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QUANTITATIVE AND QUALITATIVE CHANGES IN MONOCYTE SPROUT AND RED BONE MARROW MICROENVIRONMENT CELLS UNDER LONG-TERM ADMINISTRATION OF TRYPTORELIN WITH QUERCETIN IN THE EXPERIMENT

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The study of red bone marrow features and morphological and functional characteristics is relevant because it plays the role of a central organ of haemopoiesis and immunopoiesis. In our study, we focused on the course of monocytopoiesis, since the monocyte sprout is a source of monocytes that proliferate and transform into macrophages in the tissues of the body. In turn, the cells of the environment of RBM consist of many cell types, and the relative impact of each of these cells on the maintenance of a haematopoietic clone remains largely unclear under the influence of various endogenous and exogenous factors on these cells. In contemporary urological practice and according to European Association of Urology's suggestions, Tryptorelin is extensively employed for androgen deprivation therapy. Quercetin, a flavonoid present in fruits and vegetables possesses distinctive biological properties. The administration of tryptorelin with a long-term effect (1 year) and oral administration of the riboflavonoid "Quercetin" leads to statistically significant changes in the quantitative and qualitative composition of RBM, in particular, the studied cells of monocyte sprout and cells of environment. The maximum quantitative changes in MS cells were observed at 3 and 6 months of the study followed by a gradual recovery to the control group. Cells of environment had a significant variability of changes: the quantitative characteristics of macrophages and reticular cells remained constant, but the NSC index for macrophages was lower at all observation periods compared to the control group due to a decrease in nucleus area. Adipocytes responded with a positive increase in quantitative and qualitative characteristics.

Key words: *red bone marrow, monocytopoiesis, environment cells, tryptorelin, quercetin.*

Connection of the publication with planned research works.

The study is a fragment of the research project of the Department of Histology, Cytology and Embryology “Experimental morphological study of cryopreserved placenta transplants action diphereline, ethanol and 1% methacrylic acid on the morphofunctional status in a number of internal organs”, state registration No. 0119U102925.

Introduction.

The red bone marrow (RBM) is one of the largest organs in area and most dynamic in functional activity [1]. The study of its features and morphological and functional characteristics is relevant because it plays the role of a central organ of haemopoiesis and immunopoiesis.

In our study, we focused on the course of monocytopoiesis, since the monocyte sprout (MS) is a source of monocytes that proliferate and transform into macrophages in the tissues of the body [2, 3, 4, 5, 6].

In turn, the cells of the environment (ECs) of RBM consist of many cell types, and the relative impact of each of these cells on the maintenance of a haematopoietic clone remains largely unclear [7] under the influence of various endogenous and exogenous factors on these cells.

In contemporary urological practice and according to European Association of Urology’s suggestions [8], Tryptorelin, a synthetic analogue of gonadotropin-releasing hormone, is extensively employed for androgen deprivation therapy. This substance, a polypeptide in its chemical composition, was produced by the laboratory of the French company Beaufour Ipsen in the 1980s [9].

Quercetin, a flavonoid present in fruits and vegetables possesses distinctive biological properties. It exhibits a wide range of effects including anticancer, anti-inflammatory, antiviral, reduction in lipid peroxidation, platelet aggregation, capillary permeability, and stimulation of mitochondrial biogenesis [10, 11, 12].

Thus, the effect of tryptorelin with quercetin on monocyte sprout cells and cells of the RBM microenvironment is a topical issue of the present and is not well represented in the scientific literature.

The aim of the study.

To determine the qualitative and quantitative changes in MS and ECs after chemical castration of male rats of central origin by administration of tryptorelin solution in combination with a quercetin-enriched diet for one year.

Object and research methods.

The study was conducted on 35 adult male white rats. The rats were divided into 2 groups: Group I – control (10), which were injected with saline solution [13], animals of Group II (25) were subcutaneously injected with tryptorelin acetate at a dose of 0.3 mg of active substance per kg and Quercetin 100 mg per kg of body weight 3 times a week [14, 15].

Animals were kept in standard accommodation at the vivarium of Poltava State Medical University.

The euthanization of experimental animals was carried out in strict accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) as well as the General Ethical Principles for Experiments on Animals adopted by the First National Congress on Bioethics (Kyiv, 2001). Animals were euthanised (n=35) in accordance with the relevant terms through an overdose of ether anaesthesia.

Using standard methods, the material was embedded in paraffin blocks, and 4 μm thick sections were prepared and then stained with haematoxylin and

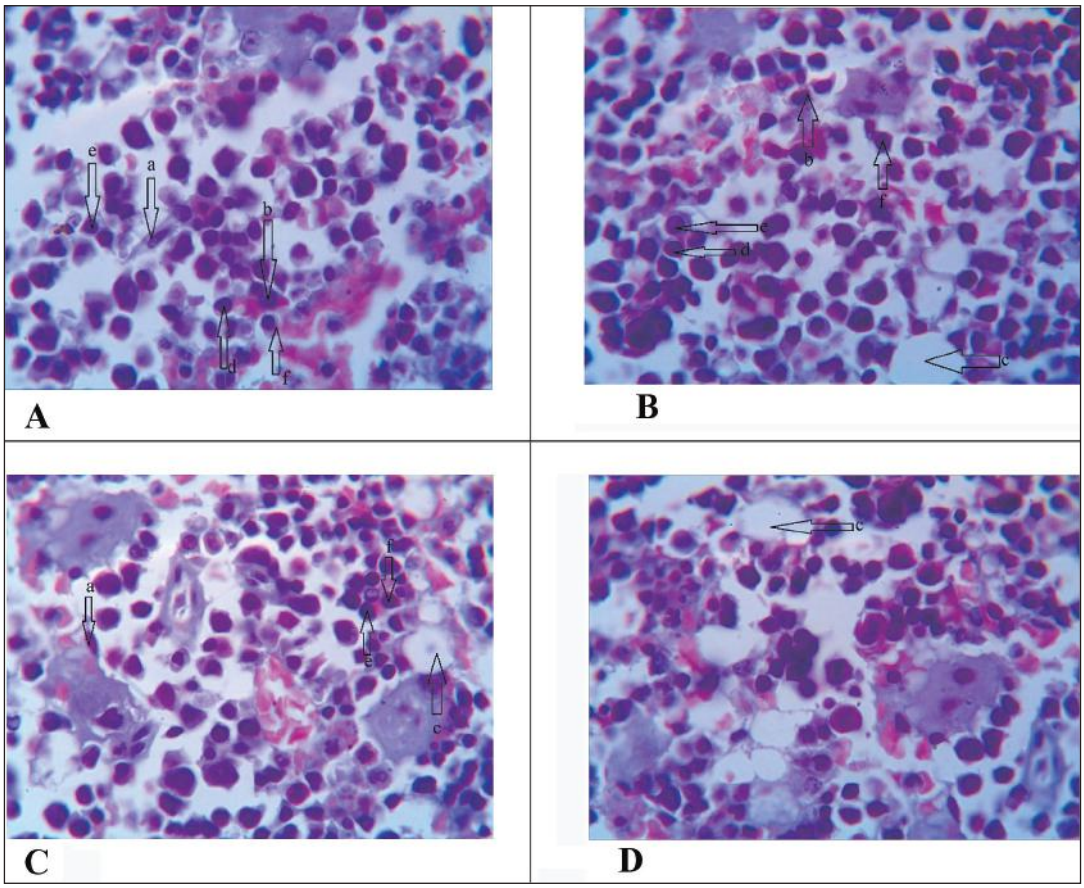

Figure – RBM. A – first month, B – third month, C – sixth month, D – ninth month. Hematoxylin and eosin staining; Magnification: ocular 10; lens 100 (oil immersion). Marking: a – reticular cell; b – macrophage; c – adipocyte; d – monoblast; e – promonocyte; f – monocyte.

eosin [16]. The histological preparations were investigated using a Biorex 3 light microscope with a digital microfilter with software adapted for these studies (serial number 5605) .

Statistical processing of the results was performed using Microsoft Office Excel and the Real Statistics 2019 extension to it. The non-parametric Mann-Whitney test was used to determine the statistical difference between the groups.

Research results and their discussion.

The obtained histological preparations of RBM were evaluated by examining the cells of MS and ECs at various stages of the experiment, including monoblasts, promonocytes, monocytes, adipocytes, reticular cells, and macrophages. Monocyte cells were at different stages of differentiation and were surrounded by ECs. Furthermore, RBM preparations were heavily vascularised by sinusoidal capillaries, which turned out to be statistically significant. The sinusoidal capillaries comprised fenestrae, enabling mature blood cells to enter the bloodstream.

There were no significant differences between the control and experimental groups when morphological changes in all cells were evaluated. The reticular cells (marked as (a) on **fig.**) exhibited consistent morphological characteristics and were eccentrically arranged. The nucleus was either rounded or oval, occasionally with pointed edges, and possessed a laced, unevenly netted structure resembling that of a monocyte nucleus. It was typically positioned at the cell centre or close to the wall and contained 1-2 nucleoli. The cytoplasm was greyish-blue or pale blue in colour with indistinct contours. It often had a dusty azurophilic granularity **(fig.).**

On the histological preparations of the RBM, the visual features of macrophages (marked as (b) on **fig.**) remained unchanged at different time points. The resident macrophages displayed diverse shapes, including round, oval, bean-shaped, elongated, butterfly, loop, mushroom, horseshoe, and ring-shaped. The nucleus occupied a smaller portion of the cell and was either oval, elongated, or rounded, bearing a dark purple colour. Chromatin had a loose, reticular structure. The cytoplasm was abundant, light grey in colour, lacked clear borders and contained multiple inclusions **(fig.).**

The morphological structure of the adipocytes (marked as (c) on **fig.**) remained constant throughout the study. Only changes in morphometric parameters were observed. The cells appeared as empty spaces and varied significantly in size, exhibiting either round or oval shapes. A large lipid vacuole occupied almost the entirety of each cell without inclusions. The cytoplasm appeared as a thin blue border with a relatively uniform structure, the small nucleus was pushed to the periphery and was round shaped and purple coloured **(fig.).**

Monoblasts (marked as (d) on **fig.**) represent the progenitor cells of the monocyte lineage. The nucleus had a finely woven network of chromatin, accompanied by numerous nucleoli of a light red or light purple colour, and was either spherical, round, ovoid or, rarely, bean-shaped. The cell demonstrated a spherical morphology. The cytoplasm of the monoblast was pale blue or blue and lacked granularity, it was thin and often had an in-

tensely coloured periphery and a distinct bright rim surrounding the nucleus **(fig.).**

Promonocytes (marked as (e) on **fig.**) were round with occasional elongation and remained morphologically consistent at all-time points. Their nuclei exhibited an oval, bean-like outline, appearing purple or dark blue in colour with slightly undulating edges. The chromatin structure of promonocytes was coarser compared to that of monoblasts, sometimes consisting of nucleolar remnants. The cytoplasm appeared greyish-blue, at times taking on a light blue hue and forming a rim around the nucleus, which may contain a delicate, dusty azurophilic granularity **(fig.).**

Monocytes (marked as (f) on **fig.**) exhibited a consistent shape and colour and were identified as mature cells of the monocyte lineage. The nucleus displayed a range of shapes, including bean-shaped, rounded, oval, butterfly, loop, mushroom, horseshoe-shaped, and ring-shaped, occasionally appearing segmented. The contour was uneven and wavy. The chromatin structure was coarse-meshed and looped, with the nucleus's colour ranging from light purple to blue. The nucleus of a monocyte appears lighter than those of neutrophils and lymphocytes due to the presence of oxychromatin within its structure. Typically, the nucleus occupies an equal or larger proportion of the cell compared with the cytoplasm. The cytoplasm of a monocyte is characterised by a smoky, greyish-blue colour and often shows azurophilic granularity and vacuoles **(fig.).**

The structural elements of the MS and ECs exhibit different responses to the administration of tryptorelin and quercetin at distinct study periods. The quantitative and morphometric parameters analysed showed dynamic alterations in both the control and experimental groups.

We studied certain morphometric parameters of monocytic sprout cells and environment cells, namely, cell size, nucleus area and nuclear-cytoplasmic ratio (NCR) at different time points.

The obtained data on changes in the morphometric parameters of MS are represented in **table 1.**

Morphological changes of monocyte sprout cells and their environment cells during examination showed that in the group with tryptorelin and quercetin administration at different time points of the experiment, we did not observe any significant changes in any of the cell components, their structure and colour.

The statistical analysis of morphometric data showed that monocyte cells, as well as cells of the environment, reacted differently and asynchronously when administered tryptorelin with quercetin at different study periods. It was found that the quantitative index of monocytes was significantly lower than that of monoblasts and promonocytes of MS in the study groups.

When tryptorelin was administered with quercetin, it resulted in a significant increase in the quantitative index of monoblasts (12.69±0.419), promonocytes (12.33±0.403), and monocytes (12.15±0.366) at the end of 3 months of observation. Additionally, there was a maximum decrease in the number of monoblasts at the end of the first month (8.84±0.291) and the promonocytes at the end of 9 months (8.92±0.202), with the minimum value of monocytes in the control group (5.65±0.175). At month 12, the quantitative indicators of monoblasts and promonocytes were not significantly different from control, but the number of monocytes increased to 8.11±0.239.

Comparison of the characteristics of cell area and nuclear area in the studied MS cells indicated that both the control group and the group receiving tryptorelin with quercetin administration at different study periods

Table 1 – Quantity and Characteristics of NCR of MS Cells Following Introduction of Tryptorelin with Quercetin

Period/cell	Monoblasts	NCR	Promonocyte	NCR	Monocytes	NCR
Control group	10,84±0,222	0,608±0,023	10,74±0,184	0,636±0,024	5,65±0,175	0,571±0,021
1 month	8,84±0,291 ***/###	0,641±0,024	9,33±0,297 **	0,682±0,024	6,25±0,321 *	0,559±0,022
3 months	12,69±0,419 ***/###	0,659±0,024 #	12,33±0,403 ***/###	0,672±0,021	12,15±0,366 ***/###	0,559±0,022
6 months	11,89±0,264 */##	0,645±0,023	11,14±0,226 */#	0,645±0,021	10,53±0,171 **/###	0,558±0,023
9 months	9,53±0,264 **/##	0,715±0,029 */##	8,92±0,202 ***/###	0,651±0,024	7,35±0,251 ***/##	0,556±0,025
12 months	10,35±0,271 *	0,703±0,022 ##	10,01±0,196 **/#	0,687±0,026 #	8,11±0,239 */###	0,566±0,024

Notes: significance of the difference between the previous study period: * <0,05; ** <0,01; *** <0,001; significance of the difference between the control group and different study periods: # <0,05; ## <0,01; ### <0,001.

Table 2 – Quantification and characterisation of NCR ECs after administration of tryptorelin and quercetin

Period/cell	Adipocytes	NCR	Reticular cells	NCR	Macrophages	NCR
Control group	4,69±0,171	0,0503±0,0086	4,86±0,147	0,312±0,0163	1,91±0,076	0,183±0,0166
1 month	4,49±0,251	0,0626±0,00405 */#	4,29±0,249 *	0,302±0,0212	1,79±0,101	0,124±0,0096 ##
3 months	4,28±0,241	0,0734±0,0045 */##	4,11±0,182 #	0,321±0,022	1,77±0,107	0,126±0,0091 ##
6 months	4,84±0,237 *	0,0673±0,00335 #	4,94±0,245 **	0,285±0,015	1,82±0,086	0,131±0,0074 ##
9 months	5,63±0,209 **/##	0,0597±0,0027 *	4,82±0,191	0,266±0,013 #	1,88±0,104	0,126±0,00619 ##
12 months	6,82±0,214 **/###	0,06013±0,00258	5,11±0,183	0,295±0,015 *	1,79±0,087	0,124±0,00536 ##

Notes: the significance of the difference between the previous study period: * <0,05; ** <0,01; *** <0,001; significance of the difference between the control group and different study periods: # <0,05; ## <0,01; ### <0,001.

had no significant or reliable changes at p>0.05. It can only be concluded that these parameters remained considerably lower for promonocytes in MS.

The morphometric analysis of the NCR showed that the introduction of tryptorelin with quercetin resulted in a significant increase in this metric for monoblasts (0.715±0.029) in the 9 month of the study (p<0.01) in comparison to the control group. The highest increase in the monoblast index was observed between month 6 (0.645±0.023) and month 9 (0.715±0.029), although the difference was only significant at p<0.05. An increase or decrease in this indicator is not considered reliable or significant for promonocytes and monocytes.

The changes in the ECs RBM are presented in **table 2.**

After administration of tryptorelin with quercetin, the quantitative parameter of the environment cells significantly increased only for adipocytes (6.82±0.214) at month 12 of the study at p<0.001. There were no significant changes in the quantitative parameter of reticular cells and macrophages at p<0.05.

The analysis of morphometric parameters environment cells revealed a significant increase in the area (570.17±22.434) for adipocytes at the 12th month of the experiment (p<0.05). Additionally, a significant decrease in area was observed for macrophages during month 9 of the study (697.09±21.249, p<0.05), while reticular cells exhibited an area increase of 216.17±12.095 at the 6th month (p<0.01).

The results of morphometric analysis show that there was a significant increase in the area of adipocyte nuclei at month 3 of the study (34.19±1.982) with a p<0.001. The same trend was observed for reticular cells at month 6 of the study (57.32±3.392) with a p<0.05. Comparing the area of macrophage nuclei at all study periods with the control group (126.94±9.146), a significant decrease was observed. The minimum value of macrophages (85.04±3.321) occurred at 9 months of observation with a p<0.001.

In turn, the analysis of NCR shows that this indicator does not change equally with the administration of tryptorelin with quercetin. Adipocyte NCR increased the most (0.0734±0.0045) after 6 months of the study at p<0.01, and reticular cells increased (0.321±0.022) after 3 months of the study at p<0.05. Conversely, macrophage NCR decreased significantly throughout the study periods in comparison to the control group (0.183±0.0166), with a minimum value (0.124±0.0096) at 1 month of the study at p<0.01.

Conclusions.

The administration of tryptorelin with a long-term effect (1 year) and oral administration of the riboflavanoid "Quercetin" leads to statistically significant changes in the quantitative and qualitative composition of RBM, in particular, the studied cells of the MS and ECs.

The maximum quantitative changes in MS cells were observed at 3 and 6 months of the study, followed by a gradual recovery to the control group. ECs had a significant variability of changes: the quantitative characteristics of macrophages and reticular cells remained constant, but the NSC index for macrophages was lower at all observation periods compared to the control group due to a decrease in nucleus area. Adipocytes responded with a positive increase in quantitative and qualitative characteristics.

Prospects for further research.

We are planning to carry out an immunohistochemical study of the monocyte sprout and the microenvironment cells of the red bone marrow under the long-term administration of tryptorelin with quercetin in the experiment, namely Ki67 and CD 68.

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КІЛЬКІСНІ ТА ЯКІСНІ ЗМІНИ МОНОЦИТАРНОГО ПАРОСТКУ ТА КЛІТИН МІКРООТОЧЕННЯ ЧЕРВОНОГО КІСТКОВОГО МОЗКУ, ПРИ ДОВГОТРИВАЛОМУ ВВЕДЕНІ ТРИПТОРЕЛІНУ З КВЕРЦЕТИНОМ В ЕКСПЕРИМЕНТІ

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Резюме. *Вступ.* Використання андрогендеприваційної терапії, а саме речовини Трипторелiну, має істотний вплив на функціональний стан організму. В нашому експерименті ми вивчали якісні та кількісні зміни клітин моноцитарного паростка та клітин оточення під час хімічної кастрації щурів-самців центрального походження, викликаних введенням розчину трипторелiну з додавання в раціон кверцетину протягом року.

Об'єкт і методи. Дослідження проводили на 35 дорослих самцях білих щурів. Щури були поділили на 2 групи: I група – контрольна (10), яким вводили фізіологічний розчин, тваринам II групи (25) підшкірно вводили трипторелін ацетат у дозі 0,3 мг активної речовини на кг та «Кверцетин» 100 мг на кг маси тіла 3 рази на тиждень. Підготовку матеріалу для мікроскопічного дослідження структур червоного кісткового мозку проводили за загальноприйнятною методикою.

Результати і обговорення. Довготривала хімічна кастрація Трипторелiном призводить до кількісних та якісних змін клітин моноцитарного паростка на всіх стадіях диференціювання та проліферації з комплементарною реакцією клітин мікрооточення. Введення рибофлаваної «Кверцетину», корегує цей вплив.

Максимальні кількісні зміни клітин моноцитарного ряду спостерігалися на 3 місяць дослідження, з послідуючим поступовим відновленням до показників контрольної групи. Зміни клітин мікрооточення асинхронні та не мають спільних алгоритмів.

Висновки. Введення трипторелiну з довготривалою дією (термін експерименту – 1 рік) та перорального введення рибофлаваноїду «Кверцетин» призводить до статистично достовірних змін кількісного та якісного складу червоного кісткового мозку, а саме досліджуваних клітин моноцитарного паростка та клітин оточення.

Ключові слова: червоний кістковий мозок, моноцитопоез, клітини оточення, трипторелін, кверцетин.

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Abstract. *Introduction.* The use of androgen deprivation therapy, specifically Tryptorelin, has a noteworthy impact on the functional state of the body. Our experiment investigated the qualitative and quantitative changes in monocyte sprout cells and surrounding cells during central chemical castration in male rats that were administered a tryptorelin solution with quercetin added to their diet for a year.

Object and Methods. The study involved 35 adult male white rats, which were separated into two groups. The first group (10) received saline injections and served as a control, while the second group (25) received subcutaneous injections of tryptorelin acetate (0.3 mg active substance per kg body weight) and quercetin (100 mg per kg body weight) three times a week. The material for microscopic examination of the red bone marrow structures was prepared in the conventional way.

Results and Discussion. Prolonged chemical castration using Tryptorelin induces quantitative and qualitative alterations in monocyte sprout cells throughout all stages of differentiation and proliferation, alongside a complementary reaction in microenvironmental cells. This effect is corrected by administering the riboflavonoid «Quercetin».

The greatest quantitative changes were observed in monocytes after three months of the study. These were followed by a gradual recovery towards the control group. Alterations in microenvironment cells occur asynchronously and do not share a common algorithm.

Conclusions. The administration of long-acting tryptorelin (1 year of the experiment) and oral administration of the riboflavonoid «Quercetin» leads to statistically significant changes in the quantitative and qualitative composition of the red bone marrow, namely, the studied monocyte sprout cells and environment cells.

Key words: red bone marrow, monocytopoiesis, environment cells, tryptorelin, quercetin.

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Conflict of interest:
The authors of the article confirm the absence of conflict of interests.

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