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Реферати

ПОКАЗНИКИ ОКИСНО-НІТРОЗАТИВНОГО СТРЕСУ У ТКАНИНАХ ПАРОДОНТА ЩУРІВ ПРИ МІСЦЕВОМУ УШКОДЖЕННІ ЯСЕН НА ТЛІ ЛІПОПОЛІСАХАРИД-ІНДУКОВАНОЇ СИСТЕМНОЇ ЗАПАЛЬНОЇ ВІДПОВІДІ

Єлінська А.М., Костенко В.О.

В експерименті на 40 білих щурах досліджено показники окисно-нітрозативного стресу в тканинах пародонта за умов дії на ясна місцевого патогенного фактора (5% розчину гідроксиду натрію) при моделюванні системної запальної відповіді (СЗВ). Останню моделювали шляхом внутрішньочеревного введення ліпополісахариду *Salmonella typhi* (пірогенал) у дозі 0,4 мкг/кг маси протягом 1-го тижня 3 рази, протягом наступних 7-ми тижнів – 1 раз у тиждень. Показано, що відтворення СЗВ супроводжується збільшенням у тканинах пародонта продукції супероксидного аніон-радикала ($\cdot O_2^-$), дизрегуляцією циклу оксиду азоту з одночасною активацією його NO-синтазної (NOS) і нітрат / нітритредуктазної складових, збільшенням концентрації пероксинітрит-йонів. Нанесення лугу на ясна підвищує в тканинах продукцію $\cdot O_2^-$ і активність NOS без порушення функціонування циклу оксиду азоту (NO) і збільшення вмісту пероксинітрит-йонів. Аплікація на ясна 5% розчину гідроксиду натрію на тлі СЗВ викликає збільшення в тканинах пародонта генерації $\cdot O_2^-$ НАДН- і НАДФН-залежними джерелами, дизрегуляцію циклу NO з підвищенням концентрації пероксинітрит-йонів, що перевершує такі при окремому впливі зазначених системного і місцевого чинників.

Ключові слова: системна запальна відповідь, гострий гінгівіт, окислювально-нітрозативного стрес, пародонт.

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INDICATORS OF OXIDATIVE-NITROSATIVE STRESS IN PERIODONTAL TISSUES OF RATS EXPOSED TO LOCAL IRRITATION OF GUMS DURING LIPOPOLYSACCHARIDE-INDUCED SYSTEMIC INFLAMMATORY RESPONSE

Yelinska A.M., Kostenko V.O.

The study carried out on 40 white rats was aimed at assessing the indices of oxidative-nitrosatitistic stress in periodontal tissues under the condition of combined effect of both local and systemic factors. Local irritation of the gums was induced by the local pathogenic factor (5% sodium hydroxide solution) during the simulation of systemic inflammatory response (SIR) induced by intraperitoneal administration of lipopolysaccharide *Salmonella typhi* (pyrogenalum) in a dose of 0.4 μg/kg of weight 3 times during the first week, and once a week for the following 7 weeks. It has been shown that simulation of SIR is accompanied by an increase in the production of superoxide anion-radical ($\cdot O_2^-$) in periodontal tissues, by the dysregulation of the nitrogen oxide cycle with the simultaneous activation of its NO-synthase (NOS) and nitrate / nitrite reductase components, as well as by the increase in peroxinitrite ion concentration. The application of alkali onto the gum increases the $\cdot O_2^-$ production and the activity of NOS without disturbing the functioning of the nitric oxide (NO) cycle and increasing in the peroxynitrite ion content in the tissues. The application of 5% sodium hydroxide solution onto the gums against the background of SIR results in an increase in $\cdot O_2^-$ generation by NADH- and NADPH-dependent sources in periodontal tissues, the dysregulation of the NO cycle with the increase in peroxinitrite ion concentration that exceeds relevant indices obtained at separate action of these systemic and local factors.

Key words: systemic inflammatory response, acute gingivitis, oxidative-nitrosative stress, periodontium.

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G.A. Yeroshenko, M.M. Koptev, S.M. Bilash, K.V. Shevchenko, A.I. Yachmin
Ukrainian Medical Stomatological Academy, Poltava

RESTRUCTURING OF RAT LUNGS IN ACUTE IMMOBILIZATION STRESS AND ITS CORRECTION

e-mail: gala_umsa@ukr.net

Currently, the search for advanced effective and safe methods of preventing and treatment of stress disorders is one of the priority tasks of medical science. The paper was aimed at the morphological study of the corrective effect of torasemide on rat lungs in experimental acute immobilization stress compared to the stress-protective effect of mexidol. Taking into account the norms of bioethics, the study was involved 30 mature albino male rats. Group I (controls) (n=10) involved animals, exposed to stress without correction; Group II (n=10) involved rats, exposed to stress after administration of mexidol; Group III (n=10) involved rats, exposed to stress that was corrected with torasemide. Correction with pharmacological drugs, especially torasemide, reduces stress effect on the lungs. Histologically, torasemide contributes to retention of the epithelial lining integrity, local visualization of intra-alveolar erythrocytes, and plethora in the capillaries of the interalveolar septa. In the epithelial lining of the bronchi, desquamation of individual cells was sometimes observed. The findings of the study have shown that torasemide has a pronounced stress-protective effect on the lung tissue, which is more powerful than correction with mexidol.

Key words: lungs, stress, correction, torasemide, mexidol, morphology.

The work is a fragment of the research project "Regularities of morphogenesis of organs, tissues and neurovascular formations in normal conditions, pathology and under the effect of exogenous factors", state registration No. 0118U004457.

Notwithstanding the long-time history of stress existence and continuous search for novel solutions, the problem of stress remains extremely relevant for the medical and scientific community to date. The issue on finding and choosing the advanced effective and safe methods of the prevention and treatment of stress disorders is one of the priorities for medical investigators [2, 3, 5, 6, 8, 9, 13, 14]. Studies conducted at the Ukrainian Medical Stomatological Academy show that acute immobilization stress causes structural changes

in the lungs of rats that can be a precursor of the development of pulmonary edema [10, 12, 15]. Therefore, in our opinion, torasemide, a loop diuretic with long-lasting effect, widely used in cardiogenic pulmonary edema, can be used as a potential stress protector. The study of the corrective effect of torasemide on rat lung in acute stress was performed in comparison with mexidol, known as a stress- protective drug.

The purpose of the work was to study the stress-protective effect of torasemide on lungs in experimental acute immobilization stress.

Materials and methods. The total of 30 Wistar albino male rats of 240-260 g body weight aged 8-10 months old were involved into morphological study. Group I (controls) (n=10) involved animals, exposed to simulated acute immobilization stress without correction; Group II (n=10) involved rats, exposed to simulated acute immobilization stress after intraperitoneal administration of stress protective drug mexidol; Group III (n=10) involved rats, exposed to simulated acute immobilization stress after administration of torasemide. Simulated acute immobilization stress was induced by six-hour immobility of rats, lying on the back. Correction procedure included intraperitoneal administration of 100 mg/kg/ body weight mexidol or 0,1 mg torasemide 20 minutes prior the period of immobilization.

The rats were killed by decapitation under thiopentone anesthesia within 2 hours after immobilization. Macroscopic analysis of the lungs and collection of material for histological study has been carried out after dissection of rats' chest. Subsequently, pieces of pulmonary tissue were fixed in 10% neutral formalin and after dehydration in spirits of the ascending densities they were embedded into paraffin according to conventional technique. The microtome specimens were stained with hematoxylin and eosin [1, 7].

The study was performed in compliance with the requirements of international principals of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg,1985) and corresponding Law of Ukraine "For the Protection of Pet Animals" (No.3446-IV, as of 21.02.2006, Kyiv) [4, 11].

Results of the study and their discussion. Examination of lungs after dissection of the chest wall has shown that in experimental animals, exposed to simulated acute immobilization stress without pharmacocorrection (Group I), numerous pleural hemorrhages were observed under the visceral pleura, the number of which was particularly significant on the diaphragm surfaces. The lungs of the rats from Group I had an elastic dough consistency and smooth surfaces. The color of the lungs was red pink with marked numerous polymorphic hemorrhages on its surfaces and in the lung tissue.

Macroscopically, less pronounced stress effect on the lungs with scarce hemorrhages under the visceral pleura and in the lung tissue was noted in rats, administered with mexidol and torasemide.

The histological study showed the adverse effect of acute stress on the lung tissue. The acute stress reaction without pharmacocorrection caused a significant enlargement of alveoli, thinning of interalveolar septa, destruction and detachment of the alveolar epithelium. Numerous alveolar macrophages were noted, sometimes forming continuous chains, their cytoplasm was densely filled with phagocytic material (fig. 1A).

Destructive phenomena were also noted in the intrapulmonary bronchi, manifested by disintegrated epithelial layer with accumulation of cellular detritus and red blood cells in the bronchial lumens, hyperhydration of the mucous membrane, peribronchial leukocytic infiltration. Blood stasis was noted in all sections of the microvasculature; lumens were filled with red blood cells; numerous foci of erythrocyte diapedesis in the interstitium of the lungs and lumens of alveoli were observed (fig. 1B).

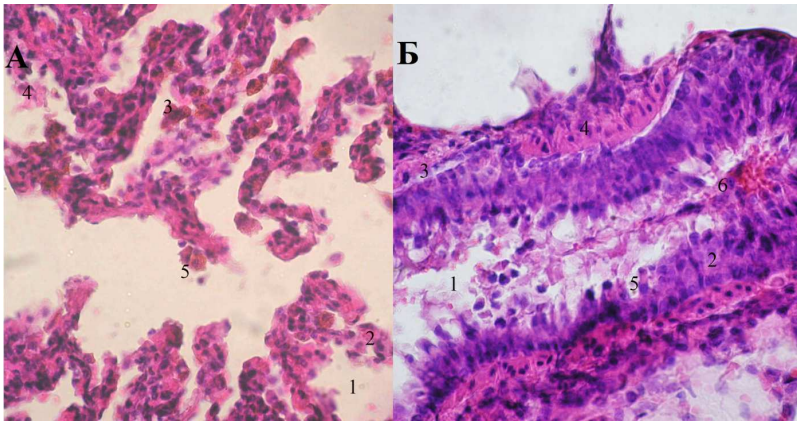

Fig. 1. Alveoli (A) and bronchiole (B) of rat after exposure to simulated acute immobilization stress. Microimage. H&E stain: Lens: 40: Ocular lens:15. A: 1 – alveolar lumen; 2 – alveolar wall; 3 – intra-alveolar macrophage; 4 – desquamated alveolocytes in the alveolar lumens; 5 – macrophages with phagocytic material; B: 1 – lumen of the bronchiole; 2 – epithelium of the bronchiole mucosa; 3 – hyperhydrated lamina propria of the bronchial mucosa; 4 – adventitious membrane; 5 – enlargement of the lateral intracellular fissures; 6 – cellular detritus in the lumen.

Histologically, correction with pharmacological drugs, torasemide, in particular, showed less apparent stress effect on the lungs.

The correction of the outcomes of the effect of acute stress reaction with mexidol showed no desquamation of the alveolar epithelium. Interalveolar septa were thickened and impregnated with erythrocytes, which were also found in the lumens of the alveoli (fig. 2A).

In the wall of small- and medium-sized bronchi a local desquamation of epithelial cells was noted; leukocytic infiltrates

were visualized peri-bronchially (fig. 2B). The arteries were spasmodic; the nuclei of the endothelial cells protruded into the lumens, where hemocytes were not found. Blood stasis and sludge of the red blood cells was detected in the veins.

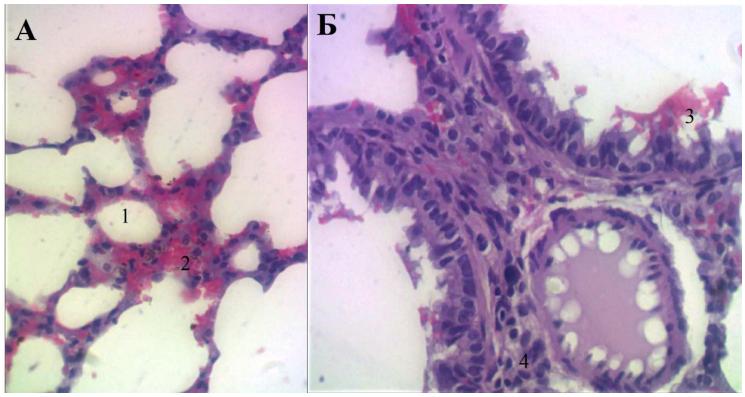

Fig. 2. Alveoli (A) and bronchi (B) of rat after exposure to simulated acute immobilization stress and correction with mexidol. Microimage. H&E stain: Lens: 40: Ocular lens:15: *1* – alveolar lumen; *2* – plethora of the vessels and diapedesis of the red blood cells in the interalveolar septum; *3* – local desquamation of the epithelial cells of the bronchial mucosa; *4* – peribronchial leukocytic infiltration.

Histological study has found that epithelial lining of alveoli retained its integrity in a group of animals, administered with torasemide in simulated immobilization stress. The capillaries of interalveolar septa were plethoric, intra-alveolar erythrocytes were locally visualized (fig. 3A). The epithelial lining of the bronchi was solid, but sporadic desquamation of individual cells in the lumen was noted. Groups of leukocytes, containing mainly macrophages, were detected peri-bronchially.

Hemocytes were found in the arterial lumens. Plasma had heterogeneous optic density. Blood perfusion was unhampered in the venules (fig. 3B).

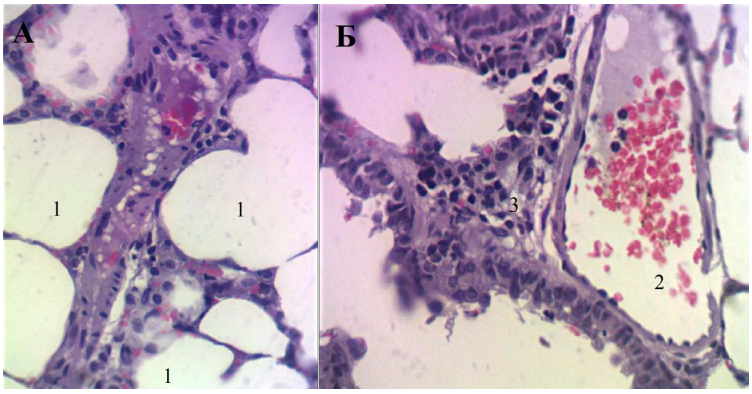

Fig. 3. Alveoli (A) and bronchi (B) of rat after exposure to simulated acute immobilization stress and correction with torasemide. Microimage. H&E stain: Lens: 40: Ocular lens:15: *1* – alveolar lumen; *2* – red blood cells in the arterial lumen; *3* – peribronchial leykocytic infiltration.

Apparently, stress causes alterations in rat organs with geodynamic disorders in the adjacent stroma. Subsequently, multiple perivascular hemorrhages and leukocyte infiltrates occurs [3, 10]. The response is typical for many organs, including lungs, and confirmed by the findings of previous studies [12, 15].

Accordingly, the findings [6, 13] showed that administration of mexidol to laboratory animals prevented the development of Selye's triad, namely, it increased the relative weight of the thymus, normalized the relative weight of the adrenal glands and prevented ulceration in the gastric mucosa. The findings of the study revealed the stress-protective effect of mexidol on the lung tissue, which prevented desquamation of alveolocytes and reduced the intensity of leukocytic infiltration. However, disturbances of blood perfusion in the microvasculature and the local desquamation of epithelial cells in the bronchial tree were noted.

Torasemide contributes to minimization of the development of edema of the stroma of the lungs, prevents the development of hypoxia in the tissue and alterations of organs, thereby providing support for homeostasis of the lung tissue.

Thus, the study shows that torasemide has a pronounced stress-protective effect on the lung tissue, more powerful than mexidol. The use of torasemide as an anti-stress drug may be possible after the follow up thorough studies on the effect of torasemide on other organs, in particular, thymus, adrenal glands, stomach, liver and kidneys.

Conclusion

Torasemide, a loop diuretic with long-lasting effect, has a pronounced stress-protective effect on the lungs, more powerful than the corrective effect of mexidol. Further research is needed to resolve the issue of the use of torasemide as a stress-protector.

Prospects for further research will encompass the study of stress protective effect of torasemide on other target organs in experimental stress.

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Реферати

СТРУКТУРНІ ЗМІНИ ЛЕГЕНЬ ЩУРІВ ПРИ ГОСТРОМУ ІММОБІЛІЗАЦІЙНОМУ СТРЕСОВІ ТА ЇХ КОРЕКЦІЯ

Єрошенко Г.А., Коптев М.М., Білаш С.М., Шевченко К.В., Ячмінь А.І.

У наш час пошук сучасних ефективних і безпечних методів профілактики та лікування стресових розладів продовжує залишатися одним із пріоритетних завдань для медичної науки. Метою роботи було вивчення на морфологічному рівні корегувального впливу торасеміду на легені щурів при експериментальному гострому іммобілізаційному стресі у порівнянні із стресспротекторною дією мексидолу. Із урахуванням норм біоетики, дослідження було виконане на 30 дорослих білих щурах-самцях. І, контрольну групу, склали 10 тварин, які зазнали стресового впливу без корекції, до ІІ групи увійшло 10 щурів, дія стресу у яких відбувалася після введення мексидолу, у ІІІ групи (10 особин) перебіг стресової реакції проходив на тлі корекції торасемідом. Корекція фармакологічними засобами, особливо торасемідом, зменшує стресовий вплив на легені. На гістологічному рівні при використанні торасеміду епітеліальна вистилка альвеол зберігала цілісність, локально візуалізувалися інтраальвеолярні еритроцити, у капілярах міжальвеолярних перегородок визначалося повнокров'я. В епітеліальній вистилці бронхів подекуди спостерігалася десквамація окремих клітин.Проведене дослідження свідчить, що на легеневу тканину торасемід має виразну стреспротекторну дію, потужнішу за корекцію мексидолом.

Ключові слова: легені, стрес, корекція, торасемід, мексидол, морфологія.

Стаття надійшла 16.10.2018

СТРУКТУРНЫЕ ИЗМЕНЕНИЯ В ЛЕГКИХ КРЫС ПРИ ОСТРОМ ИММОБИЛИЗАЦИОННОМ СТРЕССЕ И ИХ КОРРЕКЦИЯ

Єрошенко Г.А., Коптев М.Н., Билаш С.М., Шевченко К.В., Ячминь А.И.

В наше время поиск современных эффективных и безопасных методов профилактики и лечения стрессовых расстройств продолжает оставаться одной из приоритетных задач медицинской науки. Целью работы было изучение на морфологическом уровне корректирующего влияния торасемида на легкие крыс при экспериментальном остром иммобилизационном стрессе по сравнению со стресспротекторным воздействием мексидола. С учетом норм биоэтики, исследование было выполнено на 30 взрослых белых крысах-самцах. І, контрольную группу, составили 10 животных, подвергшихся стрессовому воздействию без коррекции, во ІІ группу вошло 10 крыс, влияние стресса на которых происходило после введения мексидола, в ІІІ группе (10 особей) стрессовая реакция протекала на фоне коррекции торасемидом. Коррекция фармакологическими средствами, особенно торасемидом, уменьшает стрессовое воздействие на легкие. На гистологическом уровне при использовании торасемида эпителиальная выстилка альвеол сохраняла целостность, локально визуализировались интраальвеолярные эритроциты, в капиллярах межальвеолярных перегородок определялось полнокровие. В эпителиальной выстилке бронхов иногда наблюдалась десквамация отдельных клеток. Выполненное исследование показывает, что на легочную ткань торасемид оказывает выраженное стресспротекторное воздействие, более мощное, чем коррекция мексидолом.

Ключевые слова: легкие, стресс, коррекция, торасемид, мексидол, морфология.

Рецензент Старченко І.І.