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THE ROLE OF OXIDATIVE STRESS IN THE PATHOGENESIS AND MANAGEMENT OF GRAVES' DISEASE

Abstract: Reactive oxygen species (ROS) are highly reactive molecules as they contain unpaired electron(s). They are formed by different cellular processes are involved in intracellular signalling, and are tightly regulated. A large number of antioxidant systems act as protective mechanism. Situation in which balance between oxidants and antioxidants is disturbed in favour of the oxidants is termed 'oxidative stress' (OS). OS is associated with autoimmune disease, cancer, cardiovascular and neurodegenerative diseases. Graves' disease is an autoimmune disease characterized by the presence of the TSH receptor antibodies in serum. OS is increased in all tissues of Graves' disease patients, especially in retroorbital tissues of orbitopathy patients. Use of antioxidant treatment can modify disease progressions. A recent large clinical trial evaluated effect of selenium on mild Graves' ophthalmopathy and showed that it is effective in treatment of mild Graves' orbitopathy.

Key words: Graves' disease, Oxidative stress, Selenium

Apstrakt: Slobodni kiseonički radikali (SKR) su veoma reaktivni molekuli jer sadrže nesparene elektrone. Oni nastaju u ćeliji u različitim procesima, uključeni su u intracelularnu signalizaciju I veoma su dobro regulisani. Kao zaštitni mehanizam služi veliki broj antioksidantnih sistema. Kada je odnos između oksidanata I antioksidanata poremećen u korist oksidanata nastaje oksidativni stres (OS). OS je udružen sa autoimunim, malignim, kardiovaskularnim i neurodegenerativnim bolestima. Grejvsova bolest je autoimuna bolest koju karakteriše prisustvo TSH receptorskih antitela u serumu. OS je prisutan u svim tkivima obolelih od Grejvsove bolesti, a posebno u retroorbitalnom tkivu obolelih od Grejvs orbitopatije. Primena antioksidanata može da modifikuje tok bolesti. Skorašnje veliko kliničko istraživanje je pokazalo da je selen efikasan u lečenju blagog oblika Grejvsove orbitopatije.

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Introduction

Reactive oxygen species (ROS) are highly reactive molecules as they contain unpaired electron(s). They include partially reduced forms of oxygen, such as hydrogen peroxide, hydroxyl radicals and superoxide anions, and lipid peroxides. ROS act as oxidizing agents. They are formed by cellular respiration, metabolic reactions and protein folding. ROS are involved in intracellular signalling, and are tightly regulated (1). A large number of antioxidant systems act as protective mechanism. Among them are superoxide dismutase which catalyses dismutation of superoxide to peroxide, catalase which catalyses the decomposition of hydrogen peroxide to water and oxygen, while glutathione peroxidase reduces lipid hidroperoxides while simultaneously oxidizing glutathione (2). Situation in which balance between oxidants and antioxidants is disturbed in favour of the oxidants is termed 'oxidative stress' (OS) (3). OS is associated with autoimmune disease, cancer, cardiovascular and neurodegenerative disease (4).

Graves' disease is a most common cause of hyperthyroidism in iodine sufficient areas (5). It is an autoimmune disease characterized by the presence of the TSH receptor antibodies in serum (6). Orbitopathy represents orbit involvement in Graves' disease and in 3 to 5% of the patients orbitopathy is severe (7).

Oxidative stress and the thyroid gland

Synthesis of thyroid hormones requires formation of the hydrogen peroxide, a highly reactive oxidant (8). To protect thyroid cells from ROS a potent antioxidant system exists in thyroid. Peroxiredoxin, glutathione peroxidase, thioredoxin, and catalase are involved in this antioxidant system (9). Peroxiredoxins belong to a family of antioxidant proteins that are well conserved during evolution. Peroxiredoxin 5 (PRDX5) is expressed in the thyroid, mostly in the cytoplasm. The level of expression is correlated with the functional status of thyroid cells, being higher in multinodular goitres, and even higher in hyperthyroid tissues (10). It is obvious that some level of oxidative load is necessary for thyroid function and proliferation. In a healthy thyroid, ROS are produced in an area that is located at the apical pole of the cell in microvilli. However, autoimmune induced ROS production causes ROS accumulation both in the cytoplasm and in nuclei, where it can become toxic. Interestingly, i*n vivo*, both the antioxidant N-acetylcysteine (NAC) and the anti-inflammatory prostaglandin 15deoxy-^{12,14}-prostaglandin J2 (15dPGJ2) protect the thyroid against toxic effects of the OS. It seems, that NAC and 15dPGJ2 mainly act on infiltrating inflammatory cells, reducing the extrafollicular ROS load (11).

It is important to note that tobacco smoke contains thyocyanate that blocks iodine transport into thyrocite. This could increase, H2O2 production and oxidative load, especially when associated with other environmental factors (12, 13).

Poncin and co-workers suggested that thyroid interstitial inflammation depends on the balance of the OS and the antioxidative defences (AOD). In basal, healthy conditions, both OS and AOD are low, and there is no inflammation. Increase in OS balanced by the increase in AOD would lead to minimal inflammation, but unopposed increase in OS would lead to strong inflammation and cell necrosis. Reducing OS would lead to inflammation reduction and vice versa (11, 14).

Oxidative stress in organs and tissues in Graves' disease patients

In Graves' disease increased OS is found in all tissues. Increased markers of OS and decrease in AOD are found in erythrocytes of patients with Graves' disease. Methimazole treatment normalizes all OS markers, but not radioactive iodine treatment (15). Increased markers of OS can be found in plasma of Graves' disease patients, even when they are euthyroid. Levels of OS and AOD markers in thyroid tissue and plasma are higher in Graves' disease patients treated shorter than 6 months, compared to longer treatment (16). Even in subclinical hyperthyroidism oxidative stress and antioxidative response seem to be increased (17). It seems that that the oxidative stress-induced activation of the NF-kappaB pathway might play a role in the autoimmune response in hyperthyroidism (18, 19). It seems that the level of OS is increased in subjects with Graves' ophthalmopathy compared to the other subjects with the Graves' disease. Methimazole treatment normalizes markers of oxidative stress in plasma in subject with Graves' disease, but not in subjects with Graves' ophthalmopathy (20).

Hyperthyroidism is also associated with increased oxidative stress and oxidative damage to lipids and genomic DNA in the aortic wall (21). During hyperthyroidism, there is an increase in myocardial oxidative stress that is associated with lipid peroxidation and protein oxidation. Myocardial antioxidant enzyme activities elevation accompanied by protein expression induction in occurs after four week of hyperthyroidism (22). It seems that oxidative stress plays an important role in cardiac hypertrophy, by the redox activation of AKT1 and JUN/FOS signalling pathways (23). Redox imbalance due to hyperthyroidism induce adaptation of antioxidant systems, also inducing ERK1/2 activation and leading to development of cardiac hypertrophy (24). It is interesting to note that although long-term thyroxin administration causes cardiac hypertrophy it is also associated with enhanced tolerance of the myocardium to ischemia and reperfusion. This response may involve the thyroid hormone induced upregulation of HSP70 (25). In skeletal muscle, hyperthyroidism causes increased oxidative stress associated with oxidative modification in myosin heavy chain causing the decrease in force production (26).

Oxidative stress in Graves' disease and retroorbital tissues

Graves' orbitopathy is caused by inflammation in the orbital connective tissue. Enhanced adipogenesis and overproduction of glycosaminoglycans causes an increase in orbital volume and fibrosis of the extraocular muscles (27). Among the other factors, OS is involved in proliferation of orbital fibroblasts. In orbital fibroblasts, obtained from subjects with severe grave orbitopathy superoxide radicals induce a dose-dependent cellular proliferation. This effect is not observed in fibroblast cultures obtained from control subjects (28). However, superoxide induced fibroblast proliferation could be prevented by methimazole, the xanthine oxidase inhibitor allopurinol, and nicotinamide (28, 29). It is interesting to note that strong negative correlation exists between the ophthalmopathy index and glutathione level (30).

IL-1β is produced by activated macrophages and is an important mediator of the inflammatory response. Adding IL-1 β to cultures of retroorbital fibroblasts causes an increased oxygen free radical production in a dose-dependent manner. This is observed both in Graves' and in control cultures. Total intracellular superoxide dismutase (SOD) activity was stimulated by IL-1 β, both in control and in Graves' cultures. However, in Graves' cultures SOD activity was increased at rest and less responsive to IL-1βstimulation. IL-1β was a potent stimulator of glycosaminoglycan (GAG) accumulation in both normal and GO retroocular fibroblasts. IL-1β significantly stimulated the GAG synthesis in both normal and Graves' fibroblasts cells in a dose-dependent manner. Adding SOD and catalase partially blocked accumulation of the GAG induced by IL-1β (31).

HSP72 is a stress inducible form of cytosolic HSP70. Its expression is induced by the environmental stress, such as heat shock, anoxia, and ischemia. HSP72 has cytoprotective effects and functions as a molecular chaperone in protein folding, transport, and degradation. Moreover, HSP72 can inhibit apoptosis by several different mechanisms. In addition, HSP are potent activators of the innate immune system and they stimulate the production of proinflammatory cytokines. In retroorbital fibroblasts obtained from GO patients, both H_2O_2 and heat stress significantly increased HSP72 expression. Antioxidants, methimazole and PTU reduced H2O2 induced HSP72 expression, and to a lesser degree heat-induced HSP72 expression (32-34).

Oxidative DNA damage was found to be significantly elevated in cultured orbital fibroblasts, but only slightly increased in fibroadipose tissues of patients with Graves' orbitopathy. In patients with Graves' orbitopathy, there was significant correlation between TSH receptor antibody levels and 8-hydroxy-2'-deoxyguanosine (a biomarker of DNA damage) content (35). The presence of oxidative stress parameters in cultured orbital fibroblasts and its correlation with TSH receptor antibody levels represents a good indication that oxidative stress exerts action in GO. Antioxidant have protective effect on OS induced proliferation or damage of orbital fibroblasts (36).

Urinary 8-hydroxy-2'-deoxyguanosine (8-OhdG) is also a marker of oxidative DNA damage. The study by Tsai et al. found that the urinary level of 8-OHdG was significantly increase in GO patients (1.9-fold compared with normal subjects). This increase was pronounced in patients with active GO (2.4-fold compared with normal subjects). Moreover, urinary 8-OhdG level significantly correlated with both clinical activity score and ophthalmopathy index. However, this association become non-significant after adjustment for other parameters, particularly the smoking status. It should be noted that smoker had higher urinary 8-OhdG level than never-smokers, and that smoking was significant factor in multivariate analysis (37). It is well known, from epidemiological studies, that strong evidence for a causal association between smoking and development of Graves' orbitopathy exists (38). Study by Tsai et al. implies that smoking induced oxidative stress contributes to the pathogenesis of Graves' orbitopathy (37). Cigarette smoke extract induces adipogenesis in Graves' orbital fibroblasts, what can be inhibited by antioxidants (39).

One of the major forms of DNA damage induced by OS is 7,8-dihydro-8-oxoguanine, referred in an abbreviated way as 8-oxoguanine (8-oxoG). This type of DNA damage is repaired by the base excision repair pathway. This pathway is initiated by the recognition and excision of the oxidized guanine by a DNA glycosylase. In humans, the major glycosylase is 8-oxoG DNA N-glycosylase 1 (hOGG1). The hOGG1 s located on chromosome 3p25/26 and is highly polymorphic. The C to G substitution at position 1245 in exon 7, results in substitution of serine with cysteine in codon 326, is one of the most important and has been associated with a reduced capacity to repair oxidative DNA damage. Tanrikulu et al. assessed hOGG1 Ser326Cys polymorphism (rs1052133) as a candidate risk factor for GD. They found that Cys/Cys genotype had a 3.5-fold (95% CI: 2.10–6.01, $p < 0.001$) the Cys allele had 1.83-fold (95% CI: 1.43–2.34, $p < 0.001$) increase in the risk for developing Grave's disease in their population (40). The Ser326Cys polymorphism in hOGG1 gene was shown to reduce the hOGG1 activity in both in vitro and in vivo studies (40). As the production of 8-oxoG is increased both in retroorbital fibroblasts and in urine of patients with GD, and correlates with the disease activity it could be argued that reduced hOGG1 activity causes increased DNA damage and increased OS making subject more susceptible to development of Graves' orbitopathy (35, 37).

Antioxidants as treatment for Graves' disease

Treatment of the Graves' disease reduces OS both by rendering patients euthyroid and by the direct effect of antithyroid drugs, particularly methimazole, on OS. Methimazole completely normalized parameters of OS in peripheral erythrocytes, while radioactive iodine did not (15). In cultured fibroblasts methimazole prevented superoxide-induced fibroblast proliferation, while propylthiouracil had little efect

(28). Other forms of treatment for Graves' disease also influence parameters of OS. In euthyroid patients treatment of Graves' ophthalmopathy with oral glucocorticoids significantly reduced urinary level of 8-OhdG (a marker of oxidative DNA damage). It was noted that in patients who had recurrence of GO urinary level of 8-OhdG was high (41). In the study by Akarsu et al. serum levels of serum level malondialdehyde (MDA, a product of ROS degradation of degrade polyunsaturated lipids) was higher in patients with GO, compared to controls and Graves' disease patients without GO. On the other hand, level of glutathione (GSH, a nonenzymatic antioxidant) was decreased in GO patients. Treatment with intravenous or oral methylprednisolone reduced MDA level. However, intravenous methylprednisolone induced more rapid therapeutic response and more rapid reduction in MDA level (in 4 weeks). Twelve weeks after the end of the treatment, clinical activity score and serum level of MDA were the same in both methylprednisolone treated groups (42).

Treatment of Graves' disease with antioxidants is based on a premise of role of the OS in its' pathogenesis. A small trial using allopurinol and nicotinamide showed effectiveness of antioxidant treatment of mild and moderately severe Graves' ophthalmopathy (43).

Selenium is a trace element and is essential for selenoproteins synthesis where selenium functions as a redox centre. Some of selenoproteins like thioredoxin reductase and glutathione peroxidases play the key role in antioxidative defences. Most of the European countries are selenium deficient (44). Previous clinical trials showed some effect of selenium on thyroid autoimmunity (45). A recent large clinical trial evaluated effect of selenium on mild Graves' ophthalmopathy. Patients from several European countries were treated with sodium selenite in a dose of 100 µg twice daily. Selenium treatment was associated with an improved quality of life, less eye involvement and slowed the progression of Graves' orbitopathy, compared to placebo (46).

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