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АНТОЦИАНИНЫ КАК ФАКТОР АЛИМЕНТАРНОГО ВОССТАНОВЛЕНИЯ КЛЕТОЧНОГО ИММУНИТЕТА ПРИ ИНДУЦИРОВАННОМ ДИЕТОЙ ОЖИРЕНИИ У КРЫС

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Резюме. В статье представлены результаты исследования влияния антоцианинов на клеточный иммунитет у крыс на модели алиментарного ожирения. Целью исследования являлось изучение влияния рациона, обогащенного антоцианинами, на клеточный иммунитет при индуцированном диетой ожирении у крыс. Работа выполнена на крысах самцах линии Wistar с исходной массой тела 108±2 г. Животные были рандомизированы по массе тела на 3 группы (по 8 крыс в группе). В течение 12 недель крысы 1-й (контрольной) группы получали полноценный модифицированный рацион AIN93M; крысы 2-й группы потребляли высококалорийный холинодефицитный рацион (ВКХДР), содержание жира в котором составляло 45%, фруктозы – 20% от энергетической ценности рациона; крысы 3-й группы получали ВКХДР с добавлением стандартизованных экстрактов черники и черной смородины (30% антоцианинов) в суточной дозе 11 мг антоцианинов/кг массы тела. Животных содержали по 2 особи в пластиковых клетках на подстилке из древесных стружек при искусственном освещении с равной продолжительностью ночного и дневного периодов и не ограничивали в употреблении воды. Экспрессию дифференцировочных маркеров лимфоцитов периферической крови крыс определяли методом проточной цитофлуориметрии. В результате исследования установлено, что у крыс 2-й группы с алиментарным ожирением повышено ($p < 0,05$) в периферической крови относительное содержание Т-хелперов ($CD3^+CD4^+$) (75,75±1,11% против 70,07±0,49% – 1-я группа, 72,14±0,91% – 3-я группа) и снижено ($p < 0,05$) содержание Т-цитотоксических лимфоцитов ($CD3^+CD8^+$) (22,54±1,14% против 28,09±0,72% – 1-я группа, 26,07±0,87% – 3-я группа). Соотношение CD3/CD4 у крыс 2-й группы превысило ($p < 0,05$) данный показатель у крыс 1-й и 3-й групп

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(3,44±0,25 против 2,47±0,09 – 1-я группа, 2,79±0,13 – 3-я группа). Обогащение ВКХДР экстрактами черники и черной смородины привело к нормализации указанных параметров клеточного иммунитета. Число В-лимфоцитов ($CD45R^+$), Т-лимфоцитов ($CD3^+$) и NK-клеток ($CD161^+$) в периферической крови крыс всех экспериментальных групп не имело статистически достоверных различий. Результаты исследования клеточного иммунитета у крыс с алиментарным ожирением свидетельствуют о наличии метавоспаления. Добавление в рацион крыс антоцианинов обеспечило восстановление изученных показателей адаптивного клеточного иммунитета до уровня крыс контрольной группы. Полученные результаты свидетельствуют о перспективе применения биологически активных веществ – антоцианинов в диетотерапии больных ожирением и другими алиментарно-зависимыми заболеваниями.

Ключевые слова: алиментарное ожирение, клеточный иммунитет, лимфоциты, NK-клетки, антоцианины, метавоспаление

ANTHOCYANINS AS A FACTOR IN THE ALIMENTARY RESTORATION OF CELLULAR IMMUNITY IN DIET INDUCED OBESITY IN RATS

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Abstract. The article presents the results of a study of the effect of anthocyanins on cellular immunity in rats on a model of alimentary obesity. The aim of the study was to study the effect of an anthocyanin-enriched diet on cellular immunity in diet induced obesity in rats. The study was carried out on male Wistar rats with an initial body weight of 108±2 g. The animals were randomized by body weight into 3 groups (8 pcs. in group). For 12 weeks, rats of the 1st (control) group received a complete modified diet of AIN93M; rats of the 2nd group consumed a high-calorie choline-deficient diet (HCChDD), the fat content of which was 45%, fructose – 20% of the energy value of the diet; rats of the 3rd group received HCChDD with the addition of standardized blueberry and blackcurrant extract (30% anthocyanins) at an average daily dose of 11 mg anthocyanins/kg body weight. The expression of differentiation markers of peripheral blood lymphocytes was carried out by flow cytofluorimetry. As a result of the study, it was found that in rats of the 2nd group with alimentary obesity, the relative content in the peripheral blood of T helpers ($CD3^+CD4^+$) was increased ($p < 0.05$) (75.75±1.11% *versus* 70.07±0 49% – group 1, 72.14±0.91% – group 3) and reduced ($p < 0.05$) content of T cytotoxic lymphocytes ($CD3^+CD8^+$) (22.54±1.14% *versus* 28.09±0.72% – 1st group, 26.07±0.87% – 3rd group). The CD3/CD4 ratio in rats of the 2nd group exceeded ($p < 0.05$) this index in rats of the 1st and 3rd groups (3.44±0.25 *versus* 2.47±0.09 – 1st group, 2.79±0.13 – 3rd group). Enrichment of the HCChDD with the blueberry and blackcurrant extract led to the normalization of these parameters of cellular immunity. The number of B lymphocytes ($CD45R^+$), T lymphocytes ($CD3^+$) and NK cells ($CD161^+$) in the rat peripheral blood of all experimental groups had no statistically significant differences. The results of the study of cellular immunity in rats with alimentary obesity indicate the presence of metainflammation. The received data indicate the prospect of using biologically active substances, anthocyanins, in the diet therapy of patients with obesity and other alimentary-dependent diseases.

Keywords: alimentary obesity, cell immunity, lymphocytes, NK cells, anthocyanins, metainflammation

Introduction

Obesity is a multifactorial widespread disease that is a consequence of excess intake of calories and insufficient degree of their utilization. Obesity is accompanied by chronic inflammation and is a pathogenetic basis for the development of cardiovascular pathology, metabolic syndrome, type 2 diabetes mellitus, and insulin resistance [10]. Obesity causes dysregulation throughout the immune system, affecting the balance and levels of cytokines, adipokines and innate and adaptive immunity [2, 5]. Currently, the problem of metainflammation, which is metabolic in nature, chronic, associated with moderate expression of pro-inflammatory mediators and accompanied by modification of the structure of metabolic tissues with infiltration by immune cells, is being actively studied [4, 10]. In the genesis of the disease, the main role is played by oxidative stress and chronic inflammation in metabolically active tissues: adipose tissue, liver, intestines, muscles, pancreas, and others [9]. There are increases in pro-inflammatory cytokines and organ infiltration by increased numbers of tissue macrophages, B lymphocytes, T lymphocytes and mast cells with decreased numbers of regulatory T lymphocytes, MAIT cells (mucosal-associated invariant T cells), ILC2 (innate lymphoid cell) and invariant NKT cells and accompanied by changes in immune cell content in peripheral blood [5]. The problem of immunometabolism is being actively studied on rats and mouse models of diet induced obesity.

Excessive accumulation of body fat, disorders in the insulin-dependent signaling pathway, and hyperlipidemia lead to tissue hypoxia and the development of oxidative stress [12]. One of the components of therapy for insulin resistance, immunodeficiency in obesity has been the use of natural and synthetic antioxidants [3]. Currently, the effectiveness of the use of anthocyanins, a water-soluble subclass of flavonoids, has been proven in the treatment of a number of metabolic disorders, including glucose tolerance, insulin resistance, abdominal obesity, dyslipidemia, and arterial hypertension [7]. It has been established that they have vasoprotective properties, have antioxidant, anti-inflammatory, antiatherogenic and vasodilating effects [4].

The aim of the study was to study the effect of an anthocyanin-enriched diet on cellular immunity in diet induced obesity in rats.

Materials and methods

The study was carried out on male Wistar rats with an initial body weight of 108±2 g. Rats were obtained from the nursery of the “Stolbovaya” branch of the Federal State Budgetary Institution of Science “Scientific Center for Biomedical Technologies of the FMBA”. The study was approved by the Ethics Committee of the “Federal Research Center for Nutrition and Biotechnology” (meeting No. 11 dated December 15, 2021) and was carried out in accordance with the recommendations of GOST 33216-2014 “Guidelines for accommodation and care of animals. Species-specific provisions for laboratory rodents and rabbits”.

The animals were randomized by body weight into 3 groups (8 pcs. in group). For 12 weeks, rats of the 1st (control) group received a complete modified diet of AIN93M [13]; rats of the 2nd group consumed a high-calorie choline-deficient diet (HCChDD), the fat content of which was 45%, fructose – 20% of the energy value of the diet; rats of the 3rd group received HCChDD with the addition of standardized blueberry and blackcurrant extract (30% anthocyanins, Healthberry 865, Evonik Nutrition & Care GmbH, Germany) at an average daily dose of 11 mg anthocyanins/kg body weight. Animals were kept in 2 individuals in plastic cages on a bed of wood shavings under artificial lighting with equal duration of the night and day periods and were not limited in the use of water. Withdrawal from the experiment was carried out by decapitation with a preliminary (16 hours) weaning of feed. Expression of CD45R, CD3, CD4, CD8a, CD161 receptors on rat peripheral blood lymphocytes and negative control IgG1/IgG2a was determined by direct immunofluorescent staining of whole blood cells using a panel of monoclonal antibodies conjugated to fluorescein: APC, FITC, PE (manufactured by “Miltenyi Biotec GmbH”, Germany). The samples were analyzed by flow cytometry using Cytomics FC 500 and CXP software (“Beckman Coulter”, USA). The leukocyte subsets were defined by forward- and side-scatter pattern. The negative control value was determined by a fluorescence background and antibody-nonspecific staining. The statistical analysis was performed to assess differences between groups using 1-way ANOVA. The hypothesis about the difference in the distribution function of data in the compared groups was additionally tested using the nonparametric Mann-Whitney test. Differences were considered significant at $p < 0.05$. The calculations

TABLE 1. LYMPHOCYTE SUBPOPULATIONS IN RAT PERIPHERAL BLOOD (M±m, %)

Group number	$CD45R^+$	$CD3^+$	$CD3^+CD4^+$	$CD3^+CD8^+$	CD4/CD8	$CD161^+$
	B lymphocytes	T lymphocytes	T helpers	T cytotoxic lymphocytes	ratio of CD4/CD8	NK cells
1st	22.63±1.77	67.43±2.10	70.07±0.49	28.09±0.72	2.47±0.09	3.26±0.29
2nd	22.15±1.33	62.10±1.99	75.75±1.11* **	22.54±1.14* **	3.44±0.25* **	3.15±0.49
3rd	23.66±1.75	62.39±3.34	72.14±0.91	26.07±0.87	2.79±0.13	3.30±0.58

Note. 1st gr., control, diet AIN 93M; 2nd gr., high-calorie choline-deficient diet (HCChDD), 3rd gr., HCChDD+ blueberry and blackcurrant extract. * $p < 0{,}05$, compared with the control group; ** $p < 0{,}05$, compared with the 3rd group.

were performed using the SPSS 20.0 software package. Data are presented as M±m.

Results and discussion

The study results of lymphocyte subpopulations in the rat peripheral blood are presented in the Table 1. Our analysis demonstrated that in the rats of the 2nd gr with diet induced obesity an increase in the relative content of T helpers ($CD3^+CD4^+$) and a decrease in the percentage of T cytotoxic ($CD3^+CD8^+$) lymphocytes were found relative to these subpopulations in rats of the control group and the 3rd group ($p < 0.05$). The ratio of CD3/CD4 in rats of the 2nd group exceeded this indicator in rats of control group and the 3rd group ($p < 0.05$). Enrichment of the HCChDD with the blueberry and blackcurrant extract led to the normalization of the relative content of T helpers and T cytotoxic lymphocytes, as well as the ratio of CD4/CD8 in relation to the control group ($p < 0.05$). The number of B lymphocytes ($CD45R^+$) lymphocytes, T lymphocytes ($CD3^+$) and NK cells ($CD161^+$) in the rat peripheral blood of all experimental groups had no statistically significant differences (Table 1).

The currently obtained results of studies of the mechanisms of metabolic disorders in obesity convincingly show that the immune system takes an active part in the regulation of metabolism. On the one hand, this is the preservation of the integrity of organs and tissues that control metabolism, on the other hand, the influence of the metabolic status of the body on the effector abilities of the immune cells themselves [6]. This study demonstrates, that antigen-specific lymphocytes which are fundamental to immune function, providing for the nature of the immune response ($CD3^+CD4^+$) and direct cytotoxicity ($CD3^+CD8^+$) are affected by obesity. The increased amount of T helpers ($CD3^+CD4^+$) and ratio of CD3/CD4 increase testify to the development metainflammation. The presence of metainflammation in obesity rats is confirmed in our previous work, which demonstrated significant increase in plasma levels of pro-inflammatory cytokines IFNγ, MIP-3α, and RANTES and a decrease in the content of most immunoregulatory cytokines [14].

With the development of the inflammatory process, cells innate immunity: leukocytes, macrophages, dendritic cells, mast cells and others enhance the production and release of reactive oxygen species through "respiratory burst". Activated lymphocytes in adaptive immunity generate cytokines, chemokines, growth factors and other inflammatory mediators that stimulate signal transduction cascades in addition to alterations in transcription factors. Cellular stress reactions are mediated by changes in the expression of nuclear factor of activated T cells, hypoxia-inducible factor-1α (HIF1-α), nuclear factor kappa B (NF-κB), activator protein-1, NF-E2 related factor-2. In addition, an important role in the development of stress-induced metainflammation is assigned to changes in the expression of specific microRNAs, initiation of cyclooxygenase-2 (COX-2), inducibility of nitric oxide synthase (iNOS) [3].

Antioxidants have been used in the diet therapy of obesity and other nutritionally dependent diseases for quite a long time. Anthocyanins are a water-soluble subclass of flavonoids with endogenous antioxidant properties [15]. It has been established that anthocyanins reduce cellular oxidative damage through a number of cellular mechanisms, including the Keap1/Nrf2/ARE redox-sensitive signaling system, the expression of antioxidant enzymes, such as superoxide dismutase, catalase [1, 11], and suppress the expression of the transcription factor NF-kB in activated cells and activator protein AP-1 [8]. Thus, the efficiency of consumption of anthocyanin-rich foods is due to the activation of various cellular pathways that contribute to the creation of a dynamic cellular antioxidant/anti-inflammatory microenvironment capable of responding to fluctuations in redox potential.

Conclusions

Based on the study of cellular immunity in diet induced obesity in rats, the presence of meta-inflammation was established. Enrichment of high-calorie choline-deficient diet rats with anthocyanins in the composition of blueberry and blackcurrant extracts ensures the restoration of the studied indicators of adaptive cellular immunity to the level of control group rats. The received data indicate the prospect of using biologically active substances – anthocyanins in the diet therapy of patients with obesity and other alimentary-dependent diseases.

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References

1. Aboonabi A, Singh I. Chemoprotective role of anthocyanins in atherosclerosis via activation of nrf2-ARE as an indicator and modulator of redox. *Biomed Pharm., 2015, Vol. 72, pp. 30-36.*

2. Amin M.T., Fatema K., Arefin S., Hussain F., Bhowmik D.R., Hossain M.S. Obesity, a major risk factor for immunity and severe outcomes of COVID-19. *Biosci Rep., 2021, Vol. 41, no. 8, BSR20210979.* https://doi.org / 10.1042/BSR20210979.

3. Arulselvan P., Fard M.T., Tan W.S., Gothai S., Fakurazi S., Norhaizan M.E., Kumar S.S. Role of antioxidants and natural products in inflammation. *Oxid. Med. Cell. Longev., 2016, Vol. 2016, 5276130.* doi: 10.1155/2016/5276130.

4. Fairlie-Jones L., Davison K., Fromentin E., Hill A.M. The effect of anthocyanin-rich foods or extracts on vascular function in adults: A systematic review and meta-analysis of randomised controlled trials. *Nutrients, 2017, Vol. 9, no. 8, 908.* doi: 10.3390/nu9080908.

5. Fang X., Henao-Mejia J., Henrickson S.E. Obesity and immune status in children. *Curr. Opin. Pediatr., 2020, Vol. 32, no. 6, pp. 805-815.*

6. Hotamisligil G.S. Foundations of immunometabolism and implications for metabolic health and disease. *Immunity, 2017. Vol. 47, no. 3, pp. 406-420.*

7. Koldaev V.M., Kropotov A.V. Anthocyanins in practical medicine. *Pacific Medical Journal, 2021, no. 3, pp. 24-28.* (In Russ)

8. Kuntz S., Asseburg H., Dold S., Rompp A., Frohling B., Kunz C., Rudloff S. Inhibition of low-grade inflammation by anthocyanins from grape extract in an *in vitro* epithelial-endothelial co-culture model. *Food Func., 2015, Vol. 6, pp. 1136-1149.*

9. Lee Y.S., Wollam J., Olefsky J.M. An integrated view of immunometabolism. *Cell, 2018, Vol. 172, no. 1-2, pp. 22-40.*

10. Lercher A., Baazim H., Bergthaler A. Systemic immunometabolism: challenges and opportunities. *Immunity, 2020, Vol. 53, no. 3, pp. 496-509.*

11. Li L., Wang L., Wu Z., Yao L., Wu Y., Huang L., Liu K., Zhou X., Gou D.. Anthocyanin-rich fractions from red raspberries attenuate inflammation in both RAW264.7 macrophages and a mouse model of Colitis. *Sci. Rep., 2014, Vol. 4, pp. 6234-6245.*

12. Monserrat-Mesquida M., Quetglas-Llabrés M., Capó X., Bouzas C., Mateos D., Pons A., Tur J.A., Sureda A. Metabolic syndrome is associated with oxidative stress and proinflammatory state. *Antioxidants (Basel), 2020, Vol. 9, no. 3, 236.* doi: 10.3390/antiox9030236.

13. Reeves P.G., Nielsen F.H., Fahey G.C. Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr., 1993, Vol. 123, no. 11, pp. 1939-1951.*

14. Riger N.A., Trushina E.N., Timonin A.N., Mustafina O.K., Aksenov I.V., Guseva G.V., Tutelyan V.A. Effect of carnosine and alpha-lipoic acid on the cytokine profile in obese male Wistar rats. *Allergology and Immunology. 2022, Vol. 23, no. 1, pp. 22-28.* (In Russ.)

15. Thornthwaite J.T., Thibado S.P., Thornthwaite K.A. Bilberry anthocyanins as agents to address oxidative stress. In: Pathology. Oxidative stress and dietary antioxidants. Ed. Preedy V.R. London: Academic Press, 2020, pp. 179-187.

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