REVIEWS

Dent. Med. Probl. 2012, **49**, 1, 52–56
ISSN 1644-387X

Halina Kwapińska[1], Wirginia Krzyściak[2], Maria Chomyszyn-Gajewska[3]

The Role Played by Selected Parameters of the Antioxidant Defense System in Diabetes Mellitus – Based on the Literature

Rola wybranych parametrów systemu antyoksydacyjnego w cukrzycy – na podstawie piśmiennictwa

[1] Specialist Dental Practice, Cracow, Poland
[2] Department of Medical Diagnostics, Pharmacy Faculty CM UJ, Cracow, Poland
[3] Department of Periodontology and Oral Medicine, Institute of Dentistry CM UJ, Cracow, Poland

Abstract

Free radicals, which are highly reactive and thereby destructive molecules, are known increasingly for their importance as factors in human health and disease. The main problem affecting studies on the role of reactive oxygen species (ROS) in *in vivo* conditions is the short life of these elements. Different biochemical methods are used to examine the effects of the action of ROS on cells and tissue. The effects of free radicals can be studied using a number of different methods, such as the activity of superoxide dismutase (SOD; EC-1.15.1.1), catalase (CAT; EC-1.11.1.6) and glutathione peroxidase (GSH-Px; EC-1.11.1.9). Many studies on free radicals focus on the negative role of oxidative stress on the human body, but do not specify their physiological role. Free radicals in low concentrations can exert a positive physiological influence on the induction of cellular response against infection agents, on signal transmission, and act positively as factors of the response to mitogens. In appropriate concentrations ROSs protect cells participating in the preservation of homeostasis. After exceeding a certain limit we can speak about their destructive role, hence the participation of ROS in inducing oxidative stress. Reactive oxygen species exert an influence on different systemic cells and almost all of them can be damaged under appropriate conditions. This happens through activation of signal transduction, damage to gene expression, or as a result of oxidization modifications of macromolecular compounds. The above complex process is still the subject of numerous studies. Reactive oxygen species can induce cellular changes and affect the development of diabetes and subsequent complications of the disease. The level of oxidative stress in diabetes increases in connection with the course of the disease process and disturbances in oxidative-antioxidative equilibrium. The products of protein and lipid peroxidation in patients with diabetes increase significantly, while levels of antioxidants are considerably reduced. Changes in antioxidative enzymes and glutathione levels in diabetic patients suggest that these parameters may be helpful in the diagnosis and prognosis of this disease and its manifestations (**Dent. Med. Probl. 2012, 49, 1, 52–56**).

Key words: oxidative stress, diabetes mellitus, free radicals.

Streszczenie

Wolne rodniki, wysoko reaktywne i destrukcyjne związki, są znane z powodu swej istotnej roli, jaką odgrywają w ludzkim zdrowiu i rozwoju stanów chorobowych. Głównym problemem w badaniach dotyczących roli reaktywnych form tlenu (RFT) dla zdrowia i choroby w warunkach *in vivo* jest krótki okres półtrwania tych związków. Badając skutki działania RFT w komórkach i tkankach, stosuje się różne metody biochemiczne. Rezultaty działania wolnych rodników mogą być badane z użyciem wielu metod, takich jak: aktywność dysmutazy ponadtlenkowej (SOD; EC-1.15.1.1), katalazy (CAT; EC-1.11.1.6), peroksydazy glutationowej (GSH-Px; EC-1.11.1.9). Wiele badań związanych z wolnymi rodnikami dotyczy negatywnego wpływu stresu oksydacyjnego na organizm ludzki, lecz nie definiuje ich fizjologicznej roli. Wolne rodniki w małych stężeniach mogą wywierać korzystny fizjologiczny wpływ na indukcję odpowiedzi komórkowej przeciw czynnikom infekcyjnym, na transmisję przekazywania sygnału oraz jako czynniki odpowiedzi na mitogeny. W odpowiednich stężeniach RFT chronią komórki, biorąc udział w zachowaniu hemostazy. Po przekroczeniu pewnej granicy można mówić o ich niszczycielskiej roli, stąd udział RFT w indukcji stresu oksydacyjnego. Reaktywne formy tlenu wywierają wpływ na różne komórki układowe

i prawie wszystkie z nich w odpowiednich warunkach mogą zostać uszkodzone. Dzieje się to na drodze aktywacji transdukcji sygnału, uszkodzenia ekspresji genów lub też oksydacyjnych modyfikacji związków wysokocząsteczkowych. Powyższe, złożone procesy są wciąż przedmiotem wielu badań naukowych. Reaktywne formy tlenu mogą indukować zmiany komórkowe i mają wpływ na rozwój cukrzycy oraz następujące powikłania choroby. Poziom stresu oksydacyjnego w cukrzycy zwiększa się w związku z trwającym procesem chorobowym i zaburzeniem równowagi oksydacyjno/antyoksydacyjnej. Produkty peroksydacji białek oraz lipidów u chorych na cukrzycę są znacząco zwiększone, a stężenie antyoksydantów znacząco zmniejszone. Zmiany w stężeniu enzymów antyoksydacyjnych i glutationu u chorych na cukrzycę sugerują, że te wskaźniki mogą być pomocne w rozpoznawaniu i prognozowaniu rozwoju schorzenia (**Dent. Med. Probl. 2012, 49, 1, 52–56**).

Slowa kluczowe: stres oksydacyjny, cukrzyca, wolne rodniki.

Diabetes mellitus (DM) is a widespread disease which affects all nationalities and ages. According to experts' estimates, the diabetic world population is increasing at an alarming rate and will grow from 171 to 366 million patients by 2030, according to the World Health Organisation (WHO) [1]. The number of patients in the overall Polish population will rise to 2.2–2.5 million people. In 2030, 4.5% of the global population will be diabetics [1]. The incidence of diabetes mellitus in Poland varies from region to region from 5.3% to 9% among men and from 4.2% to 7.5% among women [1]. The number of diabetic patients increases with age [2]. To this alarming trend must be added the fact that chronic complications of diabetes micro- and macroangiopathies are causes of a mortality rate in diabetes mellitus patients that is 4 times higher than in the case of healthy individuals. Therefore, the great social importance of the disease is determined not only by the millions of patients it has affected, but also by its high rate of mortality. This explains the intensive studies that have been conducted on this disease.

Oxidative Stress and Diabetes Mellitus

In recent years, the role of oxidative stress in diabetes mellitus as a possible link between metabolic control and vascular complications has been a subject of great interest. There are a number of tenable biochemical pathways connecting hyperglycemia with enhanced production of reactive oxygen species [3–5]. The antioxidant defense of diabetic subjects is impaired, suggesting a disturbed capacity of scavenging harmful free radicals. Hence, patients are exposed to continuously increasing oxidative stress concomitant with prolonged hyperglycemia. The mechanisms by which hyperglycemia causes endothelial dysfunction were unknown for many years. Contemporary clinical observations of diabetics show that the earliest occurring vessel changes include increased vasodilatation and a corresponding increase in the blood stream. Cosentino et al. [5] established that the prolonged action of high glucose concentrations intensifies the synthesis of nitric oxide and superoxide anions in human aorta. These two free radicals interact to form peroxynitrate. The release of arachidonic acid from membrane phospholipids is stimulated and a synthesis of vasoconstrictors prostaglandins PGF_{2a} and TXA_2 is reinforced. Ultimately, endothelial dysfunction occurs. To a large extent these confirm previous hypotheses regarding the inactivation of nitric oxide by the excessive presence of superoxide anions in hyperglycemia [6, 7]. The interaction of advanced glycation end products (AGEs) with their main receptor RAGE in endothelial cells induces intracellular generation of reactive oxygen species (ROS) and vascular cell adhesion molecule (VCAM)-1 expression. The inhibition of Cu/Zn superoxide dismutase inhibited both ROS and VCAM-1 induction, indicating that H_2O_2 by this source is involved as a mediator of VCAM-1 expression by AGEs [8, 9]. The resultant chronic hyperglycemia leads to chronic oxidative stress for all tissues because in abnormally high concentrations glucose forms reactive oxygen species. It has been repeatedly emphasized that this can lead to oxidative damage in the classical secondary targets of diabetes, such as the eyes, kidneys, nerves, and blood vessels. However, it has been much less appreciated that the beta cell itself is also a prime target, which is a case of double jeopardy. This situation is all the more pernicious, because pancreatic islets contain the lowest levels of antioxidant enzyme activities compared to other tissues. This adverse effect of high glucose concentrations is referred to as glucose toxicity. Major manifestations of glucose toxicity in beta cells are defective insulin gene expression, diminished insulin content, and defective insulin secretion [10, 11].

Free Radicals and Antioxidants

Therapeutic strategies against free radicals have mostly focused on augmenting the antioxidant defense system. A novel approach is to pre-

vent free radical generation through antioxidant defense system superoxide dismutase (SOD). The SOD enzyme plays an important role as a marker of oxidative stress in diabetes mellitus [12–14].

The superoxide dismutase catalyzes the conversion of superoxide (O_2^-) to H_2O_2 and O_2 according to the following reaction: $2O_2^- + 2H_2 \rightarrow H_2O_2 + O_2$. SOD enzymes are thought to limit the steady-state concentration of superoxide (O_2^-) formed as a by-product of electron transport chain activity as well as mono-oxygenase enzymatic activity. Superoxide is a weak oxidant but an excellent reductant; therefore, if steady state concentrations of O_2^- are not held in check via the action of SOD enzymes, it is believed that O_2^- could reduce redox active metal ions such as Fe^{3+} and Cu^{2+} to potent oxidants such as Fe^{2+} and Cu^{1+}, which could promote excessive damage to critical biomolecules (e.g., lipids, proteins, nucleic acids) as well as lead to the formation of other reactive oxygen species such as hydroxyl radicals (-OH), organic hydroperoxides (ROOH), alkoxy radicals (RO), and hydroperoxyl radicals (ROO). In addition, O_2^- can react with another biologically significant free radical, nitric oxide (NO·), to form peroxynitrite (ONOO), which can act as a potent oxidant capable of causing damage to critical biomolecules. For these reasons SOD enzymes are generally thought to play a protective role in cellular physiology, and the regulation of these enzymatic activities is thought to promote mammalian cellular responses to a wide variety of biologically significant stresses of toxicological importance [14].

Lipid peroxidation is common to all biological systems, appearing both in developmentally and environmentally regulated processes. During the lipid peroxidation process, polyunsaturated fatty acids (PUFA) in biomembranes, especially linolenic acid, arachidonic acid, and docosahexaenoic acid, are degraded to a wide variety of water-soluble, short-chain carbonyl compounds [15, 16]. Malonaldehyde and other aldehydes, such as alkaneals, 2-alkenals, hydroxyalkenals [17, 18] and phospholipid-bound aldehydes, are generated in the lipid peroxidation process. The major representative of 4-hydroxyalkenals, 4-hydroxynonenal (4-HNE), is the main product formed from omega 6-PUFA. 4-HNE, a highly toxic aldehyde product of lipid peroxidation, a sensitive marker of oxidative damage and lipid peroxidation, can be evaluated by immunohistochemical staining using an anti-4-HNE monoclonal antibody (MAb) [19, 20]. Increased levels of MDA and/or 4-HNE may be a useful marker of oxidative stress in diabetes mellitus [21–24]. Enhanced lipid peroxidation leads to an increase in free-radical activity in type 1 diabetics. This increase in free-radical activity in type 1 diabetes mellitus along with insulin-dependent diabetes mellitus (IDDM) can lead to the activation of stress-sensitive pathways, which may play an important role in complications of diabetes [17, 25, 26].

Glutathione (GSH) has been shown to be a major key player in reduction processes in the following ways: maintaining thiol groups of intracellular proteins; providing reducing power for cysteine, dihydrolipoate, coenzyme A ascorbate, and vitamin E; and as a factor reducing nucleotide monophosphates (NTPs) to dinucleotide monophosphates (dNTPs). GSH is involved in detoxification of endogenous and exogenous compounds, participates in the synthesis of leukotrienes and prostaglandins, serves as a cofactor of various enzymes, stores and transports cysteine, and may even be involved in cell cycle regulation and thermotolerance. It is widely accepted that the two major functions of GSH are as a substrate for the GSH peroxidase-mediated reduction of oxygen free radicals, formed either naturally (as a consequence of aerobic metabolism) or through the metabolism of foreign compounds that are known to oxidized/ /reduced states, and in the biotransformation of exogenous compounds catalyzed by glutathione-S-transferases (GSTs). Oxidative stress is defined as an imbalance between the production and detoxification of oxygen free radicals, and can be of exogenous or endogenous origin. Oxidative stress can cause oxidation of cellular constituents, such as GSH protein thiols, and lipid peroxidation. If oxygen is incompletely reduced, superoxide (O_2^-), hydrogen peroxide (H_2O_2) singlet oxygen ($^1O_2^-$), and hydroxyl radicals (HO^-) are produced. The major protective system against naturally occurring reactive oxygen species is the glutathione redox cycle, which comprises GSH peroxidase, GSH reductase, and a source of NADPH. The cycle uses NADPH and indirectly NADH reducing equivalents in the mitochondrial matrix as well as in the cytoplasm to provide a recycling supply for GSH through the GSH reductase–catalyzed reduction of glutathione disulfide (GSSG). The GSH redox cycle is also involved in the detoxification of reactive drug intermediates, which are generated by bioreduction and cause oxidative stress through redox cycling [26, 27]. Glutathione has also been shown to enhance insulin secretion in elderly subjects with impaired glucose tolerance. There are a number of other preliminary indications that glutathione might be helpful in some diabetic patients, but more research is needed before any meaningful conclusions can be made. Since a number of functionally critical proteins within a cell possess accessible Cys residues, glutathionylation may be considered an important post-trans-

lational modification in the pathogenesis of complex diseases such as diabetes.

In conditions of increased oxidative stress and changes in glutathione levels, many critical parameters of glutathione pathways are liable to undergo glutathionylation in patients with diabetes and its associated complications [27–29]. In recent years, glutathione pathways including GSH, GSSG, GST and glutathione peroxidase (GPx), products of lipid peroxidation such as MDA and 4-HNE, as well as antioxidant enzymes like superoxide dismutase (SOD; EC-1.15.1.1) have been investigated as possible biomarkers of oxidative stress in many diseases, including diabetes mellitus [25, 26, 29–31].

Conclusions

The results of numerous studies suggest that increased lipid peroxidation and NO levels reduce levels of enzymatic and non-enzymatic antioxidants and play a major role in diabetic complications. It still remains a point of discussion whether oxidative stress precedes or merely reflects diabetic complications such as atherosclerosis and nephropathy. The present studies suggest that diabetes is an altered metabolic state of oxidation-reduction and that a convenient approach is to provide therapeutic interventions with antioxidants.

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Address for correspondence:

Maria Chomyszyn-Gajewska
Department of Periodontology and Oral Medicine
Jagiellonian University, Collegium Medicum
Montelupich 4
31-155 Kraków
Poland
Tel.: +48 12 424 54 20
E-mail: mdgajews@cyf-kr.edu.pl

Received: 21.07.2011
Revised: 4.11.2011
Accepted: 24.01.2012

Praca wpłynęła do Redakcji: 21.07.2011
Po recenzji: 4.11.2011 r.
Zaakceptowano do druku: 24.01.2012 r.