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Effect of Long-Term Reduction of Deuterium Content in the Body on Hemoglobin Production and Parameters of Erythropoiesis

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Anemia is the most widespread hematological disease, therefore the search for new approaches to erythropoiesis regulation in the body remains an extremely urgent problem. We studied the effect of long-term reduction of deuterium level in the internal milieu of the body on hemoglobin production and parameters of erythropoiesis in sexually mature male Wistar rats. The animals consumed water with deuterium content decreased to 10 ppm for 2 months. After 1 month, an increase of hemoglobin synthesis in erythrocytes was detected, and after 2 months we observed intensification of erythropoiesis. Since the observed processes occurred in healthy animals with initially normal indices of hematopoiesis, the obtained data allow us to consider the reduction of deuterium level in the internal milieu of the body as a factor of erythropoiesis regulation and a possible option of its alternative non-pharmacological regulators.

Key Words: *deuterium; hydrogen isotopes; erythrocytes; erythropoiesis; hemoglobin*

Anemia is the most common blood disorder affecting about $^{1}/_{3}$ of the world's population [1]. Alimentary anemia and anemia associated with chronic diseases occupy one of the leading positions among this kind of disease [2-4]. The great variety of factors underlying anemia development makes it important to find optimal treatment based on the causes of the disease.

Despite significant advances in the prevention and treatment of anemia, this problem remains relevant, and prevention and correction of this disease remain a priority area in modern hematology.

The biological effects of changes in the isotopic composition of the internal milieu of the body are now intensively studied [5-7]. Numerous scientific reports show evidence that bilateral shifts in deuterium content result in a wide range of functional changes, including those in the neuroendocrine and immune systems [8-10]. Reduction of deuterium level in the internal milieu of the body as a factor of regulation of erythropoiesis and hemoglobin synthesis in erythrocytes is still poorly studied. Moreover, the sensitivity of hematopoietic cells to changes in deuterium concentration remains an open question.

Our aim was to study the effect of prolonged reduction of deuterium level in the body on hemoglobin production and the parameters of erythropoiesis.

MATERIALS AND METHODS

The experiments were performed on adult male Wistar rats (n=20; body weight 340-370 g) and approved by the Local Ethics Committee (Protocol No. 23 of March 25, 2020). The rats were housed in local vivarium and received standard laboratory chow. The control group rats (n=10) received tap water with normal deuterium content (146 ppm). Experimental rats (n=10) received water with reduced deuterium content (10 ppm; individual entrepreneur Selivanenko). The

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deuterium concentration in water samples was verified using a T-LWIA-45-EP isotope analyzer (Los Gatos Research), which determines deuterium content with an accuracy of 1 ppm. Water consumption by the rats was measured and the volume per 100 g body weight was calculated; no differences between the control and the experimental groups were found.

The blood from the caudal vein was collected into tubes with K_2-EDTA for prevention of clotting. Microscopic examination of blood smears stained with azure and eosin according to Romanovsky–Giemsa revealed no pronounced changes in the structure of erythrocytes (swelling, shrinkage, and fragmentation). Hemoglobin concentration, erythrocyte count, and erythrocyte distribution width were determined using a BC-2800 Vet hematological analyzer (Mindray). The above parameters determined before the experiment corresponded to normal values for animals of this age [11]. Further measurements were conducted 1 and 2 months after the beginning of consumption of water with low deuterium content.

The data were statistically processed using the Statistica 7.0 software (StatSoft, Inc.). Quantitative data with approximately normal distribution were presented as *M*±*SEM* and were compared using the Student's *t* test. The differences were considered statistically significant at $p<0.05$.

RESULTS

One month after the start of the experiment, hemoglobin concentration in rats consuming deuterium-depleted water was increased by ~11.2% in comparison with the control (Fig. 1). At the same time, the number of erythrocytes and the width of their volume distribution remained within the control values (Figs. 2 and 3).

In 2 months after the start of the experiment, the indices of erythropoiesis in control rats did not significantly differ from the previous term. In rats receiving deuterium-depleted water, the hemoglobin concentration in the blood at this term was significantly higher than in the control (by 15.2%; Fig. 1), the number of erythrocytes increased by 10.1% compared to the previous term and was also significantly higher than in the control (by 14.8%; Fig. 2). The width of erythrocyte volume distribution decreased by 9.7% compared to the previous term and was below the control by 12.6% (Fig. 3).

Hemoglobin synthesis is known to continue in the erythrocyte throughout its early development from the stage of proerythroblast to the reticulocyte in the bone marrow. After loss of the nucleus, residual ribosomal RNA provides further hemoglobin synthesis until the reticulocyte loses its RNA shortly after entering the vasculature. Thus, the hemoglobin concentration in mature erythrocytes remains constant throughout its life cycle [12]. According to the published data, the consumption of water with a deuterium content reduced by 15 times compared to the natural level for 1 month is sufficient to reduce its concentration in the blood plasma, as well as

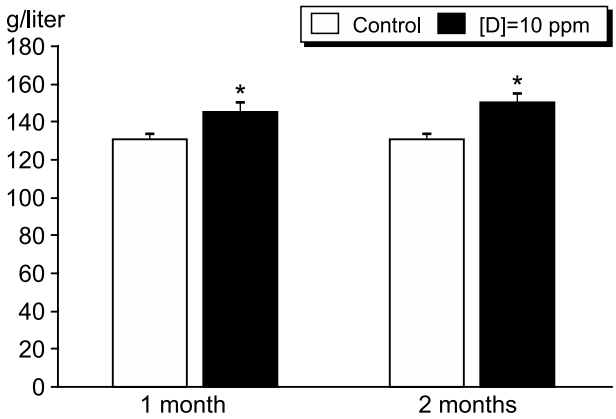

Fig. 1. Hemoglobin concentration in the blood of rats receiving deuterium-depleted water ([D]) for 1 and 2 months. *$p<0.05$ in comparison with the control.

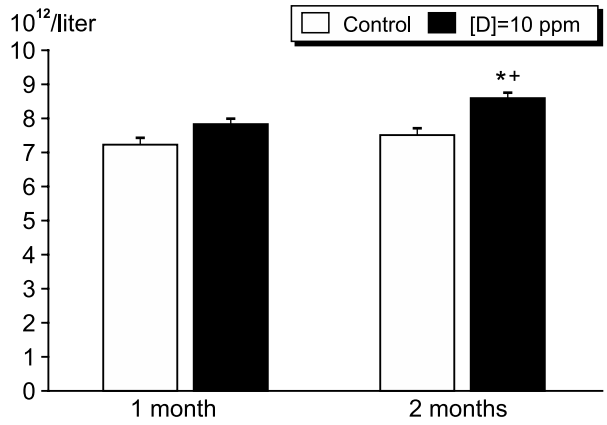

Fig. 2. Erythrocyte count in the blood of rats receiving deuterium-depleted water ([D]) for 1 and 2 months. Here and in Fig. 3: $p<0.05$ in comparison with *control, +previous term.

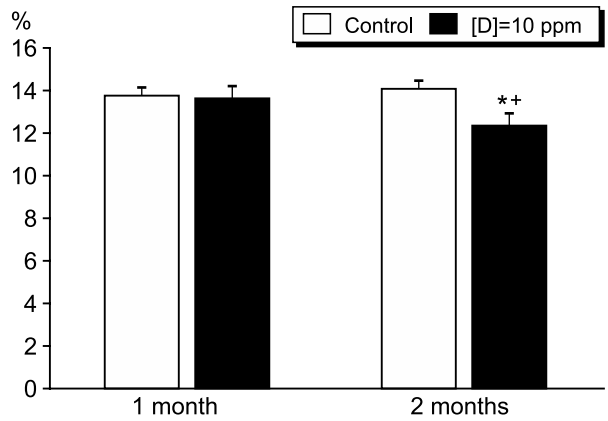

Fig. 3. Erythrocytes volume distribution width in the blood of rats receiving deuterium-depleted water ([D]) for 1 and 2 months.

in internal organs [13]. At this term (1 month), we observed an increase in hemoglobin concentration, but without appreciable changes in erythropoiesis, which was confirmed by the absence of differences in the erythrocyte count and erythrocyte volume distribution width in the control and experimental groups. Hence, higher hemoglobin level was a result of intensification of its synthesis at the early stages of erythrocyte development.

Reduction of deuterium content in the body over 2 months led to more significant changes in erythropoiesis parameters. Thus, hemoglobin concentration increased in comparison with not only the control group, but also experimental group at the previous term. The number of erythrocytes increased significantly and the erythrocyte volume distribution width decreased. It is known that the erythrocyte volume distribution width is a quantitative measure of anisocytosis, *i.e.* variability of the size of circulating erythrocytes [14]. The decrease in this index along with increased erythrocyte count indicates a pronounced stimulating effect of long-term reduction of deuterium level in the body on erythropoiesis.

In addition to stimulation of erythropoiesis, deuterium reduction may also contribute to the prevention of erythrocyte death. It is known that consumption of water with deuterium concentration of 10 ppm for 60 days resulted in stimulation of the antioxidant defense system of erythrocytes and prevented their LPO-induced death [15], which may also be one of the reasons for the increase in erythrocyte count in the blood observed in our study.

Thus, decreased deuterium level in internal milieu of the body initiates intensification of hemoglobin synthesis in erythrocytes and further leads to activation of erythropoiesis. Since the observed processes occurred in healthy animals with normal hematopoiesis, the reduction of deuterium level in the body can be considered as a possible option of alternative non-pharmacological erythropoiesis regulation.

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Conflicts of interest. The authors have no conflicts of interest to declare.

REFERENCES

1. Safiri S, Kolahi AA, Noori M, Nejadghaderi SA, Karamzad N, Bragazzi NL, Sullman MJM, Abdollahi M, Collins GS, Kaufman JS, Grieger JA. Burden of anemia and its underlying causes in 204 countries and territories, 1990-2019: results from the Global Burden of Disease Study 2019. J. Hematol. Oncol. 2021;14(1):185. doi: 10.1186/s13045-021-01202-2
2. Trukhan DI, Yurenev GL, Chusova NA. Iron deficiency anemia: actual aspects of diagnostics and treatment in real clinical practice of therapist. Terapiya. 2019;5(1):172-181. Russian. doi: 10.18565/therapy.2019.1.172-181
3. Cappellini MD, Musallam KM, Taher A.T. Iron deficiency anaemia revisited. J. Intern. Med. 2020;287(2):153-170. doi: 10.1111/joim.13004
4. Pasricha SR, Tye-Din J, Muckenthaler MU, Swinkels DW. Iron deficiency. Lancet. 2021;397:233-248. doi: 10.1016/S0140-6736(20)32594-0
5. Kozin SV, Lyasota OM, Kravtsov AA, Chikhirzhina EV, Ivlev VA, Popov KA, Dorohova AA, Malyshko VV, Moiseev AV, Drozdov AV, Dzhimak SS. Shift of Prooxidant–Antioxidant Balance in Laboratory Animals at Five Times Higher Deuterium Content in Drinking Water. Biophysics. 2023;68(2):289-294. doi: 10.1134/S0006350923020112
6. Syroeshkin AV, Antipova NV, Zlatska AV, Zlatskiy IA, Skylska MD, Grebennikova TV, Goncharuk VV. The effect of the deuterium depleted water on the biological activity of the eukaryotic cells. J. Trace Elem. Med. Biol. 2018;50:629-633. doi: 10.1016/j.jtemb.2018.05.004
7. Yaglova NV, Timokhina EP, Obernikhin SS, Yaglov VV. Emerging Role of Deuterium/Protium Disbalance in Cell Cycle and Apoptosis. Int. J. Mol. Sci. 2023;24(4):3107. doi: 10.3390/ijms24043107
8. Yaglova NV, Obernikhin SS, Timokhina EP, Nazimova SV, Yaglov VV. Reactive alterations in thymic lymphocytopoiesis to short-term decrease in deuterium content in the body. Bull. Exp. Biol. Med. 2022;173(4):494-496. doi: 10.1007/s10517-022-05568-2
9. Kozin S, Skrebitsky V, Kondratenko R, Kravtsov A, Butina E, Moiseev A, Malyshko V, Baryshev M, Elkina A, Dzhimak S. Electrophysiological Activity and Survival Rate of Rats Nervous Tissue Cells Depends on D/H Isotopic Composition of Medium. Molecules. 2021;26(7):2036. doi: 10.3390/molecules26072036
10. Yaglova NV, Obernikhin SS, Timokhina EP, Yaglov VV. Response of pituitary–thyroid axis to a short-term shift in deuterium content in the body. Bull. Exp. Biol. Med. 2021;171(2):262-264. doi: 10.1007/s10517-021-05208-1
11. Mustafina OK, Trushina EN, Shumakova AA, Arianova EA, Tyshko NV, Pashorina VA. Hematologic indices in different age Wistar rats, receiving a balanced semisynthetic vivary diet. Voprosy Pitaniya. 2013;82(2):10-16. Russian.
12. Kafina MD, Paw BH. Intracellular iron and heme trafficking and metabolism in developing erythroblasts. Metallomics. 2017;9(9):1193-1203. doi: 10.1039/c7mt00103g
13. Dzhimak SS, Basov AA, Fedulova LV, Didikin AS, Bikov IM, Arcybasheva OM, Naumov GN, Baryshev MG. Correction of Metabolic Processes in Rats during Chronic Endotoxicosis using Isotope (D/H) Exchange Reactions. Izv. Akad. Nauk Ser. Biol. 2015;5:518-527.
14. Frentiu AA, Mao K, Caruana CB, Raveendran D, Perry LA, Penny-Dimri JC, Ramson DM, Segal R, Bellomo R, Smith JA, Liu Z. The Prognostic Significance of Red Cell Distribution Width in Cardiac Surgery: A Systematic Review and Meta-Analysis. J. Cardiothorac. Vasc. Anesth. 2023;37(3):471-479. doi: 10.1053/j.jvca.2022.11.015
15. Olariu L, Petcu M, Tulcan C, Chis-Buiga I, Pup M, Florin M, Brudiu I. Deuterium Depleted Water-Antioxidant or Prooxidant. Lucrari Stiintifice Medicina Veterinara. 2007;XL:265-269.