely coordinated and multicomponent process that includes: 1 - capture of native (unchanged) antigenic material by phagocytosis, pinocytosis or receptor-mediated endocytosis; 2 - partial proteolysis (processing) of antigenic material in endosomes (or lysosomes) with the release of epitopes of antigens (antigenic determinants); 3 - under these conditions, the synthesis of Major Histocompatibility Complex (MHC) molecules and the binding of the synthesized MHC molecules to the antigen epitopes; 4 - transport of complexes of MHC molecule antigen epitope

to the surface of the dendritic cell, where they are represented by their recognizing lymphocytes. 5 - secretion of soluble mediators (preferably IL-1) that cause lymphocyte activation. To determine the critical threshold of reactive and destructive changes of dendritic cells and lymphocytes of Peyer's patches revealed by us in the conditions of burn disease is difficult, but the ordering of the course of this complex process of presentation of antigenic material from the lumen of the small intestine is, at least, impaired and impaired hyperosmolar solutions.

We found structural changes of organelles of protein-synthesizing apparatus in cells of Peyer's patches in rats with experimental burn disease, which coincides with the scientific literature [16] about the characteristic of burn disease syndrome of hypermetabolism and its component - hypercatabolism. Our study found that the most sensitive organelle of Peyer's patches cells is a granular endoplasmic reticulum, structural changes of which can be regarded as a manifestation caused by functional overload of the granular endoplasmic reticulum (ER-stress) [25, 26, 30], which results in a response to unfolded protein response. Under the norm [25], protein folding occurs in the tubules of the granular endoplasmic reticulum, in which the synthesized proteins undergo post-translational modification, acquiring a characteristic three-dimensional configuration. Unfolded protein response occurs due to the accumulation in the lumen of the tubules of the granular endoplasmic reticulum of uncoiled or misfolded proteins [27, 28].

The sequence of structural and molecular manifestations of unfolded protein response [2] indicates: an adaptive attempt to restore normal cell function by arresting protein translation and subsequent degradation of misfolded proteins, as well as activation of signaling pathways leading to increased production of molecular chaperones, proteins. Thus, unfolded protein response, under normal conditions, is a stress pathway to homeostatic regulation [27]. If the constant overvoltage of unfolded protein response is not eliminated within a certain period of time, apoptotic degradation of the corresponding cell occurs, leading to the development of various pathological conditions [26, 28, 30].

Manifestations of the typical crystalline solution, which is an isotonic solution of sodium chloride, lactoprotein with sorbitol (protein-saline colloidal-hyperosmolar solution) and HAES-LX-5% (infusion colloid-hyperosmolar plasma replacement of the new generation, created on the basis of third generation hydroxyethyl starch HES 130/0.4) have been the subject of our previous research [20, 21], as well as of other scientists [6, 9, 11, 12, 17], and have proved their differences in influencing the course of experimental burn disease. The study of reactive and destructive changes in Peyer's patches of rats with experimental burn disease, as well as the impact of ER-stress on the dynamics of necrotic and apoptotic cell changes, was updated by a new wave of controversy regarding the official assessment of possible limitations on the use of colloidal solutions based

on hydroxyethyl starch. The divergent opinions of pharmacology scientists and experts found their symbolic reflection in a name published [1] in the 2019 scientific review "Starch Wars - New Episodes of the Saga. Changes in Regulation on Hydroxyethyl Starch in the European Union". At the same time, it should be noted that, for the first time in the 1960s, various infusion solutions based on synthetic hydroxyethyl starch became widely known in intensive care units and veterinary medicine worldwide (for example, over 60 drugs were registered in 2010 in Europe and 4 in the United States). The flow of "crystalloid-colloidal debate" and "starch wars" in the scientific literature does not stop until this time, it has a wavy chain character (when one article causes the appearance of several articles with opposite conclusions, which, in turn, stimulates the publications of the authors-opponents).

Conclusions

The question about the benefits and feasibility of "use - not use" infusion solutions based on hydroxyethyl starch of different generation is still open, but there is no doubt reliable experimental evidence that demonstrates the ability of colloidal infusion vessels improve intravascular osmotic pressure improve hemomicrocirculation, limit swelling of tissues of primary intact organs in burn disease [5, 14]. We agree with the opinion of many researchers that the answer to this question should be further experimental and clinical studies of the action of specific crystalloid and colloidal infusion solutions, judiciously applied on the principle of "What? Where? When? What dose?" The results of our study indicate that timely administration of colloid-hyperosmolar solutions such as lactoprotein with sorbitol and HAES-LX-5%, in a timely manner, positive effects on the manifestations of reactive and destructive changes in Peyer's patches in burn disease caused by severe skin burns.

Conclusions

1. Intravenous infusion of colloidal-hyperosmolar solutions (lactoprotein with sorbitol and HAES-LX-5%) promotes suppression of inflammatory response, inhibits necrosis, and optimizes apoptosis of lymphocytes and dendritic cells in Peyer's patches of rats with experimental burn disease. Apoptotic lymphocytes and their apoptotic bodies are effectively phagocytosed by macrophages and are digestible in heterophagolysosomes.

2. Osmophilic cytoplasm and a nucleus with high electron density amorphous nucleoplasm are characteristic of apoptotic altered dendritic cells in Peyer's patches. In the cytoplasm are located mitochondria with enlightened matrix and destroyed cristae, irregularly expanded tubules of variable configuration of a granular endoplasmic reticulum with electronically transparent content and numerous derivatives of their vacuole transformation, which are sharply darkened. The fusion of vacuoles leads to the formation of large electron-luminous

cavities filled with various residues of compacted degraded cellular structures. Vacuolization promotes segmental segmentation of condensed cytoplasm of apoptotic dendritic cells, and the formation of apoptotic blebs, which are subject to entrapment and subsequent sequential degradation with the participation of neighboring macrophages.

3. Structural changes of organelles of protein-

synthesizing apparatus found in Peyer's patches cells in rats with experimental burn disease can be regarded as a manifestation of functional overload of the granular endoplasmic reticulum (ER-stress). The consequence of optimal development of ER-stress and subsequent unfolded protein response is the apoptotic degradation of the corresponding cell, the course of which is modified by the use of colloid-hyperosmolar solutions.

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РЕАКТИВНІ ТА ДЕСТРУКТИВНІ ЗМІНИ БЛЯШОК ПЕЙЄРА У ЩУРІВ З ЕКСПЕРИМЕНТАЛЬНОЮ ОПІКОВОЮ ХВОРОБОЮ ЗА УМОВ ІНФУЗІЇ ДЕЗІНТОКСИКАЦІЙНИХ РОЗЧИНІВ

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Патогенез опікової імунної дисфункції та опікової ентеропатії потребує подальшого уточнення з огляду на те, що клітинні пошкодження лімфоїдної тканини, асоційованої зі слизовими оболонками, є найменш вивченими. Мета дослідження - встановити можливі реактивні та деструктивні зміни в бляшках Пейєра щурів після опікової травми шкіри за умов застосування внутрішньовенної інфузії ізотонічного розчину хлориду натрію та комбінованих колоїдно-гіперосмолярних розчинів. Білі щурі-самці масою 160-200 г віком 6 місяців були розділені на 4 групи (по 18 тварин у кожній групі): I, II, III - щури з опіковою травмою шкіри (опік II-III ступеня площею 23% поверхні тіла та розвитком шокового стану середнього ступеня важкості), котрим проводили окрему внутрішньовенну інфузію 1 раз на добу протягом перших 7 днів експерименту ізотонічного розчину натрію хлориду, лактопротеїну із сорбітолом та HAES-LX-5%, у кожному випадку у дозі 10 мл/кг; IV - інтактні тварини. Забір матеріалу від щурів проводили за умов глибокого тіопенталового внутрішньоочеревинного наркозу через 1, 3, 7, 14, 21 та через 30 днів після нанесення опікової травми шкіри. Біоптати із бляшок Пейєра для гістологічного та електронно-мікроскопічного дослідження обробляли за допомогою загальноприйнятих методів. Дослідження гістологічних препаратів, забарвлених гематоксилином та еозином, виконували на мікроскопі Olympus BX51. Ультратонкі зрізи контрастували на мідних опорних сіточках ураніацетатом і цитратом свинцю за Рейнольдсом та вивчали на електронному мікроскопі ПЕМ-125К. Одержані методами електронної та світлової мікроскопії дані свідчили, що внутрішньовенна інфузія колоїдно-гіперосмолярних розчинів (лактопротеїну із сорбітолом та HAES-LX-5%) сприяє пригніченню запальної реакції, гальмує некроз та оптимізує апоптоз лімфоцитів та дендритних клітин в бляшках Пейєра щурів з експериментальною опіковою хворобою, викликану опіковою травмою шкіри площею 21-23% поверхні тіла. Апоптозні лімфоцити та їхні апоптозні тілії ефективно фагоцитуються макрофагами та підлягають перетравленню в гетерофаголізосомах. Для апоптозної зміни дендритних клітин в бляшках Пейєра характерною є осміофільна цитоплазма і ядро з аморфною нуклеоплазмою високої електронної щільності. В цитоплазмі локалізовані мітохондрії з просвітленим матриксом і зруйнованими кристами, нерівномірно розширені каналці варіабельної за конфігурацією гранулярної ендоплазматичної сітки з електронно прозорим вмістом та численні похідні їхньої вакуольної трансформації, які різко виділяються у вигляді світлих плям на темному фоні цитоплазми. Злиття вакуолей призводить до формування великих електронно світлих порожнин, заповнених різноманітними залишками ущільнених деградованих клітинних структур. Вакуолізація сприяє ділянковій сегментації конденсованої цитоплазми апоптозних дендритних клітин і утворенню апоптозних блебів (apoptotic blebs), які підлягають захопленню і наступній послідовній деградації за участі сусідніх макрофагів. Виявлені структурні зміни органел білок-синтезуючого апарату в клітинах бляшок Пейєра у щурів з експериментальною опіковою хворобою, можуть бути розцінені як прояв, викликаний функціональним перенавантаженням гранулярної ендоплазматичної сітки, стресу ендоплазматичного ретикулу (ER-стресу). Наслідком оптимального розвитку ER-стресу і подальшої відповіді на незгорнуті білки (unfolded protein response) є апоптозна деградація відповідної клітини, перебіг котрої модифікується за умов використання застосованих колоїдно-гіперосмолярних розчинів.

Ключові слова: бляшки Пейєра, структурні зміни, опікова хвороба, дезінтоксикаційні розчини.

РЕАКТИВНЫЕ И ДЕСТРУКТИВНЫЕ ИЗМЕНЕНИЯ БЛЯШЕК ПЕЙЕРА У КРЫС С ЭКСПЕРИМЕНТАЛЬНОЙ ОЖГОВОЙ БОЛЕЗНЬЮ В УСЛОВИЯХ ИНФУЗИИ ДЕЗИНТОКСИКАЦИОННЫХ РАСТВОРОВ

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Патогенез ожоговой иммунной дисфункции и ожоговой энтеропатии требует дальнейшего уточнения с учетом того, что клеточные повреждения лимфоидной ткани, ассоциированной со слизистыми оболочками, наименее изучены. Цель исследования - установить возможные реактивные и деструктивные изменения в Пейеровых бляшках крыс после ожоговой травмы кожи в условиях применения внутривенной инфузии изотонического раствора хлорида натрия и комбинированных коллоидно-гиперосмолярных растворов. Белые крысы-самцы массой 160-200 г в возрасте 6 месяцев были разделены на 4 группы (по 18 животных в каждой группе): I, II, III - крысы с ожоговой травмой кожи (ожог II-III степени площадью 23% поверхности тела и развитием шокового состояния средней степени тяжести), которым проводили отдельную инфузию 1 раз в сутки в течение первых 7 дней эксперимента изотонического раствора натрия хлорида, лактопротеина с сорбитолом и HAES-LX-5%, в каждом случае в дозе 10 мл/кг; IV группа - интактные животные. Забор материала от крыс проводили в условиях глубокого тиопенталового внутрибрюшинного наркоза через 1, 3, 7, 14, 21 и через 30 суток после нанесения ожоговой травмы кожи. Биоптаты из Пейеровых бляшек для гистологического и электронно-микроскопического исследования обрабатывали с помощью общепринятых методов. Исследование гистологических препаратов, окрашенных гематоксилином и еозином, проводили на микроскопе Olympus BX51. Ультратонкие срезы контрастировали на медных

опорных сеточках уранилацетатом и цитратом свинца по Рейнольдсу и изучали на электронном микроскопе ПЭМ-125K. Полученные методами электронной и световой микроскопии данные свидетельствуют о том, что внутривенная инфузия коллоидно-гиперосмолярных растворов (лактопротеина с сорбитолом и HAES-LX-5%) способствует угнетению воспалительной реакции, тормозит некроз и оптимизирует апоптоз лимфоцитов и дендритных клеток в бляшках Пейера крыс с экспериментальной ожоговой болезнью, которая вызвана ожоговой травмой кожи площадью 21-23% поверхности тела. Апоптозные лимфоциты и их апоптозные тельца эффективно фагоцитируются макрофагами и подлежат перевариванию в гетерофаголизосомах. Для апоптозно измененных дендритных клеток бляшек Пейера характерной является осмиофильная цитоплазма и ядро с аморфной нуклеоплазмой высокой электронной плотности. В цитоплазме локализуются митохондрии с просветленным матриксом и разрушенными кристами, неравномерно расширенные канальцы переменной по конфигурации гранулярной эндоплазматической сетки с электронно прозрачным содержимым и многочисленные производные их вакуольной трансформации, которые резко выделяются в виде светлых пятен на темном фоне цитоплазмы. Слияние вакуолей приводит к формированию больших электронно светлых полостей, которые заполнены разными остатками уплотненных деградированных клеточных структур. Вакуолизация способствует участковой сегментации конденсированной цитоплазмы апоптозных дендритных клеток и образованию апоптозных бляшек (apoptotic blebs), которые подлежат захвату и последующей деградации при участии соседних макрофагов. Обнаруженные структурные изменения органелл белоксинтезирующего аппарата в клетках бляшек Пейера у крыс с экспериментальной ожоговой болезнью могут быть оценены как проявление, вызванное функциональным перенапряжением гранулярной эндоплазматической сетки, стресса эндоплазматического ретикулума (ER-стресса). Следствием оптимального развития ER-стресса и последующего ответа на несвернутые белки (unfolded protein response) является апоптозная деградация соответствующей клетки, ход которой модифицируется в условиях использования примененных коллоидно-гиперосмолярных растворов.

Ключевые слова: бляшки Пейера, структурные изменения, ожоговая болезнь, дезинтоксикационные растворы.