розширенням просвітів та кровонаповненням судин, набряком сполучної тканини слизової та підслизової оболонок, потовщенням та деформацією ворсинок. На 7 добу експерименту гістологічні зміни в структурних компонентах дванадцятипалої кишки наростають, що проявляється пошкодженням та десквамацією стовпчастих епітеліоцитів з облямівкою, набряком строми, лейкоцитарною інфільтрацією, крово-наповненням та деструкцією стінки судин мікроциркуляторного русла, гіпертрофією кінцевих секреторних відділів дуоденальних залоз. На 14 добу експерименту деструктивні зміни структур стінки дванадцятипалої кишки менш виражені, ніж у попередній термін. Зменшується кровонаповнення судин, набряк сполучної тканини та лейкоцитарна інфільтрація, пошкодження клітин епітеліальної пластинки. Встановлені гістологічні зміни дванадцятипалої кишки за умов експериментального панкреатиту необхідні для пошуку ефективних коригуючих чинників, що призведуть до нормалізації її структурних компонентів.

Ключові слова: гістологічні зміни, дванадцятипала кишка, експериментальний панкреатит.

Стаття надійшла 3.11.2017 р.

и кровенаполнением сосудов, отеком соединительной ткани слизистой и подслизистой оболочек, утолщением и деформацией ворсинок. На 7 сутки эксперимента гистологические изменения в структурных компонентах двенадцатиперстной кишки нарастают, что проявляется повреждением и десквамацией столбчатых эпителиоцитов с каемкой, отеком стромы, лейкоцитарной инфильтрацией, кровенаполнением и деструкцией стенки сосудов микроциркуляторного русла, гипертрофией конечных секреторных отделов дуоденальных желез. На 14 сутки эксперимента деструктивные изменения структур стенки двенадцатиперстной кишки менее выражены, чем в предыдущий срок. Уменшается кровенаполнение сосудов, отек соединительной ткани и лейкоцитарная инфильтрация, повреждения клеток эпителиальной пластинки. Установленные гистологические изменения двенадцатиперстной кишки в условиях экспериментального панкреатита необходимые для поиска эффективных корректирующих факторов, которые приведут к нормализации структурных компонентов этого отдела пищеварительной системы.

Ключевые слова: гистологические изменения, двенадцатиперстная кишка, экспериментальный панкреатит.

Рецензент Білаш С.М.

DOI 10.26724 / 2079-8334-2017-4-62-104-108
UDC (616.341:611.018.1):616.5-001.37

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INDICATORS CELL CYCLE AND DNA FRAGMENTATION IN CELLS OF SMALL INTESTINE MUCOSA 14, 21 AND 30 DAYS AFTER SKIN BURNS ON THE BACKGROUND OF PRELIMINARY INFUSION OF SOLUTION LACTOPROTEIN WITH SORBITOL OR HAES-LX 5%

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In analyzing the data of cell cycle and DNA fragmentation of the cells of the mucous membranes of the small intestine of rats in the late stages after the thermal burn of skin 2-3 degrees, in the area of 21-23% of the body surface, on the background of the previous use of infusion solutions, it was found that "lactoprotein with sorbitol" or HAES-LX-5% have a positive effect on cell cycle performance: after 14 days, the S-phase data and the index of proliferation were increased compared with those in the burn group + 0.9% NaCl solution in the same period; after 21 days, the S-phase data and the index of proliferation of these two groups were significantly higher than those in the burns + 0.9% NaCl solution, and at the same time, the values of the SUB-G0G1 interval in both groups were lower than those in the group where was used 0,9% NaCl solution on the background of burn. After 30 days in the burn + HAES-LX-5% group, all cell cycle indices have no significant or trend-specific differences compared to those in the non-burning group, and with "lactoprotein with sorbitol", the G0G1 and the proliferation phases have been significantly lower than indicators in a group without skin burns.

Key words: cell cycle, DNA cytometry, small intestine, rats, thermal burn skin, "lactoprotein with sorbitol", HAES-LX 5%.

The small intestine is the target organ in a burn disease, which is caused by violations of its functioning, with increasing damage on the background of toxemia and microbiocenosis [6]. The peculiarity of these violations is their long-term negative impact. It has been established [12] that dysbiosis is observed 21 days after thermal damage, especially against the background of antibiotic therapy, which is the standard method of treating severe burns.

Particularly important data are violations in the light of the hypothesis that the small intestine is the motor of the development of syndrome of polyorganic dysfunction with burn disease [4]. In particular, in this hypothesis, there is a significant role of the violations of enterocyte apoptosis [8] as a trigger mechanism for activation of multiple organ failure syndrome, along with cytokine stimulation, dysregulation of intercellular interaction, activation of microbial flora and other factors. It is dangerous to have abdominal hypertension syndrome [1], which can develop at the background of elevated intraperitoneal pressure, requiring accurate calculation of the volume of infusion, modification of the volume of infusion by monitoring diuresis, but completely eliminate this complication is impossible without the use of active infusion solutions, preferably with hyperosmolar effect [5].

Our attention was attracted by a number of works by domestic authors [3, 16, 19] on the established effectiveness of the application of the HAES-LX 5% developed in Ukraine in the early and late terms of burn disease with a protective effect on various organs and systems, which became the basis for the experimental research.

The purpose of the work is to study the cell cycle indexes in the small intestine mucus cells using lactoprotein with sorbitol solutions or HAES-LX 5% 14, 21 and 31 days after thermal burns of the skin.

Materials and Methods. Experimental study of the effect of infusion drugs "lactoprotein with sorbitol" and HAES-LX-5% on the structure of the ileum in later periods (14, 21 and 30 days) after burn skin lesions were performed on 60 laboratory white rats males weighing 150-160 g received from the vivarium of the State University "Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine". The animals were kept at the Scientific-Experimental Clinic of the National Pirogov Memorial Medical University, Vinnitsa on a standard diet, with free access to water and food. The temperature in the room where the animals were kept was 24-25 °C. Bioethics Committee of National Pirogov Memorial Medical University, Vinnitsa found that the experiments were carried out taking into account the recommendations of the European Commission on medical and biological research using animals, medical recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine and "Rules for the clinical evaluation of safety of pharmacological agents (GLP)" [14, 21].

The rats were divided into 7 groups, which previously, under the conditions of propofol anesthesia 60 mg/kg internally, catheterization of the femoral vein and depilation of the lateral surfaces of the trunk were performed. Group 1 - intact rats (only catheterization and shaving of the lateral surfaces of the body are performed). 2, 3, 4 groups - rats without thermal trauma, which once a day for the first 7 days were administered intravenous infusion of 0.9% solution of NaCl, "lactoprotein with sorbitol" and HAES-LX-5% in a dose of 10 ml per kg. In groups 5, 6, and 7, rats were also given once a day with the first 7 days of infusion of 0.9% NaCl solution, lactoprotein with sorbitol and HAES-LX-5% at a dose of 10 ml per kg after skin burn. A burnout shock was caused by applying four copper plates (two plates on each side) to the shaved lateral surfaces of the trunk of the rats, which were preheated for 6 minutes in water at a constant temperature of 100 °C. [9, 18]. The surface area of each plate was 13.86 cm^2. The total area of burns, calculated by the formula M. O. Lee [15], was 21-23% of the body surface of rats. Such an area at an exposure of 10 seconds is sufficient for the formation of 2-3 degree burns (according to the classification adopted at the 20th Congress of Surgeons of Ukraine in September 2000 in Ternopil) and causing a medium-gravity shock state [20], which has been confirmed jointly with the team of scientific workers of the research center of the National Pirogov Memorial Medical University, Vinnitsa [10]. Shaving of rats, burns, catheterization of major vessels and decapitation (after 14, 21 and 30 days) were carried out under conditions of propofol anesthesia (60 mg/kg i/v). The material was collected for flow cytometric analysis in rats grom the small intestine, similar to those selected for histological examination (colon). The removed portion of the small intestine, about 20 mm in length, was cut lengthwise, washed with 0.9% NaCl solution, placed on the glass, and under the control of the binocular microscope, with acute microsurgical spoon, scratches of the mucous membrane were performed in sufficient quantities. DNA content in the nuclei of the mucous membranes of the small intestine of rats was determined by flow DNA cytometry. The suspensions of the cell nuclei from the mucosal cells of the small intestine of rats were obtained using a set of CyStain DNA Step 2 DNA samples (Partec, Germany), according to the manufacturer's protocol. This kit allows extraction of nuclei and the labeling of DNA by 4'-6-diamidino-2-phenylindole (DAPI). CellTrics 50 μm disposable filters (Partec, Germany) were used in the production of nucleic suspensions. The flow analysis was carried out at the multifunctional flow-through flow cytometer "Partec PAS" (Partec, Germany), at the research center of the National Pirogov Memorial Medical University, Vinnitsa. UV radiation was used to stimulate DAPI fluorescence. From each sample of the nucleic suspension of the analysis, 10 thousand events were subject to. Cellular analysis of the cells was carried out using FloMax software (Partec, Germany) in full numeric matching according to the mathematical model, which determined: G0G1 - percentage ratio of G0G1 phase cells to all cells of the cell cycle (DNA content = 2c); S - percentage ratio of the phase of DNA synthesis to all cells of the cell cycle (DNA content> 2c and <4c); G2 + M - percentage ratio of the G2 + M phase to all cells of the cell cycle (DNA = 4c); IP - the index of proliferation, which was determined by the sum of the indices S + G2 + M; BP - block of proliferation that was evaluated by the ratio S/(G2 + M) (an increase in the number of cells in the G2 + M phase at low values of the S-phase indicates a delay in proliferation in the G2 + M stage). Determination of DNA fragmentation (apoptosis) is accomplished by isolating the SUB-G0G1 site on the DNA histograms-RN2 before the peak G0G1, indicating cell nuclei containing DNA <2c. Statistical processing of cytoflowmetric results of the study was performed in the license package "STATISTICA 5.5" using nonparametric estimation methods. Average values for each

sign and standard deviations were determined. The reliability of the difference between independent quantitative values was determined using the nonparametric Man-Whitney U-criterion.

Results and its discussion. Results of cell cycle and fragmentation of DNA of mucosal cells of the small intestine after burn of the skin on the background of infusion solutions are presented in Table 1.

When using "lactoprotein with sorbitol" and HAES-LX-5% after 14 days of skin burn, the S-phase and the index of proliferation are significantly higher than in the burn group + 0.9% NaCl solution at the same time (see Table 1). Also established the differences in the effects of these two drugs on the division of cells. If the use of "lactoprotein with sorbitol" more clearly ($p < 0.05$) increased the index of proliferation, then a more pronounced positive effect is established on the indicator of proliferation in the application of HAES-LX-5% compared with the parameters of the group of 0.9% NaCl solution without burns. Confirmation of this fact is an insignificant tendency ($p = 0.076$) to the higher values of the index of proliferation in the application of HAES-LX-5% compared to a similar indicator for the time period in the group of 0.9% NaCl solution + burn (Table 1). Regarding the parameters of the G0G1 and G2 + M phases against the background of the use of lactobacillus solution with sorbitol after 14 days after thermal damage, they were similar to those obtained in the burn group + 0.9% NaCl solution and were in their lower values ($p < 0.05$) in comparison with the indicators of the group without skin burns (Table 1), which indicates a more active involvement of cells in reparative processes against the background of the use of the studied infusion solutions.

Table 1

Indicators of the cell cycle in the mucosal cells of the small intestine using 0.9% NaCl solution, lactoprotein with sorbitol or HAES-LX 5% 14.21 and 31 days after thermal burn of the skin (M±σ)

Day	Drug	Indicators of the cell cycle					
		G0G1	S	G2+M	IP	BP	SUB-G0G1
14	0,9 % NaCl	89,11±1,86	4,572±1,717	6,320±0,271	10,89±1,86	0,720±0,251	9,968±2,118
	0,9 % NaCl+ burn	89,13±4,04	2,684±0,618*	6,790±1,463	9,474±1,438	0,416±0,155*	11,11±1,58
	LP with s. + burn	85,81±3,56*	4,534±1,305**	9,660±3,885	14,19±3,56**,*	0,556±0,302*	12,33±3,90
	HAES-LX 5% + burn	88,24±1,86	4,618±1,211**	7,136±1,590	11,75±1,86**	0,680±0,271*, tt	12,02±2,88
21	0,9 % NaCl	88,71±1,09	4,800±1,109	6,488±0,183	11,29±1,10	0,740±0,179	8,452±2,977
	0,9% NaCl+ burn	88,31±1,73	4,262±0,622	7,430±1,710	11,69±1,73	0,600±0,168	16,69±4,04*
	LP with s. + опік	85,12±3,84	4,590±0,617**	10,29±4,21	14,88±3,84**,*	0,542±0,289	11,94±5,38**
	HAES-LX 5% + burn	86,16±1,98*	5,478±0,807**	8,362±1,260*	13,84±1,98*	0,658±0,061	12,00±1,51**
30	0,9 % NaCl	89,36±2,23	4,596±1,634	6,042±1,162	10,64±2,22	0,768±0,266	8,868±3,033
	0,9% NaCl+ burn	83,78±3,37*	4,130±1,300	12,09±2,44*	16,22±3,37*	0,340±0,080*	9,174±3,284
	LP with s. + burn	85,45±3,93	4,628±0,877	9,916±3,321*	14,54±3,93	0,504±0,184*	8,688±2,535
	HAES-LX 5% + burn	84,68±3,64	5,180±1,960	10,14±3,55	15,32±3,65	0,592±0,341	9,716±3,879

Notes: * – $p<0,05$ compared to data of group 0.9% NaCl without a skin burn; ** - $p<0,05$ in comparison with the parameters of the group 0,9% solution NaCl + burn; t - trend compared with the 0.9% NaCl group without skin burn; tt - trend compared to the 0.9% NaCl + burn group.

21 days after the thermal damage, differences in the effects of solutions of "lactoprotein with sorbitol" or HAES-LX-5% on the parameters of the cell cycle of the mucous membrane of the small intestine remain. Thus, the S-phase and the index of proliferation in these two groups are significantly higher than the similar indicators in the burn group + 0.9% NaCl solution ($p < 0.05$) (Table 1). At the same time, the interval of SUB-G0G1 in both groups was lower ($p < 0.05$) from the same indicator in the group where 0.9% NaCl solution was used against the background of skin burn (Table 1). Differences in the effect of HAES-LX-5% in comparison with "lactoprotein with sorbitol" on mucosal cells of the small intestine appeared in a more pronounced increase in G2+M and a decrease in G0G1 ($p < 0.05$), indicating a higher cell mobilization for activation of the separation and restoration of the mucous membrane, when using the HAES-LX-5% solution (Table 1).

The results indicate a more distinct reparative process when using these drags, especially HAES-LX-5%, as compared to 0.9% NaCl solution. It was established that the indicator of proliferation unit after 30 days in the application of "lactoprotein with sorbitol" was approaching ($p = 0,059$) to similar values of the indicator in the group of 0.9% solution NaCl + burn and significantly differed from the indicator in the intact group ($p < 0,05$) when using "lactoprotein with sorbitol". Also, G0G1 and the proliferation index in the lactoprotein with sorbitol group after skin burn had a similar tendency ($p = 0.076$) to differences in indices without burns where the same drug was used (Table 1). In the burn + HAES-LX-5% group, all the figures did not differ significantly from those in the group without burn injury, only G2M had a slight tendency ($p = 0.758$) to differ from the same indicator in the non-burnout group (Table 1).

The issue of correction of damage to the epithelium of the small intestine is rather undeveloped and needs further refinement, especially taking into account the cell cycle disturbances established by us in the delay of the time of burn disease. The proposed methods of correction with a solution of pefturane [17] proved to be quite effective at the level of recovery of blood flow in the early period after burn injury. However, in our opinion, the authors did not consider the possibility of development of remote organ damage as a result of

reperfusion [2] and re-intensification of the pathological factors of burn disease. We have found only isolated data [22] concerning the effect of reperfusion and oxidative stress on the background of burn disease on the parameters of the cell cycle of individual organs and systems, but not of the small intestine. The activation of reperfusion damage is directly dependent on inflammation mediators, accumulation of toxins, products of peroxidation, and the development of subsequent systemic damage [13]. Damaged tissue initiates prolonged inflammation and hypermetabolic condition, which becomes a risk factor for the development of long-term damage to all organs and systems. It is proved that even non-specific reperfusion is an activator of apoptosis in the small intestine cells [11], and in the case of burn reperfusion in 24 hours is associated with an elevated level of apoptosis, however, there was no direct relationship between these phenomena. Experimentally try to solve this problem by introducing trimetazidine [23], whose action leads at a histologic level to protect the damage of the epithelium and significantly reduce peroxides in the epithelial cells of the small intestine. However, this study showed a positive effect only 5 hours after injury, which is insufficient for conclusions about long-term projective effects. The data obtained by us testifies precisely to the positive distant effect of infusion of solutions of "lactoprotein with sorbitol" or HAES-LX-5% on processes of apoptosis and DNA synthesis.

In particular, the introduction of solutions of "lactoprotein with sorbitol" or HAES-LX-5% on the background of thermal damage to the skin positively affects the parameters of the S-phase interval and the index of proliferation of the cells of the mucous membrane of the small intestine, increasing the proliferative activity since 14 days after burning the skin and restoring balance between cell cycle performance. This effect is realized 21 and 30 days after thermal damage, which allows to confirm the confirmed and long-lasting effect of the use of these drugs. We can assume that it is in this period that there is a gradual restoration of the integrity of the mucous membrane of the small intestine by increasing the reparative processes with simultaneous increase in the synthesis of DNA and apoptosis. The obtained data coincide with the received histological research methods in the same period [7].

However, it should be noted that the results of DNA cytometry more thoroughly testify the existence of remote damage of cells of the mucous membrane of the small intestine, even with the use of infusion therapy. For the HAES-LX-5%, certain benefits were found to have a more pronounced effect on all cell cycle indices against skin burns compared with the use of lactoprotein with sorbitol, especially after 30 days of experiment. Summing up, we can state that the use of "lactoprotein with sorbitol" or HAES-LX 5% in order to correct the damage to the small intestine epithelium significantly improves the cell cycle, in terms of normalizing the balance of apoptosis and DNA synthesis, improving the reparative processes.

Conclusion

1. The use of solutions of "lactoprotein with sorbitol" or HAES-LX 5% after skin burn has a positive effect on the cell cycle parameters of the mucous membranes of the small intestine: after 14 and 21 days, the S-phase and the proliferation index are increased ($p < 0.05$) in comparison with similar skin burns and application of 0.9% NaCl solution; after 21 days the interval of the SUB-G0G1 in both groups was lower ($p < 0,05$) from similar figures in the group where 0.9% NaCl solution was used against the background of burn.
2. After 30 days, using HAES-LX 5%, all cell cycle indices have no significant or trendy differences compared to those in the non-burning group, while using "lactoprotein with sorbitol", the parameters of the G0G1 phase and the proliferation unit remain lower (respectively $p = 0.076$ and $p < 0.05$) compared to similar indices in non-burning skin rats.

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Реферати

ПОКАЗНИКИ КЛІТИННОГО ЦИКЛУ І ФРАГМЕНТАЦІЇ ДНК КЛІТИН СЛИЗОВОЇ ОБОЛОНКИ ТОНКОЇ КИШКИ ЧЕРЕЗ 14, 21 ТА 30 ДІБ ПІСЛЯ ОПІКУ ШКІРИ НА ФОНІ ПОПЕРЕДНЬОЇ ІНФУЗІЇ РОЗЧИНІВ ЛАКТОПРОТЕЇНУ З СОРБІТОЛОМ АБО HAES-LX 5%

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При аналізі показників клітинного циклу і фрагментації ДНК клітин слизової оболонки тонкої кишки щурів у пізні терміни після термічного опіку шкіри 2-3 ступеню, площею 21-23 % поверхні тіла, на фоні попереднього застосування інфузійних розчинів встановлено, що “лактопротеїну з сорбітолом” або HAES-LX-5% позитивно впливають на показники клітинного циклу: через 14 діб показники S-фази і індексу проліферації виявились збільшеними у порівнянні із аналогічними в групі опік + 0,9 % розчин NaCl в цей же термін; через 21 добу показники S-фази і індексу проліферації цих двох груп були суттєво вищими від аналогічних показників в групи опік + 0,9 % розчин NaCl і одночасно показники інтервалу SUB-G0G1 в обох групах виявились нижчими від аналогічних показників в групі де використовувався 0,9 % розчин NaCl на фоні опіку. Через 30 діб в групі опік + HAES-LX-5% всі показники клітинного циклу не мають достовірних або тенденцій відмінностей порівняно з аналогічними показниками в групі без опікового ушкодження, а при використанні “лактопротеїну з сорбітолом” показники фази G0G1 та блоку проліферації виявились достовірно нижчими від показників у групі без опіку шкіри.

Ключові слова: клітинний цикл, ДНК цитометрія, тонка кишка, щури, термічний опік шкіри, “лактопротеїн з сорбітолом”, HAES-LX 5%.

Стаття надійшла 5.11.2017 р.

ПОКАЗАТЕЛИ КЛЕТОЧНОГО ЦИКЛА И ФРАГМЕНТАЦИИ ДНК КЛЕТОК СЛИЗИСТОЙ ОБО-ЛОЧКИ ТОНКОЙ КИШКИ ЧЕРЕЗ 14, 21 И 30 ДНЕЙ ПОСЛЕ ОЖОГА КОЖИ НА ФОНЕ ПРЕДВА-РИТЕЛЬНОЙ ІНФУЗИИ РАСТВОРОВ ЛАКТОПРО-ТЕИНА С СОРБИТОЛОМ ИЛИ HAES-LX 5%

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При анализе показателей клеточного цикла и фрагментации ДНК клетки слизистой оболочки тонкой кишки крыс в поздние термины после термического ожога кожи 2-3 степени, площадью 21-23 % поверхности тела, на фоне предварительного использования инфузионных растворов установлено, что “лактопротеин с сорбитолом” или HAES-LX-5% положительно влияют на показатели клеточного цикла: через 14 дней показатели S-фазы и индекса пролиферации выявились увеличенными в сравнении с аналогичными в группе ожег + 0,9 % раствор NaCl в этот же срок; через 21 день показатели S-фазы и индекса пролиферации этих двух групп были значительно выше нежели аналогичные показатели в группе ожег + 0,9 % раствор NaCl и одновременно показатели интервала SUB-G0G1 в обеих группах выявились ниже нежели аналогичные показатели в группе где использовался 0,9 % раствор NaCl на фоне ожога. Через 30 дней в группе ожег + HAES-LX-5% все показатели клеточного цикла не имеют достоверных или тенденций отличий в сравнении с аналогичными показателями в группе без ожогового повреждения, а при использовании “лактопротеина с сорбитолом” показатели фазы G0G1 и блока пролиферации выявились достоверно меньшими нежели показатели в группе без ожога кожи.

Ключевые слова: клеточный цикл, ДНК цитометрия, тонкая кишка, крысы, термический ожог кожи, “лактопротеин с сорбитолом”, HAES-LX 5%.

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