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

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, Chornobyl 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”...
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 (NGAIT) in the earliest postoperative period while being treated with alpha-lipoic acid

<table>
<thead>
<tr>
<th>Values</th>
<th>Donors (n = 30)</th>
<th>1st day</th>
<th>3rd day</th>
<th>5th day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I (n = 30)</td>
<td>Group II (n = 50)</td>
<td>Group I (n = 30)</td>
<td>Group II (n = 50)</td>
</tr>
<tr>
<td>MA, μmol/l of erythrocytes</td>
<td>8.17 ± 1.21</td>
<td>13.27 ± 1.46</td>
<td>P1-2***</td>
<td>9.86 ± 1.17</td>
</tr>
<tr>
<td>OMP, μg/d of protein</td>
<td>39.61 ± 1.23</td>
<td>56.63 ± 1.77</td>
<td>P1-2***</td>
<td>49.93 ± 1.63</td>
</tr>
</tbody>
</table>

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).
The activity of CP in blood plasma of patients in the control group was found to decrease progressively from the 1st to the 5th day of the postoperative period — from 77,2 ± 5,61 to 59,32 ± 4,42 μmol/g of protein, and in patients of the experimental group, it increased reliably from 77,20 ± 5,61 to 97,31 ± 4,42 μmol/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 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).

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

<table>
<thead>
<tr>
<th>Values</th>
<th>Donors (n = 30)</th>
<th>Group I (n = 30)</th>
<th>Group II (n = 50)</th>
<th>1st day</th>
<th>3rd day</th>
<th>5th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT, μmol/min • n</td>
<td>168.73 ± 14.40</td>
<td>152.51 ± 12.69</td>
<td>162.27 ± 11.07</td>
<td>149.88 ± 10.19</td>
<td>179.28 ± 16.33</td>
<td>138.96 ± 11.43</td>
</tr>
<tr>
<td>GPX, μmol/min • n</td>
<td>205 ± 17</td>
<td>181.32 ± 12.68</td>
<td>197.81 ± 13.73</td>
<td>183.55 ± 11.81</td>
<td>219.83 ± 13.67</td>
<td>201.13 ± 10.34</td>
</tr>
<tr>
<td>CP, uod/g of protein</td>
<td>77,20 ± 5,61</td>
<td>62.12 ± 3.87</td>
<td>81.64 ± 4.16</td>
<td>61.88 ± 3.42</td>
<td>85.62 ± 3.78</td>
<td>58.16 ± 2.78</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Values</th>
<th>Unaltered thyroid tissue (n = 32)</th>
<th>The tissue altered due to goiter (control group) (n = 30)</th>
<th>The tissue altered due to goiter (experimental group) (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OMP, uod/g of protein</td>
<td>46.19 ± 2.75</td>
<td>60,68 ± 2.92</td>
<td>44,02 ± 3.96</td>
</tr>
<tr>
<td>GPX, μmol/min • g of tissue</td>
<td>191.55 ± 14.55</td>
<td>166,65 ± 15,85</td>
<td>178,49 ± 10,54</td>
</tr>
<tr>
<td>G-S-transferase, μmol/min • g of tissue</td>
<td>24.65 ± 1.82</td>
<td>12.72 ± 1.43</td>
<td>18.61 ± 1,88</td>
</tr>
</tbody>
</table>

Notes: * — probability coefficient P between the groups — < 0.05; ** — < 0.01; *** — < 0.001 (only statistically reliable differences are given).
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 ($H_2O_2$) (Nekrasova T.A. et al., 2011).

Specifically, observations indicate that GPX is involved in the degradation of fairly low $H_2O_2$ levels, whereas CT is required to degrade $H_2O_2$ at higher concentrations. It is thus possible that the lower activities of GPX and CAT lead to $H_2O_2$-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 $H_2O_2$ in cultured thyroid pig cells aggravated the apoptic 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 $H_2O_2$-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 defenses. 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 (Oliynyk 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 leads 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-
References


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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.