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INDICATORS OF THE CARDIOMYOCYTES' CELLS CYCLE UNDER INFUSION OF BLOOD SUBSTITUTES AND IN THE CORRECTION OF EXPERIMENTAL BURN INJURY BY 0.9% NaCl SOLUTION

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According to the WHO, the thermal trauma is on the third place among other injuries. Burned injury is not only damage to the skin, but also the traumatization of all organs and systems of the body as a result of the stress response of the vascular system and the effects of toxic products coming from the area of burn injury. Firstly, such damages affect cardiomyocytes and the microcirculation vessels of the heart. The purpose of our study was to evaluate the changes in the cell cycle of myocardial cells in the left ventricle of rats under conditions of blood substitutes infusion and in the correction of experimental burn injury with a 0.9% solution of NaCl. The burn trauma was modeled using the Regas' method and placed a catheter into the lower vena cava for intravenous infusion. The following solutions were used for infusion: 0.9% NaCl solution, lactoproteinum with sorbitol (Lactoproteinum-C) and colloidal-hyperosmolar HAES-LX-5% solution. Flow cytometry of the nuclear suspension of left ventricular cardiomyocytes was performed on the 1st, 3rd, and 7th days of the experiment. The statistical analysis of the results was carried out using the "STATISTICA 6.1" program package. The results of the performed study show a fairly stable picture of cell cycle parameters in myocardial cells of animals without burn injury with a predominance, on the one hand, of cells present in the G0G1 phase and the presence of a certain balance between the processes of creation of nuclear DNA synthesis and apoptosis. Changes in the phase of cardiac myocyte cell cycle against the background of the thermal injury of the skin throughout the observation time indicate a prolonged, uncorrected cell cycle disorder and a lack of effective normalization on the background of the physiological solution usage in the first 7 days after burning trauma of the skin. The protective effect of HAES-LX-5% prevents over-strain of cells, as evidenced by the lower synthetic activity of nuclei of cardiomyocytes at all times of the experiment.

Keywords: burn trauma, myocardium, cell cycle, rats, cardiomyocytes, 0.9% NaCl solution, lactoproteinum with sorbitol, HAES-LX-5%.

Introduction

A large burn injury causes significant hemodynamic and cardiodynamic changes that support the development of sepsis, multiple organ failure, and leads to death [8]. Cardiogenic stress is a sign of the acute phase of response, and the negative results of the treatment of thermal damage are associated, in particular, with severe cardiac dysfunction [11, 12, 19]. Compromised cardiac function leads to hypoperfusion of organs, disturbance of peripheral microcirculation, increase of burn area and decrease of resistance to bacterial infection in the wound area [10, 11, 13]. Infusion therapy for burn injury is designed to compensate for the amount of fluid lost, with subsequent

maintenance of the volume of circulating blood at a constant level, reduction of edema syndrome, normalization of the acid-base balance, electrolyte balance, and also the enhancement of perfusion of organs and tissues [2, 14, 15, 16, 18]. Literary data indicate that the problem of adequate use of infusion-transfusion solutions in the case of burn injury is far from the solution [2, 5, 14, 15, 18]. First of all, it concerns the changes in the parameters of the cardiomyocyte cell cycle, which are almost not covered in modern literature.

The aim of our study was to evaluate changes in the cell cycle of myocardial cells in the left ventricle of rats under conditions of infusion of blood substitutes and correction of

experimental burn injury by a 0.9% solution of NaCl.

Materials and methods

Experimental research was carried out on the basis of vivarium, research laboratory of functional morphology and genetics of the development of the research center (certificate of State Pharmacological Center of Ministry of Health of Ukraine No. 003/10 dated January 11, 2010) and the chemical scientific laboratory of the Department of Pharmacology (certificate of the State Pharmacological Center of Ministry of Health of Ukraine No. 000679 from January 11, 2008) National Pirogov Memorial Medical University, Vinnytsya.

All manipulations with animals and their keeping were conducted in accordance with the "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001), and also guided by the recommendations of the "European Convention on the Protection of Vertebrate Animals used for Experimental and Other Scientific goals" (Strasbourg, 1985) and the provisions of the "Rules of preclinical safety assessment of pharmacological agents (GLP)", fully complied with the rules of humane treatment of experimental animals that cough Department of Bioethics, National Pirogov Memorial Medical University, Vinnytsya (Minutes No. 1 dated January 14, 2010).

Experiments were performed on 77 white male rats weighing 160-180 g obtained from the vivarium of the State Institution "Institute of Pharmacology and Toxicology of the National Academy of Medical Sciences of Ukraine".

The control group consisted of intact rats, which throughout the study period were infusion therapy with physiological solution.

All other rats under the general anesthesia of propofol (60 mg/kg of animal weight) were placed into the lower vena cava of the intravenous infusion catheter and modeled the burns of II-III degrees using the Regas method (fourth group of experimental animals) [17].

By the nature of infusion therapy, all experimental animals were randomly assigned to four groups: to group 1 - rats, which were given a 0.9% NaCl solution in a dose of 10 ml/kg; group 2 - rats, which were injected with a solution of lactoproteinum with sorbitol (Lactoprotein-C, issued by Kiev Closed Joint-Stock Company "Biopharm", Certificate of state registration of the Ministry of Health of Ukraine No. 464/09-300200000 dated March 12, 2009) at a dose of 10 ml/kg; group 3 - rats, which were injected with a colloid-hyperosmolar HAES-LX-5% solution (developed at the SI "Institute of Blood Pathology and Transfusion Medicine of the National Academy of Medical Sciences of Ukraine", Lviv) at a dose of 10 ml/kg; group 4 - rats that were injected by 0.9% NaCl solution at a dose of 10 ml/kg against a burn injury.

The first injection was carried out 1 hour after the simulation of burn injury, the subsequent infusions were performed 1 time per day during the first 7 days of the experiment.

Animals were withdrawn from the experiment on 1, 3, and 7 days by overdose of propofol anesthesia in accordance with the basic requirements for euthanasia (Annex 4 "Rules for carrying out work using experimental animals", approved by order number 755 dated August 12, 1977, Ministry of Health of the USSR "On measures to further improve organizational forms of work using experimental animals", Helsinki Declaration of the World Medical Association (2000).

To detect the peculiarities of changes, cell cycle indices, and determination of DNA content in the nuclei of myocardial cells of rats, we used a flow-through DNA-cytofluorometry method.

After the heart was removed from the body of the rat, suspensions of the nuclei from the left ventricular myocardial cells of rats were prepared. The suspension was prepared using a CyStain DNA Step 1 for DNA dilution solution from Partec, Germany, in accordance with the manufacturer's protocol. This solution allows for the extraction of nuclei and the labeling of nuclear DNA by 4.6-Diamino-phenylindole (DAPI). In the manufacture of nucleic suspensions, we used CellTrics 50 μ m disposable filters (Partec, Germany).

Flow analysis was performed on a multi-functional flow-through flow cytometric analyzer "Partec PAS" from Partec (Germany), at the CRC of National Pirogov Memorial Medical University, Vinnytsya. We used UV radiation to stimulate DAPI fluorescence. From each sample of a nuclear suspension, 10,000 events were subject to analysis. The distribution of DNA reflecting the cell cycle and fragmentation of DNA is presented on a page with one histogram using a linear scale. Calculation, plotting, cyclic analysis of cells were performed using FloMax software application (Partec, Germany), which was provided by the manufacturer to the equipment, in full digital equivalence according to the mathematical model, which determined:

G0G1 (G1%) - percentage ratio of G0G1 phase cells to all cells in the cell cycle (DNA content = 2c);

S (S%) - percentage of cells in the phase of DNA synthesis to all cells of the cell cycle (DNA content > 2c and < 4c);

G2 + M (G2M%) - percentage of cells in the G2 + M phase to all cells in the cell cycle (DNA = 4c), or cells containing DNA = 4c;

Determination of DNA fragmentation is accomplished by isolating the SUB-G0G1 site on the DNA histograms-RN1 before the peak G0G1, which indicates the nuclei of the cells containing DNA < 2c. This is the percentage of cell nuclei in the state of apoptosis.

IP is an index of proliferation (a proliferative index), which is determined by the sum of the indices S + G2 + M. The larger its value, the more intensive proliferation and vice versa - the smaller the value, the less proliferative activity.

BP - block of proliferation. An increase in the number of cells in the G2 + M phase at low values of the S-phase indicates a delay (cell proliferation) of the cell cycle in the G2 + M stage. This indicator is rated by the ratio: S / (G2 + M).

The statistical analysis of the results was carried out using

the "STATISTICA 6.1" program package (license number BXXR901E246022FA).

Results

In the study of DNA histograms of a nuclear suspension of rat myocardial cells without skin burn on 1 day after application of 0.9% NaCl solution, lactoproteinum with sorbitol or HAES-LX-5%, we did not find any significant difference between the cell cycle and fragmentation of myocardial cell DNA rats.

In all groups, most of the cells were in the G0G1 and G2 + M-phase, phase S parameters were different in the HAES-LX-5% group, while in groups with administration of lactoproteinum with sorbitol and 0.9% NaCl solution, we did not find any reliable differences, the SUB-G0G1 interval was the highest in the group of animals that were infused with lactoproteinum with sorbitol (Fig. 1).

For the third day of the experiment, cell cycle parameters, such as the number of cells in the G0G1 and G2 + M-phases, were almost identical for the experimental groups of animals 1, 2 and 3, with the lowest data being when using HAES-LX-5%.

At the same time, with the application of HAES-LX-5%, the S-phase and SUB-G0G1 intervals were the highest.

On the seventh day, we observed that when infusions of blood substitutes in groups 1, 2 and 3, the bulk of cardiomyocytes were also in the phases G0G1 and G2 + M.

Infusion of HAES-LX-5% resulted in a decrease in the parameters of the SUB-G0G1 and S-phase (Fig. 2) compared to similar groups in which 0.9% NaCl solution ($p < 0.01$) and lactoproteinum with sorbitol ($p < 0.05$) were used.

Therefore, as a control, we selected a group of rats that we injected a 0.9% NaCl solution without burn injury (group 1) and compared with those obtained in a group of rats, which injected a 0.9% NaCl solution for 7 days at the background of burn injury (group 4).

It was established that the difference in the number of nuclei of cardiomyocytes located in the resting phase and the presynthetic phase of the CC (G0-G1 interval) was higher in animals with burn injury at the 1 and 7 day of the experiment and amounted to 2%, on 3 day the difference in the rate in 1 and 4 groups was 0.5% and was not reliable (Fig. 3).

We considered this trend as a mobilization of cardiomyocytes reserves (G0 phase) to provide regeneration. At the same time, the percentage of nuclei that were in the phase of DNA synthesis (S-phase) was

Fig. 1. S-phase indices and SUB-G0G1 intervals with 0.9% NaCl, lactoproteinum with sorbitol and HAES-LX-5% in rats without burn injuries on 1 day.

Fig. 2. Indicators of the interval SUB-G0G1 in the application of physiological solution of 0.9% NaCl, lactoproteinum with sorbitol and HAES-LX-5% in rats without burn injury at 1, 3 and 7 days.

Fig. 3. Indicators of interval G0-G1 during using 0.9% NaCl physiological solution in rats without burn injury and after skin burn on 1, 3 and 7 days.

larger by almost 2 times ($p < 0.05$) on 3 day in rats with burn injury compared with group 1, which indicated an increase in synthetic processes in the nuclei of cardiomyocytes against the background of dystrophic changes that were detected histologically with a burn injury (Fig. 4).

Fig. 4. S-phase indices with the use of 0.9% NaCl solution in rats without burn injury and after skin burn on 1, 3 and 7 days.

Fig. 5. Indicators of the interval G2M and the index of proliferation using 0.9% NaCl physiological solution in rats without burn injury and after skin burn on 1, 3 and 7 days.

Fig. 6. Indicators of the interval SUB-G0G1 with the use of a 0.9% NaCl solution in rats without burn injury and after burning of skin on 1, 3 and 7 days.

We observed a significantly lower percentage of nuclei in the post-synthetic and mitotic phases (G2M interval) in group 4, with the largest difference in group 1 (in 1.71 times) found on the first day of the experiment. At the same time, the index of proliferation (IP - the sum of the indicators of phases S and G2M) at that time was also lower by 13,74% ($p < 0.05$), on the 7 day - by 9.1%, which we considered as a decrease the ability for reparative regeneration of cardiomyocytes in response to their damage by burn injury

and use of a physiological solution (Fig. 5).

In the case of a burn injury in group 4, we established a significantly higher percentage of nuclei of cardiomyocytes in the S-phase: in 2 times on 3 day of the experiment, in 1.5 and 1.35 times on 1 and 7 days of the experiment, respectively, compared to the animals in group 1. The foregoing indicated an increase in the synthesis of DNA, which we considered as a compensatory-adaptive reaction, aimed at restoring the mass of the damaged organ. In animals of group 4 with burn injury, the interval SUB-G0G1 (apoptosis score) on the third and seventh day of the experiment was in 2.19 and 1.55 times higher than that of animals of group 2 ($p < 0.05$) (Fig. 6). Such a significant increase in the DNA fragmentation of the nuclei of cardiomyocytes, in our opinion, is the main indicator of pathogenically induced apoptosis, which, in the future, can lead to the development of irreversible damage to cells.

In rats with burn injury, which were given with a physiological solution, the percentage of diploid cells with a set of chromosomes 2c was only 2.45% higher in the first day of the experiment, almost the same - on 3 day and 1.29% higher - on the seventh day of the experiment comparatively with group 1.

This indicates slight fluctuations in the percentage of nuclei that are in the range of G0-G1 and pass into the following phases of the CC, which prepare the cell for division to implement reparative regeneration. Larger values of the index of the interval of SUB-G0G1 of cardiomyocytes against the background of thermal damage to the skin during the entire observation time indicate a violation of the time values of the cell cycle.

A higher percentage of diploid nuclei in animals with burn injury and the introduction of the physiological solution was combined with a lower percentage of tetraploid - almost twice (Fig. 7), which was reflected in an increase of the ratio 2c/4c, which was 10.97 against 6.22 in animals of group 1. The change in the ratio between percentage 2c and 4c nuclei in this case indicates an increase in the ability to regenerate a damaged organ.

Discussion

Summarizing the results of the study, analyzing the results and comparing them with the results of researchers who studied post-mortem changes in other organs, we can conclude that there is a possibility of violations of the phases of the cell cycle of cardiomyocytes in later time (14, 21 and 30 days) after burn injuries of skin in rats [1, 3,

Fig. 7. Indicators of the intervals G0G1, G2M and the ratio 2c/4c in the use of a 0.9% NaCl solution in rats without burn injury and after skin burn on 1, 3 and 7 days.

6, 7, 9]. We did not find literary data on analogous studies of the cardiomyocyte cell cycle in the long term after burn infections of the skin. We can assume that in the remote post-mortem period, the processes of myocardial rehabilitation are carried out by increasing the synthesis of cellular material against the background of amplification of apoptosis of damaged cells. Despite the presence of views on the protective role of apoptosis after thermal damage, comparing the clinical data of other researchers and the results obtained by us suggests that heart damage can occur precisely on the background of amplification of apoptosis [4, 20, 21]. This may also indicate an increase in the S-phase, set 7 days after the thermal trauma, which, in turn, indicates a lack of cardiomyocyte reparation processes in the early

stages of the burn injury. It is also important to note that a comparable increase in the population of cells with fragmented nuclear DNA may indicate an imbalance of reparative processes in the cardiac muscle 7 days after the thermal loss. In favor of the hypothesis of the violation of reparative processes in the myocardium indicates the dynamics of indexes and the block of proliferation recorded during the study. The foregoing suggests that the normalization of the leading indicators of the cellular cycle of cardiomyocytes against the background of burn injury can occur only in the long term after burn injury.

In our opinion, a promising further study of morphophysiological indicators of cardiac activity subject to a thermal trauma and its adjustment with various infusion drugs.

Conclusions

1. Infusion of 0.9% solution of NaCl, lactoproteinum with sorbitol or HAES-LX-5% for 7 days in rats without skin burns does not cause significant changes in cell cycle and fragmentation of myocardial cell DNA.

2. The long-term, unregulated changes in the cell cycle and its insufficiently effective normalization against the background of the use of saline solution for 7 days after burn injury to the skin have been determined.

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ПОКАЗНИКИ КЛІТИННОГО ЦИКЛУ КАРДІОМІОЦИТІВ В УМОВАХ ІНФУЗІЇ КРОВОЗАМІННИКІВ ТА ПРИ КОРЕКЦІЇ ЕКСПЕРИМЕНТАЛЬНОЇ ОПІКОВОЇ ТРАВМИ 0,9% РОЗЧИНОМ NaCl

Радьога Р.В.

Згідно даних ВООЗ, опікова травма посідає третє місце серед інших видів травм. Термічна травма - це не тільки ушкодження шкірних покривів, але й травматизація всіх органів і систем організму внаслідок стресової реакції судинної системи та впливу токсичних продуктів, які надходять із ділянки опікової пошкодження. У першу чергу такі ушкодження впливають на кардіоміоцити та судини мікроциркуляторного русла серця. Метою нашого дослідження ми обрали оцінку змін показників клітинного циклу клітин міокарда лівого шлуночка щурів в умовах інфузії кровозамінників та при корекції експериментальної опікової травми 0,9% розчином NaCl. Опікову травму моделювали за методикою Regas та катетеризували нижню порожнисту вену для внутрішньовенної інфузії. Для інфузії використовували наступні розчини: 0,9% розчин NaCl, лактопротеїн з сорбітолом (Лактопротеїн-С) та колоїдно-гіперосмолярний розчин HAES-LX-5%. Проточну цитометрію ядерної суспензії кардіоміоцитів лівого шлуночка виконували на 1, 3, та 7 добу експерименту. Статистичний аналіз отриманих результатів проводили за допомогою пакету програм "STATISTICA 6.1". Результати здійсненого дослідження вказують на досить стабільну картину показників клітинного циклу у кардіоміоцитах тварин без термічної травми з переважанням, з однієї сторони, клітин, що перебувають у фазі G0G1, і присутністю певного балансу між процесами утворення ядерної ДНК і апоптозу. Зміни показників фаз клітинного циклу кардіоміоцитів на фоні опікової травми шкіри протягом усього часу спостереження вказують на некореговане, стійке порушення та недостатність ефективної нормалізації клітинного циклу на фоні застосування фізіологічного розчину протягом 7 діб після опікової травми шкіри.

Ключові слова: опікова травма, клітинний цикл, щурі, кардіоміоцити, 0,9% розчин NaCl, лактопротеїн з сорбітолом, HAES-LX-5%.

ПОКАЗАТЕЛИ КЛЕТОЧНОГО ЦИКЛА КАРДИОМИОЦИТОВ В УСЛОВИЯХ ИНФУЗИИ КРОВЕЗАМЕНТЕЛЕЙ И ПРИ КОРРЕКЦИИ ЭКСПЕРИМЕНТАЛЬНОЙ ОЖГОВОЙ ТРАВМЫ 0,9% РАСТВОРОМ NaCl

Радёга Р.В.

Согласно данным ВОЗ, ожоговая травма занимает третье место среди других видов травм. Термическая травма - это не только повреждения кожных покровов, но и травматизация всех органов и систем организма в результате стрессовой реакции сосудистой системы и воздействия токсических продуктов, поступающих из участка ожогового повреждения. В

первую очередь такие повреждения влияют на кардиомиоциты и сосуды микроциркуляторного русла сердца. Целью нашего исследования мы выбрали оценку изменений показателей клеточного цикла клеток миокарда левого желудочка крыс в условиях инфузии кровезаменителей и при коррекции экспериментальной ожоговой травмы 0,9% раствором NaCl. Ожоговую травму моделировали по методике Regas и катетеризировали нижнюю полую вену для внутривенной инфузии. Для инфузии использовали следующие растворы: 0,9% раствор NaCl, лактопротеин с сорбитолом (Лактопротеин-С) и коллоидно-гиперосмолярный раствор HAES-LX-5%. Проточную цитометрию ядерной суспензии кардиомиоцитов левого желудочка выполняли на 1, 3, и 7 сутки эксперимента. Статистический анализ полученных результатов проводили с помощью пакета программ "STATISTICA 6.1". Результаты проведенного исследования указывают на достаточно стабильную картину показателей клеточного цикла в кардиомиоцитах животных без термической травмы с преобладанием, с одной стороны, клеток, находящихся в фазе G0G1, и присутствием определенного баланса между процессами образования ядерной ДНК и апоптоза. Изменения показателей фаз клеточного цикла кардиомиоцитов на фоне ожоговой травмы кожи в течение всего времени наблюдения указывают на некорректированное, стойкое нарушение и недостаточность эффективной нормализации клеточного цикла на фоне применения физиологического раствора в течение 7 дней после ожоговой травмы кожи.

Ключевые слова: ожоговая травма, клеточный цикл, крысы, кардиомиоциты, 0,9% раствор NaCl, лактопротеин с сорбитолом, HAES-LX-5%.