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Research Article

# Pathogenetic Approach to the Use of a Non-hormonal Drug for Correction of Experimental Thyroid Pathology

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## Abstract

Today, hypothyroidism of various origins affects about 10% of the Earth's population. The relevance of this work is due to the absence of original harmless and effective natural products on the domestic and global pharmaceutical market, which can be used both to normalize the immune system and to restore the functional activity of the thyroid gland and reduce the level of associated complications. In modern endocrinology, fetal biological products, including cord blood products, can be a certain alternative to replacement therapy (or in combination with it). The aim of the work was to find new therapeutic approaches for the correction of the most common thyropathies. The study was conducted on 75 sexually mature male Wistar rats in a model of autoimmune hypothyroidism, which developed in rats against the background of autoimmune thyroid damage, which was caused by immunizing animals with thyroid antigen in combination with Freund's complete adjuvant. The animals were injected with a cryopreserved cord blood preparation "Cryocell-Cryocord" (CC) and a reference preparation "L-thyroxine" (LT4) (Berlin-Chemie, Germany). The AIH model leads to persistent pathological changes in the structure and function of the thyroid gland and immunological disorders. It was determined that at all times of the study, the biological preparation of CC had a positive effect on the restoration of the hormone-producing function of the thyroid gland in rats with AIH, increasing the content of both fractions of thyroid hormones, i.e. the biological preparation of CC showed itself as a powerful stimulator of the hormone-producing function of the thyroid gland. In addition, the use of the cord blood preparation significantly reduced the titer of antithyroid antibodies. The normalizing effect of CC on the indicators of specific humoral and cellular immunity was maintained throughout the experiment, which indicated the effectiveness of its use in thyroid pathologies. The multipotent properties of CC allow us to consider this biological preparation as a promising potential tool in the complex therapy of hypothyroidism of various genesis. The use of such approaches in the therapy of thyroid dysfunction is pathogenetically justified.

## Keywords

Thyroid Gland, Hypothyroidism, Thyroid Hormones, Antithyroid Antibodies, Biological Preparations, Cord Blood

## 1. Introduction

Hypothyroidism is a disease characterized by a persistent, prolonged deficiency of thyroid hormones in the human body or a decrease in their biological effects at the tissue level. It is important to know that thyroid hormones, such as triiodothy-

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ronine (T3) and thyroxine (T4), are key regulators of metabolism and development, which have numerous pleiotropic effects. Almost all tissues of our body have receptors for thyroid hormones.

The relevance of this work is due to the lack of original harmless and effective natural products on the domestic and global pharmaceutical market, which can be used both to normalize the immune system, and to restore the functional activity of the thyroid gland (thyroid) and reduce the level of associated complications.

Global trends in the treatment of hypothyroidism traditionally boil down to replacement therapy, the means of which are various versions of the dosage forms of thyroid hormones [1, 2]. A significant amount of research is devoted to the use of selenium-containing drugs, in particular L-selenomethionine-based drugs, as auxiliary drugs in the treatment of hypothyroidism. [3, 4].

At present, a modern promising direction is the measures of regenerative medicine using new compounds and pharmaceutical compositions of biological origin, which are able to restore the affected links of both the immune system and metabolism as a whole. The effectiveness of biological drugs is confirmed by the results of experimental and clinical studies [5, 6].

In modern endocrinology, fetal biological drugs, including cord blood drugs, the use of which is rapidly expanding [7, 8]. Human placental (cord) blood cells do not have strain and species specificity, which contributes to the overall increase in the body's resistance to airborne infections. In addition, this bioobject has great potential in the treatment of diseases with an immune component that are widespread today [9, 10].

The aim of the work was to find new therapeutic approaches for the correction of the most common thyropathies and associated complications.

## 2. Materials and Methods

Experimental studies were conducted in the laboratory of pharmacology of the department of experimental pharmacology and toxicology of the State Institution "V.Ya. Danilevsky Institute of Endocrine Pathology of the National Academy of Medical Sciences of Ukraine".

The study was conducted on 75 sexually mature male rats with an initial body weight of 144 g - 195 g at the beginning of the experiment and 348 g - 403 g at the end of the observations. Autoimmune hypothyroidism (AIH), which developed in rats against the background of autoimmune thyroid disease, was induced by immunizing animals with thyroid antigen in combination with Freund's complete adjuvant, Imject TM Freund's Complete Adjuvant. REF 77140; LOT VI313055 (Thermo Fisher Scientific Inc, USA) [9]. The hypothyroid state of animals with simulated AIT was verified by determining the content of total and free forms of thyroid hormones, as well as antibodies to thyroglobulin (AB TG) and thyroperoxidase (AB TPO) using standard commercial test kits for enzyme immunoassay manufactured by the company “Hema” (Ukraine) using a microplate enzyme immunoassay analyzer “Stat Fax 3200” (Awareness Technology inc., USA).

The drug “Cryocell-Cryocord” (CC) was provided by the State Enterprise “Interdepartmental Scientific Center for Cryobiology and Cryomedicine of the National Academy of Sciences, the Academy of Medical Sciences and the Ministry of Health of Ukraine”. “Cryocell-Cryocord” was diluted with saline in a ratio of 1:10. (0.3 ml of CC + 3 ml of saline). The drug was administered intramuscularly in a course of 10 injections every other day at the rate of 0.1 ml of diluted solution per 100 g of body weight.

The reference drug "L-thyroxine" (LT4) (Berlin-Chemie, Germany, series 02057A, 05/2022) tablets were carefully ground in a mortar with the addition of 2% starch solution, administered to rats at a dose of 10 μg/kg b.w. for 10 days, daily.

The distribution of animals by groups is given in Table 1.

***Table 1.*** *Distribution of animals into groups.*

| Group № | Group | Daily amount of substance mg/kg body weight | Number of animals in the group |
|---|---|---|---|
| 1-3 | Intact control, 1, 3 and 6 months. | - | 5+5+5 |
| AIH modeling | | | |
| 4-6 | Induced AIH, 1, 3 and 6 months (negative control) | four times administration of 150μl of thyroid antigen with PAF | 5+5+5 |
| substance administration, 1, 3 and 6 months after the effect | | | |
| 7-9 | Intact control | - | 5+5+5 |
| 10-12 | AIH+ CC | 300 mg/kg b.w. | 5+5+5 |
| 13-15 | AIH+ LT4 | 10 mcg/kg b.w. | 5+5+5 |

At one, three and six months after the end of the administration of the studied drugs, the animals were removed from the experiment. In all series of studies, the method of instantaneous cutting of the spine at the base of the skull under light ether anesthesia was used to euthanize the rats. The studies were conducted in accordance with the national "General Ethical Principles of Animal Experiments" [10].

The obtained data were presented as the mean ± standard error of the mean. Standard deviations between experimental groups were estimated using the Student's t-test. Values were considered significantly different at $p < 0.05$. The analysis was performed using the SigmaPlot statistical program.

## 3. Results

It was shown that in the dynamics of hypothyroidism development against the background of AIT, a progressive decrease in thyroid hormone levels and an imbalance of individual links of nonspecific humoral and cellular immunity were noted, which was confirmed by an increase in specific autoantibodies (Table 1). The detected changes in thyroid status indicated the development of a dysfunctional state of the gland in animals, which is a characteristic feature of AIG.

The use of the cord blood biopreparation "Cryocell-Cryocord" (CC) already at an early stage of observation significantly reduced the titer of antithyroid antibodies, but their content was at a sufficiently high level compared to the control.

The reference drug had practically no effect on the indicator of blood TG, at the same time it significantly reduced the content of blood AB-TPO in the circulating blood - even to a greater extent than the biopreparation (Table 2).

In the early post-treatment period, the biological preparation CC had a positive effect on the restoration of the thyroid hormone-producing function of rats with AIH. The content of both T4 fractions increased, a greater increase was observed in the free form of this hormone - the increase had significant differences from the AIH-control group. In parallel, a proportional increase in the concentration of free and total T3 was noted (in all cases $P<0.05$, see Table 1). In the long term - after 3 months and 6 months. post-treatment, the biological preparation CC also showed itself as a powerful stimulator of the thyroid hormone-producing function: (see Table 1). Against the background of high levels of both AB-TG and AB-TPO blood levels in rats with AIH, the use of both CC and LT4 led to the normalization of this link in immunity and to the inhibition of the production of autoantibodies to the own organ. The most significant and prolonged effect aimed at normalizing immunity was observed in the group of rats that were injected with cryopreserved biological preparation CC. The effect of LT4 on the state of the immune system was more moderate - the content of AB-TG in the blood was significantly higher than in the control ($P<0.05$). A similar pattern was observed when analyzing changes in the AB-TG index (Table 2).

***Table 2.*** *Changes in the thyroid status of male rats in the dynamics of the development of experimental AIH and its correction after the end of immunization,* $\overline{X} \pm S_{\overline{x}}$ .

| Name | Intact control | AIH | AIH +CC | AIT+LT |
|---|---|---|---|---|
| 1 month | | | | |
| T3 total, nmol/l | 1,38±0,15 | 0,96±0,02[1)] | 1,60±0,22[2)] | 1,40±0,12[2)] |
| T3 free, pmol/l | 5,43±0,24 | 3,18±0,28 [1)] | 5,18±0,33[2)] | 6,30±0,58 [2)] |
| T4 total, nmol/l | 56,45±5,31 | 45,86±3,04[1)] | 58,60±4,23[2)] | 66,52±4,23[2)] |
| T4 free, pmol/l | 11,56±0,83 | 10,15±1,07[1)] | 15,42±1,07[2)] | 26,42±1,87[1) 2)] |
| AB-TG, U/ml | 38,40±2,71 | 80,30±5,25[1)] | 52,57±4,77[2)] | 64,42±5,52[1)] |
| AB-TPO, U/ml | 23,55±1,13 | 52,70±2,03[1)] | 44,72±3,05[1)] | 29,50±1,87[2)] |
| 3 month | | | | |
| T3 total, nmol/l | 1,51±0,16 | 0,88±0,06[1)] | 1,73±0,15[2)] | 1,35±0,19[2)] |
| T3 free, pmol/l | 6,14±0,41 | 2,97±0,22 [1)] | 5,55±0,53[2)] | 4,53±0,35[2)] |
| T4 total, nmol/l | 71,12±5,24 | 40,71±3,18[1)] | 66,80±4,36[2)] | 62,54±4,51[2)] |
| T4 free, pmol/l | 19,31±1,12 | 10,44±1,01 [1)] | 16,84±1,24[2)] | 16,73±1,33[2)] |
| AB-TG, U/ml | 42,56±2,88 | 104,84±6,36 [1)] | 48,21±3,06[2)] | 54,67±4,07[2)] |
| AB-TPO, U/ml | 18,08±1,10 | 58,58±2,03[1)] | 24,16±2,05[2)] | 25,53±1,07[2)] |

| Name | Intact control | AIH | AIH +CC | AIT+LT |
|---|---|---|---|---|
| 6 month | | | | |
| T3 total, nmol/l | 2,34±0,21 | 0,78±0,03 [1)] | 1,91±0,18[2)] | 1,18±0,20[1) 2)] |
| T3 free, pmol/l | 8,03±0,50 | 4,54±0,28 [1)] | 7,07±0,52[2)] | 5,40±0,38 |
| T4 total, nmol/l | 82,40±5,22 | 42,51±2,84[1)] | 71,4±5,49[2)] | 59,77±4,38[1) 2)] |
| T4 free, pmol/l | 23,53±1,08 | 13,74±1,17[1)] | 19,56±1,14[2)] | 16,28±1,17 |
| AB-TG, U/ml | 35,52±2,21 | 148,80±8,82 [1)] | 45,71±4,03[2)] | 70,24±4,82[1) 2)] |
| AB-TPO, U/ml | 17,66±1,13 | 61,38±4,03[1)] | 28,55±3,05[1) 2)] | 37,64±2,17[1)2)] |

[1)] – Significance of changes in indicators of the corresponding intact control, (P<0.05);
[2)] – Significance of changes in indicators according to the AIH control, (P<0,05)

LT4 replacement therapy in the early period expectedly increased their concentration in the blood, especially this applied to free forms. It should be noted separately that in more distant terms in the group of rats that were injected with LT4, a moderate decrease in the level of thyroid hormones was observed, which may be due to the gradual elimination of their excess from the bloodstream.

In the long term of the studies, it was determined that the most effective and prolonged effect on the pituitary-thyroid system and the state of specific humoral immunity was demonstrated by the biological preparation CC. The action of LT4 was quite effective, it maintained the hormonal balance of rats with hypothyroidism, but did not restore the hormone-producing properties of the gland.

## 4. Discussion

It was determined that the AIH model leads to persistent pathological changes in the structure and function of the thyroid gland, immunological disorders and concomitant metabolic disorders, which was confirmed by hormonal and immunological studies. The detected changes in thyroid status indicated the development of a dysfunctional state of the gland in animals, which is a characteristic feature of AIH.

A decrease in the titer of antithyroid antibodies after the use of the cord blood biopreparation “Cryocell-Cryocord” (CC) indicates the presence of an immunomodulatory effect of CC. This effect can be explained by the immunosuppressive properties of this drug on the one hand, and regenerative properties on the other [11, 12]. As evidenced by the restoration of the level of total and free forms of thyroid hormones Based on the fact that it significantly influenced the increase in the level of free forms of hormones, it can be assumed that cord blood serum, due to a large number of biologically active substances, can additionally realize its effect at the level of deposition and binding of hormones to blood proteins. It should be noted that such restoration has a prolonged effect, unlike replacement therapy, which must be used throughout life with a gradual increase in dose. Which can contribute to the load on the cardiovascular system.

In our experiment, we administered the reference drug for a course of 10 days, so the effect observed after a month was significantly reduced in the long term. This fact once again emphasizes the need to search for agents with a more prolonged effect with the possibility of restoring hormone-producing properties.

## 5. Conclusions

1) The AIH model leads to persistent immunological disorders, pathological changes in the structure and function of the thyroid gland. In rats, in the dynamics of hypothyroidism development against the background of AIT, an imbalance of nonspecific humoral and cellular immunity was noted, which was confirmed by an increase in specific autoantibodies.
2) The action of the biological preparation CC potentiated the normalization of the thyroid hormone profile at all study periods and led to a decrease in the titer of AB- TG and AB -TPO compared to the AIH-control group. LT4 at an early stage after administration increased the level of thyroid hormones in the blood of rats with induced AIH, but did not change the high titer of AB- TG. Over time, its effect decreased compared to the action of CC.
3) The multipotent properties of CC allow us to consider this biological preparation as a promising potential agent in the complex therapy of hypothyroidism of various genesis. The use of this type of biological products in the treatment of thyroid dysfunction is pathogenetically justified and may be appropriate for triggering an immunomodulatory mechanism and correcting immunosuppression that occurs in this pathology.

## Abbreviations

| | |
|---|---|
| AIH | Autoimmune Hypothyroidism |
| AIT | Autoimmune Thyroiditis |

| | |
|---|---|
| TG | Thyroid Gland |
| TSH | Thyroid-stimulating Hormone |
| fT4 | Free Thyroxine |
| fT3 | Free Triiodothyronine |
| AB-TPO | Antibodies to Thyroid Peroxidase |
| AB TG | Thyroglobulin Antibodies |
| CC | Cord Blood Bioproduct "Cryocell-Cryocord" |
| T3 | Total Triiodothyronine |
| T4 | Total Thyroxine |
| TSH | Thyroid Stimulating Hormone |
| LT4 | "L-thyroxine" |
| PAF | Freund's Complete Adjuvant |

## Author Contributions

**Natalia Malova:** Conceptualization, Writing – review & editing

**Larysa Syrotenko:** Supervision, Investigation

**Irina Komarova:** Data curation

**Yuriy Karachentsev:** Project administration, Formal Analysis

**Vadym Khaziyev:** Validation, Methodology

**Vitaly Varavin:** Software

## Data Availability Statement

The data supporting the outcome of this research work has been reported in this manuscript.

## Conflict of Interest

The authors declare that there is no conflict of interest in relation to this paper, as well as the published research results, including the financial aspects of conducting the research, obtaining and using its results, as well as any non-financial personal relationships.

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