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STRUCTURAL MANIFESTATIONS OF ER-STRESS IN THE NEPHRON HAEMOCAPILLARY ENDOTHELYCYTES IN EXPERIMENTAL BURN DISEASE IN RATS

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Studies on structural manifestations of ER-stress, apoptosis and necrosis in endothelial cells of nephron hemocapillaries in experimental burn disease in rats (caused by burn injury of the skin with an area of 21-23% of the body surface) have shown indisputable protector and therapeutic advantages of intravenous colloidal hyperosmolar solutions infusion over the isotonic solution infusion, which was 0.9% NaCl solution. The most vulnerable organelle in endothelial cells of nephron hemocapillaries in experimental burn disease and in detoxification therapy performed is a granular endoplasmic reticulum, therefore an important evidence of the used detoxification solutions efficacy is the nature of the ER-stress course. Under the conditions of intravenous colloidal hyperosmolar solutions infusion (lactoprotein with sorbitol and HAES-LX-5%), normalization of ER stress is observed, accompanied by apoptotic changes of endothelial cells, but it is not accompanied by necrosis of endothelial cells. The consequence of the endothelial cells necrosis in the kidney hemocapillaries is the formation of paravasal hemorrhages and lympho-leukocyte infiltrates, which is evidence of impaired filtration and reabsorption functions of the kidneys, as well as of the inflammatory process progress in the kidneys.

Key words: burn disease, detoxification solutions, nephron hemocapillaries.

The work is a fragment of the research project "Morphological features and changes of the digestive tract organs in experimental skin burn injury", state registration No. 0119U101618.

It is recognized that the main factors of burn disease (BD) are endogenous intoxication, dyscirculatory hypoxia and histotoxic ischemia [17], therefore, in the BD treatment, intravenous detoxification infusion is used to prevent the development of severe toxic, hypoxic, and reperfusion damage to cells and tissues, which efficacy is the subject of clinical and experimental studies [4, 10, 7, 8].

For this purpose, different types of crystalloid and colloidal solutions are used. Ongoing discussions on the use of colloidal and crystalloid solutions have now turned to the debate on the optimal type of colloidal solutions, which solutions based on hydroxyethyl starch of different generation are attributed to, but even they are subject to debate on the presence or absence of hydroxyethyl starch nephrotoxic effects [10,1]. Determining the mechanisms of reactive and destructive changes of kidney cells under the action of toxicants and factors of various diseases led to the revision in many aspects on the problem of nephrotoxic damage to the kidney and involvement of hemomicrocirculation changes, stress of endoplasmic reticulum (ER-stress) in cells, necrosis, apoptosis, autophagy in this process. [2, 3, 9, 11, 12]. Meanwhile, the study of molecular and cellular manifestations of ER-stress, apoptosis, and necrosis in nephron hemocapillaries endothelial cells (HE) in burn disease (BD) under the effect of various detoxification solutions infusion has not recently been the subject of special studies.

The purpose of the work was to study the structural manifestations of ER-stress, apoptosis and necrosis in nephrons HE in experimental burn disease in rats under the effect of infusion: a typical isotonic crystalloid solution (0.9% NaCl solution), complex protein-saline hyperosmolar solution (lactoprotein with sorbitol) and the recently developed hydroxyethyl starch of the third generation colloidal-hyperosmolar HAES-LX-5% solution.

Materials and methods. Structural manifestations of ER-stress, apoptosis and necrosis in HE of nephrons with BD in rats were studied under the effect of various detoxification solutions infusion, namely: 0.9% NaCl solution, lactoprotein with sorbitol and HAES-LX-5%, on 105 white rat- males weighing 155-160 grams.

The experiment on the burn injury, infusion of solutions and a number of related study procedures was performed on the basis of the problematic research laboratory of functional morphology and development genetics at Vinnytsia Pirogov Memorial National Medical University, which is certified by the State Pharmacological Center at the MOH of Ukraine (Certificate No. 003/10 dated 11.01. 2010) and the laboratory of the Pharmacology Department at Vinnitsa Pirogov Memorial National Medical University, which is certified by the State Pharmacological Center at the MOH of Ukraine (certificate No. 000679 dated 11.01.2008).

Keeping in the vivarium and all manipulations with rats were carried out in full compliance with the provisions of the "General Ethical Principles for Animal Experiments", approved by the First National Congress on Bioethics (Kyiv, 2001), with strict adherence to the recommendations of the "European

Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes"(Council of Europe, Strasburg, 1986).

The experimental rats were divided into seven groups (15 animals in each): group I - intact rats; groups II, III and IV - rats without BD induction, which were administered a separate intravenous infusion of 0.9% NaCl solution, lactoprotein with sorbitol and HAES-LX-5% at the dose of 10 ml/kg; groups V, VI and VII - rats with BD, which under the same scheme were administered an intravenous infusion of the studied solutions.

For BD modeling, an experimental burn skin injury (T) was performed by pressing four heated copper plates (two plates on each side, each area being 13.86 cm^2) for 10 minutes to the shaved lateral surfaces of the rat's body, which were previously held for 6 minutes in water at a permanent temperature of 100C^o C. To determine the damage degree in T, the severity index was used, which took into account the parameters of the burns area and depth, as well as the total skin area of the burned. The calculation data show that T covered 21-23% of the animal body surface, which was quite sufficient for the formation of II-III degree burns, the development of moderate-severity burn shock, and the BD initiation.

Intravenous infusion of solutions at the dose of 10 ml/kg was performed within 5 minutes into the caudal vena cava after the catheter was inserted under aseptic conditions through the femoral vein. The course of infusion therapy lasted 7 days (the first intravenous infusion was performed 1 hour after the induction of T, subsequent injections were carried out once a day). Induction of T, catheterization of the great vessels was performed under anesthesia caused by intraperitoneal administration of propofol at the dose of 60 mg/kg. Biological sampling from rats for morphological examination of the kidneys was performed under conditions of deep thiopental intraperitoneal anesthesia in 14, 21 and 30 days after T. For histological and electron microscopic studies, the sampled biopsies were processed according to the conventional method. Hemotoxylin-eosin stained histological specimens were examined on an Olympus BX51 microscope. Ultra-thin sections were made using LKB-3 ultramicrotome (Sweden), contrasted on copper support meshes with uranyl acetate and lead citrate according to Reynolds. Electron microscopic study was performed using PEM-125K electron microscope.

Results of the study and their discussion. The performed study showed that T and BD caused by it lead to significant structural changes in hemocapillaries of the vascular glomerulus (component of the filtration apparatus) and hemocapillaries of the pretubular vascular network (PH) (component of the reabsorption apparatus) of nephrons after 14, 21 and 30 days (at the stages of late BD toxemia and septicotoxemia, which manifestations were largely offset by timely infusion therapy). During this period of time, the process of destruction and structural restructuring in nephrons of experimental burned rats does not attenuate, but is only modified.

It is established that already 14 days after T under the conditions of 0.9% NaCl solution infusion (rats of group V) manifestations of mosaic reactive and destructive changes in blood vessels walls, kidney stroma and parenchyma are registered. The dilatation and / or collapse of the blood-filled and / or hollow lumens of the peritubular circulatory network vessels and hemocapillaries of the vascular glomerulus were detected. The phenomena of interstitial edema and erythrocytes diapedesis are observed. Particular destruction of PH is recorded, as well as the formation of paravasal edema, hemorrhage, lympho-leukocyte infiltrates.

After 21 days of observation in the renal cortex of group V rats an interstitial edema was revealed, which had mainly perivascular localization. Deformation and destruction of the granular endoplasmic reticulum (GER), vacuolation of the cytoplasm, and partial exfoliation of the EH PH are observed. Sometimes subtotal or even total destruction of the wall in individual PH is observed, followed by the formation of paravasal hemorrhages.

The lumen of most peritubular blood vessels is filled with sludged erythrocytes. The presence of PH with a destroyed wall and paravasal interstitial hemorrhages is combined with the occurrence of hemolyzed erythrocytes and cellular detritus in the lumen of the nephron tubules with the damaged wall. The erythrocyte hemorrhage site contains the remnants of the destroyed cells, therefore it is difficult in most cases to determine the extent of damage to the wall of peritubular blood vessels located in that area. Attention is drawn to the hemocapillaries of the vascular glomerulus, whose endothelial cover is locally completely disintegrated by apoptosis (anoikis) of the EH. The lumen of such microvessels is clouded by apoptotic altered EH that have completely or partially lost contact with the basement membrane. The nucleoplasm and cytoplasm in such apoptotic EH are condensed, the nuclear membrane is in many places invaginated, chromatin in the state of marginal aggregation (fig. 1).

Under the conditions of intravenous infusion of lactoprotein with sorbitol (group VI rats), 14 days after the T, nephrons PH are structurally preserved. The EH is characterized by the cytoplasmic zonation, which arises due to the organelle hypertrophy of the protein-synthesizing apparatus. In particular,

hypertrophied, long, branched, but prone to parallel placement to each other, expanded and filled with small-globular contents granular endoplasmic reticulum (GER) localized in the adjacent to the nucleus, elongated and thickened area of organelles. The peripheral, refined area of fenestra localization is also long and, as a rule, contralateral to the nuclear zone of the EH cytoplasmic organelles (fig. 2.).

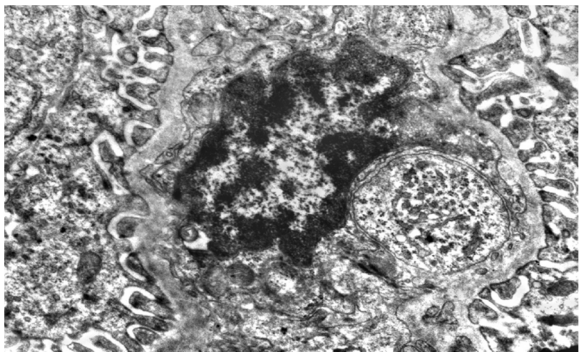

Fig. 1. Hemocapillary of the vascular glomerulus with disintegrated endothelial cover of the rat renal cortex 21 days after T under the conditions of administering 0.9% NaCl. 1 - nucleus of apoptotic EH; 2- apoptotic body; 3- basement membrane; 4- cytotrabecule; 5- podocyte cytopodia. Electronic micrograph. Magn. 35000.

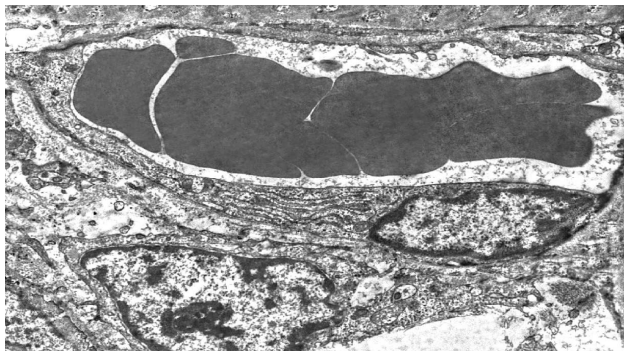

Fig. 2. Hypertrophy of the parallel tubes of the GER (marked with a single arrow) in the cytoplasm of the peritubular vascular network EH in the rat renal cortex 14 days after T under the conditions of administering lactoprotein with sorbitol. Electronic micrograph. Magn. 10000.

Electron microscopic studies of the renal cortex in animals of group VI 14 days after T showed that changes in all components of the filtration barrier occur in renal corpuscles, but they are less pronounced than in rats administered 0.9% NaCl solution. The enlargement of hemocapillary lumens of the vascular glomerulus and their moderate blood flow were observed. In the nuclear zone, the EH cytoplasm looks swollen, vacuolated and cleared, but a clear fenestration of the cytoplasmic areas remains.

In some peritubular areas (fig. 3), signs of interstitial edema (in the form of enlightenment of the basic amorphous substance) are noted, as well as intracellular edema, autophagic changes, vacuolation, necrosis of the interstitial cells and individual EH. Vacualization and intracellular edema of EH are also observed without concomitant signs of perivascular edema and are the result of the vacuum transformation of the tubules of the GER, whose membranes lose their attached ribosomes. This is combined with manifestations of hemomicrocirculatory disorders.

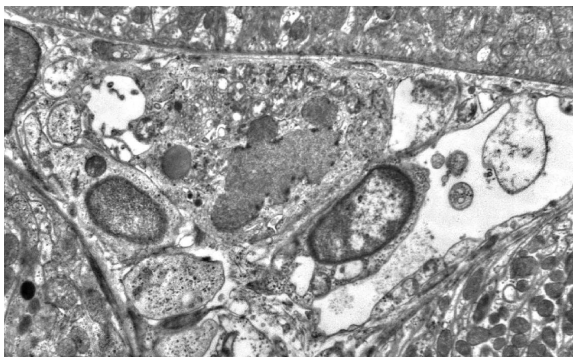

Fig. 3. Intracellular edema and vacuolation of EH in the rat renal cortex 21 days after T under the conditions of lactoprotein with sorbitol administration. Electronic micrograph. Magn. 25000.

The results of the morphological study on the structural components of the renal cortex 30 days after the T in group VI, indicate their high survivability. Perivascular edema is insignificant, PH are partially enlarged and blood filled. In the renal cortex there are hypertrophied renal corpuscles, some of them having small sizes. The hemocapillaries of the vascular glomerulus in the enlarged renal corpuscles are located tightly and blood-filled. In hemocapillaries of vascular glomeruli, apoptotic (anoikis) changes of EH are noted, but most of the renal corpuscles in their ultrastructure are similar to those in the control rats' kidney.

Morphological study of the rat kidneys in group VII 14 and 21 days after T showed that administration of HAES-LX-5% solution prevents the development of destructive changes in nephrons. Morphological study of the rat kidneys in group VII with T under the condition of HAES-LX-5% administration after 30 days of the experiment sometimes permitted to detect areas of renal cortex that do not differ from those in the control (unburned) animals. The prevalence and mutual arrangement of mitochondria, ribosomes, polysomes, GER tubules and Golgi complex in the EH indicate their coordinated and adjusted functioning.

The studies performed have shown the absolute positive advantages of intravenous infusion of colloidal-hyperosmolar solutions over infusion of isotonic solution, which is 0.9% NaCl solution. There are also differences in the influence on the renal cortex structure of lactoprotein with sorbitol and HAES-LX-5% in burned rats. We have found that the most vulnerable organelle of EH in nephrons with BD and detoxification therapy is GER. According to current concepts, GER (endoplasmic reticulum) is a membrane network that extends throughout the cell cytoplasm and is adjacent to the nuclear membrane.

It is a site of protein synthesis and transport, its assembly and coagulation, protein degradation, lipid and steroid synthesis, carbohydrate metabolism and calcium storage. GER consists of functional subdomains that have unique biophysical structure, which requires coordination in response to changes in the intracellular environment. Proteins are synthesized in ribosomes that are attached to the membranes of the GER tubules. Once inside the GER tubules, proteins undergo post-translational modification and acquire their correct three-dimensional shape (collapsed). Excessive increase in protein synthesis can lead to overloading of protein coagulation mechanisms in the GER tubules lumens, which results in imbalance of coagulation and accumulation of misfolded (folded) proteins [1, 13], which causes the stress state of GER (endoplasmic reticulum stress or ER-stress) [2].

Recent kidney studies [15] have found that acute kidney injury caused by renal ischemia-reperfusion leads to accumulation of unfolded and misfolded proteins in the lumen of the kidney GER and to development of ER stress. Under these conditions, prolonged ER stress activates the apoptotic cell death pathway, eliminating dysfunctional cells. According to M. Yanetall [15], modulation of ER stress in renal cells can be ensured by successful implementation of a therapeutic strategy for treatment of acute renal disease. This is why it is now widely accepted that ER stress plays a major role in acute and chronic structural damage to the kidneys, but also promotes cellular adaptation and nephroprotection [3, 15].

In our previous studies [8], the role of mitochondria in providing reactive rearrangement of nephron epithelial cells in BD was established, as well as the role of such phenomenon as mitophagy in this process [11]. The latest (as of publication date) literature review [9] on cellular and molecular mechanisms of renal toxicity discusses the role of mitochondria and GER in the signaling pathways of renal cell death, but the role of ER stress in the regulation of renal EH death under the conditions of toxicant exposure remains beyond the researchers'attention.

We have found that under the conditions of BD development (which component and factor is endogenous intoxication), structural changes of the GER occur in the EH of the vascular glomerulus and the pretubular vascular network of nephrons. Under the conditions of the applied detoxification solutions, hypertrophy (expansion of the lumen and the branching degree) of GER tubules overfilled with electron-dense content, which, taking into account the research literature data, indicates the tension of protein synthesis structural mechanisms and creation of conditions for the adaptive [ER] stress development. The second stage of the ER-stress development is the loss of attachment of most ribosomes to the tubule membranes of hypertrophied GER (which may indicate an attempt to restore normal EH function by stopping protein translation).

Such a scenario of ultrastructural transformations is not a preventer of EH apoptosis, but it is a preventer of necrosis. In the worst case of the ER-stress scenario, GER tubules released from the attached ribosomes are fragmented into vacuoles that merge with each other. The caused excessive vacuolation of the cytoplasm results in the necrolytic death of EH, which is caused by both the deficiency of correctly folded proteins and an excess of misfolded proteins that provide a cytotoxic effect.

It should be noted that there is a continuing debate in the research literature about the presence or absence of nephrotoxic effects produced by infusion colloid-electrolyte-hyperosmolar solutions based on hydroxyethyl starch of different generation [5, 10, 14]. The data obtained indicate the nephroprotective properties of a new (based on third generation hydroxyethyl starch HES 130 / 0.4) balanced HAES-LX-5% plasma substitute, which provides a cytoprotective effect on the EH structure of nephrons in rats with BD.

Conclusion

The studies performed have shown the indisputable positive advantages of intravenous infusion of colloid-hyperosmolar solutions over infusion of isotonic solution, which is 0.9% NaCl solution. There are also differences in the influence of lactoprotein with sorbitol and HAES-LX-5% on the structure of nephrons in burned rats.

The most vulnerable organelle of nephron EH in BD and in the detoxification therapy performed is GER, therefore an important evidence of the used detoxification solutions' efficacy is the nature of the ER-stress in them. Under the conditions of intravenous infusion of colloid-hyperosmolar solutions (lactoprotein with sorbitol and HAES-LX-5%), ER-stress is normalized, accompanied by apoptotic changes in the EH, but it is not accompanied by the EH necrosis (which, by definition, is unprogrammed, randomized, and prone to unmanaged distribution of the process).

The consequence of renal EH necrosis is the formation of paravasal hemorrhages and lympho-leukocyte infiltrates, which is evidence of impaired filtration and reabsorption functions of the kidneys, as well as the progress of the nflammatory process in the kidneys. Such structural changes are particularly dangerous because, under the conditions of burn endogenous intoxication, the detoxification function of the kidneys is impaired.

The prospects for further scientific research in this field are to study the effects of different colloidal infusion solutions on the structure of nephrons in various pathological conditions.

References

1. Adams C J, Kopp MC, Larburu N, Nowak PR, Ali MMU. Structure and Molecular Mechanism of ER Stress Signaling by the Unfolded Protein Response Signal Activator IRE1. Front. Mol. Biosci, 2019; 6, 11. doi: 10.1111/fmolb.2019.00011
2. Cybulsky AV. Endoplasmatic reticulum stress, the unfolded protein response and autophagy in kidney diseases. Nat Rev Nephrol. 2017; 13 (11), 681-696. doi: 10.1038/nrneph.2017.129
3. Gallazzini V, Pallet N. Endoplasmic reticulum stress and kidney dysfunction. Biol Cell. 2018; 110 (9), 205-216. doi: 10.1111/boc.201800019
4. Huai-Wu He. Colloids and the Microcirculation. Anesthesia and Analgesia. 2017; 126 (5): 1. doi: 10.1213/ANE.0000000000002620
5. Kansir AS, Johansen JK, Pedersen EB. The effect of 6% hydroxyethyl starch 130/0.4 on renal function, arteriol blood pressure, and vasoactive hormones during radical prostatectomy: a randomized controlled trial. Anesth Analg. 2015; 120 (3),608-618. doi: 10.1213/ANE.0000000000000596
6. Keck M, Herdon D, Komolz LP. Pathophysiologyofburns. WienMedWochenschr. 2009; 159, 327-336.doi: 10.1136/bmj.328.7453.1427
7. Kovalchuk O, Cherkasov E, Dzevulska I, Raminsky R, Korsak A, Sokurenko L. Dynamics of morphological changes of rats adenohypophysis in burn disease. Georgian Medical News. 2017; (270),104-108. PMID:28972493
8. Lachtadyr TV. Structural changes of the rat kidney cortical substance in the long-term period after burn injury of the skin under conditions of HAES-LX_5% infusion. Emergency Medicine. 2019; 5(100), 96-100. DOI: https://doi.org/10.22141/2224-0586.5.100.2019.177023
9. Lillie Marie A, Barnett BS, Brain S, Cummings, PhD. Cellular and Molecular Mechanisms of Kidney Toxicity. Seminars in Nephrology. 2018; 39(2), 141-151. DOI: https://doi.org/10.1016/j.semnephrol.2018.12.004
10. Mohanan M, Rajan S, Kesavan R, Mohamed ZU, Ramaiyar, SK, Zumar L. Evaluation of renal function with administration of 6% hydroxyethyl starch and 4% gelatin in major abdominal surgeries: a pilot study. AnesthEssays Res. 2019 Apr-Jun, 13(2), 219-224. doi:10.4103/aer.AER_25_
11. Pickles S, Vigie P, Youle RJ. Mitophagy and quality control mechanisms in mitochondrial maintenance. Curr Biol. 2018 Feb 19, 28(4), R170-R185. doi:10.1016/j.cub.2018.01.004
12. Taniguch M, Yoshida H. Endoplasmic reticulum stress in kidney function and disease. 2016 Opin Nephrol Hypertens, 2016; 24 (4), 345-350. doi: 10.1097/MNH.0000000000000141
13. Wang M, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature. 2015; 529, 326-335. doi: 10.1038/nature17041
14. Weiskopf RB. Lack nephrotoxicity of hydroxyethyl starch 130/0.4 when used in surgery. Anesthesiology. 2015; 123(2), 482-483. doi: 10.1097/ALN.0000000000000719
15. Yan M., Sho S, Guo C, Tang C, Dong Z. Endoplazmic reticulum stress in ischemic and nephrotoxic acute kidney injury. AnnMed, 2018; 50 (5), 381-390. doi: 10.1080/07853890.2018.1489142

Реферати

СТРУКТУРНІ ПРОЯВИ ER-СТРЕСУ В ЕНДОТЕЛІОЦИТАХ ГЕМОКАПІЛЯРІВ НЕФРОНІВ ПРИ ЕКСПЕРИМЕНТАЛЬНІЙ ОПІКОВІЙ ХВОРОБІ У ЩУРІВ

Лахтадир Т.В., Черкасов Е.В., Яременко Л.М., Грабовий А.Н., Шепітько К.В.

Проведені дослідження структурних проявів ER-стресу, апоптозу і некрозу в ендотеліальних клітинах гемо капілярів нефронів при експериментальній опіковій хворобі у щурів (викликаної шляхом нанесення опікової травми шкіри площею 21-23% поверхні тіла) засвідчили безумовні протекторні та лікувальні позитивні переваги внутрішньовенної інфузії колоїдно-гіперосмолярних розчинів над інфузією ізотонічного розчину, яким є 0,9% розчин NaCl. Найбільш вразливою органелою ендотеліальних клітин гемокапілярів нефронів при експериментальній опіковій хворобіта здійсненій дезінтоксикаційній терапії є гранулярна ендоплазматична сітка, тому важливим свідченням ефективності застосованих дезінтоксикаційних розчинів є характер перебігу в них ER-стресу. За умов внутрішньовенної інфузії колоїдно-гіперосмолярних розчинів (лактопротеїну з сорбітолом та HAES-LX-5%) відбувається нормалізація ER-стресу, що супроводжується апоптозними змінами ендотеліальних клітин, але не супроводжується некрозом ендотеліальних клітин. Наслідком некрозу ендотеліальних клітин гемокапілярів нирок є утворення паравазальних крововиливів та лімфо-лейкоцитарних інфільтратів, що є свідченням порушень фільтраційної та реабсорбційної функцій нирок, а також прогресу запального процесу в нирках.

Ключові слова: опікова хвороба, дезінтоксикаційні розчини, гемокапіляри нефронів.

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СТРУКТУРНЫЕ ПРОЯВЛЕНИЯ ER-СТРЕССА В ЭНДОТЕЛИОЦИТАХ ГЕМОКАПИЛЛЯРОВ НЕФРОНОВ ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ОЖОГОВОЙ БОЛЕЗНИ У КРЫС

Лахтадыр Т.В., Черкасов Э.В., Яременко Л.М., Грабовой А.Н., Шепитько К.В.

Проведенные исследования структурных проявлений ER-стресса, апоптоза и некроза в эндотелиальных клетках гемокапилляров нефронов при экспериментальной ожоговой болезни у крыс (вызванной нанесением ожоговой травмы кожи площадью 21-23% поверхности тела) показали безусловные протекторные и лечебные положительные преимущества инфузии коллоидно-гиперосмолярных растворов над инфузией изотонического раствора, которым является 0,9% раствор NaCl. Наиболее уязвимой органеллой эндотелиальных клеток гемокапилляров нефронов при экспериментальной ожоговой болезни в условиях осуществленной дезинтоксикационной терапии является гранулярная эндоплазматическая сеть, поэтому важным свидетельством эффективности применяемых дезинтоксикационных растворов является характер течения в них ER-стресса. При инфузии коллоидно-гиперосмолярных растворов (лактопротеин с сорбитолом и HAES-LX-5%) происходит нормализация ER-стресса, которая сопровождается апоптозных изменениями эндотелиальных клеток, но не сопровождается некрозом эндотелиальных клеток. Результатом некроза эндотелиальных клеток гемокапилляров почек становится образование паравазальных кровоизлияний и лимфо- лейкоцитарных инфильтратов, что свидетельствует о нарушении фильтрационной и реабсорбционной функции почек, а также о прогрессе воспалительного процесса в почках.

Ключевые слова: ожоговая болезнь, дезинтоксикационные растворы, гемокапилляры нефронов.

Рецензент Єрошенко Г.А.