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MONTHLY RATES OF CELL CYCLE OF RAT ADRENAL GLANDS AFTER BURN AND IN ADMINISTRATION OF 0.9% NACL SOLUTION, LACTOPROTEIN WITH SORBITOL AND HAES-LX-5%

The paper presents the results of the analysis of monthly rates of cell cycle of rat adrenal glands and DNA-fragmentation after the II-III degree burn with superficial burn surface of 21-23 % in administration of 0.9 % NaCl solution, lactoprotein with sorbitol and HAES-LX-5%. Burn injuries along with infusion of 0.9 % NaCl solution during the first 7 days of the experiment is accompanied by a significant increase of rates of SUB-GOG1 interval and S-phase of adrenal cells after 1, 3, 7 and 14 days after burn. Following the 21 and 30 days after burn no significant difference in the rate of S-phase in the adrenal cells against the similar rate in groups without burn is found, whereas the rate of SUB-GOG1 interval remains reliably higher than those in groups without burn. Thermal burn-related administration of lactoprotein with sorbitol and HAES-LX-5% solutions during the first 7 days of the experiment is accompanied by the positive effect on the rates of SUB-GOG1 interval and S-phase of adrenal cells following the 1, 3, 7 and 14 days after burn. And following the 21 and 30 days after burn the value of the abovementioned rates was almost the same as compared with similar rates in groups where infusion therapy was carried out to animals without burn.

Key words: cell cycle, DNA fragmentation, adrenal glands, rats, burn, 0.9 % NaCl solution, lactoprotein with sorbitol, HAES-LX-5%.

The paper draws from the planned joint research work (regulated by the agreement on scientific cooperation between Bogomolets National Medical University and National Pirogov Memorial Medical University, Vinnytsya) entitled "Experimental Substantiation of the Efficacy of Composite Infusion Agents in Simulated Burn Disease in Animals", which is the fragment of planned research work "Development of the Novel Composite Colloidal Blood Substitutes of Polyfunctioning Effect and Solution for Red Blood Cells Resuspending (laboratory and experimental rationale of their application in transfusiology)" (KPKV6561040, National registration № 0107U001132).

Immediate enhanced infusion therapy is one of the promising approach to burn disease treatment [7]. It is evident that such active infusion therapy leads to improving the outcomes of burn disease treatment, reduces mortality and shortens the duration of hospitalization. However, the proposed infusion solutions have a number of fairly significant drawbacks and limitations that narrows the range of therapeutic possibilities. Therefore, the development and testing of the novel agents for infusion therapy of burn disease remains the urgent issue of contemporary medicine, especially when taking into consideration the growing incidence of this pathology accompanied by the military operations, conducted on the territory of Ukraine.

One of the most burn-induced detrimental damages is the adrenal lesion, confirmed by the numerous experimental and clinical research; however, pathogenetic mechanisms of damage of this important part of body functioning remain to be understood, that essentially impedes the development of the novel and effective approaches of therapy aimed at this link of the pathological process [10].

Current data relative to the adrenal glands functioning in burn injury are obtained mainly using cytological, histological and biochemical approaches [4, 8, 9]. However, no publications, elucidating the state of adrenal glands functioning using the DNA-cytometry, which is considered as one of the most scrupulous approach in evaluation of synthetic processes and apoptosis (DNA degradation) on the cellular level, have been found [13]. It is apoptosis that is the main mechanism of cell damage, caused by the burn disease, and is involved in many areas of the pathological process and requires intensive monitoring and effective rehabilitation to prevent systematic destruction of the whole body [6].

The aim was the comparative analysis of the rates of rat adrenal cell cycle and DNA-fragmentation following the 1, 3, 7, 14, 21 i 30 days after thermal burn in rehabilitation with 0.9 % NaCl solution, lactoprotein with sorbitol and HAES-LX-5%.

Material and Methods. The experimental study has been made on 180 Wistar white male rats, weighted 160-180 g, provided by the vivarium at the Institute of Pharmacology and Toxicology of NAMS of Ukraine, and carried out on the basis of the Research Laboratory of Functional Morphology and Genetics of Research Center at National Pirogov Memorial Medical University, Vinnytsya, certified by the MOH of Ukraine (Certificate № 003/10 issued on 11.01.2010).

Animal housing and experiments on them have been carried out in compliance with the "General Ethic Rules for Conducting Experiments on Animals", adopted by the I National Congress on Bioethics (Kyiv, 2001) and the requirements of international principles of the "European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1985), as well as the principles of "Good laboratory practice for safety tests on chemicals", rules of

humane attitude to the experimental animals, approved by the Committee on Bioethics at National Pirogov Memorial Medical University, Vinnytsya (Protocol No 1issued on 14.01.2010); International requirements for humane treatment of animals, guidelines of NPC MOH of Ukraine "Preclinical studies of drugs" [5].

Before simulation of pathological state the lateral surfaces of animals' bodies were shaved with shaver and safety razor. The burn injury was induced by placing of four copperplates (two plates per each side), pre-soaked in water at 100°C for 6 min [11, 12]. The total superficial burn surface of rats with specified weight accounted for 21-23% in 10 sec exposure that was sufficient for initiation of the II-III degree burn and the development of moderate shock [1].

The infusion of 0.9% NaCl solution, lactoprotein with sorbitol or HAES-LX-5% was conducted in the lower hollow vein after its catheterization through the femoral vein, made in aseptic conditions. Catheter was sewed subcutaneously, its lumen was filled with heparin titrating solution (0,1 ml heparin per 10 ml 0.9% NaCl solution) after each administration of agents. Infusions were performed once a day during the first 7 days.

Catheterization of great vessels and animals' decapitation were made under 60 mg/kg intravenous propofol anesthesia.

DNA content in nuclei of rat adrenal cells was determined by flow cytometry. After animals' decapitation the adrenal glands were removed and capsules were extracted and all its contents were used to make nuclear suspension for flow cytometry. Nuclear suspension from adrenal cells was obtained using the CyStain DNA solution, intended for the analysis of the nuclear DNA, produced by the Partec company, Germany, in compliance with the protocol-instruction of the manufacturer. The solution enables rapid extraction of nuclei and marking of nuclear DNA by diamidino phenylindole (DAPI). During the preparation of nuclear suspension the CellTrics 50 mcm (Partec, Germany) disposable filters were used. Flow analysis was made on multifunctional research flow cytometer "Partec PAS" (Partec, Germany) in the Research Center at N.I. Pirogov Vinnytsya National Medical University.

UV- radiation was used for DAPI fluorescence excitation. 10 thousand events had to be analyzed from each sample. The analysis of cell cycle was made by means of FloMax (Partec, Germany) software in full digital compliance with mathematical model, where: S - percentage ratio of DNA synthesis phase to all cells of cell cycle (DNA content > 2s and < 4s). Determination of DNA fragmentation (apoptosis) was made by SUB-G0G1 area extraction on DNA-histograms RN1 before the G0G1peack, indicating about cell nuclei with DNA content < 2s.

Statistical processing of the results was carried out in the STATISTICA 6.1 license package using the nonparametric methods of the evaluation of the obtained results. Reliability of distribution of criteria for each of the obtained variational series, the average values of each studied criterion and standard quadratic deviation were assessed. Reliability of value difference between noncontiguous quantitative values was determined using the Mann-Whitney U-test criterion.

Results and Discussion. It has been established that administration of 0.9% NaCl solution in 1 day after burn injury does not prevent excessive activation of cellular processes in the form of activation of the synthetic phase (almost by 2.8 times as compared with a similar group without burn $-0.662 \pm 0.197\%$, p < 0.01), and apoptosis (by 1.8 times as compared with a similar group without burn $-3.362 \pm 0.237\%$, p < 0.01) (Fig. 1, 2), which leads, in its turn, to the onset of wide range of pathological processes, specific to dermal burn injury consequences. Activation of adrenal glands creates conditions for activation of the main links of the pathological process that develops in the body in irritation of the adrenal hormone receptors due to significant increase of synthetic processes in the gland itself.

This response allows the body to overcome the direct impact of burn injury. It cannot be definitely established whether this hyperactivation is the manifestation of pathological damage, or is the activation of protective mechanisms. However, a comparison of data obtained by the method of DNA-cytometry and histological and cytological approaches [2, 3] allows us to assert that this condition at the later time periods after the burn injury leads to a significant decrease in the activity of the adrenal glands and, consequently, to the development of organ's hypofunction.

Maximum increase of rates of SUB-G0G1 interval (almost by 3 times higher as compared to a similar group without burn $-5.514 \pm 0.851\%$, p < 0.01) and S-phase (4.8 times higher as compared to a similar group without burn $-1.203 \pm 0.439\%$, p < 0.01) (see Fig. 1, 2) was noted following the 3 days after burn injury along with administration of 0.9% NaCl solution, indicating about the highest activation of adrenal glands and coincides with the data, obtained by other researchers regarding the functioning of the adrenal glands concomitant with severe burn injury [10].

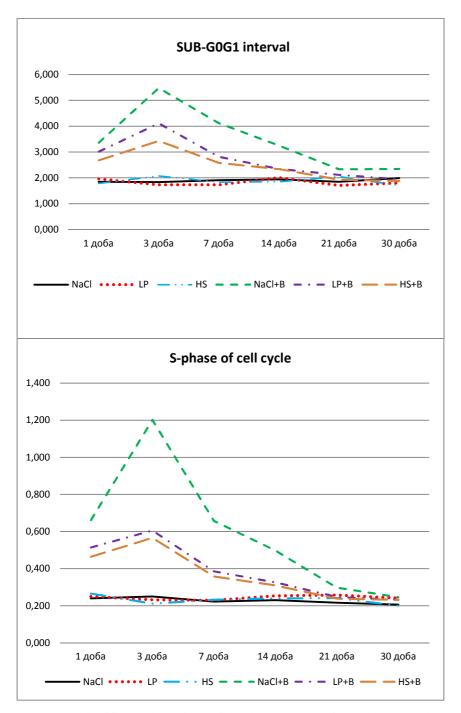


Fig. 1. The dynamic change in SUB-G0G1 interval (apoptosis) in the adrenal cells without and after the burn injury along with administration of 0.9% NaCl solution, lactoprotein with sorbitol and HAES-LX-5%.

Fig. 2. The dynamic change in S-phase interval (phase of DNA synthesis) in the adrenal cells without and after the burn injury along with administration of 0.9% NaCl solution, lactoprotein with sorbitol and HAES-LX-5%.

A significant lowering of both SUB-G0G1interval rate (almost 2.2 times higher values, as compared with a similar group without burn $-5.514 \pm 0.851\%$, p < 0.01) and S-phase rate (almost 3 times higher values as compared to a similar group without burn $-5.514 \pm 0.851\%$, p < 0.01) (see Fig. 1, 2) was noted after 7 days after burn injury along with administration of 0.9% NaCl solution, reflecting the decrease in proliferative activity and apoptosis in adrenal cells and inhibition of their response to burn injury. Notably, while comparing the data of histological studies, obtained in this time period, with data from publications [4] it was established that it is exactly in this term (7-14 days), along with the signs of the functional activity of cells, when the signs of degradation of cell elements, i.e., nuclei, membranes, organelles, become apparent. These signs represent the transition from the state of activation to the depletion of adrenal glands in severe burn injury, which is the precondition for reducing the resistance of the body and the development of numerous complications.

A gradual lowering of the rate of SUB-G0G1 interval (almost 1.7 times higher values as compared to a similar group without burn $-3,248 \pm 0,866\%$, p < 0.05) and S-phase rate (almost 2.2 times higher values, as compared with a similar group without burn $-0,498 \pm 0,099\%$, p < 0.01) was observed

and in 14 days after burn injury along with administration of 0.9% NaCl solution during the first seven days (see Fig. 1, 2), indicating about prolongation of pathological damage of the adrenal glands.

Following the 21 and 30 days after burn injury along with administration of 0.9% NaCl solution during the first seven days the S-phase rate in the adrenal cells remained insignificant and without differences against the similar rate in groups without the burn injury (0,298 \pm 0,141% and 0,246 \pm 0,188%, respectively, p > 0.05 in both time periods of observation), and rate of SUB-G0G1 interval remained statistically significantly higher (p < 0.05) than in groups without the burn injury (2,332 \pm 0,251% and 2,344 \pm 0,236%, respectively) (see Fig. 1, 2). Thus, hyperactivity of adrenal glands is observed even in the later time periods after the burn injury, which is also recorded by other researchers in the analysis of the level of adrenal hormones [10].

The resulting data indicated that application of lactoprotein with sorbitol and HAES-LX-5% solutions concomitant with dermal burn injury has a positive effect on the rates of SUB-G0G1 interval and S-phase of adrenal cells, reducing their activity right on day 1 after burn injury (lactoprotein with sorbitol group: SUB-G0G1 is almost 54% higher values (p < 0.01), as compared with a similar group without burn and 12% lower values (p < 0.05) as compared with a burn group + 0.9% NaCl solution – 3,012 \pm 0,135%; S-phase is almost 2 times higher values (p < 0.01), as compared with a similar group without burn and almost 29% lower values (p > 0.05) as compared with a burn group + 0.9% NaCl solution – 0,514 \pm 0,096%; HAES-LX 5% group: SUB-G0G1 is 50% higher values (p < 0.05) as compared with a similar group without burn and 36.5% lower values (p < 0.05) as compared with a burn group + 0.9% NaCl solution – 2,682 \pm 0,454%; S-phase is 74.4% higher values (p < 0.05) as compared with a similar group without burn and almost 42.6% lower values (p > 0.05) as compared with a burn group + 0.9% NaCl solution – 0,464 \pm 0,137%) (see Fig. 1, 2).

Such effect is observed in the follow-up periods of the experimental study (see Fig. 1, 2): after 3 days – lactoprotein with sorbitol group: SUB-G0G1 is almost 2,4 times higher values (p<0,01), as compared with a similar group without burn and 33.2% lower values (p < 0.05) as compared with a burn group + 0.9% NaCl solution -4.114 ± 0.773 %; S-phase is 2,6 times higher values (p < 0.01), as compared with a similar group without burn and almost 2 times lower values (p<0,01) as compared with a burn group + 0.9% NaCl solution - 0,606±0,127 %; HAES-LX 5% group: SUB-G0G1 is 65,2 % higher values (p < 0.01) as compared with a similar group without burn and almost 58 % lower values (p < 0.01) as compared with a burn group + 0.9% NaCl solution – 3,432±0,720 %; S-phase is almost 2,7 times higher values (p < 0.01) as compared with a similar group without burn and 2.1times lower values (p<0,01) as compared with a burn group + 0.9% NaCl solution - 0,566±0,141 %; after 7 days - lactoprotein with sorbitol group: SUB-G0G1 is 62.3% higher values (p < 0.01), as compared with a similar group without burn and 46,3 % lower values (p < 0.05) as compared with a burn group + 0.9% NaCl solution - 2.816 ± 0.373 %; S-phase is 67.8 % higher values (p < 0.05), as compared with a similar group without burn and 70,4 % lower values (p < 0.05) compared with a burn group + 0.9% NaCl solution -0,386±0,075 %; HAES-LX 5% group: SUB-G0G1 is 40,5 % higher values (p>0,05) as compared with a similar group without burn and almost 60 % lower values (p < 0.01) as compared with a burn group + 0.9% NaCl solution -2.578 ± 0.338 %; S-phase is 54.3 % higher values (p < 0.01) as compared with a similar group without burn and almost 84 % lower values (p<0,01) as compared with a burn group + 0.9% NaCl solution – 0,358±0,034 %; after 14 days– lactoprotein with sorbitol group: SUB-G0G1 is only 16,9 % higher values (p>0,05), as compared with a similar group without burn and 38,3 % lower values (p>0.05) as compared with a burn group +0.9% NaCl solution $-2.348\pm0.415\%$; S-phase is only 28,3 % higher values (p>0,05), as compared with a similar group without burn and almost 53 % lower values (p<0.01) as compared with a burn group + 0.9% NaCl solution - 0,326±0,063 %; HAES-LX 5% group: SUB-G0G1 is only 26,2 % higher values (p>0,05) as compared with a similar group without burn and 38,9 % lower values (p>0.05) as compared with a burn group + 0.9% NaCl solution $- 2.338\pm0.939$ %; Sphase is only 29,2 % higher values (p>0,05) as compared with a similar group without burn and 60,6 % lower values (p<0.05) as compared with a burn group + 0.9% NaCl solution $- 0.310\pm0.065\%$.

And after 21 and 31 days after burn injury we found the recovery of the S-phase and SUB-G0G1 interval rates to the level of similar rates in groups where infusion therapy was carried out to animals without burn (see Fig. 1, 2): after 21 days—lactoprotein with sorbitol group: SUB-G0G1 is 24 % higher values (p>0,05), as compared with a similar group without burn and only 10,2 % lower values (p>0,05) as compared with a burn group + 0.9% NaCl solution – 2,116±0,571 %; S-phase is almost the same (p>0,05), as compared with a similar group without burn and 20,1 % lower values (p>0,05) as compared with a burn group + 0.9% NaCl solution – 0,248±0,062 %; HAES-LX 5% group: SUB-G0G1 is almost the same (p>0,05) as compared with a similar group without burn and 20,7 % lower values (p<0,05) as

compared with a burn group + 0.9% NaCl solution - 1,932 \pm 0,229 %; S-phase is almost the same (p>0,05) as compared with a similar group without burn and 25,2 % lower values (p>0,05) as compared with a burn group + 0.9% NaCl solution - 0,238 \pm 0,058; after 30 days - lactoprotein with sorbitol group: SUB-G0G1 is almost the same (p>0,05), as compared with a similar group without burn and shows 20,3 % lower values (p>0,05) as compared with a burn group + 0.9% NaCl solution - 1,948 \pm 0,668 %; S-phase is almost the same (p>0,05), as compared with a similar group without burn and the burn group + 0.9% NaCl solution - 0,232 \pm 0,089 %; HAES-LX 5% group: SUB-G0G1 is only 10,2 % higher values (p>0,05) as compared with a similar group without burn and 24,7 % lower values (p>0,05) as compared with a burn group + 0.9% NaCl solution - 1,880 \pm 0,408 %; S-phase is only 16 % higher values (p>0,05) as compared with a similar group without burn and almost the same (p>0,05) as compared with a burn group + 0.9% NaCl solution - 0,232 \pm 0,041 %.

The findings confirmed the long-term effect of infusion with the abovementioned agents. It should be noted that HAES-LX 5% had more apparent rehabilitating effect on the rates of SUB-G0G1 interval accompanied by the burn injury. In our opinion, the DNA-cytometry approach provides with more accurate determination of the precise mechanisms of synthesis activation and apoptosis initiation, which enables to find out the critical points of adrenal glands damage and possible prospects of therapeutic rehabilitation.

Conclusions

- 1. Dermal burn injury along with infusion of 0.9% NaCl solution during the first 7 days of the experiment is accompanied by a significant increase of rates of SUB-G0G1 interval and S-phase of adrenal cells in 1 day (by 1.8 times and almost 2.8 times, respectively, as compared with a similar group without burn, p < 0.01), 3 days (by almost 3 times and 4.8 times, respectively, as compared with a similar group without burn, p < 0.01), 7 days (by almost 2.2 times and almost 3 times, respectively, as compared with a similar group without burn, p < 0.01) and 14 days (by almost 1.7 times and almost 2.2 times, respectively, as compared with a similar group without burn, p < 0.05-0.01) after burn injury. Following the 21 and 30 days after burn injury the S-phase rate in the adrenal cells remained insignificant with no reliable differences as compared with similar rate in groups without burn (only 38.0% and 19.4% higher values, respectively, p > 0.05), and rate of SUB-G0G1 interval remains significantly higher than in groups without the burn injury (26.3% and 18.0% higher values, respectively, p < 0.05).
- 2. Burn-related administration of lactoprotein with sorbitol solutions and HAES-LX-5% during the first seven days of the experiment is accompanied by the positive effect on the rates of SUB-G0G1interval and i S-phase of adrenal cells after 1 day (12,0 and 36,5 % lower, respectively, for the SUB-G0G1, as compared with the 0.9 % NaCl + burn group, p<0,05; 29,0 and 42,6 % lower, respectively, for the S-phase, as compared with the 0.9 % NaCl + burn group, p>0,05), 3 days (33,2 and 58,0 % lower, respectively, for the SUB-G0G1, as compared with the 0.9 % NaCl + burn group, p<0,05-0,01; 200 and 210 % lower, respectively, for the S-phase, as compared with the 0.9 % NaCl + burn group, p<0,01), 7 days (46,3 and 60,0 % lower, respectively, for the SUB-G0G1, as compared with the 0.9 % NaCl + burn group, p<0.05-0.01; 70.4 and 84.0 % lower, respectively, for the S-phase, as compared with the 0.9 % NaCl + burn group, p<0,05-0,01) and 14 days (38,3 and 38,9 % lower, respectively, for the SUB-GOG1, as compared with the 0.9 % NaCl + burn group, p>0.05; 53,0 and 60,6 % lower, respectively, for the S-phase, as compared with the 0.9 % NaCl + burn group, p<0,05-0.01) after burn injury. And following the 21 and 30 days after the burn injury the value of the rates in most cases was almost the same as compared with similar rates in the groups where infusion therapy was carried out to animals without burn. Administration of HAES-LX 5% solution showed lower rates of SUB-G0G1 interval, indicating about a more expressive antiapoptotic effect of the agent onto the adrenal cells concomitant with dermal burn injury.

Perspectives of further research will encompass the enhancement of activation of compensatory mechanisms of the whole body due to cytological studies of rehabilitation activities using the infusion solutions of lactoprotein with sorbitol and HAES-LX-5%, contributing to reduction of the damage and mortality by the total activation of reparatory processes in pathologically impaired organs and systems after the severe dermal burns.

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Реферати

ПОКАЗНИКИ КЛІТИННОГО ЦИКЛУ КЛІТИН НАДНИРКОВИХ ЗАЛОЗ У ЩУРІВ ПРОТЯГОМ МІСЯЦЯ ПІСЛЯ ОПІКУ ШКІРИ І ВВЕДЕННЯ 0,9% РОЗЧИНУ NACL, ЛАКТОПРОЕІНУ З СОРБІТОЛОМ ТА HAES-LX-5%

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В статті представлені результати дослідження показників клітинного циклу надниркових залоз та фрагментації ДНК у щурів протягом місяця після термічного опіку шкіри ІІ-ІІІ ступеня площею 21-23 % поверхні тіла на фоні корекції 0,9 % розчином NaCl, лактопротеїном з сорбітолом та HAES-LX 5%. Опікове ушкодження шкіри на фоні інфузії перших 7 діб експерименту 0,9 % розчином NaCl супроводжується суттєвим підвищенням показників інтервалу SUB-G0G1 і S-фази клітин надниркових залоз через 1, 3, 7 та 14 діб після опіку. Через 21 та 30 діб після опіку шкіри показник S-фази в клітинах надниркових залоз не має достовірних відмінностей порівняно з аналогічним показником у групах без опіку шкіри, а показник інтервалу SUB-GOG1 залишається достовірно більшим, ніж у групах без опікового пошкодження шкіри. Застосування перших 7 діб експерименту розчинів лактопротеїну з сорбітолом та HAES-LX 5% на фоні термічної травми шкіри супроводжується позитивним впливом на показники інтервалу SUB-G0G1 і S-фази клітин надниркових залоз через 1, 3, 7 та 14 діб після опіку. А через 21 та 30 діб після опіку шкіри величина даних показників практично не відрізняється аналогічних показників у групах де проводилася інфузійна терапія тваринам без опіку шкіри.

Ключові слова: клітинний цикл, фрагментація ДНК, надниркові залози, щури, опік шкіри, 0,9 % розчин NaCl, лактопротеїн з сорбітолом, HAES-LX 5%.

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ПОКАЗАТЕЛИ КЛЕТОЧНОГО ЦИКЛА КЛЕТОК НАДПОЧЕЧНИКОВ У КРЫС ВТЕЧЕНИЕ МЕСЯЦА ПОСЛЕ ОЖОГА КОЖИ И ВВЕДЕНИЯ 0,9% РАСТВОРА NaCl, ЛАКТОПРОЕИНА С СОРБИТОЛОМ И HAES-LX-5%

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В статье представлены результаты исследования показателей клеточного цикла надпочечников фрагментации ДНК у крыс в течение месяца после термического ожога кожи ІІ-ІІІ степени площадью 21-23% поверхности тела на фоне коррекции 0,9% раствором NaCl, Лактопротеином с сорбитолом и HAES-LX-5 %. Ожоговое повреждение кожи на фоне инфузии первых 7 суток эксперимента 0,9% раствором NaCl сопровождается существенным повышением показателей интервала SUB-G0G1 и S-фазы клеток надпочечников через 1, 3, 7 и 14 суток после ожога. Через 21 и 30 суток после ожога кожи показатель S-фазы в клетках надпочечников не имеет достоверных различий по сравнению с аналогичным показателем в группах без ожога кожи, а показатель интервала SUB-G0G1 остается достоверно больше, чем в группах без ожогового повреждения кожи. Применение первых 7 суток эксперимента растворов Лактопротеина с сорбитолом и HAES-LX-5% на фоне термической травмы кожи сопровождается положительным влиянием на показатели интервала SUB-G0G1 и S-фазы клеток надпочечников через 1, 3, 7 и 14 суток после ожога. А через 21 и 30 суток после ожога кожи величина данных показателей практически не отличается от аналогичных показателей в группах, где проводилась инфузионная терапия животным без ожога кожи.

Ключевые слова: клеточный цикл, фрагментация ДНК, надпочечники, крысы, ожог кожи, 0,9% раствор NaCl, Лактопротеин с сорбитол, HAES-LX-5%.

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