ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIII, N 4, 27 SECTIO DDD 2010

Ivano-Frankivsk National Medical University, Physiology Department, Ukraine

NATALIYA VORONYCH-SEMCHENKO, YURIY BORTNYK,
LYUBOV NYKOLYSHUN, TETYANA GURANYCH,
MYKOLA BAGRIY, SVITLANA VORONYCH

The influence of iron, selenium and copper deficiencies on the thyroid status, processes of protein and lipid peroxidation in rats in conditions of thyroid hypofunction

Wpływ niedoborów żelaza, selenu i miedzi na status tarczycy oraz procesy peroksydacji białek i lipidów u szczurów z niedoczynnością tarczycy

INTRODUCTION

One of the priority medical and social directions of health care is to defeat the iodine deficiency, because there is a well known concept about the key role of iodine in structural and functional homeostasis of the thyroid gland. But functional capability of thyroid could also depend on the income of other essential chemical elements to the organism [3]. That is why the aim of this work was to study the influence of iron, selenium and copper deficiencies on the thyroid status of animals with experimental hypothyroidism.

MATERIAL AND METHODS

The experiment was carried out on 120 male white not purebred rats weighing 80–150 g that were divided into four research groups: first (n=30) – animals with mono iodine deficiency, second (n=30) – with combined iodine and iron deficiencies, third (n=30) – with combined iodine and selenium deficiencies, fourth (n=30) – with combined iodine and copper deficiencies. All animals were kept on the basic iodine deficient diet [8]. With the aim of preventing the income of even small doses of iodine, methimazole ("Zdorovya", Ukraine; dose 7.5 mg/100 g of weight) was added to the animals' drinking water during 45 days [8]. Iron deficiency was induced by daily intraperitoneal administration of iron chelating agent deferoxamine (desferal, "Novartis Pharma", Switzerland) in a dose 10 mg/100 g of weight during the last 15 days of methimazole administration [6]. Selenium deficiency was induced by keeping the animals on the half-synthetic balanced diet developed by the Institute of nutrition of the Russian Academy of Medical Sciences [1]. Copper deficiency was induced by the administration of cuprenil (d-penicillamine, "Polfa", Poland; dose 100 mg/100 g of weight) during the last three weeks of

the experiment [7]. The functional state of thyroid was studied by the serum levels of thyroid stimulating hormone (TSH), free triiodthyronine (fT_3), thyroxin (fT_4), by calculation of the integral thyroid index (ITI), index of peripheral conversion (fT_3/fT_4) and TSH/fT_4 ratio [4]. Lipid peroxidation was studied by the levels of dien conjugates, malondialdehyde in serum and tissues of thyroid and cerebrum [10]. Free-radical oxidation of proteins was characterized by the indexes of their oxidative modifications in blood serum, tissues of thyroid and cerebrum using spectrophotometry at wave lengths of 356, 370, 430, 530 (nm) [5]. The content of the stable metabolite of nitrogen monoxide nitrite ion was also measured in blood serum [9]. The income of microelements into the organism was estimated by their content in red blood cells' mass, tissues of thyroid and cerebrum. Also, the indexes of hemoglobin, serum ferritin, level of transferrin saturation with iron were studied in order to evaluate iron status of the organism, level of ceruloplasmin to evaluate copper status of the organism, iodine content in urine to evaluate iodine status of the organism. In comparison, the analogous indexes were studied in 30 control animals. During the experiment the international ethic principles for the conduction of experiments on animals were kept (Strasbourg 1986, Kyiv 2000).

Statistical analysis of the results was done on PC programmes Microsoft Excel and Statistica with the use of the methods of variative statistics and correlation. Significant values were those with $p<0.05$.

RESULTS AND DISCUSSION

The conducted experiment showed that in blood serum of rats that were fed the basic iodine deficient diet on the background of methimazole administration (first research group) the level of fT_3 decreased by 75.33% ($p<0.001$), the level of fT_4 decreased by 70.68% ($p<0.001$), the index of peripheral conversion impaired in comparison with the analogous indexes in intact animals. In such conditions the blood serum level of TSH in rats of this group increased almost 2.5 times ($p<0.05$) and ITI decreased by 69.16% ($p<0.01$). The abovementioned changes are characteristic of the state of hypothyroidism and correspond to the literature data [4]. More significant changes of thyroid status manifested in animals with combined deficiencies of iodine and iron, selenium or copper (second to fourth research groups). In such a case emphasis should be placed on the disturbance of the balance in the system of thyroid hormones in animals of second-fourth research groups that is pointed by the decrease of indexes fT_3/fT_4 by 18.09–44.56%, ITI – on 25.11–30.62% and the increase of TSH/fT_4 by 24.55–39.15% ($p<0.05$) against the analogous indexes in animals with mono iodine deficiency (first research group).

Such changes of the thyroid status were observed on the background of decreased iodine content in urine of rats of all research groups, which confirms the formation of iodine deprivation in animals. The decreased content of iron, selenium and copper in all researched tissues indicated a limited income of these microelements into the organism. The analysis and ranging of the content of chemical elements in blood, tissues of thyroid and cerebrum was performed. The highest accumulation of iron in blood serum, selenium – in thyroid, copper – in cerebrum was revealed in animals of the first research group. The development of iron deficiency in the rats of the second research group is demonstrated by the decrease of levels of hemoglobin by 35.28% ($p<0.05$) and serum ferritin by 85.71% ($p<0.05$) against the control, which corresponds to the age and sex of the animals. It should be pointed that iodine deficiency was also followed by the decrease of hemoglobin and serum ferritin levels which could characterize the relationship between iodine and iron supply of the

organism of animals. A significant decrease of the level of transferrin saturation with iron and the level of ceruloplasmin in the rat blood serum in all research groups was also determined. This could characterize the depression of the antioxidant defence not only in animals with the deficiencies of copper or iron, respectively. The levels of transferrin saturation with iron and ceruloplasmin in the blood serum were the lowest in animals with combined iodine and copper deficiency.

As a result of the analysis of the indexes of lipid peroxidation, an increase of the content of dien conjugates and malondialdehyde was revealed in all research tissues in comparison with the analogous control data (Fig. 1).

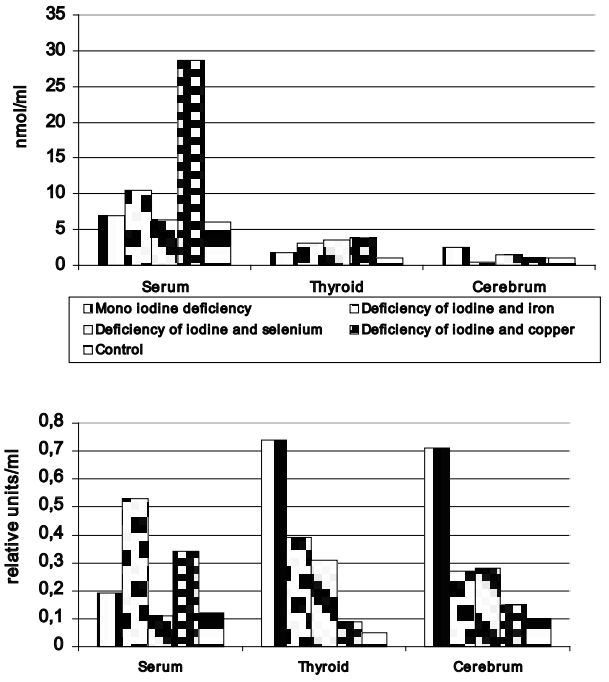

Fig. 1. Content of dien conjugates, malondialdehyde in blood serum, tissues of thyroid and cerebrum in animals with combined deficiencies of the microelements

It is important to point out that the combined deficiency of the essential microelements led to a more significant elevation of lipid peroxidation products in blood serum in comparison with mono iodine deficiency. In particular, the content of dien conjugates in blood serum as compared with the first research group (mono iodine deficiency) was 2.79 times higher ($p<0.05$) in animals of the second research group (combined deficiency of iodine and iron) and 1.79 times higher ($p<0.05$) in the rats of the fourth research group (combined deficiency of iodine and copper). The content of dien conjugates in the thyroid tissue of animals in second-fourth research groups was slightly smaller than in rats of the first research group. At the same time the content of malondialdehyde in the blood serum of the animals in these research groups exceeded by 1.81–2.21 times the analogous value of the animals in

conditions of mono iodine deficiency. In this case the most significant variations were observed in the animals with the combined deficiency of iodine and copper.

According to the data of the literature, the role of lipid peroxidation in the pathology of cells and tissues has been proved, but besides the major subject of peroxidation – biological molecules of membranes and nuclear chromatin the active forms of oxygen also induce the oxidative modification of proteins [5]. As a result of the present experiment, the activation of protein peroxidation was revealed and it was testified to by the increase of the products of the oxidative modification of proteins (Fig. 2). As it is seen in the figure, the oxidative modification of proteins has selective and specific character (being the most significant in conditions of combined deficiency of iodine and iron), although in general its level exceeds the control data in animals of all research groups.

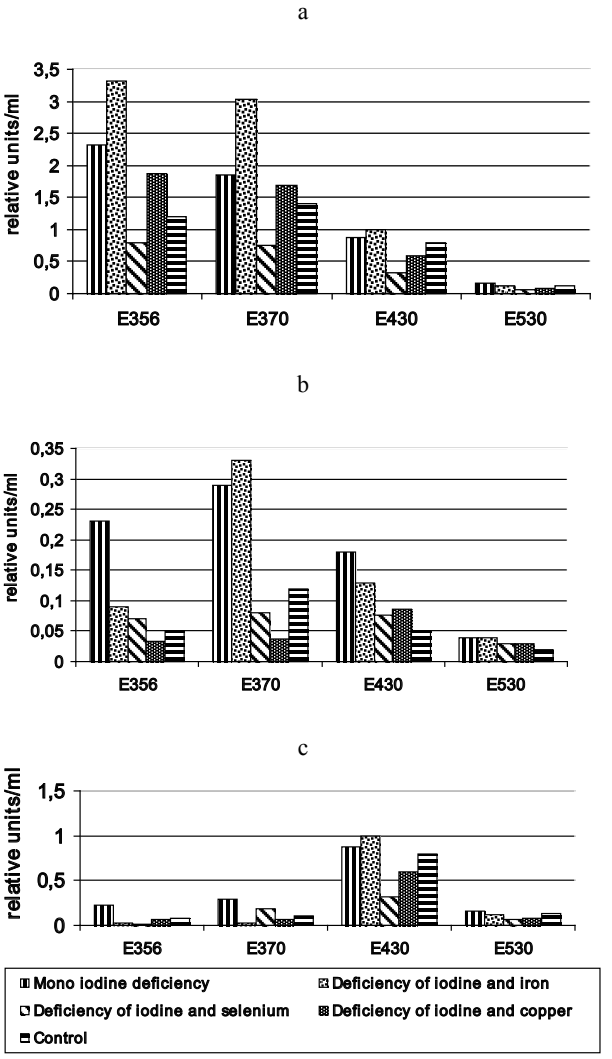

Fig. 2. Levels of oxidative modifications of proteins in blood serum (a), tissues of thyroid (b) and cerebrum (c) in animals with combined deficiencies of microelements

Special attention should be paid to the analysis of the changes of nitrite ion in blood serum of research animals. In rats of the first research group this index was lower by 38.55% ($p<0.05$) in comparison to the control. At the same time the combined deficiency of microelements led to the decrease of level of nitrite ion additionally by 33.94–49.52% ($p<0.05$) against the data in animals with mono iodine deficiency which in general could be especially dangerous for the cells and tissues [9].

It has to be stressed a that correlative link (mainly of medium significance) between the accumulation of the essential microelements (iron, selenium and copper) and the indexes of the thyroid status (levels of TSH, fT_3, fT_4 in blood serum) was established. The relationship between the income to the organism of iron, selenium, copper and iodine supply of the organism of research animals was revealed.

CONCLUSIONS

A combined deficiency of iodine and iron, selenium or copper induced a decrease of the functional capability of thyroid and the disturbance of the balance in the system of thyroid hormones. That is why it is possible to prove the protective influence of these chemical elements on the functional properties of thyroid. Among one of the probable reasons for the revealed disturbances could be the changes in the system of nitric oxide, the activation of the processes of lipid and protein peroxidation and also the depression of the antioxidant defence.

REFFERENCES

1. Baruscheva E.S., Lebedev S.V., Notova S.V.: Strukturno-funkcionalnaja reorganizacija schitovidnoj gelezi pri razlichnom soderjanii mikroelementov v racione. Morfologia, 129, 19, 2006.
2. Charnosch S.M.: Porivnjalna kharakterustuka trox eksperumentanukh modelej hypotireozy. Visnik Naykovikh Doslidgen, 2, 113, 2007.
3. Farhutdinova L.M.: Vozmognosti rescheniya problemu endemicheskogo zoba z pozicij mediciny i geologii. Kazanskij Med. Gurn., 87, 73, 2006.
4. Gzhegotskyj M.P., Chupaschko O.I.: Osoblivosty kusenzalejhnukh procesiv u krovi ta miokardi schuriv pri gypotureozi, Archiv Klin. i Eksper. Med., 12, 32, 2003.
5. Khodorovskij V.M.: Zminy tyreoidnogo gomeostazy pri eksperimentalnij zalizodeficitnij anemii. Bukovinskij Medichnij Visnik, 10, 123, 2006.
6. Koputova T.V., Dmitrieva O.N., Khimkina L.N. et al.: Okislitelnaja modifikacija belkov i oligopeptidov u bolnikh chronicheskimi dermatozami s syndromom endogennoj intoksikoscii. Fundamentalnue Issledovanija, 6, 15, 2009.
7. Maftines-Galan J.R., Pedraza P., Santacana M.: Early effect of iodine deficiency on radial glial cells of the hippocampus of the ret fetuset. J. Clin. Invest., 99, 2901, 1997.
8. Masahiko Yamamo et al.: D-penicillliamine - induced copper deficiency in suckling mice: neurogical abnormalities and brain mitochondrial enzyme activities., Division of Ultrastructural Research, National Institute of Neuroscience, NCNP, 123, Tokyo 1990.

9. Sklyarov O.Y., Fedorovuch I.P., Korobov V.M. et al.: Zminy koncentracii NO v biologichnych ridunah pru zahvoryuvanni na rak shlunka, Med. Himiya, 6, 55, 2004.
10. Timirbulatov T.A., Seleznjov S.A.: Metod povischenija intensivnosti svobodnoradikalnogo okislenija lipidosoderjhaschih komponentov krovi i ego diagnosticheskoe znachenie. Lab. Delo, 4, 209, 1988.

SUMMARY

In an experiment on rats changes of the indexes of thyroid status (increase of the level of TSH, decrease of the levels of fT_3, fT_4) and the balance in the system of thyroid hormones in animals with mono iodine deficiency, a combined deficiency of iodine and iron, selenium or copper were studied. Such disturbances of the functional capability of thyroid were observed on the background of the increase of indexes of lipid and protein peroxidation which was manifested by the increase of the content of dien conjugates, malondialdehyde, oxidative modifications of proteins in the blood serum, tissues of thyroid and cerebrum. In this case the combined deficiency of iodine and iron, selenium or copper was followed by more significant changes of the thyroid status, by the increase of lipoperoxidation, by the decrease of content of nitrite ion in blood serum in comparison to the condition of mono iodine deficiency.

Keywords: thyroid status, iodine, iron, selenium, copper deficiencies, processes of protein and lipid peroxidation, system of nitric oxide

STRESZCZENIE

W eksperymencie na szczurach oceniono zmiany we wskaźnikach stanu tarczycy (wzrost poziomu TSH, spadek poziomów fT_3, fT_4) oraz równowagę w systemie hormonalnym tarczycy u zwierząt z niedoborem jodu oraz złożonym niedoborem jodu, żelaza, selenu i miedzi. W prostych niedoborach jodu obserwowano zaburzenia potencjału funkcjonalnego tarczycy, manifestujące się wzrostem zmian wartości wskaźników peroksydacji lipidów, wzrostem zawartości sprzężonych dienów, malondialdehydu oraz oksydacyjnie zmodyfikowanych białek w surowicy, tarczycy i mózgu. W przypadku złożonych niedoborów jodu i żelaza, selenu lub miedzi zaburzenia te były bardziej nasilone.

Słowa kluczowe: status tarczycy, jod, żelazo, selen, miedź, niedobór, peroksydacja białek i lipidów, tlenek azotu