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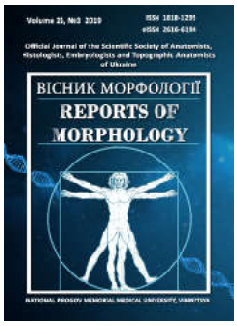

Indicators of the cell cycle in the thyroid gland in rats when using infusion of 0.9% NaCl solution on the background of thermal skin burns

Tiron O.I.
Odessa National Medical University, Odessa, Ukraine

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CORRESPONDING AUTHOR
e-mail: chekina.o@ukr.net
Tiron O.I.

Systemic damage of the organs, including the thyroid gland, is one of the key factors in the pathogenesis of burn disease due to thermal skin burns. The aim of this study was to investigate the indices of the cell cycle and DNA fragmentation of thyroid gland cells in rats with the use of infusion of 0.9% NaCl solution against the background of thermal skin burns. Experimental studies were conducted on 60 white male rats weighing 160-180 g, which was subjected to thermal burns of the skin of 2-3 degrees with a total area of 21-23% of the body surface. The first 7 days rats were infused with 0.9% NaCl solution into the inferior vena cava. Animals were removed from the experiment by decapitation (after 1, 3, 7, 14, 21, and 30 days). DNA content in the nuclei of the cells of the thyroid gland of rats was determined by flow cytometry. The statistical processing of the obtained results was carried out in the license package "STATISTICA 6.1" using nonparametric estimation methods. After 1 day after thermal skin damage and using 0.9% NaCl solution, lower ($p<0.05$) values of the S-phase index (0.234±0.094) were found compared to the control group without burn (0.652±0.134). The maximum decrease ($p<0.01$) of S-phase indicators (0.622±0.110 and 0.214±0.105, respectively) and a significant increase ($p<0.01$) of the SUB-G0G1 interval (5.288±0.840) compared to similar control group values (2.594±0.628) is observed after 3 days. The S-phase against the background of the introduction of 0.9% NaCl solution and thermal skin burn remained significantly lower than those of the similar control groups at 7 ($p<0.01$), 14 ($p<0.05$) and 21 days ($p<0.05$). At 14 days after thermal skin injury, the SUB-G0G1 interval ($p<0.05$) was lower than in the control group of rats. After 30 days, the G0G1 phase parameters were significantly lower ($p<0.01$), and the G2+M phase values were significantly ($p<0.01$) higher than those in the control group at the same time. Thus, it was found that 0.9% NaCl solution was not effective enough to correct cell division disorders during the entire observation period after skin burns.

Keywords: *thyroid gland, thermal burns of the skin, DNA cytometry, 0.9% NaCl solution.*

Introduction

The urgency of the problem of therapy of thermal burns of the skin and burn disease (BD) is caused by the increase in the number of burn injuries in modern society, the lack of efficiency of existing methods of therapy, the high frequency of development of complications of a systemic nature [14]. The inefficiency of the proposed methods of treatment is not least due to the complex pathogenesis of this damage, the numerous factors responsible for the cascade of pathological processes in thermal burns [13]. For this reason, the worldwide study of the mechanisms and pathogenetic factors of BD at the tissue, cell, subcellular and molecular levels perform, which deepens knowledge about this process and identifies potential targets of therapy [3].

One of the key factors in the pathogenesis of burn disease due to thermal burns of the skin, many researchers [6, 10, 18] are considering systemic damage to the organs of the endocrine system, which manifests itself both functionally and at the cellular and subcellular level. A special role in the pathogenesis of BD according to modern data [18] is given to the interaction of the triangle pituitary gland - adrenal cortex, pituitary gland - thyroid gland.

It is known [8] that patients with severe thermal burns of the skin have decreased plasma triiodothyronine concentrations, low thyroxine and normal range, or slightly

decreased thyrotropic hormone concentrations. This ensemble of change is collectively known as the "non-thyroid disease" syndrome [1]. The degree of manifestation of this disease is associated with the prognosis of the disease, but there is no evidence for the causality of this association. It is assumed [18] that the development of this condition is a consequence of the acute phase response to systemic irritation and microelement constraints. Thyroid injury pathogenetically is associated with the level of endogenous intoxication and the development of general inflammatory response, established a clear link between changes in thyroid hormone metabolism and activation of various proinflammatory cytokines [15].

Today, it is believed [7] that the problem of correction of thyroid gland damage remains an open question in the treatment of BD, and a problem that requires an urgent solution, given the important role of the functioning of this gland in the metabolism of the skin and other vital tissues (bone, connective tissue) that provide the restoration of homeostasis in the body. However, the most accurate method for assessing cell division is the DNA cytometry method, which is nowadays defined as a reference for the establishment of apoptosis markers, and such that allows dividing the cell phases into separate components [2]. We did not find any data on studies of thyroid cell division by DNA cytometry against the background of burn skin damage.

The aim of the study was to investigate the indices of the cell cycle and DNA fragmentation of the thyroid gland in rats using infusion of 0.9% NaCl solution against the background of thermal skin burns.

Materials and methods

Experimental studies were conducted on 60 white male rats weighing 160-180 g, conducted at the Research Laboratory of Functional Morphology and Genetics Research Center National Pirogov Memorial Medical University, Vinnytsya. The keeping and manipulation of animals was carried out in accordance with the "General Ethical Principles of Animal Experiments" (Kyiv, 2001), also guided by the recommendations of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1985), guidelines of the State Pharmacological Center of the Ministry of Health of Ukraine on "Preclinical studies of medicinal products" (2001), as well as the rules of humane treatment for experimental animals and conditions approved the Committee on Bioethics of National Pirogov Memorial Medical University, Vinnytsya (Minutes No. 1 of 14.01.2010).

Thermal burns of the skin of 2-3 degrees were carried out by applying to the pre-shaved lateral surfaces of the trunk of rats four copper plates (each with a surface area of 13.86 cm2) for 10 seconds, which were pre-heated for 6 minutes in water with a temperature of 100?C [9]. The total area of skin lesions was 21-23%. The first 7 days of rats were infused with 0.9% NaCl solution into the inferior vena cava. Animals were removed from the experiment by decapitation (after 1, 3, 7, 14, 21, and 30 days). Shaving, catheterization of veins, staging of skin burns, and decapitation of rats were performed under the conditions of intravenous propofol anesthesia (calculated at 60 mg/kg animal weight).

Within the framework of the agreement on scientific cooperation between the Research Center of National Pirogov Memorial Medical University, Vinnytsya and the Department of Histology, Cytology and Embryology of the Odessa National Medical University, DNA content in the nuclei of thyroid cells of rats was determined by flow DNA cytometry on a multifunction flow cytometer "Partec PAS" (Partec, Germany) [17]. Determined: G0G1 is the percentage of G0G1 phase cells to all cells in the cell cycle (DNA content = 2c); S is the percentage of the phase of DNA synthesis to all cells of the cell cycle (DNA content > 2c and < 4c); G2+M is the percentage ratio of the G2+M phase to all cells in the cell cycle (DNA = 4c). Determination of DNA fragmentation (SUB-G0G1, apoptosis) was performed by isolating the RN2 regions on DNA histograms before the G0G1 peak, indicating nuclei of cells with a DNA content < 2c.

The statistical processing of the obtained results was carried out in the license package "STATISTICA 6.1" using nonparametric estimation methods. The significance of the difference in values between the independent quantitative values was determined using the Mann-Whitney U test.

Results

It is established that the background of the introduction of 0.9% NaCl solution 1 day after thermal burns of the skin marked changes in the cell cycle indices of the thyroid gland - statistically significant decrease in the number of cells in phase S ($p<0.01$), which indicates insufficient restoration of the damaged cell population. Other indicators of the cell cycle have no significant or trending differences with those of the group without skin burn (Table 1). No significant or trending differences for G0G1, G2+M, and SUB-G0G1 were identified (see Table 1).

On the presented DNA-histogram (Fig. 1) of thyroid cells 1 day after skin burn on the background of the introduction of 0.9% NaCl solution, the level of DNA fragmentation in the interval SUB-G0G1 was 3.32%, and phase S - 0.19%, indicating expressed inhibition of DNA synthesis.

The maximum reduction in the percentage of thyroid cells in phase S ($p<0.01$) and, at the same time, a peak increase in the average level of SUB-G0G1 interval ($p<0.01$) was established after 3 days of observation from the start of thermal skin burn on the background of 0.9% NaCl solution introduction (see Table 1). No significant or trending differences were found for G0G1 and G2+M indicators (see Table 1).

In the presented DNA histogram (Fig. 2) of nuclear

Table 1. Indicators of the cell cycle in the cells of the thyroid gland of rats after skin burn with the use of infusion therapy 0.9% NaCl solution according to flow cytometry DNA (M±σ).

Group	Indicators of the cell cycle (%)			
	S	SUB-G0G1	G0G1	G2+M
1 day				
0.9% NaCl	0.652±0.134	2.462±0.800	91.16±2.41	8.192±2.368
Burn + 0.9% NaCl	0.234±0.094	2.732±1.141	91.90±2.65	7.868±2.678
$p_{(0.9\%\ NaCl\ -\ burn+0.9\%\ NaCl)}$	<0.01	>0.05	>0.05	>0.05
3 day				
0.9% NaCl	0.622±0.110	2.594±0.628	90.99±2.48	8.392±2.375
Burn + 0.9% NaCl	0.214±0.105	5.288±0.840	91.46±2.80	8.328±2.711
$p_{(0.9\%\ NaCl\ -\ burn+0.9\%\ NaCl)}$	<0.01	<0.01	>0.05	>0.05
7 day				
0.9% NaCl	0.650±0.139	2.632±0.724	90.90±2.17	8.448±2.113
Burn + 0.9% NaCl	0.350±0.088	3.994±1.204	88.70±3.13	10.95±3.14
$p_{(0.9\%\ NaCl\ -\ burn+0.9\%\ NaCl)}$	<0.01	=0.076	>0.05	>0.05
14 day				
0.9% NaCl	0.562±0.153	2.304±0.835	91.29±1.49	8.146±1.520
Burn + 0.9% NaCl	0.322±0.043	3.664±0.239	89.15±3.56	10.53±3.54
$p_{(0.9\%\ NaCl\ -\ burn+0.9\%\ NaCl)}$	<0.05	<0.05	>0.05	>0.05
21 day				
0.9% NaCl	0.522±0.075	2.622±0.677	90.60±2.48	8.986±2.370
Burn + 0.9% NaCl	0.364±0.092	3.250±0.755	87.98±3.30	11.66±3.27
$p_{(0.9\%\ NaCl\ -\ burn+0.9\%\ NaCl)}$	<0.05	>0.05	>0.05	>0.05
30 day				
0.9% NaCl	0.592±0.193	2.630±0.717	91.16±1.82	8.252±1.851
Burn + 0.9% NaCl	0.408±0.063	2.900±1.078	83.11±2.14	16.50±2.18
$p_{(0.9\%\ NaCl\ -\ burn+0.9\%\ NaCl)}$	=0.060	>0.05	<0.01	<0.01

suspension of cells of the thyroid gland of rats 3 days after skin burn on the background of the introduction of 0.9% NaCl solution, SUB-G0G1 (RN2, DNA fragmentation) was 5.33%, indicating the presence of a significant group of cells who are in the state of apoptosis activation.

After 7 days after thermal skin burns and 0.9% NaCl solution, a significantly lower (S<0.01) value of S-phase was observed and a slight tendency (p=0.076) to greater values of SUB-G0G1 interval compared to the group without skin burn (see Table 1). No significant or trending differences for G0G1 and G2+M indicators were detected (see Table 1).

After 14 days after thermal skin burn, significantly lower (p<0.05) values of the number of cells in the S-phase and higher (p<0.05) values of the level of the DNA fragmentation index in the interval SUB-G0G1 against the background of the introduction of the first seven days of 0.9% NaCl solution and compared to similar indicators in animals without burns (see Table 1). No significant or trending differences were found for G0G1 and G2+M.

At day 21 after thermal skin burn, significantly lower (p<0.05) cell counts in the S-phase were observed against the background of administration of the first seven days of 0.9% NaCl solution and compared with similar indices in animals without burns (see Table 1). No significant or trending differences for G0G1, G2+M, and SUB-G0G1 were identified (see Table 1).

30 days after skin burns, against the background of the introduction of the first seven days 0.9% NaCl solution tended (p=0.060) to lower values of the number of cells in the S-phase, significantly (p<0.01) less than the value of the G0G1 phase and higher (p<0.01) value of the G2+M phase

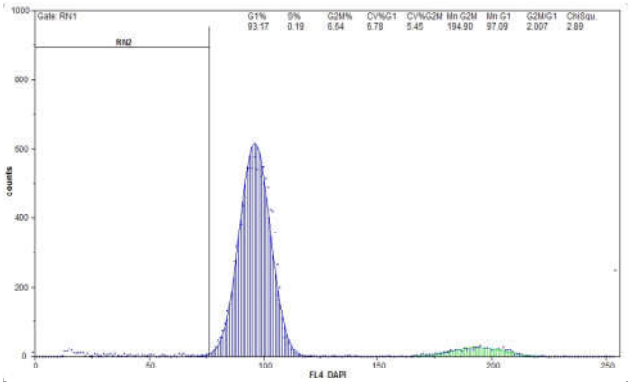

Fig. 1. DNA histogram of nuclear suspension of thyroid cells 1 day after burn injury on the background of the introduction of 0.9% NaCl solution. RN2 (SUB-G0G1, DNA fragmentation) = 3.32%.

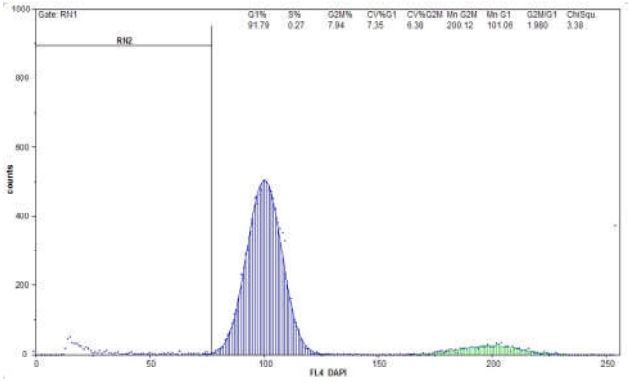

Fig. 2. DNA histogram of nuclear suspension of thyroid cells 3 days after burn injury on the background of the introduction of 0.9% NaCl solution. RN2 (SUB-G0G1, DNA fragmentation) = 5.33%.

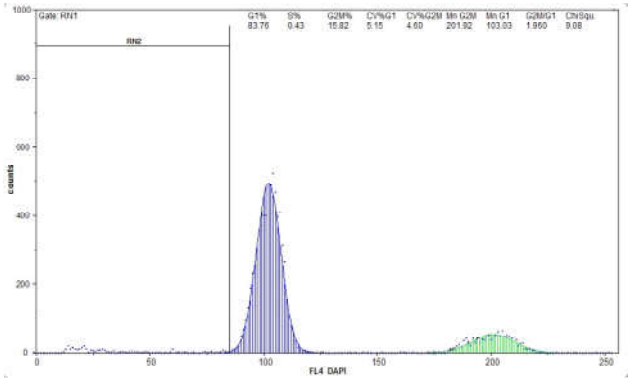

Fig. 3. DNA histogram of thyroid gland cell suspension 30 days after burn injury on the background of 0.9% NaCl solution administration. RN2 (SUB-G0G1, DNA fragmentation) = 3.60%.

compared with similar values in animals without burns (see Table 1). No significant or trending differences were observed for the SUB-G0G1 interval (see Table 1).

In the presented DNA histogram (Fig. 3) of a nuclear suspension of the cells of the thyroid gland of rats 30 days after skin burn on the background of the introduction of 0.9% solution of NaCl indicator SUB-G0G1 (RN2, DNA fragmentation) was 3.60%.

When analyzing the dynamics of changes in the cell cycle of the thyroid gland within 30 days after skin burns against the background of the introduction of 0.9% NaCl solution - G0G1 phase indicators have significantly ($p<0.05$-0.01) smaller values between 1 and 30, 3 and 21, 3 and 30, 7 and 30, 14 and 30, 21 and 30 days, as well as minor trends ($p=0.076$ in both cases) to smaller values between 1 and 21, 3 and 7 days of the experiment; phase S indicators have significantly ($p<0.05$ in all cases) greater values between 1 and 30, 3 and 21, 3 and 30, 14 and 30 days, as well as trends ($p=0.060$-0.076) to larger values between 1 and 21, 3 and 7, 3 and 14 days of experiment; G2+M phase indices have significantly ($p<0.05$-0.01) greater values between 1 and 30, 3 and 30, 7 and 30, 14 and 30, 21 and 30 days, and a slight tendency ($p=0.076$) to larger values between 1 and 21 days of the experiment; the SUB-G0G1 interval values had significantly ($p<0.01$) greater values between 1 and 3 days, and significantly ($p<0.05$-0.01) smaller values between 3 and 14, 3 and 21, 3 and 30 days of the experiment.

Discussion

Analyzing the obtained data of thyroid cell cycle indexes against the background of thermal burns of the skin and infusion of 0.9% NaCl solution and comparing the results with the data of other similar studies [12, 16] we can make some generalizations. The most pronounced cell cycle abnormalities were observed 3 days after thermal skin damage, although the first signs of these disorders in the form of a significant decrease in DNA synthesis ($p<0.01$) were observed after 1 day. However, established changes after 1 day are only the initiation of further damage to the thyroid gland, as changes at the subcellular level precede changes at the tissue and cellular levels, with subsequent disruption of the functioning of the organ. There are several factors that can be explained by the increase in the negative effect of burn injury due to increased toxicity of products, the stress of organ depletion and the potential activation of protective mechanisms, which is known to be often observed in burn skin damage [1, 11]. The thyroid gland in this case is included in the systemic damage of the organs of the endocrine system against the background of burn injury, which is manifested both functionally and at the cellular and subcellular levels and has been established in many studies [16, 18].

Perhaps the changes we recorded in the first days of observation appear due to the complex effects of burn toxins, the imbalance of hormonal regulation at the level of the hypothalamic-pituitary system, and the launch of a protective mechanism to inhibit damage, which are well-known factors of protection in critical states [5, 16]. However, our further observations in the following terms indicate insufficient protective effect and deepening of the violation of the cell cycle of the thyroid cells using 0.9% NaCl solution.

As is known [10], changes at the subcellular level precede changes at the cellular and histological levels, which corresponds to the hypofunctional state of the organ. Therefore, we can consider our indicators more sensitive marker of damage to the thyroid gland than morphological studies, which can potentially indicate the further development of deep morpho-functional damage to the gland, which is characteristic when using DNA cytometry [2, 12]. It should also be noted that these disorders were observed even in the long term of our study, which can affect the regeneration processes not only in the gland itself, but also in skin cells, the regeneration of which is controlled by the level of thyroid hormones [4, 15]. That is why damage to the thyroid gland against the background of burn injury of the skin is an important element of pathogenesis and requires effective correction [8, 14]. We can note the lack of effectiveness of using 0.9% NaCl solution to correct cell division disorders throughout the observation period.

The prospect of further research is to study the effect of other infusion solutions on thyroid cell cycle indices against the background of thermal skin burns.

Conclusions

1. 1 day after thermal skin damage and use of 0.9% NaCl solution, lower ($p<0.05$) values of the S-phase indicator were found compared to the control group of rats (0.9% NaCl solution without burn), reflecting a significant disturbance of the thyroid cell cycle.

2. The maximum decrease ($p<0.01$) of S-phase indicators and a significant increase ($p<0.01$) of the SUB-G0G1 interval compared to the same control group was observed after 3 days. The S-phase indicators against the background of the introduction of 0.9% NaCl solution and thermal skin burn remained significantly lower than those of the similar control groups at 7 ($p<0.01$), 14 ($p<0.05$) and 21 days ($p<0.05$). At 14 days after thermal skin injury, the SUB-G0G1 interval ($p<0.05$) was lower than in the control group of rats.

3. After 30 days, the G0G1 phase indicators were significantly lower ($p<0.01$) and the G2+M phase indicators were significantly ($p<0.01$) higher than those established in the control group at the same time.

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ПОКАЗНИКИ КЛІТИННОГО ЦИКЛУ В ЩИТОПОДІБНІЙ ЗАЛОЗІ У ЩУРІВ ПРИ ЗАСТОСУВАННІ ІНФУЗІЇ 0,9% РОЗЧИНУ NACL НА ФОНІ ТЕРМІЧНОГО ОПІКУ ШКІРИ

Тірон О.І.

Одним із ключових факторів патогенезу опікової хвороби внаслідок термічного опіку шкіри є системне ушкодження органів, зокрема щитоподібної залози. Мета роботи - дослідити показники клітинного циклу та фрагментації ДНК клітин щитоподібної залози у щурів при застосуванні інфузії 0,9% розчину NaCl на фоні термічного опіку шкіри. Експериментальні дослідження проведені на 60 білих щурах-самцях масою 160-180 г, котрим було нанесено термічний опік шкіри 2-3 ступеня загальною площею 21-23% поверхні тіла. Перші 7 діб щурам проводили інфузію 0,9% розчину NaCl у нижню порожнисту вену. Тварин виводили з експерименту шляхом декапітації (через 1, 3, 7, 14, 21 та 30 діб). Вміст ДНК в ядрах клітин щитоподібної залози щурів визначали методом проточної цитометрії. Статистична обробка отриманих результатів була проведена в ліцензійному пакеті "STATISTICA 6.1" із застосуванням непараметричних методів оцінки. Через 1 добу після термічного ушкодження шкіри і використання 0,9% розчину NaCl встановлено менші (p<0,05) значення показника S-фази (0,234±0,094) порівняно з контрольною групою без опіку (0,652±0,134). Максимальне зниження (p<0,01) показників S-фази (0,622±0,110 та 0,214±0,105 відповідно) та суттєве підвищення (p<0,01) показнику інтервалу SUB-G0G1 (5,288±0,840), порівняно з аналогічними показниками контрольної групи (2,594±0,628), спостерігали через 3 доби. Показники S-фази на фоні введення 0,9% розчину NaCl і термічного опіку шкіри залишались значно меншими від аналогічних показників відповідних контрольних груп через 7 (p<0,01), 14 (p<0,05) та 21 добу (p<0,05). Через 14 діб після термічного ушкодження шкіри встановлено менші (p<0,05) значення показника інтервалу SUB-G0G1 порівняно з контрольною групою щурів. Через 30 діб показники фази G0G1 виявились суттєво меншими (p<0,01), а показники фази G2+M значно (p<0,01) більшими від показників, встановлених в аналогічний термін у групі контролю. Таким чином, встановлена недостатня ефективність використання 0,9% розчину NaCl з метою корекції порушень клітинного поділу протягом всього терміну спостереження після опіку шкіри.

Ключові слова: *щитоподібна залоза, термічний опік шкіри, ДНК-цитометрія, 0,9% розчин NaCl.*

ПОКАЗАТЕЛИ КЛЕТОЧНОГО ЦИКЛА В ЩИТОВИДНОЙ ЖЕЛЕЗЕ У КРЫС ПРИ ПРИМЕНЕНИИ ИНФУЗИИ 0,9% РАСТВОРА NACL НА ФОНЕ ТЕРМИЧЕСКОГО ОЖОГА КОЖИ

Тирон О.И.

Одним из ключевых факторов патогенеза ожоговой болезни в результате термического ожога кожи является системное

повреждение органов, в том числе щитовидной железы. Цель работы исследовать показатели клеточного цикла и фрагментации ДНК клеток щитовидной железы у крыс при применении инфузии 0,9% раствора NaCl на фоне термического ожога кожи. Экспериментальные исследования проведены на 60 белых крысах-самцах массой 160-180 г, которым был нанесен термический ожог кожи 2-3 степени общей площадью 21-23% поверхности тела. Первые 7 дней крысам проводили инфузию 0,9% раствора NaCl в нижнюю полую вену. Животных выводили из эксперимента путем декапитации (через 1, 3, 7, 14, 21 и 30 суток). Содержание ДНК в ядрах клеток щитовидной железы крыс определяли методом проточной цитометрии. Статистическая обработка полученных результатов была проведена в лицензионном пакете "STATISTICA 6.1" с применением непараметрических методов оценки результатов. Через 1 сутки после термического повреждения кожи и использования 0,9% раствора NaCl установлено достоверно меньшее ($p<0,05$) значение показателя S-фазы (0,234±0,094) по сравнению с контрольной группой без ожога (0,652±0,134). Максимальное снижение ($p<0,01$) показателей S-фазы (0,622±0,110 и 0,214±0,105 соответственно) и существенное повышение ($p<0,01$) показателя интервала SUB-G0G1 (5,288±0,840), по сравнению с аналогичными показателями контрольной группы (2,594±0,628), наблюдали через 3 суток. Показатели S-фазы на фоне введения 0,9% раствора NaCl и термического ожога кожи оставались значительно меньшими, чем аналогичные показатели соответствующих контрольных групп через 7 ($p<0,01$), 14 ($p<0,05$) и 21 день ($p<0,05$). Через 14 суток после термического повреждения кожи установлено меньшее ($p<0,05$) значение показателя интервала SUB-G0G1 по сравнению с контрольной группой крыс. Через 30 суток показатели фазы G0G1 оказались существенно меньше ($p<0,01$), а показатели фазы G2+M значительно ($p<0,01$) больше показателей, установленных в аналогичный срок в группе контроля. Таким образом, установлена недостаточная эффективность использования 0,9% раствора NaCl с целью коррекции нарушений клеточного деления в течение всего срока наблюдения после ожога кожи.

Ключевые слова: *щитовидная железа, термический ожог кожи, ДНК-цитометрия, 0,9% раствор NaCl.*