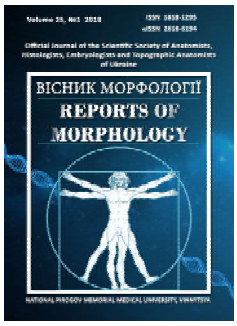
DOI: 10.31393/morphology-journal-2019-25(1)-09 Reports of Morphology, Vol. 25, №1, Pages 62-67

ISSN 1818-1295 eISSN 2616-6194

REPORTS OF MORPHOLOGY

Official Journal of the Scientific Society of Anatomists, Histologists, Embryologists and Topographic Anatomists of Ukraine

journal homepage: https://morphology-journal.com



Indicators of the cell cycle in the thyroid gland in rats when applying infusion of 0.9% solution of NaCl, lactoprotein with sorbitol or HAES-LX 5%

Tiron O.I., Appelhans O.L., Gunas I.V.[1], Chereshniuk I.L.[1]
Odessa National Medical University, Odessa, Ukraine
[1]National Pirogov Memorial Medical University, Vinnytsya, Ukraine

ARTICLE INFO
Received: 20 December, 2018
Accepted: 28 January, 2019

UDC: 616.441:599.323.4:615.456/.384

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

The thyroid gland is an important organ that is involved in the regulation of homeostasis and adaptation in various pathological conditions. However, the question of the study of the proliferative activity of thyroid cells by flow cytometry is still poorly understood. Purpose of study: to investigate the indices of the cell cycle and DNA fragmentation of thyroid cells in rats against the background of infusion of 0.9% NaCl solution, lactoprotein with sorbitol or HAES-LX 5%. Experimental studies were performed on 90 white male rats weighing 160-180 g. Infusion of 0.9% NaCl solution, lactoprotein with sorbitol or HAES-LX 5% was performed in the inferior vena cava after its catheterization in aseptic conditions through the femoral vein. The infusions were performed once a day for the first 7 days. Trunk catheterization and decapitation of animals (after 1, 3, 7, 14, 21, and 30 days) were performed under propofol anesthesia (60 mg/kg i/v). 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 (from 01/01/2018), DNA content in the nuclei of thyroid cells of rats was determined by flow DNA cytometry. Cell cycle analysis was performed using the software FloMax (Partec, Germany) in full digital accordance with the mathematical model, which determined: G0G1 - the percentage of cells of the phase G0G1 to all cells of the cell cycle (DNA content = 2c); S - the percentage of the phase of DNA synthesis to all cells of the cell cycle (DNA content > 2c and < 4c); G2+M - 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 region 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 data obtained showed a virtually identical pattern of rat cell cycle and DNA fragmentation of the thyroid gland cells at all study times against the use of 0.9% NaCl solution, lactoprotein with sorbitol or HAES-LX 5%. Thyroid cells in rats are predominantly in the inactive phase of DNA synthesis (G0G1) (90.32% - 91.88%), significantly fewer cells are in the G2+M phase (7.56% - 9.17%), and there is a small percentage of cells in the S-phase (DNA synthesis) (0.52% - 0.67%) and the SUB-G0G1 interval (DNA fragmentation, apoptosis) (2.23% - 2.81%). We can state that the activity of the main part of the thyroid gland is rather low without pathological irritation.

Keywords: *thyroid gland, DNA cytometry, cell cycle, 0.9% NaCl solution, lactoprotein with sorbitol, HAES-LX 5%.*

Introduction

The thyroid gland is an important organ that is involved in the regulation of homeostasis and adaptation in various pathological conditions [5, 13]. Numerous data on the morphological, biochemical and hormonal features of the thyroid gland in norm and pathology have been accumulated today [3]. However, the question of the

proliferative activity of organ cells remains poorly understood. Publications are devoted to the study of the proliferative activity of thyroid cells, mainly devoted to tumor and pre-tumor diseases [19, 20], which does not allow to extrapolate the obtained data to other pathological conditions.

It is known that the proliferative activity of the thyroid gland is a manifestation of physiological regeneration at the cellular level, with the number of cells in the state of mitotic division is normally in a small percentage [12]. Changes in the proliferative activity of cells in the thyroid gland depend on age, overall regeneration process and seasonal influences [11]. There is a significant increase in the proliferation of thyroid cells in pathology, which is associated with systemic activation of the body's neuroendocrine system by various factors [15]. It is known that the division of thyrocytes is controlled by central hormones, in particular thyroid tropic hormone and local modulators (growth factors, cytokines), which can stimulate both normal and pathological proliferation [17].

Changes in thyroglobulin in the colloid of the thyroid follicles are closely correlated with the phases of accumulation and evacuation of thyroid hormones having age, sex and circadian features. The relationships of proliferative activity, synthesis and resorption of thyroglobulin in thyroid cells are quite complex and mediated by a whole group of regulating factors of the gland itself and the hypothalamic-pituitary system [7].

A feature of cell proliferation in the thyroid gland, according to many researchers, is the process of desquamation of the follicular epithelium [9]. They argued that the basis of desquamation as a cellular destructive process may be a phenomenon such as apoptosis, and concluded that in the normal thyroid gland, the proliferation and desquamation of the follicular epithelium are antagonistic regulatory mechanisms that provide the necessary balance norms and pathologies.

Given the complexity of histological and morphological life-long study of the thyroid gland, there is a need to involve more accurate methods of studying cell division disorders. More than 60 imaging and evaluation markers have been proposed to determine apoptosis and cell proliferative activity. Proliferating cells that are at different stages of the mitotic cycle are immunohistochemically determined (Ki-67, PCNA, pl05, CDK-2, cdE) [8, 18]. In particular, these markers identify not only cells that have entered the mitosis, but also cells that are in the process of preparation for division, which allows to evaluate their proliferative potential [4]. However, the most informative method for assessing cell division is the flow cytometry method, which is nowadays defined as a reference for determining DNA fragmentation (apoptosis), and such that allows dividing the cell cycle into separate phases [1]. Flow cytometry method allowed estimation of changes in cell division and DNA fragmentation in various organs, in particular, endocrine ones, without pathological influence on the background of the use of infusion solutions [6, 10, 14].

Data on the study of indicators of the cell cycle of thyroid cells by flow DNA cytometry in non-tumor pathology, we have not revealed.

The *aim* of the study was to investigate the indices of the cell cycle and DNA fragmentation of thyroid cells in rats against the background of infusion of 0.9% NaCl solution, lactoprotein with sorbitol or HAES-LX 5%.

Materials and methods

Experimental studies were performed on 90 white male rats weighing 160-180 g (obtained from the vivarium of the Institute of Pharmacology and Toxicology of the National Academy of Medical Sciences of Ukraine), conducted on the basis of the Research Laboratory of Functional Morphology and Genetics of the Research Center of 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", adopted by the First National Congress on Bioethics (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).

The infusion of 0.9% NaCl solution, lactoprotein with sorbitol or HAES-LX 5% was performed into the inferior vena cava after catheterization in aseptic conditions through the femoral vein. The catheter was sewn under the skin, its lumen filled along the entire length with a titrated heparin solution (0.1 ml heparin per 10 ml 0.9% NaCl solution) after each substance administration. The infusions were performed once a day for the first 7 days. Trunk catheterization and decapitation of animals (after 1, 3, 7, 14, 21, and 30 days) were performed under propofol anesthesia (60 mg/kg i/v).

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 (from 01.01.2018), DNA content in the nuclei of thyroid cells of rats was determined by flow DNA cytometry. Samples were prepared using a CyStain DNA Step 1 (Partec, Germany) nuclear DNA test solution according to the manufacturer's instructions. The use of this solution allows rapid extraction of nuclei and labeling nuclear DNA with 4`,6-diamidino-2-phenylindole (DAPI). CellTrics 50 μm disposable filters (Partec, Germany) were also used for sample production. Flow analysis was performed on a multifunctional flow cytometer "Partec PAS" (Partec, Germany), at the Research Center of

National Pirogov Memorial Medical University, Vinnytsya.

Ultraviolet radiation was used to excite DAPI fluorescence. 10 thousand events were subjected to each test sample. Cell cycle analysis was performed using the software FloMax (Partec, Germany) in full digital compliance according to a mathematical model, which 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 for the independent samples.

Results

The data obtained showed an identical pattern of cell cycle and DNA fragmentation of rat thyroid cells at all study times against the use of 0.9% NaCl solution, lactoprotein with sorbitol, or HAES-LX 5% in rats (Table 1). Among all cell cycle indices studied, only the S-phase indicator in the group with the introduction of 0.9% NaCl solution after 1 and 21 days had a slight trend of differences (p = 0.076).

The results of a study of DNA content in the cells of the thyroid gland of animals against the introduction of 0.9% NaCl solution, lactoprotein with sorbitol or HAES-LX 5% showed the predominance of cells in the G0G1 phase (see Table 1). A much smaller number of cells were in a state of proliferation (S-phase, G2+M phase) (see Table 1). The level of cells with signs of apoptosis (SUB-G0G1) was negligible (see Table 1).

Figure 1 shows an example of a DNA histogram of thyroid cells after 3 days against the background of the introduction of 0.9% NaCl solution, in which the SUB-G0G1 interval was 2.44% of the total cellular events.

Figure 2 shows an example of a DNA histogram of thyroid cells after 7 days against the background of the introduction

Table 1. Indicators of the cell cycle in the thyroid gland of rats against the background of 0.9% NaCl solution, lactoprotein with sorbitol or HAES-LX 5% according to flow cytometry DNA (M±σ).

Indicators of the cell cycle	Groups of animals			p	p_1	p_2
	0.9% NaCl (n=5)	LPS (n=5)	HAES-LX 5% (n=5)			
After 1 day from the beginning of the experiment						
G0G1	91.16±2.41	90.87±1.69	90.68±1.93	>0.05	>0.05	>0.05
S	0.652±0.134	0.548±0.118	0.638±0.162	>0.05	>0.05	>0.05
G2+M	8.192±2.368	8.576±1.759	8.682±1.855	>0.05	>0.05	>0.05
SUB-G0G1	2.462±0.800	2.814±0.707	2.688±0.870	>0.05	>0.05	>0.05
After 3 days from the beginning of the experiment						
G0G1	90.99±2.48	90.39±2.11	90.21±1.78	>0.05	>0.05	>0.05
S	0.622±0.110	0.600±0.047	0.616±0.134	>0.05	>0.05	>0.05
G2+M	8.392±2.375	9.008±2.129	9.174±1.811	>0.05	>0.05	>0.05
SUB-G0G1	2.594±0.628	2.410±0.825	2.480±0.812	>0.05	>0.05	>0.05
After 7 days from the beginning of the experiment						
G0G1	90.90±2.17	91.06±1.68	90.32±1.78	>0.05	>0.05	>0.05
S	0.650±0.139	0.672±0.133	0.592±0.076	>0.05	>0.05	>0.05
G2+M	8.448±2.113	8.276±1.647	9.084±1.757	>0.05	>0.05	>0.05
SUB-G0G1	2.632±0.724	2.510±1.006	2.662±0.711	>0.05	>0.05	>0.05
After 14 days from the beginning of the experiment						
G0G1	91.29±1.49	90.54±1.69	91.24±1.85	>0.05	>0.05	>0.05
S	0.562±0.153	0.658±0.168	0.586±0.146	>0.05	>0.05	>0.05
G2+M	8.146±1.520	8.798±1.736	8.176±1.881	>0.05	>0.05	>0.05
SUB-G0G1	2.304±0.835	2.812±0.772	2.326±1.096	>0.05	>0.05	>0.05
After 21 days from the beginning of the experiment						
G0G1	90.60±2.48	91.88±1.74	90.60±2.11	>0.05	>0.05	>0.05
S	0.522±0.075	0.556±0.166	0.594±0.157	>0.05	>0.05	>0.05
G2+M	8.986±2.370	7.558±1.595	8.804±2.187	>0.05	>0.05	>0.05
SUB-G0G1	2.622±0.677	2.742±0.513	2.266±0.623	>0.05	>0.05	>0.05
After 30 days from the beginning of the experiment						
G0G1	91.16±1.82	90.84±1.94	91.31±2.49	>0.05	>0.05	>0.05
S	0.592±0.193	0.590±0.216	0.582±0.133	>0.05	>0.05	>0.05
G2+M	8.252±1.851	8.570±1.767	8.110±2.409	>0.05	>0.05	>0.05
SUB-G0G1	2.630±0.717	2.600±1.013	2.232±0.417	>0.05	>0.05	>0.05

Notes: LPS - lactoprotein with sorbitol; p - indicator of significance differences between similar groups of 0.9% NaCl solution and lactoprotein with sorbitol; p_1 -index of significance differences between similar groups of 0.9% NaCl solution and HAES-LX 5%; p_2 - indicator of significance of differences between similar indicators of lactoprotein with sorbitol groups and HAES-LX 5%.

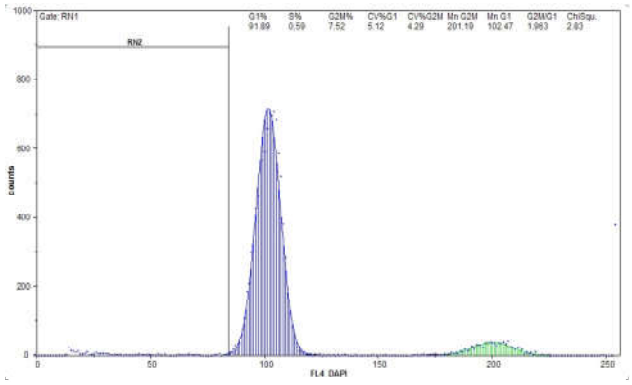


Fig. 1. DNA histogram of the nuclear suspension of thyroid cells after 3 days against the background of the introduction of 0.9% NaCl solution. RN2 (SUB-G0G1, DNA fragmentation) = 2.44%.

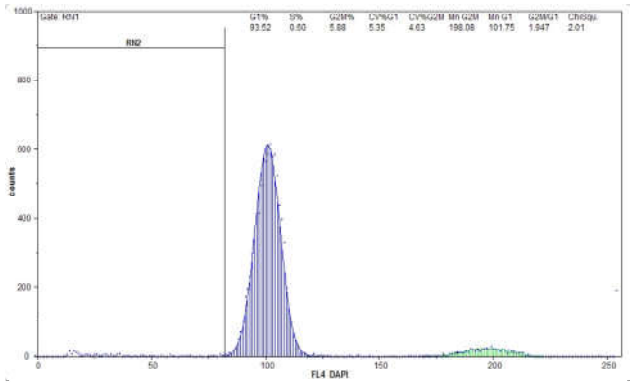


Fig. 2. DNA histogram of the nuclear suspension of thyroid cells after 7 days on the background of infusion with lactoprotein with sorbitol. RN2 (SUB-G0G1, DNA fragmentation) = 2.69%.

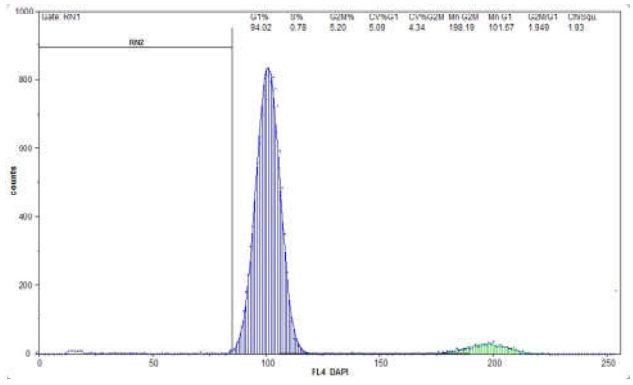


Fig. 3. DNA histogram of nuclear suspension of thyroid cells after 21 days without thermal skin burn on the background of infusion with HAES-LX 5%. RN2 (SUB-G0G1, DNA fragmentation) = 1.91%.

of lactoprotein with sorbitol, in which the interval SUB-G0G1 was 2.69% of the total number of cellular events.

Figure 3 shows an example of a DNA histogram of thyroid cells after 21 days on the background of the introduction of the first seven days of HAES-LX 5% solution, in which the SUB-G0G1 interval was 1.91% of the total cellular events.

According to our study, when using 0.9% NaCl solution, lactoprotein with sorbitol or HAES-LX 5%, it is established that the overwhelming percentage of cells is in the inactive phase G0G1 (range from 90.32±1.78% to 91.88±1.74%), significantly fewer number cells are in the G2+M phase (range from 7.558±1.595% to 9.174±1.811%), there is a small percentage of cells with signs of apoptosis (SUB-G0G1) (range from 2.232±0.417% to 2.814±0.707%) and in S-phase (DNA synthesis) (range from 0.522±0.075% to 0.672±0.133%).

Discussion

The study of thyroid cell cycle indices in rats by flow cytometry without pathological effects on the background of infusion of 0.9% NaCl solution, lactoprotein with sorbitol or HAES-LX 5% revealed that their use has no significant effect on the cell cycle fragmentation indices DNA of cells. Also, the determination of cell cycle indices and DNA fragmentation of thyroid cells against the background of infusion of 0.9% NaCl solution, lactoprotein with sorbitol or HAES-LX 5%, allowed to eliminate the potential impact on the normal cycle of gland cells, which was found in similar studies application of these solutions [6, 14]. It should be noted that the data obtained by us generally correspond to similar studies on the proliferative activity of thyroid cells in animals and humans [12, 21]. In studies of the processes of DNA synthesis and apoptosis in thyroid cells carried out by other methods [16], similar data were obtained regarding the relatively low level of synthesis and apoptosis of thyroid cells in animals without pathology.

It is also worth noting that other researchers [21] found a negative dependence of cell proliferation and increased induction of apoptosis in the thyroid gland as animals age increase, which is consistent with a general tendency to decrease the rate of cell renewal with age. There are also similar data regarding the age-related changes in the microstructure of the human thyroid gland [2]. We did not find signs of active apoptosis in this organ and little activity of DNA synthesis against the background of using the investigated solutions, which suggests that there is a balance between the processes of death and the renewal of the cell population of the thyroid gland. We can say that the activity of the main part of the cells is rather low without pathological irritation, which, in general, is in line with current views on the thyroid cell population and its functioning [13].

Our study has complemented the current understanding of the state of proliferative activity of thyroid cells under conditions of infusion solutions using flow cytometry. Given that we have not identified data on studies of the cell cycle and DNA fragmentation of thyroid cells and this technique is one of the benchmarks for the evaluation of apoptosis and cell division phases, we can state its priority in this area of research.

Conclusions

1. Infusion of 0.9% solution of NaCl, lactoprotein with sorbitol or HAES-LX 5% for duration of 7 days does not

affect the cell cycle and DNA fragmentation of the thyroid gland cells.

2. Thyroid gland cells in rats are predominantly in the inactive phase of DNA synthesis (G0G1) (90.32% - 91.88%), significantly fewer number of cells are in the G2+M phase (7.558% - 9.174%), there is a small percentage of cells in the S-phase (DNA synthesis) (0.522% - 0.672%) and the interval SUB-G0G1 (DNA fragmentation, apoptosis) (2.232% - 2.814%).

References

[1] Adan, A., Alizada, G., Kiraz, Y., Baran, Y., & Nalbant, A. (2017). Flow cytometry: basic principles and applications. *Critical reviews in biotechnology,* 37(2), 163-176. doi: 10.3109/07388551.2015.1128876

[2] Ageenko, K. I., Gorbachev, A. L., & Shubert, E. E. (2011). Features of the thyroid gland histostructure in residents of the city of Magadan. *Basic research,* 2(9), 191-195.

[3] Arzhanov, I. Y., Buniatov, M. R., & Ushakova, G. A. (2017). The thyroid status of a conditionally healthy adult population of Prydniprovia. *Regulatory Mechanisms in Biosystems,* 8(4), 554-558. https://doi.org/10.15421/021785

[4] Behbehani, G. K. (2018). Cell cycle analysis by mass cytometry. *Methods Mol. Biol.,* 1686, 105-124. doi: 10.1007/978-1-4939-7371-2_8

[5] Benvenga, S., Tuccari, G., Leni, A., & Vita, R. (2018). *Thyroid Gland: Anatomy and Physiology. In book: Reference Module in Biomedical Sciences.* Elsevier Inc. doi: 10.1016/B978-0-12-801238-3.96022-7

[6] Cherkasov, V. G., Cherkasov, E. V., Kaminsky, R. F., Pastukhova, V. A., Kovalchuk, O. I., & Trofimenko, Yu. Yu. (2017). Influence of HAES-LX-5% infusion solution on the DNA content of endocrine glands cells against the background of thermal burn of skin in rats. *Світ медицини та біології*, 13, 4(62), 168-173. doi: 10.26724/2079-8334-2017-4-62-168-173

[7] Davis, P. J., Leonard, J. L., Lin, H. Y., Leinung, M., & Mousa, S. A. (2018). Molecular basis of nongenomic actions of thyroid hormone. *Vitamins and hormones,* 106, 67-96. doi: 10.1016/bs.vh.2017.06.001

[8] Kaczmarzyk, T., Kisielowski, K., Koszowski, R., Rynkiewicz, M., Gawelek, E., Babiuch, K. … Drozdzowska, B. (2018). Investigation of clinicopathological parameters and expression of COX-2, bcl-2, PCNA, and p53 in primary and recurrent sporadic odontogenic keratocysts. *Clinical oral investigations,* 22(9), 3097-3106. doi: 10.1007/s00784-018-2400-7

[9] Khmel'niczkij, O. K., & Gorbachev, A. L. (2005). To the question of desquamation of thyroid epithelium. *Human ecology*, 2, 10-16.

[10] Kim, K. H., & Sederstrom, J. M. (2015). Assaying cell cycle status using flow cytometry. *Current protocols in molecular biology,* 111 (28.6), 1-11. doi: 10.1002/0471142727.mb2806s111

[11] Konovalov, S. S., & Polyakova, V. O. (Eds.) (2009). *Verification of expression of the P53 protein in the human thyroid gland during aging.* Belgorod. Abstracts are presented in the materials of the "Spring Gerontological Conference", Belgorod (pp. 27-28). Belgorod: [b. i.].

[12] Myalin, A. N., Mozerov, S. A., Chekushkin, A. A., & Sokolov, I. A. (2007). The effect of burn shock on the morphofunctional state of the thyroid gland. News of higher educational institutions. Volga region. Medical sciences, 4, 22-29.

[13] Nilsson, M., & Fagman, H. (2017). Development of the thyroid gland. *Development,* 144(12), 2123-2140. doi: 10.1242/dev.145615

[14] Ocheretna, N. P., Guminskiy, Yu. I., & Gunas, I. V. (2018). Indicators of cell cycle and dna fragmentation of spleen cells in early terms after thermal burns of skin on the background of using "lactoprotein with sorbitol" or HAES-LX-5%. *Вісник наукових досліджень,* 1, 141-146. doi: 10.11603/2415-8798.2018.1.8627

[15] Ortiga-Carvalho, T. M., Chiamolera, M. I., Pazos-Moura, C. C., & Wondisford, F. E. (2016). Hypothalamus-Pituitary-Thyroid Axis. *Comprehensive Physiology,* 6(3), 1387-1428. doi: 10.1002/cphy.c150027

[16] Pavlov, A. V., & Bezdenezhny'kh, A. V. (2018). Proliferative and secretory activity of follicular thyrocytes in various modes of muscle activity. *Bulletin of new medical technologies*, 25(3), 202-208.

[17] Rashmi, M., Liu, Y.-Y., & Brent, G. A. (2014). Thyroid hormone regulation of metabolism. *Physiological reviews,* 94.2, 355-382. doi: 10.1152/physrev.00030.2013

[18] Sun, X., & Kaufman, P. D. (2018). Ki-67: more than a proliferation marker. *Chromosoma,* 127(2), 175-186. doi: 10.1007/s00412-018-0659-8

[19] Wang, X., Lu, X., Geng, Z., Yang, G., & Shi, Y. (2017). LncRNA PTCSC3/miR-574-5p governs cell proliferation and migration of papillary thyroid carcinoma via Wnt/β-catenin signaling. *Journal of cellular biochemistry*, 118(12), 4745-4752. https://doi.org/10.1002/jcb.26142

[20] Zhou, Q., Chen, J., Feng, J., & Wang, J. (2016). Long noncoding RNA PVT1 modulates thyroid cancer cell proliferation by recruiting EZH2 and regulating thyroid-stimulating hormone receptor (TSHR). *Tumor Biol.*, 37(3), 3105-3113. doi: 10.1007/s13277-015-4149-9

[21] Zugairova, O. N., Polyakova, V. O., & Konovalov, S. S. (2009). Dynamics of processes of proliferation and apoptosis of human and rat thyroid follicular cells in natural and radiation-induced aging. *Medico-biological and socio-psychological problems of safety in emergency situations,* 4, 54-58.

ПОКАЗНИКИ КЛІТИННОГО ЦИКЛУ В ЩИТОПОДІБНІЙ ЗАЛОЗІ У ЩУРІВ ПРИ ЗАСТОСУВАННІ ІНФУЗІЇ 0,9% РОЗЧИНУ NACL, ЛАКТОПРОТЕЇНУ ІЗ СОРБІТОЛОМ АБО HAES-LX 5%

Тірон О.І., Аппельханс О.Л., Гунас І.В., Черешнюк І.Л.

Щитоподібна залоза є важливим органом, який бере участь у регуляції гомеостазу та адаптації при різних патологічних станах. Однак, питання вивчення проліферативної активності клітин щитоподібної залози методом проточної ДНК-цитометрії залишається маловивченим. Мета роботи: дослідити показники клітинного циклу та фрагментації ДНК клітин щитоподібної залози у щурів на фоні інфузії 0,9% розчину NaCl, лактопротеїну із сорбітолом або HAES-LX 5%. Експериментальні дослідження проведені на 90 білих щурах-самцях масою 160-180 г. Інфузію 0,9% розчину NaCl, лактопротеїну із сорбітолом або HAES-LX 5% проводили у нижню порожнисту вену після її катетеризації в асептичних умовах через стегнову вену. Інфузії виконували раз на добу на протязі перших 7 діб. Катетеризацію магістральних судин та декапітацію

тварин (через 1, 3, 7, 14, 21 та 30 діб) здійснювали в умовах пропофолового наркозу (60 мг/кг в/в). У рамках угоди про наукову співпрацю між науково-дослідним центром Вінницького національного медичного університету ім. М.І.Пирогова та кафедрою гістології, цитології та ембріології Одеського національного медичного університету (від 01.01.2018), вміст ДНК в ядрах клітин щитоподібної залози щурів визначали методом проточної ДНК-цитометрії. Аналіз клітинного циклу виконували засобами програмного забезпечення FloMax (Partec, Німеччина) у повній цифровій відповідності згідно математичної моделі, де визначали: G0G1 - відсоткове співвідношення клітин фази G0G1 до всіх клітин клітинного циклу (вміст ДНК = 2с); S - відсоткове співвідношення фази синтезу ДНК до всіх клітин клітинного циклу (вміст ДНК > 2с та < 4с); G2+M - відсоткове співвідношення фази G2+M до всіх клітин клітинного циклу (ДНК = 4с). Визначення фрагментації ДНК (SUB-G0G1, апоптоз) було виконано шляхом виділення ділянки RN2 на ДНК-гістограмах перед піком G0G1, яка вказує на ядра клітин з вмістом ДНК < 2с. Статистична обробка отриманих результатів була проведена в ліцензійному пакеті "STATISTICA 6.1" із застосуванням непараметричних методів оцінки. Отримані дані засвідчили практично ідентичну картину показників клітинного циклу та фрагментації ДНК клітин щитоподібної залози щурів в усі терміни дослідження на фоні використання 0,9% розчину NaCl, лактопротеїну з сорбітолом або HAES-LX 5%. Клітини щитоподібної залози в щурів переважно знаходяться в неактивній фазі синтезу ДНК (G0G1) (90,32% - 91,88%), значно менша кількість клітин перебувають в фазі G2+M (7,56% - 9,17%), наявний незначний відсоток клітин в S-фазі (синтез ДНК) (0,52% - 0,67%) та показника інтервалу SUB-G0G1 (фрагментація ДНК, апоптоз) (2,23% - 2,81%). Можемо стверджувати про досить невисоку активність основної частини клітин щитоподібної залози без патологічного подразнення.

Ключові слова: *щитоподібна залоза, ДНК-цитометрія, клітинний цикл, 0,9% розчин NaCl, лактопротеїн з сорбітолом, HAES-LX 5%.*

ПОКАЗАТЕЛИ КЛЕТОЧНОГО ЦИКЛА В ЩИТОВИДНОЙ ЖЕЛЕЗЕ У КРЫС ПРИ ПРИМЕНЕНИИ ИНФУЗИИ 0,9% РАСТВОРА NACL, ЛАКТОПРОТЕИНА С СОРБИТОЛОМ ИЛИ HAES-LX 5%

Тирон О.И., Аппельханс Е.Л., Гунас И.В., Черешнюк И.Л.

Щитовидная железа является важным органом, который принимает участие в регуляции гомеостаза и адаптации при различных патологических состояниях. Однако, вопрос изучения пролиферативной активности клеток щитовидной железы методом проточной ДНК-цитометрии остается малоизученным. Цель работы: исследовать показатели клеточного цикла и фрагментации ДНК клеток щитовидной железы у крыс на фоне инфузии 0,9% раствора NaCl, лактопротеина с сорбитолом или HAES-LX 5%. Экспериментальные исследования проведены на 90 белых крысах-самцах массой 160-180 г. Инфузию 0,9% раствора NaCl, лактопротеина с сорбитолом или HAES-LX 5% проводили в нижнюю полую вену после ее катетеризации в асептических условиях через бедренную вену. Инфузии выполняли раз в сутки в течение первых 7 дней. Катетеризацию магистральных сосудов и декапитацию животных (через 1, 3, 7, 14, 21 и 30 суток) осуществляли в условиях пропофолового наркоза (60 мг/кг в/в). В рамках соглашения о научном сотрудничестве между научно-исследовательским центром Винницкого национального медицинского университета им. Н.И. Пирогова и кафедрой гистологии, цитологии и эмбриологии Одесского национального медицинского университета (от 01.01.2018), содержание ДНК в ядрах клеток щитовидной железы крыс определяли методом проточной ДНК-цитометрии. Анализ клеточного цикла выполняли средствами программного обеспечения FloMax (Partec, Германия) в полном цифровом соответствии согласно математической модели, где определялись: G0G1 - процентное соотношение клеток фазы G0G1 ко всем клеткам клеточного цикла (содержание ДНК = 2с); S - процентное соотношение фазы синтеза ДНК ко всем клеткам клеточного цикла (содержание ДНК>2с и <4с); G2+M - процентное соотношение фазы G2+M ко всем клеткам клеточного цикла (ДНК = 4с). Определение фрагментации ДНК (SUB-G0G1, апоптоз) было выполнено путем выделения участка RN2 на ДНК-гистограммах перед пиком G0G1, указывающая на ядра клеток с содержанием ДНК < 2с. Статистическая обработка полученных результатов была проведена в лицензионном пакете "STATISTICA 6.1" с применением непараметрических методов оценки. Полученные данные показали практически идентичную картину показателей клеточного цикла и фрагментации ДНК клеток щитовидной железы крыс во все сроки исследования на фоне использования 0,9% раствора NaCl, лактопротеина с сорбитолом или HAES-LX 5%. Клетки щитовидной железы у крыс преимущественно находятся в неактивную фазу синтеза ДНК (G0G1) (90,32% - 91,88%), значительно меньшее количество клеток находятся в фазе G2+M (7,56% - 9,17%), имеется незначительный процент клеток в S-фазе (синтез ДНК) (0,52% - 0,67%) и показателя интервала SUB-G0G1 (фрагментация ДНК, апоптоз) (2,23% - 2,81%). Можем утверждать о достаточно невысокой активности основной части клеток щитовидной железы без патологического раздражения.

Ключевые слова: *щитовидная железа, ДНК-цитометрия, клеточный цикл, 0,9% раствор NaCl, лактопротеин с сорбитолом, HAES-LX 5%.*