Evaluation of the Effectiveness of Bound Forms of Iodine and Zinc in Experimental Models of Iodine Deficiency and Immunodeficiency

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ABSTRACT
The most common microelementoses are diseases that occur with iodine and zinc deficiency. The purpose of this work was to evaluate the biological effectiveness of a developed additive containing bound forms of iodine and zinc on experimental models of iodine deficiency and immunodeficiency. The experiments were carried out on white outbred male rats weighing 150–180 grams. The iodine deficiency model was induced by the introduction of the thyrostatic compound tyrosol for 14 days. The immunodeficiency model was induced by the introduction of azathioprine for 7 days. The levels of thyroid hormones and immunoglobulins A, M and G were determined by solid-phase ELISA using standard sets. The intensity of lipid peroxidation and antioxidant status was assessed by conventional methods. A form of iodine and zinc was developed, associated with peptides from soy protein hydrolysate. The existence of an ionic bond with iodine and a covalent bond with zinc was experimentally demonstrated. This additive stimulated the synthesis of thyroid gland hormones and immunoglobulins in the sera of experimental animals, increased the antioxidant status of the organism and reduced the oxidative stress caused by iodine deficiency. The developed additive maybe used for the development of mass-produced foods enriched with iodine and zinc to prevent microelementoses.

Keywords: iodine deficiency, zinc deficiency, immunodeficiency

1. INTRODUCTION
The problem of preserving health and increasing the life expectancy of a person has been and remains one of the most urgent tasks of our time. Changes in the way of life of a person can cause changes in the specificity of diseases, which are characterised by a predominance of alimentary dependent ones. At present, the insufficient supply of micronutrients in food is a common problem in all countries. The International Conference on Nutrition (Rome, 1992), organised by the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO), called the widespread micronutrient deficiency a major nutritional problem in both developing and developed countries, and stressed the need for large-scale measures at state levels [1].

One of the reasons for the growth of alimentary-dependent diseases is the deficiency of micronutrients such as microelements. The most common microelements include iodine deficiency (in particular, the frequency of occurrence in the Russian Federation is more than 30 %) and zinc (a frequency of 20–40 % according to the data of the Russian Federation) [2].

Iodine is a vital element; it has important physiological activity and is a structural component of the thyroid hormones of the thyroid gland. Thyroid hormones regulate the intensity of biochemical reactions in tissues as well as the exchange of vitamins, a number of microelements, fats, carbohydrates and proteins. Triiodothyronine (T3) and thyroxine (T4) are also necessary for normal water-electrolyte metabolism. Thyroid hormones increase the sensitivity of the cells of the cardiovascular and nervous systems to the action of catecholamines. Iodine deficiency is endemic, so it cannot be eradicated completely, but it is possible to provide rational prophylaxis [3].

Zinc deficiency is also mainly a result of inadequate food intake. Zinc is a component of many enzymes (more than 300) and participates in a wide variety of processes involved in cell division, growth and function. Zinc also regulates the functions of the immune system. When there is a lack of zinc, the signs of insufficient activity of the immune system (immunodeficiency) first appear. The effect of zinc on the differentiation of T lymphocytes is due to the fact that it is part of thymulin, which is required for the differentiation of T cells. If there is a lack of zinc in animals, thymus involution is observed, and the number of Thelper cells, CD⁴⁺/CD₂⁵⁺ regulatory T cells ratio, the number of cytotoxiclymphocytes (CD⁸⁺) and the...
production of B-lymphocytes and immunoglobulins decrease [4–8].

Thus, iodine and zinc are essential microelements coming derived from food and water. They are involved in many metabolic processes, and a lack of these elements is areas on for widespread elementoses. Microelement deficiency is associated with the absence of mass prophylaxis, as well as imbalances and inadequacy of the diet [9, 10]. One of effective way of preventing and correcting the diet of the population is the development of products enriched with microelements. Here, we developed a biologically active food additive containing bound forms of iodine and zinc. Bound forms of microelements are preferable because they are characterised by better bioavailability, lower toxicity and volatility, higher efficiency [11]. The purpose of this work was to evaluate the biological effectiveness of the developed additive containing bound forms of iodine and zinc in experimental models of iodine deficiency and immunodeficiency.

2. MATERIALS AND METHODS

Zinc and iodine salts immobilised on low molecular weight peptides of soy protein hydrolysate were the object of the study. We carried out enzymatic hydrolysis of the soy protein isolate in one stage at a temperature of 37–40 °C for 12 hours at pH 2.8–3.2 under the action of pepsin. After fermentation, the solution was neutralised to pH 7.0, centrifuged at 3000 rpm for 20 minutes. We used a solution of the obtained hydrolysate at a concentration of 30 mg/ml by protein. The immobilisation of salts of zinc sulphate and potassium iodide was carried out sequentially. We separated organic forms of microelements from mineral salts by dialysis through a semipermeable membrane in a stream of water for 24 hours [12].

The experiments were carried out on white outbred male rats weighing 150–180 g. Animal handling was performed according to the Laboratory Practice Rules (GLP) and Order No. 708H of the Ministry of Health of the Russian Federation of August 23, 2010 “On the Approval of the Rules of Laboratory Practice”. The experiments were carried out with the observance of the principles of humanity in accordance with the “Rules for the performance of work using experimental animals” (Annex to the order of the Ministry of Health of the USSR No. 755 of 12.08.77) and the principles of the “European Convention for the Protection of Vertebrates Animals used for Experimental and Other Scientific Purposes” (Strasbourg, 1986). Euthanization of animals was carried out by instantaneous decapitation under light ether anaesthesia.

The iodine deficiency model was induced by treatment with the thyrostatic compound tyrosol at a dose of 25 mg/kg body weight for 14 days. The model of immunodeficiency was reproduced by the introduction of azathioprine at a dose of 25 mg/kg body weight for 7 days [13].

The animals were divided into three experimental groups: group I – intact, group II – control and group III – experimental. The intact group of animals received water. The control group received tyrosol as a model of iodine deficiency and azathioprine for the simulation of experimental immunodeficiency. The experimental group of animals received the developed additive against the background of experimental microelement deficiency. The introduction of the bound forms of microelements was carried out orally in 0.5 ml of a solution containing 1.5 μg/ml of iodine and 75 μg/ml of zinc for 21 days against a background of hypothyroidism caused by introduction of tyrosol, and for 14 days against the background of immunodeficiency caused by the introduction of azathioprine. In experiments modelling iodine deficiency, the level of thyroid-stimulating hormones in the blood serum of intact and experimental animals was recorded on the background of hypothyroidism (control group) and with subsequent correction using organic forms of iodine and zinc (experimental group). We used the method of solid-phase enzyme-linked immunosorbent assay (ELISA) with a kit of reagents from LLC NPO Diagnostic Systems [14]. In experiments modelling immunodeficiency and its subsequent correction, we determined the content of immunoglobulins in serum of animals by ELISA using standard immunoassay kits (China).

The concentration of malondialdehyde (MDA) in the liver and the serum was determined by a method based on the reaction of MDA with 2-thiobarbituric acid (TBA) (Sigma-Aldrich). The concentration of TBA-active products in samples was measured at a wavelength of 532 nm, indicated by the degree of formation of the coloured complex with TBA [15].

The determination of reduced glutathione (GSH) was based on the interaction of GSH with DTNB (5,5'-dithio-bis-2-nitrobenzoic acid) by producing yellow-colour edanion of 2-nitro-5-thiobenzoate. The increase in the concentration of the yellow anion was recorded spectrophotometrically at a wavelength of 412 nm during this reaction [16].

Determination of glutathione peroxidase (GSH-Px) activity was based on the ability of glutathione peroxidase to catalyse the reaction of the interaction of GSH with tert-butyl hydroperoxide (HTTB). Enzyme activity can be assessed by measuring the content of GSH in samples before and after incubation with the model substrate during a colour reaction with DTNB (5,5'-dithio-bis-2-nitrobenzoic acid) [17].

Assessment of the total content of water-soluble antioxidants in the blood serum of experimental animals was conducted using a Tsvetyauza 01-AA amperometric detector (GOST R 54037-2010).

For the statistical analysis, we determined the arithmetic mean values and the standard errors of the arithmetic means (M ± m). The significance of the differences in the average arithmetic ranked criteria for the normal distribution was estimated using Student’s t-test. A statistically significant difference was set at p<0.05.
3. RESULTS AND DISCUSSION

Given the pathogenic mechanisms responsible for the development of hypothyroidism, a model of experimental goitre was used to reproduce its main symptoms. To this end, thyrostatic tyrosol was used at a dose of 25 mg/kg of the body weight of the animal. Tyrosol accelerates the excretion of iodides from the thyroid gland, blocks peroxidase and inhibits the iodination of tyrosine for the formation of triiodothyronine and thyroxine. It activates the synthesis and excretion of thyrotropic hormone (TTH) by the pituitary gland [15].

TTH is produced by the pituitary gland under the influence of thyrotropin-releasing factor from the hypothalamus. With iodine deficiency, the synthesis of thyroid hormones is suppressed and the production of TTH increases according to the mechanism of negative feedback. Conversely, with sufficient intake of iodine and, accordingly, thyroid hormones, the concentration of TTH decreases.

The hormone levels in the experimental animals are provided in Table 1.

The table shows that treatment with the thyrostatic compound tyrosol caused a two-fold increase in the production of TTH and a two-fold decrease in the level of both T3 and T4 relative to the corresponding indices in the intact group of animals. The introduction of the developed additive within 14 days restored the studied parameters. Thus, the TTH level was restored to 83 % of the level of the corresponding index in the intact group, the level of the hormone T3 exceeded four times the level of that in the intact group of animals, and T4 reached the corresponding level of the intact group of animals. Exceeding the level of the hormone T3 in the blood serum of the animals of the experimental group was apparently associated with the high iodine content in the developed supplement, which undoubtedly required correction; namely the selection of the most optimal concentration.

An evaluation of the effectiveness of a new supplement on the functional state of the body's immune system was carried out on the model of immunosuppression caused by the introduction of azathioprine. Azathioprine is an imidazole derivative of mercaptopurine. In the body, it undergoes enzymatic cleavage into mercaptopurine and a thioimidazole compound and has an immunosuppressive effect, essentially due to mercaptopurine. The thioimidazole compound is also known to suppress immunity. The immunosuppressive effect of azathioprine is due to the fact that it suppresses the proliferation of immunocompetent cells.

Table 2 shows the concentration of blood serum immunoglobulins in the experimental animals.

The introduction of azathioprine at a concentration of 25 mg/kg body weight caused a decrease in production of IgA by 86 %, IgM by 98 % and IgG by 83 %. The introduction of the studied additive against the background of immunodeficiency caused by the introduction of azathioprine led to a sharp increase in the production of the antibodies IgA, IgG and IgM. The level of antibodies exceeded the corresponding indices in intact animals 1.7–2 times (IgA to the level of the intact group, IgM by two-fold and IgG by 1.7-fold), which indicates stimulation of adaptive immune responses.

### Table 1

| №  | Group                                   | TTH, mIU/l | T3, pg/ml | T4, pg/ml |
|----|-----------------------------------------|------------|-----------|-----------|
| 1  | Intact                                  | 0.213±0.014| 1.156±0.065| 9.616±0.55|
| 2  | Control, tyrosol (25 mg/kg)             | 0.465±0.0283\textsuperscript{a} | 0.562±0.0316\textsuperscript{a} | 4.833±0.25\textsuperscript{a} |
| 3  | Experimental, tyrosol+ supplement (25 mg/kg + 0.5 ml) | 0.176±0.02\textsuperscript{b} | 4.833±0.25\textsuperscript{b} | 10.883±0.45\textsuperscript{b} |

\textsuperscript{a} – statistically significant difference relative to group 1
\textsuperscript{b} – statistically significant difference relative to group 2

### Table 2

| №  | Group                               | IgA. mg/ml | IgM. mg/ml | IgG. mg/ml |
|----|-------------------------------------|------------|------------|-----------|
| 1  | Intact                              | 0.058±0.004| 0.552±0.040| 3.98±0.32 |
| 2  | Control, azathioprine (25 mg/kg)    | 0.008±0.002\textsuperscript{a} | 0.01±0.002\textsuperscript{a} | 0.68±0.16\textsuperscript{a} |
| 3  | Experimental, azathioprine+ supplement (25 mg/kg + 0.5 ml) | 0.056±0.01\textsuperscript{b} | 1.160±0.06\textsuperscript{b} | 6.92±0.16\textsuperscript{b} |

\textsuperscript{a} – statistically significant difference relative to group 1
\textsuperscript{b} – statistically significant difference relative to group 2
Since active forms of oxygen are formed in the process of thyroid hormone biosynthesis, we studied the level of oxidative stress and the state of the body’s antioxidant system following introduction of the studied supplement against a background of hypothyroidism.

One of the most indicative markers for the development of oxidative stress is the level of malonic dialdehyde (MDA) in blood serum. The concentration of malonic dialdehyde in the serum reflects the activity of lipid peroxidation in the body and serves as an indicator of the degree of endogenous intoxication (Table 3).

**Table 3** Indicators of the level of malonic dialdehyde in the blood serum of experimental animals that received organic forms of iodine and zinc on a background of iodine deficiency

| Group          | Intact               | Control                      | Experimental          |
|----------------|----------------------|------------------------------|-----------------------|
| MDA of the blood serum, mmol/L | 0.0227±0.0019        | 0.0305±0.0009<sup>a</sup> | 0.024±0.0006<sup>b</sup> |

<sup>a</sup> – statistically significant difference relative to the intact group  
<sup>b</sup> – statistically significant difference relative to the control group

As follows from the presented data, the introduction of thyrostatic tyrosol for two weeks led to an increase in the intensity of the processes of lipid peroxidation, estimated by the index of malonic dialdehyde content in the blood serum, which increased 1.34-fold in the control group relative to the intact group, indicating an increase in oxidative stress. After the introduction of bound forms of microelements to the animals, the inhibiting effect of the developed additive on lipid peroxidation was revealed. The MDA content in the serum of the animals of the experimental group decreased to the level of those in the intact group. It is known that iodine-containing thyroid hormones are able to limit lipid peroxidation due to stimulating effects on the activity of superoxide dismutase and catalase, which are components of the body’s antioxidant system. An equally important part of the antioxidant system is glutathione peroxidase, which restores hydrogen peroxide and organic hydroperoxides. The role of glutathione is particularly important in the antioxidant system, as it is the main reductant in cells. Its concentration in the cell is higher than most other organic substances; it directly restores active forms of oxygen, and glutathione-dependent enzymes that work in all compartments of the cell [18].

The results of the study on the content of reduced glutathione and the activity of glutathione peroxidase in the erythrocytes of experimental animals are shown in Table 4.

**Table 4** Influence of organic forms of microelements on the antioxidant status of animals against a background of experimental iodine deficiency

| №  | Group    | Glutathione reduced erythrocytes, micromol/g • Hb | Activity of glutathione peroxidase, erythrocytes of blood, μmol/min • l |
|----|----------|--------------------------------------------------|------------------------------------------------------------------------|
| 1  | Intact   | 1.290±0.024                                       | 0.043±0.008                                                            |
| 2  | Control  | 0.328±0.019<sup>a</sup>                           | 0.033±0.006<sup>a</sup>                                                |
| 3  | Experimental | 1.310±0.031<sup>b</sup>                          | 0.039±0.005<sup>b</sup>                                                |

<sup>a</sup> – statistically significant difference relative to group 1  
<sup>b</sup> – statistically significant difference relative to group 2

The content of reduced glutathione in erythrocytes was decreased 3.93-fold on a background of thyrostatic hypothyroidism. In addition, we observed a 1.3-fold decrease in the activity of glutathione peroxidase in the erythrocytes of animals treated with tyrosol compared with the intact group (Table 4). In the experimental group of animals treated with organic forms of iodine and zinc, the level of glutathione was restored to the level of the intact group. In addition, the activity of glutathione peroxidase increased to the corresponding indices of intact animals. This indicates a correction of the antioxidant status of the animals in the experimental group, similar to that of the intact group.

The overall picture of the effect of iodine deficiency on the antioxidant status of experimental animals can be assessed by the level of the total content of water-soluble antioxidants, determined by the amperometric method in the sera of experimental animals (Table 5).

The results show that the total content of antioxidants in the blood serum of the control group of animals treated with thyrostatic tyrosol decreased by 16 %. This is due to the development of oxidative stress on the background of iodine deficiency. In turn, the total content of antioxidants in the blood serum of the experimental group of animals treated with the organic forms of microelements (zinc and iodine) under conditions of iodine deficiency was restored to the corresponding index of the intact group.
Table 5 The total content of antioxidants in the blood serum of intact and experimental animals.

| Group                      | Intact          | Control        | Experimental   |
|----------------------------|-----------------|----------------|----------------|
| The total content of antioxidants in the blood serum, mg/100 ml | 62.137±0.624    | 51.966±0.612a  | 63.473±1.564b  |

a – statistically significant difference relative to the intact group
b – statistically significant difference relative to the control group

Thus, it can be concluded that the bound form of microelements can restore not only the level of thyroid hormones in an experimental model of iodine deficiency, but also reduce the processes of lipid peroxidation, thereby restoring enzyme activity and the level of non-enzyme antioxidants. The introduction of the studied additive containing bound forms of iodine and zinc was also able to restore immune function in an experimental model of immunodeficiency.

4. CONCLUSION

Microelementoses develop because of an insufficient or excessive supply of microelements. Disturbances in the exchange of microelements do not occur in isolation, but affect other types of metabolism: protein, fatty carbohydrate, as well as the exchange of vitamins and pigments. The most common microelementoses are diseases caused by iodine and zinc deficiency, which are endemic. Microelementoses are much easier to prevent than cure. Methods of mass prophylaxis of microelementoses are needed. State programs focus on basic microelements (iron, iodine, zinc) and the most popular, affordable foods (bread, milk, etc.).

Studies in recent years have shown that organic forms of microelements are most preferable and effective [19, 20]. Their ready processability and easily controlled doses, storage and bioavailability make it possible to use them widely in the development of dietary supplements and functional food products for the prevention of microelement insufficiency.

We developed bound forms of iodine and zinc, experimentally demonstrated by the presence of an ionic bond with iodine and a covalent bond with zinc. The content of bound iodine was 1.6 μg/ml and that of bound zinc was 75 μg/ml. The experimental models of iodine deficiency and immunodeficiency demonstrated that the introduction of this additive stimulates both the synthesis of thyroid hormones and the synthesis of immunoglobulins in the sera of experimental animals.

The introduction of the developed supplement on the background of iodine deficiency increased not only the level of thyroid hormones, but also the antioxidant status of the organism and reduced the intensity of oxidative stress caused by iodine deficiency. It is known that iodine-containing thyroid hormones are able to limit the processes of lipid peroxidation by stimulating the activity of superoxide dismutase and catalase, which are components of the body’s antioxidant system [21, 22]. Replenishment of the zinc pool in the body contributes to an increase in the antioxidant status of the organism due to an increase in the activity of superoxide dismutase, which interrupts the chain of free radical processes at the initial stages, thereby preventing the formation of especially aggressive active forms of oxygen (OH•, O2•−). In addition, zinc contributes to providing the body with vitamin A, which is a lipid antioxidant [23].

Thus, the obtained data indicate to the effectiveness of the developed additive and argue in favour of its use in creating mass food products enriched with iodine and zinc to prevent microelementoses.

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