THE STUDY OF THE ANTIGOITROGENIC EFFECT OF THE EXTRACT FROM LAMINARIA ON THE MODEL OF MERCAZOLILUM-INDUCED HYPOTHYROIDISM

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The paper presents the results of the experimental study of the effect of the aqueous extract from Laminaria on the thyroid function compared to Iodomarin on the model of mercazolilum-induced hypothyroidism. It has been found that the extract from Laminaria reveals the antigoitrogenic effect determined by measuring the weight of the thyroid gland, and it exceeds the effect of Iodomarin. Introduction of the extract from Laminaria and Iodomarin does not lead to recovery of the thyroid hormone level in the blood, which is significantly reduced in experimental animals with mercazolilum-induced hypothyroidism. However, taking into account the stimulating effect previously determined on the hormone-synthetic function of the thyroid gland in intact animals the marked antigoitrogenic effect revealed on the model of experimental hypothyroidism, and improvement of thyroid hormone level at the end of the experiment give grounds to conclude about the thyroid-stimulating effect of the aqueous extract from Laminaria under study.

Medical and social significance of hypothyroidism cannot be overestimated. It depends not only on its prevalence in the population and the tendency to further increase of the number of patients, but the fact that the hypofunction of the thyroid gland (TG) leads to various organ and neuropsychiatric disorders, decreased lifeware, etc. [11].

The primary method of prevention of these diseases is the use of iodine-treated salt, bread, and the main treatment is to control iodine deficiency using drugs, including herbal formulations [7].

Today in Ukraine among the registered drugs for prevention and treatment of iodine deficiency disorders are only products based on potassium iodide in the form of tablets, among them the larger market segment is occupied by drugs of foreign manufacturers, in particular Germany and the United States [2]. Therefore, development of new drugs for correction of the thyroid hypofunction remains a pressing problem of modern medicine and pharmacy.

The search and study of herbal substances with thyroid-stimulating properties, development of dosage forms and creation of drugs on their basis are carried out in the National University of Pharmacy. The studies of the extract from Laminaria conducted allowed revealing the thyroid-stimulating effect in intact animals [3].

The aim of this work was to study the effect of the aqueous extract from Laminaria Saccharina on the thyroid function in experimental hypothyroidism.

Materials and Methods

Manipulations with animals of the experimental vivarium were performed according to the national “General ethical principles of animal experimentation” (Ukraine, 2001), which are consistent with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) [6].

The animals were kept under standard vivarium conditions with natural light, diet recommended...
Thirty-six rats weighting 115-125 g were used in the experiment. Animals were randomly divided into 4 groups: group 1 – intact control (IC); group 2 – animals treated with mercazolilum (1-methyl-2-merkaptoimidazol of the Pharmaceutical company “Zdorovye”, Ltd., Ukraine) in the dose of 0.01 g/100 g of the body weight per os in 10% starch for 33 days – the group of control pathology (CP); group 3 – animals treated with the extract from Laminaria (an aqueous residue obtained by complex processing of Laminaria thalli) per os in the dose of 1 ml/100 g of the body weight from 13 to 33 day of the experiment (21 days); group 4 – animals treated with Iodomarin 100 (Berlin-Chemie AG, Germany) in the dose of 20 μg of iodine/100 g of the body weight.

Animals were weighed on an empty stomach on the 12th, 19th, 26th and 34th days of the experiment. After 12 days the tail vein blood from the part of the experimental animals was taken for determination of serum thyroid hormones.

Table 1

| Group                                           | Statistical index  | Day of the experiment |
|-------------------------------------------------|--------------------|-----------------------|
|                                                |                    | Start of the experiment | 12-th | 19-th | 26-th | 33-d |
| **Intact control (IC)**                         | n 6                | 118.67                | 156.67* | 171.67 | 177.50 | 186.33*** |
|                                                | ±Sx                | 7.92                  | 6.67    | 4.77  | 4.61    | 4.59    |
| **Control pathology (CP)**                     | n 10               | 115.63                | 144.00* | 171.00 | 177.50 | 161.00*** |
|                                                | ±Sx                | 6.30                  | 6.27    | 6.00  | 2.83    | 5.42    |
| **Mercazolilum + extract from Laminaria**      | n 10               | 120.00                | 153.00* | 173.50 | 176.88 | 194.00*** |
|                                                | ±Sx                | 8.40                  | 7.75    | 6.63  | 8.13    | 6.86    |
| **Mercazolilum + Iodomarin**                   | n 10               | 124.38                | 159.00* | 178.50 | 186.25 | 188.00*** |
|                                                | ±Sx                | 6.64                  | 5.47    | 5.73  | 6.66    | 5.87    |

Notes:
1) * – statistically significant differences compared to the beginning of the experiment;
2) ** – statistically significant differences from the group of CP;
3) ≠ p<0.05; ≠≠ p<0.01; ≠≠≠ p<0.001.

for this type of animal and drinking regimen ad libitum.

The animals were sacrificed from the experiment by rapid decapitation. During the autopsy the blood samples were collected in rats, thyroid gland (TG), thymus, spleen, adrenals, testes, ventral portion of the prostate were selected and weighed.

The serum concentration of thyroid hormones – thyroxine (T₄) and triiodothyronine (T₃) was detected by ELISA using commercial kits.

The statistical analysis of the results was carried out using analysis the Excel 2007 software package. The data obtained are presented as the mean (x), its error (±Sx). The null hypothesis concerning the absence of difference between groups was checked using parametric methods (using Student t-test) [4,8]. In the case of multiple comparisons, the null hypothesis was checked using Q-criterion of Dunn [4]. Differences between groups were considered to be significant at the level of statistical significance accepted as p<0.05.

**Results and Discussion**

Mercazolilum is a known thyreostatic, which mechanism of action is in blocking the thyroid hormone synthesis on the level of interaction between mono- and diiodothyrosine and inhibiting the process of thyrosine iodination of thyroglobulin fragments. In addition, mercazolilum application leads to changes in the immune status that are evident in antithyroid antibody titre reduction, improvement of T-lymphocyte-suppressors. In the experiments previously conducted it was shown that the content of albumin and immunoglobulins of all classes in the blood decreased in animals treated with this thyreostatic [1].

In our study the weight of animals treated with mercazolilum after 12 days statistically significant increased compared to the baseline (Tab.1). At the same time there was the weight gain in animals from the group of the intact control. At the end of the experiment (day 33) the body weight of rats from group 2 (CP) was lower than in group 1 (IC) by 13.8%. This result may be manifestation of the lack of thyroid hormones (T₄ and T₃) in animals caused by mercazolilum. It is also well known that along with iodine-containing
thyroid hormone deficiency the growth hormone deficiency, which secretion is related to the level of thyroid hormones, develops.

Autopsy showed that the thyroid weight increased by 226.1% in animals with hypothyroidism on the 33rd day compared to the control (p<0.001), i.e. the evident goitrogenic effect of mercazolilum was observed. In addition, this group of animals had a marked decrease of the thymus and spleen weight compared to those of the intact control group. It is consistent with the literature data concerning the effects of a thyreostatic on the immune system. Other changes in the mass of the examined organs were not found (Tab. 2).

The body weight in the group of animals treated with the extract from Laminaria increased by 13.4% (p<0.05) at the end of the experiment compared to the control and intact groups of animals. In autopsy it was found that the weight of the thyroid gland in rats significantly decreased (by 3 times) when compared to animals treated with mercazolilum alone and did not differ from that of the intact control, i.e. the extract from Laminaria prevented development of the goitrogenic effect of mercazolilum.

In addition, a slight increase in the weight of the thymus and spleen (10.1% and 1.8%, respectively) was also observed in this group of rats compared to the control pathology. It may indicate a positive tendency of the effect of the extract from Laminaria on this indicator. However, the decrease in the weight of prostate (27.9%) was registered. The results obtained need further studies.

The effect of the reference drug Iodomarin used to prevent thyroid disease with insufficient iodine (i.e. diffuse nontoxic goiter, diffuse euthyroid goiter) also resulted in preventing the growth of the thyroid gland weight compared to the group of the experimental animals treated with mercazolilum alone. The thyroid gland weight increased by 82.6% compared to 226.1% in the control group of animals. It should be noted that this effect of Iodomarin was less prominent than the same effect of the extract from Laminaria (Tab. 2). At the end of the experiment the weight gain in rats treated with Iodomarin was less than in the group of animals treated with the aqueous extract from Laminaria under study (17.8% and 20.5%, respectively).

Changes in the thymus and spleen weight in the group of rats treated with Iodomarin were similar to those observed in rats from group 3. Only the prostate weight increased by 8.2% compared to the animals of group 2 and by 23% compared to the control group.

When measuring the concentration of iodine-containing thyroid hormones it was found that

| Indicator     | Statistical index | Intact control | Control pathology | Mercazolilum + extract from Laminaria | Mercazolilum + Iodomarin |
|---------------|------------------|----------------|-------------------|--------------------------------------|--------------------------|
| Thyroid gland, mg | n 6 10 10 | 0.023 0.075** 0.025*** 0.042*** | 0.004 0.020 0.020 0.043 |
| Thymus, mg     | n 6 10 10 | 0.388 0.267 0.294 0.271 | 0.040 0.019 0.020 0.020 |
| Spleen, mg     | n 6 10 10 | 0.986 0.664 0.676 0.686 | 0.155 0.073 0.061 0.029 |
| Adrenal glands, mg | n 6 10 10 | 0.038 0.031 0.032 0.035 | 0.004 0.002 0.002 0.001 |
| Testes, mg     | n 6 10 10 | 2.869 2.976 2.807 2.685 | 0.085 0.104 0.041 0.185 |
| Prostate, mg   | n 6 10 10 | 0.344 0.391 0.282** 0.423 | 0.033 0.027 0.020 0.044 |

Notes:
1) * – statistically significant differences from the group of IC;
2) ** – statistically significant difference from the group of CP;
3) 'p<0.05; **p<0.01; ***p<0.001.
already in 12 days of treatment with mercazolilum (simulation of experimental hypothyroidism) the level of T4 and T3 was significantly lower (by 43.3 and 32.7%, respectively, p<0.05) than in intact animals (Tab. 3).

At the end of the experiment (day 33) the level of hormones remained lower (T4 – by 55.0%; p<0.001 and T3 – 26.0%; p<0.01) compared to the intact control. The ratio T3/T4 representing the character of deiodination in peripheral tissues changed from 0.05 to 0.08. It exceeds the reference values almost by 1.6 times. These data confirm correctness of the experiment in creating the model of mercazolilum-induced hypothyroidism.

The levels of thyroxin and triiodothyronine in serum increased by 37.2% (p<0.01) and 22.1% (p<0.05), respectively, in animals of group 3 compared to hypothyroidism-induced animals, but remained lower compared to the intact group of rats at the end of the experiment (day 33). The ratio of T3/T4 decreased slightly from 0.08 to 0.07. The aqueous extract from Laminaria did not fully re-store the synthesis of thyroid hormones in animals with mercazolilum-induced hypofunction despite its evident antigoitrogenic effect.

The similar results were obtained in the group of animals treated with the reference drug Iodomarin (group 4). The concentrations of T4 and T3 increased by 22.6% (p<0.01) and 11.6%, respectively. Therefore, Iodomarin was inferior to the extract from Laminaria by the specific effect on the thyroid gland.

This effect can be explained by short-term introduction of the extract from Laminaria studied and the reference drug Iodomarin, dose and the mechanism of experimental hypothyroidism development. It was determined that mercazolilum revealed a strong inhibiting action on the biosynthesis of thyroid hormones (group 2 of the experimental animals). Probably the extract from Laminaria as a prophylactic agent would prevent development of the iodine deficiency state and promote earlier restoration of the hormone-synthetic function of the gland. Elucidation of the mechanisms of the thyroid-stimulating effect on the thyroid gland requires further research.

CONCLUSIONS
1. On the model of mercazolilum-induced hypothyroidism the extract from Laminaria in the dose of 1 ml/100 g of the body weight shows the antigoitrogenic effect (prevention of the thyroid weight increase), which exceeds the effect of the reference drug Iodomarin.
2. The extract from Laminaria and Iodomarin in our experiments does not show complete recovery of the thyroid hormone production function (by the level of thyroid hormones in the blood serum) in rats with mercazolilum-induced hypothyroidism compared to intact animals. However, increase of the level of thyroxin and triiodothyronine is observed in animals treated from Laminaria by 37.2% and 22.1%, respectively. When using Iodomarin the increased concentrations of T4 by 22.6% and T3 – by 11.6% were observed.
3. The thyroid-stimulating effect of the aqueous extract from Laminaria studied exceeds the effect of the reference drug Iodomarin.
REFERENCES

1. Bondar T.H. // Visnik Har'kov's'kogo nacional'noho universytetu im. V.N. Karazina. Ser. Biologiya. – 2009. – №878, вып. 10. – C. 102-108.

2. Vladimirova I.M., Goryaznyc B.A. // Farmaci. chasopis. – 2010. – №4. – C. 90-93.

3. Vladimirova I.M., Kruchenko V.M., Goryaznyc B.A. // Klinichna farmatsiya. – 2011. – Т. 15, №3. – С. 67-69.

4. Glanu S.A. Mediko-biologicheskaya statistika. – M.: Pрактика, 1998. – 459 с.

5. Dokl'inye dosl'edjenia l'kar's'kikh zasobiv: Metod. rekomend. / За ред. O.V. Stepanova. – K.: Aviatsiya, 2001. – 528 с.

6. Zagal'nye eticheskie principy eksperimentov na tvarivakh // Endokrinol. – 2003. – Т. 8, №1. – С. 142-145.

7. Kvaenchuk A.N., Kvaenchuk E.L. // Vrachebnoe delo. – 2012. – №3-4. – C. 1-4.

8. Lachin G.F. Biomertitsa: Ucheb. posobie dlja biol. spec. vuzov. – M.: Vysshi. shk., 1990. – 352 c.

9. Problema yododefiцитu v Ukraini [Elektronnyy resurs] – Rezhim dostupa: http://medstrana.com/articles/300/

10. Constantinou K., Marigoula M., Valgana T. // Molecular and Cellular Biochemistry. – 2005. – Vol. 278. – Р. 93-1005.

11. Ordooei M., Mottaghipisheh H., Fallah R., Rabiee A. // Iran J. Child Neurol. – 2014. – Vol. 8 (4). – P. 28-32.

ИССЛЕДОВАНИЕ АНТИЗОБОГЕННОГО ЭФФЕКТА ЭКСТРАКТА ЛАМИНАРИИ НА МОДЕЛИ МЕРКАЗОЛИЛОВОГО ГИПОТИРЕОЗА

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Ключевые слова: экстракт ламинарии; щитовидная железа; мерказолиловый гипотиреоз; тиреоидные гормоны

Представлены результаты экспериментального изучения влияния водного экстракта ламинарии на функцию щитовидной железы в сравнении с йодомарином на модели мерказолилового гипотиреоза. Установлено, что экстракт ламинарии имеет антизобогенный эффект, определенный по результатам измерения массы щитовидной железы, которой превышает эффект йодомарина. Введение экстракта ламинарии и йодомарина не приводит к восстановлению уровня тиреоидных гормонов в крови, который значительно снижается при экспериментальном гипотиреозе. Но, учитывая ранее установленное стимулирующее влияние на гормонсintéзирующую функцию щитовидной железы в интактных тварях, выявленный в эксперименте антизобогенный эффект на модели экспериментального гипотиреоза и определение нами повышения уровня тиреоидных гормонов в конце эксперимента дают основание сделать вывод о тиреоидстимулирующем действии исследуемого водного экстракта ламинарии.

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