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New approaches to postoperative treatment of patients with nodular goiter and autoimmune thyroiditis

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Abstract. Background. Oxidative stress is a multifactorial process involving numerous metabolic pathways in the cell. Thyroid hormones play a significant role in reactive oxygen species production due to their ability to accelerate the basal metabolism and to change respiratory rate in mitochondria. On the other hand, thyroid hormones also differ from the cell antioxidant mechanisms in different ways, thus, creating a multivariate situation whose outcome is difficult to predict. The purpose of this study was to research the activity of oxidant and antioxidant defense in the blood and in the thyroid tissue of patients with nodular goiter against the background of autoimmune thyroiditis (AIT) and their changes due to the use of alpha-lipoic acid in preoperative preparation and in the postoperative period. **Materials and methods.** The activity of oxidant and antioxidant defense in the blood and in the thyroid tissue was studied in 80 patients with nodular goiter and AIT in the pre- and postoperative periods. **Results.** The study showed that in patients with nodular goiter and AIT, there is an activation of peroxidation and an increase in the functional ability of the antioxidant defense elements in the blood and in the thyroid tissue. **Conclusions.** It has been found that inclusion of alpha-lipoic acid in the integrated treatment of such patients contributes to a decrease in the activity of peroxidation, activation of antioxidant defense systems and more rapid restoration of the thyroid function.

Keywords: nodular goiter with autoimmune thyroiditis; oxidant and antioxidant defense; alpha-lipoic acid

Introduction

Surgery of endocrine organs will always face a complex problem of combining surgical radicalism and postoperative recovery of specific hormonal homeostasis. The increase in the incidence of thyroid pathology in Ukraine, associated with iodine deficiency, carcinogenic effects, Chernobyl radionuclide radiation, and disorders of the human immune system caused an increase in surgical activity. First of all, it concerns nodular goiters and tumors, autoimmune thyroiditis (AIT) with nodule formation [1–3]. With an increase in the number of surgically treated patients, the number of cases of postoperative hypothyroidism increases as well [4]. Although using synthetic thyroid hormones solves the problem of compensating the thyroid function, it does not always work [5]. The main drawback of substitution therapy for hypothyroidism with artificial hormones is the lack of feedback in the system of hormonal regulation, which can

only be achieved by a functioning thyroid parenchyma [6–8]. The development of postoperative hypothyroidism has long been the center of attention of researchers. Most of them focus on autoimmune processes in the parenchyma of the gland and on the parenchymal volume loss [9–11]. Other pathogenic factors of hypothyroidism during resection operations remain little known, so do the mechanisms of nodule formation [12–14].

One of the reasons for this is the fact that until now, many aspects of the pathogenesis of nodule formation and, in particular, the development of postoperative complications have not been fully understood.

According to the literature, the peroxidation of lipids and proteins of the cell membranes, which occurs under the influence of excess production of active forms of oxygen (AFO) is the earliest stage in the pathogenesis of AIT. In order to describe the imbalance in the system of antioxidant — prooxidants, the term “oxidative stress”

has been used in recent years. As a result of its development, the destructive processes, that can be pathogenic factors of the disease, become stronger [4, 11, 12, 15]. The actuation of peroxide oxidation processes (PO), depending on the place of generation, distribution, type of free radicals, duration and strength of their actions, as well as on the redox status of the cell, may have different effects on the cell signaling pathways, transcription factors, and some enzyme proteins. It leads to cell survival, their proliferation, or death through apoptosis [16, 17].

Administering analgesic and desensitizing drugs after a surgery on the thyroid gland prevents from correcting other disturbances which occur in the thyroid gland itself. The lack of drugs with targeted antioxidant action in the integrated treatment contributes to uncontrolled progression and activation of peroxide oxidation processes in the blood and the thyroid tissue, which is believed to be the main cause of the development and progression of postoperative hypothyroidism.

The purpose of this study was to research some of the links of the pathogenesis of the development and progression of postoperative hypothyroidism in patients with nodular goiters secondary to autoimmune thyroiditis by investigating the activity of lipid and protein peroxide oxidation in the blood and the thyroid tissue before and after a surgical intervention, and to develop effective methods of their prevention.

Materials and methods

During 2013 to 2016, on the basis of the Chernivtsi Regional Clinical Hospital, 80 women with nodular goiter secondary to autoimmune thyroiditis (NGAIT) were screened. The control and main groups were comparable in age (34.20 ± 10.33 and 38.00 ± 10.62 years respectively, $p = 0.12$), anthropometric data (body mass index — $23.50 \pm 2, 71$ and 24.30 ± 4.88 kg/m², respectively, $p = 0.43$) and the free T₃ level (4.40 ± 0.91 and 4.40 ± 0.93 ng/l, $p = 0.93$), but they differed in the level of free T₄ (16.60 ± 2.02 and 12.90 ± 3.42 μmol/l, $p < 0.0001$), TSH (1.90 ± 0.76 and $4.90 \pm 3,51$ mU/l, $p < 0.0001$) and TPOAb (11.90 ± 13.92 and 255.70 ± 340.58 mUn/l, $p = 0.0009$). In general, the differences between the groups were regular and confirmed an autoimmune lesion and a tendency to a decreasing function against the background of AIT. The diagnosis was confirmed morphologically after a surgical treatment.

The control (I) group of patients only received analgesic drugs in the postoperative period. The experimental (II) group of patients, in addition to this treatment, received 300 units of alpha-lipoic acid intravenously a day before the surgery and every day after it (for 4–5 days), and after the discharge — alpha-lipoic acid 1 tablet (300 mg) per day for 1 month. This is a drug with a targeted anti-oxidant effect. Patients of both groups did not differ in terms of surgical intervention and the method of intraoperative anesthesia. It should be noted that patients in both groups had received substitution therapy with L-thyroxine (1.6 m/kg/day) before the surgery. 7 female patients in the experimental group were of reproductive age.

Prior to the surgery, and on the first, third and fifth days afterwards, all patients were evaluated for the activity of peroxide oxidation and the state of antioxidant systems by measuring the degree of oxidative modification of proteins (OMP) in serum, the activity of ceruloplasmin (CP); in erythrocytes — the content of malonic aldehyde (MA), the activity of Glutathione-peroxidase (GPX) and catalase (CT) according to generally accepted methods. The same blood and plasma parameters in 30 practically healthy donors were studied. The study approved by the Bioethical Commission of the Bukovinian State Medical University, Chernivtsi, Ukraine (Protocol #8, 14.06.2017).

The pro- and antioxidant activity in 5 % homogenates of the thyroid tissue altered due to goiter was investigated by determining the activity of Glutathione-peroxidase, Glutathione-S-transferase, and the degree of oxidative modification of proteins. Autopsied specimens taken from the residents of the Chernivtsi region who died in accidents and whose thyroid gland was normal in volume and structure were used as the control ones. 32 glands obtained in Chernivtsi RCMI “Department of morbid anatomy” were investigated.

Statistical processing of the data was carried out using the Statgraphics program (2010) by calculating the Student’s criterion.

Results

It has been established that in patients with NGAIT there is a significant activation of peroxide oxidation processes — the level of MA in erythrocytes in patients of group I was significantly higher (23.3 %) than that in the

Table 1. Blood antioxidant defense values in patients with nodular goiters secondary to autoimmune thyroiditis in the earliest postoperative period while being treated with alpha-lipoic acid

Values	Donors (n = 30)	1 st day		3 rd day		5 th day	
		Group I (n = 30)	Group II (n = 50)	Group I (n = 30)	Group II (n = 50)	Group I (n = 30)	Group II (n = 50)
		1	2	3	4	5	6
MA, μmol/l of erythrocytes	8,17 ± 1,21	13,27 ± 1,46 P1-2***	9,86 ± 1,17 P2-3***	12,66 ± 1,12 P1-4***	9,11 ± 1,23 P4-5***	12,17 ± 1,52 P1-6***	8,37 ± 0,96 P6-7***
OMP, uod/g of protein	39,61 ± 1,23	56,63 ± 1,77 P1-2***	49,93 ± 1,63 P1-3*** P2-3**	61,03 ± 1,72 P1-4*** P2-4*	42,97 ± 1,92 P3-5** P4-5***	59,33 ± 2,11 P1-6***	40,71 ± 1,74 P3-7** P6-7***

Notes: group I includes the patients who were not administered alpha-lipoic acid; group II consisted of the patients who were administered alpha-lipoic acid; * — probability coefficient P between the groups — < 0,05; ** — < 0,01; *** — < 0,001 (only statistically reliable differences are given).

donors. There was also an increase (by 21.8 %) in their OMP activity.

In patients from the experimental group, after a single administration of alpha-lipoic acid, the level of MA was only higher by 11.4 %, and of OMP — by 14.3 % (table 1).

The activity of antioxidant defense enzymes was found to decrease significantly in patients with NGAIT: the activity of CP — by 11.2 %, of GPX — by 3.6 % and of CT — by 8.2 %. A single administration of alpha-lipoic acid led to a reliable (by 11.5 %) increase in CP activity. At the same time, the activity of CT decreased by 8.9 %, and the GPX remained almost unchanged (table 2).

On the 1st day after the operation the patients from the control group had an increase in the level of MA by 37.3 % and OMP — by 29.7 %, while in the experimental group there was a decrease of the first value by 35.2 %, and OMP remained almost unchanged. On the 3rd day after the operation the patients from the control group had a reliable increase in the level of MA and OMB (31.1 % and 42.3 % respectively), while in the experimental patients these values remained almost unchanged compared to the first day. On the 5th day after the surgery the activity of peroxide oxidation processes in the blood of the patients in the control group remained higher than those before the operation, while in patients in the experimental group these values were significantly lower (table 1).

The activity of CP in blood plasma of patients in the control group was found to decrease progressively from 1st to the 5th day of the postoperative period — from 77,2 ± 5,61 to 59,32 ± 4,42 uod/g of protein, and in patients of the experimental group, it increased reliably from 77,20 ± 5,61 to 97,31 ± 4,42 uod/g of protein (p < 0.001). The same pattern is characteristic of the activity of CT. The GPX activity in patients of both groups decreased significantly to the 3rd day after the surgery and increased on the 5th day, and it was more pronounced in patients from the experimental group (table 2).

Studying the oxidant and antioxidant defense values in the thyroid tissue found that the severity of OMP in the tissue altered due to goiter is reliably higher (by 23.8 %) compared with healthy tissue, while GPX and Glutathione-S-transferase activity is significantly lower. A single administration of alpha-lipoic acid reduces the activity of the processes of oxidative modification of proteins, promotes the activation of antioxidant defense systems: the level of GPX and Glutathione-S-transferase in these patients was significantly (by 9.7 and 28.6 % respectively) higher compared to the patients in the control group, but lower compared with the values in the unchanged thyroid tissue (table 3).

Studying AOD values in patients of both groups showed that CT activity in the patients from Group I

Table 2. Blood antioxidant defense values in patients with nodular goiters secondary to autoimmune thyroiditis in the earliest postoperative period while being treated with alpha-lipoic acid

Values	Donors (n = 30)	1 st day		3 rd day		5 th day	
		Group I (n = 30)	Group II (n = 50)	Group I (n = 30)	Group II (n = 50)	Group I (n = 30)	Group II (n = 50)
		1	2	3	4	5	6
CT, μmol/min • n	168,73 ± 14,40	152,51 ± 12,69	162,27 ± 11,07	149,88 ± 10,19	179,28 ± 16,33	138,96 ± 11,43	181,41 ± 13,72 P6-7***
GPX, μmol/min • n	205 ± 17	181,32 ± 12,68	197,81 ± 13,73	183,55 ± 11,81	219,83 ± 13,67 P4-5*	201,13 ± 10,34	236,82 ± 14,72 P1-7* P6-7**
CP, uod/g of protein	77,20 ± 5,61	62,12 ± 3,87 P1-2*	81,64 ± 4,16 P2-3***	61,88 ± 3,42 P1-4**	85,62 ± 3,78 P4-5***	58,16 ± 2,78 P1-6***	93,39 ± 4,07 P1-7* P6-7**

Notes: group I — includes the patients who were not administered alpha-lipoic acid; group II consisted of the patients who were administered alpha-lipoic acid; * — probability coefficient P between the groups — < 0,05; ** — < 0,01; *** — < 0,001 (only statistically reliable differences are given).

Table 3. Values of oxidant and antioxidant defense in the thyroid tissue of patients with nodular goiter secondary to autoimmune thyroiditis while using alpha-lipoic acid

Values	Unaltered thyroid tissue (n = 32)	The tissue altered due to goiter (control group) (n = 30)	The tissue altered due to goiter (experimental group) (n = 50)
	1	2	3
OMP, uod/g of protein	46,19 ± 2,75	60,68 ± 2,92 P1-2***	44,02 ± 3,96 P2-3***
GPX, μmol/min • g of tissue	191,55 ± 14,55	166,65 ± 15,85 P 1-2*	178,49 ± 10,54 P1-3**
G-S-transferase, μmol/min • g of tissue	24,65 ± 1,82	12,72 ± 1,43 P1-2***	18,61 ± 1,88 P1-3*** P2-3***

Notes: * — probability coefficient P between the groups — < 0,05; ** — < 0,01; *** — < 0,001 (only statistically reliable differences are given).

(table 2) tended to decrease over the entire observation period, while in the patients from Group II it increased progressively, differing from the same value in the patients from group I on the 5–6 days after the surgery.

The activity of GPX in patients of Group I tended to increase during the immediate postoperative period and only on the 5th day after the operation its activeness approached that of the donors. In the patients from the second group the activity of the GPX grew in a more pronounced way. This value was reliably higher than that in the patients of group I on the 3rd day after the surgery, and in comparison with donors it was much higher both on the 3rd and on the 5th days of the postoperative period.

The activity of CP in the patients from Group I tended to decrease, while in the patients from Group II it increased reliably as early as beginning from the 3rd day after the surgery, exceeding the same value in the donors. On the 5th day after the surgery, the CP level was reliably higher than both in group I and in donors.

Discussion

H. Erdamar et al. (2008) found that hypothyroidism was associated with enhanced oxidative stress and lipid peroxidation, and supposed that this might lead to the development and progression of atherosclerosis. Reactive oxygen species (ROS) have been reported to induce oxidative damage to membrane lipids, proteins, and DNA, and might in cell death by necrosis or apoptosis (Hancock J.T. et al., 2001). Both glutathione peroxidase and catalase are major defenses against harmful effects of ROS in cells, and in cultured thyrocytes, both have a high capacity to degrade exogenous hydrogen peroxide (H₂O₂) (Nekrasova T.A. et al., 2011).

Specifically, observations indicate that GPX is involved in the degradation of fairly low H₂O₂ levels, whereas CT is required to degrade H₂O₂ at higher concentrations. It is thus possible that the lower activities of GPX and CAT lead to H₂O₂-induced apoptosis of thyroid cells in Hashimoto's thyroiditis patients. In an *in vitro* study by (Shen H.M. et al., 2001). Impaired capacity of GPX in degrading H₂O₂ in cultured thyroid pig cells aggravated the apoptotic response. This data and presented results suggest the possibility that reduced GPX and CAT activities in hypothyroid patients might participate in initiation of the autoimmune process might lead to H₂O₂-induced damage of thyroid cells related to cystolic oxidative stress.

The mechanism linking hypothyroidism with oxidative stress and antioxidants is unknown. The effects of hypothyroidism on antioxidants parameters have been investigated in hypothyroid patients with intellectual disability (Ai J. et al., 2003). Antioxidant deficiencies may lead to a failure to effectively combat extrinsic factors (i.e., weather, diet, drugs, and physical exercise) and intrinsic factors (i.e., injuries, weakness, and fatigue involved in oxidative stress. An extensive body of evidence now exists confirming that antioxidants are involved in the cellular defense against oxidative stress in a variety of pathological conditions.

It has been suggested that hypothyroidism lead to oxidative stress and to a reduction of antioxidant de-

fenses. In addition, previous experimental studies have reported that hypothyroidism is characterized by endothelial dysfunction of blood vessels (Andreev A.Yu. et al., 2005). In agreement with previous findings, thyroid hormones are involving in combating the toxicity of oxidative stress (Oliylyk V.A., 2006).

Thus, under normal conditions, the protective effect of thyroid hormone against oxidative stress can be explained by the function of antioxidants as a defense system. However, a chronic state of hypothyroidism is characterized by impairments in the redox potential. This lead to free radical chain reactions and to metabolic suppression on antioxidant capacity. Results from this study support the suggestion that the hypothyroidism of patients in some way is linked to the low levels of the major antioxidant molecules found in these patients. The depletion of antioxidants observed in hypo-thyroid individuals may reflected the increased free radical production in the electron transport chain in the mitochondrial inner membrane (Mishunina T.M. et al., 2009).

The increase of free radicals is not compensated, as one would expect, by a decrease of antioxidants. A high oxidative state in hypothyroid people has metabolic and biochemical characteristics such as increased mitochondrial enzyme activity.

Thus, it is likely that patient's cells are damaged by prolonged oxidative stress that far exceeds the capacity of the patient's organs to synthesize antioxidant molecules or to synthesize them from extra cellular sources (Mishunina T.M. et al., 2009).

In this regard, it can be assumed that autoimmune aggression against thyroid hormones, whose structure changes due to the activation of peroxide oxidation processes, is one of the mechanisms of hypothyroidism. Including alpha-lipoic acid in the process of treatment leads to the normalization of hormonal homeostasis. A month after the surgery, the level of TTG practically did not differ from that of donors, which is evidence of normalization of the thyroid gland function and reduction of the signs of hypothyroidism. The remote monitoring results of the experimental group of patients confirm the efficacy of using alpha-lipoic acid in the integrated treatment of postoperative hypothyroidism in patients operated for NGAIT. The 2–3 years after the surgery, the level of TTG practically did not differ from that of donors and in five women of fertile age their pregnancy and delivery were without complications.

Conclusions

1. Patients with nodular goiter secondary to autoimmune thyroiditis experience an activation of the peroxidation and an increase in the functional ability of the antioxidant defense elements in the blood and in the thyroid tissue.

2. A surgery leads to a progressive imbalance between the pro- and antioxidant systems of the blood during the earliest postoperative period.

3. Including alpha-lipoic acid in the integrated treatment of such patients causes a decrease in the activity of the peroxidation processes as well as the activation of the antioxidant systems in the blood and in the thyroid tis-

sue, contributes to a fast functional recovery in the thyroid gland.

4. The remote monitoring results of the experimental group of patients confirm the efficacy of using of alpha-lipoic acid in the integrated treatment of postoperative hypothyroidism in patients operated for NGAIT.

Conflicts of interests. Authors declares the absence of any conflicts of interests that might be construed to influence the results or interpretation of their manuscript.

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Нові підходи до післяопераційного лікування хворих на вузловий зоб і автоімунний тиреоїдит

Резюме. *Актуальність.* Оксидантний стрес — багатофакторний процес, що включає численні метаболічні шляхи в клітині. Гормони щитоподібної залози відіграють значну роль у виробництві активних форм кисню за рахунок їх здатності прискорити основний метаболізм і змінювати частоту дихання в мітохондріях. З іншого боку, гормони щитоподібної залози також відрізняються від антиоксидантних механізмів клітин різними шляхами, створюючи тим самим багатоваріантну ситуацію, результат якої важко передбачити. *Метою* даного дослідження було вивчення активності оксидантного та антиоксидантного захисту в крові та тканині щитоподібної залози в пацієнтів із вузловим зобом на тлі автоімунного тиреоїдиту та їх зміни внаслідок застосування альфа-ліпоєвої кислоти в передопераційній підготовці та в післяопераційному періоді. *Матеріали та методи.* Активність оксидантного

та антиоксидантного захисту в крові та тканині щитоподібної залози вивчалась у 80 хворих на вузловий зоб на тлі автоімунного тиреоїдиту в до- та післяопераційному періодах. *Результати.* У дослідженні було встановлено, що в пацієнтів із вузловим зобом на тлі автоімунного тиреоїдиту спостерігаються активація пероксидації та підвищення функціональної здатності антиоксидантних елементів захисту в крові та тканині щитоподібної залози. *Висновки.* Включення альфа-ліпоєвої кислоти в комплексне лікування таких хворих сприяє зниженню активності пероксидних процесів, активації антиоксидантних систем захисту та більш швидкому відновленню функції щитоподібної залози.

Ключові слова: вузловий зоб з автоімунним тиреоїдитом; оксидантний та антиоксидантний захист; альфа-ліпоєва кислота

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Новые подходы к послеоперационному лечению больных узловым и аутоиммунным тиреоидитом

Резюме. Актуальность. Оксидантный стресс — многофакторный процесс, включающий многочисленные метаболические пути в клетке. Гормоны щитовидной железы играют важную роль в производстве активных форм кислорода за счет их способности ускорить основной метаболизм и изменять частоту дыхания в митохондриях. С другой стороны, гормоны щитовидной железы также отличаются от антиоксидантных механизмов клеток различными путями, создавая тем самым многовариантную ситуацию, исход которой трудно предвидеть. **Целью** данного исследования было изучение активности оксидантной и антиоксидантной защиты в крови и ткани щитовидной железы у пациентов с узловым зобом на фоне аутоиммунного тиреоидита и их изменения в результате применения альфа-липоевой кислоты в предоперационной подготовке и послеоперационном периоде. **Материалы и методы.**

Активность оксидантной и антиоксидантной защиты в крови и ткани щитовидной железы изучалась у 80 больных узловым зобом на фоне аутоиммунного тиреоидита в до- и послеоперационном периодах. **Результаты.** В исследовании было установлено, что у пациентов с узловым зобом на фоне аутоиммунного тиреоидита наблюдаются активация пероксидации и повышение функциональной способности антиоксидантных элементов защиты в крови и ткани щитовидной железы. **Выводы.** Включение альфа-липоевой кислоты в комплексное лечение таких больных способствует снижению активности пероксидных процессов, активации антиоксидантных систем защиты и более быстрому восстановлению функции щитовидной железы.

Ключевые слова: узловой зоб с аутоиммунным тиреоидитом; оксидантная и антиоксидантная защита; альфа-липоевая кислота