Elements of oxidation/reduction balance in experimental hypothyroidism

Elementy bariery oksydacyjno-redukcyjnej w doświadczalnej hypotyreozie

Edward Kowalczyk[1], Jacek Urbanowicz[2], Maria Kopff[2], Julita Ciećwierz[1], Grzegorz Andryskowski[3]

[1]*Department of Pharmacology and Toxicology, Medical University of Łódź, Poland*
[2]*Department of Clinical Chemie and Biochemie, Medical University of Łódź, Poland*
[3]*Department of Internal Diseases and Clinical Pharmacology, Medical University of Łódź, Poland*

Abstract

Background: The aim of this study was to investigate the effect of the decreased level of thyroid hormones on selected parameters of the oxidation/reduction balance by assessing the activity of antioxidant enzymes: superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px); the level of antioxidant vitamins (A, C, and E); and the concentration of compounds reacting with thiobarbituric acid (TBARS).
Material and methods: Investigations involved 20 Belgian giant rabbits of both sexes. Hypothyroidism was induced by intragastric administration of thiamizole. Before this was done, blood was collected from the ear marginal vein (control group) and then the animals received thiamizole through an intragastric tube at a dose of 2 mg/kg b.w. for 21 days. Blood was collected again (the experimental group) and the following determinations were performed:
— in blood serum, the thyroid hormones T3, T4 and TSH;
— vitamin A, C and E blood serum concentrations;
— in erythrocytes, the concentration of compounds reacting with TBARS, SOD and GSH-Px.
Results: A 21-day exposure of rabbits to thiamazole (2 mg/kg b.w./24 h) resulted in a statistically significant decrease of TBARS, a decrease of SOD and GPH-Px activity and in a statistically insignificant decrease in the level of vitamins A, C and E.
Conclusions: Hypothyroidism decreases the level of erythrocytes oxidation/reduction balance by diminishing oxidative lipids damage and by decreasing the activity of antioxidative enzymes, but not by changes in the level of antioxidant vitamins.
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Key words: reactive oxygen species, antioxidants, hypothyroidism

Streszczenie

Wstęp: Celem badania była ocena wpływu zmniejszenia stężenia hormonów tarczycy na wybrane parametry równowagi oksydacyjno-redukcyjnej przez badanie aktywności enzymów antyoksydacyjnych (SOD, GSH-Px), stężenia witamin antyoksydacyjnych (A, C, E) oraz związków reagujących z kwasem tiobarbiturowym (TBARS).
Materiał i metody: Badania przeprowadzono na 40 królikach rasy olbrzym beligijski, obojga płci. Niedoczynność tarczycy wywołano dożołądkowym podawaniem thiamazolu. Przed podaniem thiamazolu pobrano krew żylną (grupa kontrolna), a następnie przez 21 dni zwierzęta otrzymywały dożołądkowo thiamazol w dawce 2 mg/kg mc. Po tym czasie ponownie pobierano krew (grupa badana) i oznaczano:
— stężenie hormonów tarczycy T3, T4 i TSH;
— stężenie witamin A, C i E w surowicy;
— stężenie związków reagujących z kwasem tiobarbiturowym (TBARS) i aktywność dysmutazy ponadtlenkowej (SOD) i peroksydazy glutationowej (GPH-Px) w erytrocytach.
Wyniki: Po 21 dniach dożołądkowego podawania królikom thiamazolu (2 mg/kg mc./24 h) stwierdzono statystycznie znamienne zmniejszenie stężenia TBARS, obniżenie aktywności SOD i GPH-Px oraz statystycznie nieznamienne obniżenie stężenia witaminy A, C i E.
Wnioski: Niedoczynność tarczycy obniża poziom równowagi oksydacyjno-redukcyjnej erytrocytów poprzez zmniejszenie oksydacyjnych uszkodzeń tłuszczów oraz zmniejszenie aktywności enzymów antyoksydacyjnych, a nie poprzez zmiany w stężeniu witamin antyoksydacyjnych. **(Endokrynol Pol 2011; 62 (3): 220–223)**

Słowa kluczowe: wolne rodniki tlenowe, antyoksydanty, niedoczynność tarczycy

Introduction

In mammals, thyroid hormones are humoral factors controlling the basal metabolism. They play a crucial role in the regulation of synthesis and degradation of proteins, vitamin A, E and β-caroten metabolism, tissues sensitivity to catecholamines, antioxidant enzymes activity and, above all, oxidative changes in mitochondria; they determine oxygen consumption in the respiratory chain [1, 2]. Mitochondria are the main

Edward Kowalczyk, Department of Pharmacology and Toxicology, Medical University of Łódź, Pl. Hallera 1, 90–647 Łódź, Poland, e-mail: edward.kowalczyk@umed.lodz.pl

source of reactive oxygen species (ROS) in an organism in physiological conditions owing to the chain of tissue oxidation, which is located in them. Electrons which leak from mitochondria from the respiratory chain cause a one-electron reduction of oxygen which leads to the formation of a superoxide anion radical, a very reactive form of oxygen. The magnitude of superoxide anion radical generation directly derives from oxygen consumption by mitochondria.

Hypothyroidism is a clinical entity caused by insufficient production of thyroid hormone by the thyroid gland. Depression of metabolism in hypothyroidism is described as decreased oxygen consumption and thus the production of ROS. Owing to that, hypothyroidism is believed to protect against damage caused by different active oxygen compounds [3, 4]. However, the data concerning the shift of the oxidation/reduction balance towards the latter in hypothyroidism is limited, and the topic remains controversial [5, 6].

The aim of our study was to investigate the effect of experimental hypothyroidism on elements of the oxidation/reduction barrier by determining:
— in red blood cells:
   a) activity of the antioxidant enzymes superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px);
   b) concentration of compounds reacting with thiobarbituric acid (TBARS);
— in blood serum:
   a) the content of antioxidant vitamins (A, C, and E).

Material and methods

Investigations involved 20 Belgian giant rabbits of both sexes, mean body weight 3.2 kg, fed with LSK fodder (standard granulated fodder). The animals were housed in animal quartering conditions (temperature 18°C, humidity 55%) with free access to water. Hypothyroidism was induced by intragastric administration of thiamizole (Metizol-ICN Polfa/Rzeszów, Valeant). Before this, blood was collected from the ear marginal vein (control group) and then the animals received thiamizole through an intragastric tube at a dose of 2 mg/kg b.w. for 21 days. Blood was collected again (the experimental group), and the following determinations were performed:
— the thyroid hormones T3 and TSH level by the MEIA method and T4 level by the FPIA method on immunochemical analyser (AxSYM, ABBOT) using ABBOT (IMX);
— cholesterol level using Kone Pro biochemical analyser and Bio Merieux reagents;
— in blood serum, vitamin A concentration by the Selvaraj and Sivakumar spectrofluorometric method [7, 8], vitamin C concentration by the Benzie enzymatic

method [9], and vitamin E concentration by the spectrofluorometric method according to Hashim [10];
— in erythrocytes, concentration of compounds reacting with thiobarbituric acid (TBARS) according to Rice-Evans [11];
— in erythrocytes, superoxide dismutase activity (SOD) with Ransod-Randox (cat. no. SD 125) and glutathione peroxidase activity (GSH-Px) with Ransel-Randox (cat. no. RS 505).

The results were subjected to statistical analysis: arithmetical mean and standard deviation were calculated. Where distribution was not normal, Mann-Whitney U test was used to calculate statistical significance.

The study protocol was approved by the Bioethics Committee No. 61/99.

Results

The obtained results are presented in Tables I and II. Table 1 demonstrates the effect of 21-day administration of thiamizole at the dose of 2 mg/kg b.w. on TSH, T3 and T4 hormones and on cholesterol level in rabbits. A significant increase of thyroid-stimulating hormone (TSH) $p < 0.01$ and decrease of triiodothyroxin (T3) $p < 0.001$ and thyroxin (T4) $p < 0.001$ allowed a diagnosis of hypothyroidism. Moreover, the cholesterol level was found to increase statistically significantly ($p < 0.001$) by more than 55%. Table II demonstrates the effect of experimental hypothyroidism on the selected parameters of the oxidation/reduction balance. The concentration of substances reacting with thiobarbituric acid (TBARS) decreased significantly from 24.6 ± 4.5 to 15.3 ± 3.8 μM/L ($p < 0.01$). Superoxide dismutase activity decreased significantly from 112.7 ± 6.4 to 85.7 ± 7.3 U/L, and similarly the activity of glutathione peroxidase decreased significantly from 90.8 ± 5.4 to 58.6 ± 5.6 U/L.

The concentrations of vitamins A, E and C decreased after thiamizole but did not differ statistically significantly from those before the preparation administration.

Discussion

Decreased basal metabolism is a biochemical exponent of hypothyroidism associated with the limitation of oxygen consumption by mitochondrial respiratory chain [1, 2]. The consequence is not only inhibition of the flow of electrons through the chain of tissue oxidation, and decrease of oxidative phosphorylation, but also a decrease in the production of free radicals which are responsible for oxygen toxic activity.

Some authors suggest that hypothyroidism protects tissues against accelerated lipid peroxidation, although the data concerning oxidation/antioxidation in hypothyroidism is incomplete and contradictory [3, 12]. Lipid

Table I. *The level of TSH, T3, T4 and cholesterol in plasma of rabbits exposed to thiamizole (2 mg/kg b.w./24 h) for 21 days*
Tabela I. *Stężenie tyreotreopiny (TSH), trijodotyroniny (T3), tyroksyny (T4) oraz cholesterolu w osoczu królików poddanych działaniu metizolu (2 mg/kg mc./d.)*

	Thiamizole	
	Before administration	After administration
Thyroid-stimulating hormone (TSH) [mU/L]	0.63 ± 0.13	5.14 ± 1.03
Triiodothyroxin (T3) [nmol/L]	2.39 ± 0.48	1.44 ± 0.27
Thyroxin (T4) [nmol/L]	137.8 ± 6.79	57.40 ± 5.20
Cholesterol [mg/dl]	77.10 ± 10.50	120.67 ± 14.80

Table II. *The effect of experimental hypothyroidism on selected parameters of the oxidation/reduction balance*
Tabela II. *Wpływ doświadczalnej hypotyreozy na wybrane parametry równowagi oksydacyjno-redukcyjnej*

Parameter	Thiamizole		Statistical significance
	Before	After	
TBARS [μmol/L]	24.6 ± 4.5	15.3 ± 3.8	$p < 0.01$
SOD [U/L of PRBCc]	112.7 ± 6.4	85.7 ± 7.3	$p < 0.001$
GSHPx [U/L of PRBCc]	90.8 ± 5.4	58.6 ± 5.6	$p < 0.001$
Vitamin A [mg/L]	0.8 ± 0.2	0.7 ± 0.2	No
Vitamin E [mg/L]	6.5 ± 1.8	6.1 ± 2.1	No
Vitamin C [mg/L]	4.2 ± 1.4	3.6 ± 1.1	No

PRBCc — packed red blood cells

peroxidation is the most recognised free radical process in an organism. The notion is understood as the process of unsaturated fatty acids oxidation, in consequence of which peroxides of those acids are formed [13]. In carrying out our own investigations into the decrease of lipid peroxidation, we estimated that the level of compounds reacting with thiobarbituric acid (TBARS) decreased by about 35% compared to the control group.

The results of other authors are controversial. Dariyerli et al. did not find any changes in the content of malonyldialdehyde in rats with thiamizole-induced hypothyroidism [14]. However, an increase of lipid peroxidation attributed to dislipidaemia has been observed in women with hypothyroidism and dislipidemia in investigations of oxidative stress [15]. Both qualitative and quantitative lipid disorders, hyperlipoproteinemia and hypercholesterolemia have been described for a long time in hypothyroidism [16]. In our study, cholesterol levels increased after the administration of thiamizole by 56.5% in relation to the control group. Constantini et al., testing the effect of different concentrations of thyroid hormones on low density lipoproteins, observed an increase of lipid peroxidation which strictly correlated with the level of free thyroxin. In our study, thyroxin levels decreased significantly [17].

The organism system defence against reactive oxygen species called the 'antioxidative barrier' has two kinds of compounds: enzymatic and nonenzymatic. The enzymatic compounds include: superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (Cat). In our study, the decrease of thyroxin level by about 60% caused the decrease of lipid peroxidation index (TBARS) by about 35%, and decrease of SOD activity by about 20% and GSH-Px by 35%. The data concerning SOD or GSH-Px activity in hypothyroidism is contradictory. Some authors have observed decreased SOD activity in hypothyroidism, particularly in peripheral tissues [6, 18], while others have not noticed any changes [14, 19].

A similar situation prevails with GSH-Px activity. Some authors did not find significant differences in GSH-Px activity [14, 19], while others have reported an increase of this activity in hypothyroidism [15, 18, 20]. The latter results are rather surprising, because hydrogen peroxide is a substrate for GSH-Px. This product is generated (among others) in the reaction of superoxide anion radical dismutation catalyzed by SOD, thus the availability of substrate for GSH-Px will be limited when there is no SOD activity or it is decreased.

Vitamins E, A and C belong to major nonenzymatic components of the antioxidative barrier. Vitamin E is

a mixture of tocopherols and tocotrienols. The differences between α, β, γ and δ tocopherols lie in the position of methyl groups in the chromane ring. Most cellular vitamin E is located in membranes, mainly in the lipid layer [21]. The great effectiveness of vitamin E as an oxidant results from the fact that it can inhibit the oxidation process at the initiation and the propagation stages [22]. In our study, the serum level of vitamin E after thiamizole administration decreased statistically insignificantly. However, there is data reporting an increase of vitamin E level in hypothyroidism parallel to an increase of MDA concentration, contradicting the thesis about the inhibition of peroxidation processes in hypothyroidism [23].

Similarly to vitamin E, carotens (α, β) and vitamin A (retinol) formed from β-carotene are hydrophobic antioxidants. Carotens have been demonstrated to prevent oxidation of cell membrane lipids and serum lipoprotein fractions [24]. The antioxidant mechanism of carotens activity can be defined as three processes: extinction of factors initiating peroxidation processes; singlet oxygen scavenging; and scavenging and participation in peroxyl radicals decomposition [25]. The mean serum level of vitamin A in the control group of rabbits was 0.8 mg/l and after the administration of thiamizole it decreased statistically insignificantly. In the studies carried out by Aktuna et al., retinol levels did not differ statistically significantly in hypothyreosis, hyperthyreosis or euthyreosis [26]. It should be added that vitamin A has a multi-level effect on thyroid hormones production [27].

Vitamin C (ascorbic acid) demonstrates hydrophilic properties, and owing to strong reductive properties it is considered to be the main antioxidative factor of the aqueous phase, although it can equally easily react with lipid peroxides [28]. Primates, human beings and guinea pigs have lost the ability to synthesise ascorbic acid because of the loss of the end enzyme of the ascorbate biosynthetic pathway gluconolactone oxidase (E.C.1.1.3.8). This is why they are dependent on ascorbate intake with their food. The rabbits' serum level of vitamin C after thiamizole decreased insignificantly in relation to the control group. It could have been caused by the relatively short half-life of vitamin C (in humans it is 10–20 days) [29]. On the other hand, Moncayo et al. found decreased levels of vitamin C in hyperthyroidism, hypothyroidism, carcinoma and thyroiditis [30].

Conclusions

Hypothyroidism decreases the level of erythrocytes oxidation/reduction balance by diminishing oxidative lipids damage and by decreasing the activity of antioxidative enzymes, but not by changes in the level of antioxidant vitamins.

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