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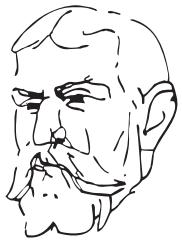

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EFFECT OF BRASSICA OLERACEA EXTRACT ON THE ERYTHRON STATE DURING CHRONIC YTTRIUM SALT INTAKE

WPŁYW EKSTRAKTU Z KAPUSTY WARZYWNEJ NA STAN ERYTROCYTÓW PODCZAS PRZEWLEKŁEGO STOSOWANIA SOLI ITRU

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ABSTRACT

Introduction: Yttrium compounds are known to be able to produce toxic effects on the body. This fact brings up the question of the development of preventive healthcare measures for those who can be exposed to the chemical element.

The aim: The purpose of this work is to study the effect of broccoli extract on the state of erythron in animals exposed to chronic intake of a water-soluble yttrium compound.

Materials and methods: The series of experiments involved 16 white male rats, divided into 3 groups: intact rats; animals, which were administered yttrium acetate in a daily dose of 175 mg / kg body weight for 10 days; animals, which were given yttrium salt and dry extract of broccoli (Brassica oleracea L. var Italica Plenck) in a dose of 25 mg / kg body weight with food for 10 days.

Results: Blood samples obtained were studied to evaluate red blood cells (RBC) count, hematocrit, total hemoglobin, RBC indices and reticulocyte content (Rt). The total number of karyocytes in the bone marrow of the femoral bone of the rats and their myelogram were investigated. The administration of yttrium acetate to the animals did not cause significant changes in "red" blood, but resulted in decreased Rt content compared with the intact control. There was a decrease in the karyocyte count in the bone marrow, the count of normoblasts and the total count of all erythroid cells. The use of the broccoli extract resulted in an increase in the blood Rt content in 1.4 times compared with the same level of yttrium loading without pharmacological correction. In the bone marrow of the animals of this group, the number of erythroid cells increased as well as the number of pro-normoblasts.

Conclusions: The broccoli extract is able to reduce the negative effects produced by excess yttrium on erythropoiesis, contributing to the restoration of normal formation of reticulocytes and their release into the blood.

KEY WORDS: salt of yttrium, extract of broccoli, erythropoiesis, red blood cells

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INTRODUCTION

The compounds of yttrium, a rare-earth element, are widely used in modern technological processes, e.g. in manufacturing cathode tubes, luminophors and lasers; they are used as catalysts and components of alloys for atomic reactors, aerospace engineering and automobile industry, ceramics for heating elements, glass for special purposes, thermoelectric elements, superconductors [1]. Compounds of radioactive yttrium are applied in medicine for treating cancer and for radionuclide synovectomy indicated in cases of joint inflammation [2, 3]. Although the biological role of yttrium is negligible, and the reports on its toxicity are controversial [4], it is considered that the long-term exposure to yttrium compounds can lead to pulmonary affection in humans, cause hypochromic anaemia and enhance carcinogenesis in experimental animals [4, 5]. Chronic uptake of yttrium can cause morph-functional disorders in the mucous membrane of the stomach and upper segments of the small intestine, and is characterized by hepatotoxicity and nephrotoxicity [6]. With regard to mentioned above, it is important to bring up the question of the development of preventive healthcare measures for people who have to be exposed to yttrium compounds in their professional activity. The search for such measures should cover primarily natural substances. For instance, a complex of biologically active substances of broccoli (Brassica oleracea L. var. Italica Plenck), a well known nutraceutical, possesses all the necessary qualities [7]. Food items and in particular food supplements made of this plant are rich in glycosides, polyphenols (quercetin, campferol and others), sulforaphane, vitamins and minerals [8] providing antioxidant, detoxification, anticancer, anti-inflammatory, hepatoprotective and cardioprotective properties [9]. In our previous work, we have shown that a dry extract from the ground part of broccoli, obtained by national scientists, is able to reduce the manifestations of oxidative stress in test animals caused by excessive yttrium intake [10], however, its effect on erythropoies under the load of yttrium is still remaining unclear.

Table I. Hematologic indices under chronic yttrium salt intake of and pharmacological correction with broccoli extract (M ± m).

Animal groups	RBC, x10^{12}/l	Hct, un.	Hb, g/l	MCH, pg	MCHC, g/dl	MCV, mkm^3	RDW, %	Rt, ‰
Intact (n=5)	8,75±0,11	0,48±0,01	177,3±5,4	20, 2±0,5	373±11	54,4±0,5	9,66±0,16	51,2±3,9
Yttrium acetate (n=5)	8,47±0,12	0,46±0,01	170,6±4,3	20,1±0,7	383±10	54,2±0,4	9,84±0,16	30,8±3,5*
Yttrium acetate + broccoli extract (n=6)	8,66±0,12	0,47±0,01	180,0±2,3	19,2±1,0	401±14	55,4±0,4	9,76±0,18	44,4±3,5**

Notes:
1. * - p <0,05 in comparison with intact animals (control).
2. ** - p <0,05 in comparison with administration of yttrium salt without pharmacological correction (control pathology).
3. n - number of animals in the group.

Table II. Effect of broccoli extract on karyocyte count and cellular composition of femoral bone marrow in rats during chronic administration of yttrium salt (M ± m).

The character of influence	Number of karyocytes, 10^6 cells					
	myelokaryocytes (total)	erythroblasts	pronormoblasts	normoblasts	erythroid cells (together)	other cells
Intact (n=5)	162,9±2,4	0,7±0,05	4,0±0,2	51,4±2,3	55,1±2,5	107,7±1,4
Yttrium acetate (n=5)	153,1±1,5*	0,6±0,05	3,2±0,3	40,6±2,2*	44,4±2,3*	108,8±3,8
Yttrium acetate + broccoli extract (n=6)	158,6±2,0	0,5±0,10	4,4±0,3**	45,2±1,9	51,2±2,2**	107,4±4,3

Notes:
1. * - p <0,05 in comparison with intact animals (control).
2. ** - p <0,05 in comparison with administration of yttrium salts without pharmacological correction (control pathology).
3. n - number of animals in the group.

THE AIM

The purpose of this research is to study the effect of dry broccoli extract on the state of erythron in animals during chronic intake of water soluble yttrium compound.

MATERIALS AND METHODS

The series of experiments involved 16 white mature male rats kept under standard vivarium conditions during the research. This study was approved by the Bioethics Commission of The Higher State Educational Establishment of Ukraine "Ukrainian Medical Stomatological Academy". The animals were divided into 3 groups: intact rats (n = 5), animals, which were administered yttrium acetate in a dose of 175 mg / kg body weight for 10 days (control pathology, n=6); animals (n=5), which were given yttrium salt and dry broccoli extract (Brassica oleracea L. var Italica Plenck) in a dose of 25 mg / kg body weight with food for 10 days. Yttrium acetate was administered to white rats with food for 10 days at a dose of 175 mg / kg (0.25 LD_{100}) [11]. For the purpose of pharmacological correction, the animals were subjected to simultaneous administration of yttrium and a dry extract from the ground part of the broccoli (Brassica oleracea L. var. Italica Plenck) obtained at the Department of Chemistry of Natural Compounds, Kharkiv National Pharmaceutical University [12]. The extract was given with food in a daily dose of 25 mg / kg per body weight for 10 days. *Blood* was *withdrawn* from the heart of the animals under urethane narcosis until the heart stopped.

The blood samples were studied to evaluate the indices of the peripheral levels of erythron: red blood cell (RBC) count, hematocrit (Hct), total haemoglobin (Hb), mean corpuscular erythrocyte volume (MCV), mean corpuscular hemoglobin concentration in erythrocyte (MCHC), mean count of haemoglobin in erythrocyte (MCH) and red cell distribution curve width (RDW) using the MicroCC-20Plus Vet automatic hematologic analyzer (High Technology Inc., USA) [13]. The content of reticulocytes (Rt) was studied by supravalent staining with methylene blue followed by microscopy of stained smears with an x100 magnification lens on an AmScope T490B-10MT microscope (United Scope LLC, USA) [14]. Karyocyte count (cytosis) in the bone marrow of the femur of the rats was investigated by the method of P. D. Horizontov et al. (1983) using the whole organ homogenized in 5% acetic acid solution [15]. Bone marrow cytosis was calculated according to the formula: $N_2 = (N_1 \times 50) \times V$, where N_2 is cytosis (106 cells); N_1 is the number of cells in five large square fields of the Goryaev chamber; V is the volume of the suspension (μl). Bone marrow smears were fixed with methanol and stained by Pappenheim [14]. The smears were used to study myelogram by counting the erythroid

cells and other cellular elements, identifying them by their morphological signs with the AmScope T490B-10MT microscope [16]. The digital data obtained were statistically processed using standard computer software packages of Statistica for Windows 8.0, calculating the mean M, its error m, and the probability of difference between groups using a single-factor ANOVA dispersion analysis with Fisher's LSD aposteriori test.

RESULTS AND DISCUSSION

Administration of yttrium acetate to animals for 10 days did not cause significant changes in RBC, Hb, Hct and erythrocyte indices, but lowered the blood Rt content compared with the intact control ($p < 0.005$) (Table I). At the same time, the suppressive effect of excess yttrium on medullary erythropoiesis was recorded. It consisted in the fact that the karyocyte count in the bone marrow of the femur of the rats decreased ($p < 0.05$) and the number of all erythroid cells ($p < 0.02$) decreased as well compared to those in the intact group of animals (Table II). The reduction of erythroid elements occurred mainly due to normoblasts, the number of which decreased ($p < 0.01$) under the unchanged number of erythroblasts and under the tendency to decrease in the pro-normoblasts ($p < 0.1$).

Thus, the loading with yttrium in the conditions of this experiment was characterized by signs of inhibition of the late stages of erythropoiesis and did not affect the basic indices of "red" blood. The indices, apparently, remained stable that can be explained by the fact that during the 10-day duration of the experiment there was the prevalence of erythrocytes released into the bloodstream prior to the development of the toxic effects of yttrium cations, because the life span of these cells in Wistar rats is 59.8 days on average [17]. The decrease in the blood Rt content and in erythroid cells in the bone marrow in response to the yttrium salts administration, obviously, can be considered as a prerequisite for anaemic condition, which is described by the more prolonged loading of the organism with yttrium [5].

The therapeutic and prophylactic application of the dry extract of the ground part of broccoli during the excessive yttrium intake resulted in a tendency to increased Hb ($p < 0.1$) in the absence of changes in RBC and Hct compared with control pathology (see Table 1). Erythrocyte indices did not undergo significant changes under the influence of the broccoli extract as well. There was only a tendency to increase MCV ($p < 0.1$). At the same time, the complex of biologically active substances of broccoli contributed to an increase in the blood Rt content in 1.4 times ($p < 0.05$) compared with the same indices for yttrium load without pharmacological correction. In the bone marrow of animals of this group, the total number of erythroid cells ($p < 0.05$) was significantly increased, among which the number of pro-normoblasts increased ($p < 0.02$) compared with the control pathology (see Table 2). Such shifts in myelogram occurred along with the tendency to increase in the count of myelocaryocytes ($p < 0.1$).

The results obtained allowed us to conclude that broccoli extract is able to reduce the negative influence of excess yttrium on the erythropoiesis in the test animals, contributing to the increase in erythroid cells of the bone marrow and to the restoration of normal formation of reticulocytes and their release into the blood. Since it has been known that flavonoids can form chelate compounds and thus regulate iron homeostasis depending on the structure and dose [18, 19], it should be assumed that together with vitamins (ascorbic acid, folate) and trace elements in the composition of the broccoli extract used they play a direct role of a positive regulator of erythropoiesis in the conditions of this experiment. The stimulation of erythropoiesis by the extract of broccoli in the animals subjected to the yttrium loading is likely mediated by the antioxidant action of the biologically active substances of broccoli [20] and by the elimination of the oxidative stress caused by yttrium cations [10]. However, regardless the mechanism of action, it can be argued that indications to use a dry extract of the ground part of broccoli cabbage are very promising in the treatment and prevention of toxic effects of yttrium compounds.

CONCLUSIONS

1. Per oral 10-day administration of yttrium acetate to white rats in a daily dose of 175 mg / kg causes inhibition of medullary erythropoiesis, reduces blood Rt content and does not affect RBC, Hb, Hct and erythrocytic indices.
2. The use of dry extract of broccoli (Brassica oleracea L. var. Italica Plenck) in a daily dose of 25 mg / kg per body weight per orally for 10 days during loading of animals yttrium increases the number of erythroid cells, and in particular pro-normoblasts in the bone marrow and elevates the blood Rt content compared with the control pathology.

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Drugs and substances:
yttrium acetate - yttrium acetate
broccoli extract - broccoli extract
Abbreviation:
Rt – reticulocytes

The work is carried out within the framework of the planned initiative research work "Finding the means and biologically active substances from the number of derivatives of 2-oxoindole and 3-hydroxypyridine for the pharmaco-correction of adaptive processes in violation of homeostasis of different etiologies" (state registration number 0111U004879).

Conflict of interest:
The Authors declare no conflict of interest

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