Oxidative stress – repair systems of oxidatively damaged biomolecules

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ABSTRACT

Molecular oxygen (O_2), constituting the basis of life on Earth, is classified as a substance with oxidizing properties. Reacting with organic compounds, it leads to their oxidation and at the same time participates in reduction processes. In aerobic organisms, over 90% of oxygen undergoes a total four-electron reduction to produce water molecules ($O_2 + 4\ H^+ + 4e^- \rightarrow 2\ H_2O$). The remaining 10% of oxygen, however, is not fully reduced, which results in the production of molecules referred to as reactive oxygen species (ROS). In high concentrations ROS can interact with cellular components (DNA, proteins and lipids), leading to the oxidation of these macromolecules. The resulting oxidation products interfere with the proper functioning of the body by influencing gene expression, intercellular signaling and apoptosis. These changes have been observed in numerous pathological conditions, such as neurodegenerative, cardiovascular, metabolic, autoimmune diseases, and cancer. However, in the context of evolution, living organisms developed specialized repair mechanisms to prevent cellular accumulation of the products of DNA, protein and lipid oxidation, including enzymatic mechanisms (e.g. nucleases, proteases, phospholipases) or removal of damaged DNA, proteins and lipids by apoptosis or autophagy. This article briefly discusses the mechanisms of oxidative modification of cell components and the main repair systems responsible for the removal of lesions in cells by oxidative damage.

Keywords: oxidative stress, oxidative damage, oxidative stress repair systems

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REACTIVE OXYGEN SPECIES AND OXIDATIVE STRESS

ROS exist in free radical and non-radical forms of oxygen [1]. A oxygen free radical is an atom or molecule capable of independent existence, having one or more unpaired electrons [2,3]. ROS are highly reactive and strive for pair electrons by ridding an excess electron or taking an electron from another molecule [4,5]. When two free radicals share unpaired electrons, a molecule is formed that is not a free radical. Non-radical forms include singlet oxygen ($^1O_2$), ozone (O_3), hydrogen peroxide (H_2O_2), and chloramines. Free radical species are demonstrated by: superoxide anion radical ($O_2^{\bullet-}$), hydroxide radical ($HO_2^{\bullet}$) and hydroxyl radical ($^{\bullet}OH$) [4-11]. Superoxide anion radical plays a major role as the initiator of biological damage as it is a precursor of other ROS. It reacts with a higher number of substances and acts faster than molecular oxygen. It can react with itself, resulting in oxidation of one radical and reduction of the other (dismutation reaction). For example, dismutation products are oxygen and hydrogen peroxide. Hydrogen peroxide is a better oxidant than a reducing agent in comparison with superoxide anion radical. It is characterized by particular reactivity to thiol, indole, imidazole, phenolic, thioester and methionyl groups, leading to oxidative damage to the polypeptide chains and loss of biological function of the proteins [12,13]. It should be emphasized that hydrogen peroxide has the ability to diffuse through a cell membrane and can appear in cellular compartments distant from the sites in which it has been formed. Cell damage by hydrogen peroxide is greater if divalent ions of iron and copper as well as cobalt, nickel, manganese or chromium are present in the environment. Hydrogen peroxide is then involved in a Fenton reaction and transformed into a hydroxyl radical [13]. Hydroxyl radical demonstrates extremely strong oxidizing properties and has the potential to react with all biomolecules, but – in contrast with superoxide anion and hydrogen peroxide – there is no antioxidant capable of neutralizing $^{\bullet}OH$, which makes this oxygen radical the most potent of all ROS [1].

Singlet oxygen reacts with most of the organic compounds present in the cells, because all electrons of these molecules are spin paired, so organic molecules are in the singlet state. The thiol groups of cysteine in proteins are the main site of ozone attack. Ozone also reacts quickly with ascorbate, tocopherols, urate and unsaturated fatty acids. Hypochlorous acid, as a strong oxidant, reacts with compounds containing amino groups, -SH groups of proteins and glutathione as well as iron and sulphur centers of proteins and ions of iron in heme proteins.

The effect of ROS on organic compounds is the formation of an alkoxy radical $RO^{\bullet}$, a peroxy radical $ROO^{\bullet}$, and a semiquinone radical and anionic radical $H\text{-}Ch^{\bullet}$ and $Ch^{\bullet-}$. These reactions may also produce non-free radical forms such as hypochlorous acid HOCl, hypobromous acid HBrO, and hypoiodous acid HOI [5], as well as oxygen and iron complexes of higher oxidation states, e.g. $Fe{=}O^{2+}$ ferryl radical and $Fe{=}O^{3+}$ perferryl radical.

It is important to emphasize that about 90% of ROS generated in the body are formed with the participation of the mitochondrial respiratory chain. The remaining 10% are produced during reactions that occur in various cell structures: cytoplasm, cell nucleus, endoplasmic reticulum. These reactions are catalyzed by: NADPH oxidase (NOX), xanthine oxidase (XO) or L-amino acid oxidase (LAAO) as well as chemical reactions with transition metals, mainly iron and copper. Exogenous sources of ROS are, inter alia, ionizing radiation, ultraviolet radiation, ultrasounds, increased temperature, or metabolism of exogenous chemical compounds, e.g. medicines [14-17].

Importantly, ROS are essential for cell functioning. In homeostasis, ROS act as mediators and regulators of metabolism [18]. They initiate cell differentiation by activating the proteins responsible for cell divisions, and regulate gene expression. ROS participate in the process of cell growth and death, and induce apoptosis. Moreover, they increase capillary permeability, stimulate the transport of glucose into cells, and participate in the formation of high-energy phosphate compounds. ROS also eliminate microbes and induce detoxification of xenobiotics [14,15,19].

Reactive oxygen species generated by the body but not used are neutralized by compounds called antioxidants. These can act preventively by not allowing for the formation of ROS; they can also serve as ROS scavengers (by interrupting chain reactions). Among the mechanisms protecting the body against ROS we can also distinguish the functioning of reparative enzymes that remove the products of ROS reactions with cellular components. Antioxidants are also commonly divided into enzymatic and non-enzymatic. Enzymatic antioxidant defenses include: superoxide dismutases, catalase, peroxidases, myeloperoxidase, and glutathione peroxidases. Non-enzymatic antioxidants (including especially low molecular weight antioxidants) are: uric acid, reduced glutathione, ubiquinone, bilirubin, lactoferrin, albumin, ascorbic acid, α-tocopherol, flavonoids, and carotenoids. Reactions of non-enzymatic antioxidants with free radicals are characterized by lower specificity than in case of enzymatic reactions, which makes these antioxidants crucial in protecting the body against free radicals. Antioxidants inhibit oxidation processes not only by reacting with oxidizing agents, but also with

indirect oxidation products such as free radicals [15,20,21].

If the body is in the homeostatic state, the production and elimination of ROS are in equilibrium. ROS concentration may, however, increase temporarily or permanently, which may lead to a phenomenon known as oxidative stress (OS) [22]. OS is a situation characterized by dysregulation of cellular metabolism and oxidative-mediated degradation of cellular components. It should be borne in mind that OS may also develop in efficient antioxidant systems. Oxidative stress can be caused by factors that we cannot control, e.g. oxygen metabolism, autoxidation of reduced forms of the compounds present in the body, medical treatment, or trauma, but it also occurs in the case of excessive physical activity, alcohol consumption, inappropriate quantitative or qualitative diet, or smoking [23]. It is widely believed that disturbances in cellular redox homeostasis as well as oxidative stress are involved in the pathogenesis of most modern pathological diseases such as genetic diseases (Ataxia Telangiectasia, Bloom syndrome, Down syndrome), metabolic diseases (obesity, insulin resistance, diabetes), and civilization diseases (depression, hypertension, coronary heart disease, atherosclerosis) [3-6]. However, a special share of oxidative stress is attributed to the neurodegenerative diseases. It has been shown that the brain is particularly sensitive to oxidative damage due to the low activity of antioxidant enzymes and high content of unsaturated fatty acids [5].

Studies of the oxidation sequence of proteins, lipids and DNA in various cell types have shown that the most probable primary object of an ROS attack are proteins. Lipids and DNA are macromolecules that are effectively protected by proteins and are therefore a secondary target of ROS attacks. According to Gębicki et all [23] the proportions of primary substrates of the $OH^{\cdot}$ radical reaction in the mammalian cell are: 75% proteins, 21% lipids, and 4% DNA, which is related to the specificity of the mechanism of $OH^{\cdot}$ radical production in the Fenton reaction [23].

OXIDATIVE DAMAGE TO PROTEINS

Oxidation of proteins leads to their denaturation, modification of amino acid residues and enzyme prosthetic groups, as well as fragmentation and aggregation of proteins, which results in modification or loss of their biological functions. Mediators of oxidative damage to proteins are the radicals: $^{\cdot}OH$, H_2O_2 and O_2^{-}. The most active radical is $^{\cdot}OH$. ROS reactions with proteins lead to the formation of alkyl, alkyl peroxide, alkyl hydroperoxide, and alkoxy radicals are formed. High susceptibility to ROS is manifested by residues of sulphur-containing (cysteine and methionine) and aromatic amino acids (tyrosine, tryptophan, phenylalanine) [24,25]. Oxidized cysteine and methionine residues lead to the formation of glutathione disulfide (GSSG) and methionine sulphoxide – reversible forms of amino acids which, due to glutathione reductase (E.C. 1.6.4.2) and methionine sulphoxide reductase (E.C. 1.8. 4.11), are transformed into reduced forms [24]. ROS also demonstrate oxidative properties towards non-protein components of complex proteins. They oxidize carbohydrates and ions of transition metals that are usually loosely bound to proteins, leading to loss of the biological function of the latter [2,23, 25].

Proteins oxidized by ROS become biologically inactive, can initiate the formation of new radicals, oxidize antioxidants (such as glutathione and ascorbate), and lead to the formation of covalent bonds between proteins and DNA [23,26,27].

LIPID PEROXIDATION

Lipid peroxidation is a free radical process of oxidizing unsaturated fatty acids or other lipids in which peroxides of these compounds are formed. It is polyunsaturated fatty acids residues contained in the phospholipids of cell membranes that are primarily oxidized. Lipid peroxidation may occur non-enzymatically or as a result of enzymatic reactions, e.g. during the formation of biologically active compounds such as prostaglandins, thromboxanes, or leukotrienes.

The process of lipid peroxidation has three stages: initiation, prolongation with the formation of peroxide radicals, and termination that causes the formation of a non-radical final product. In lipid peroxidation we can see a “snowball effect” as the reaction lasts until the entire substrate is exhausted.

One of the phenomena occurring in the process of lipid peroxidation is reinitiation, during which lipid peroxides disintegrate and free radicals are recreated. The disintegration takes place in the presence of transition metal ions of iron and copper. Subsequent transformations of peroxidation products lead to the decomposition of poly-unsaturated fatty acid residues and formation of aldehydes, hydroxy aldehydes and hydrocarbons. The final products of lipid peroxidation include: malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), and 4-hydroxyhexenal (4-HHE). They can interact with nucleic acids and proteins, causing gene expression disorders, impaired protein synthesis, altering antigenic properties of proteins, uncoupling oxidative phosphorylation in the mitochondria, and disrupting metabolic processes. Non-enzymatic lipid peroxidation markers are isoprostanes (IsoP) produced in a phospholipid membrane under the influence of ROS [28].

Lipid peroxidation disturbs the structure of the lipid layers of the cell membrane and changes their liquefaction, thus leading to increased permeability of these layers and loss of the integrity of the membrane transport. The binding affinity of receptors and antigenic determinants is altered, which results in the release of cellular lysosomal enzymes and self-destruction of cells [2,14,24-27, 29].

OXIDATIVE DNA DAMAGE

$^{\cdot}OH$ and $^{1}O_2$ cause oxidative DNA damage. $O_2^{\bullet}$ and H_2O_2 do not cause direct changes in the DNA structure. It should be noted, however, that H_2O_2, which can easily penetrate the nuclear membrane, is the substrate in the Fenton reaction which leads to the formation of hydroxyl radical.

The interaction of ROS with nuclear DNA leads to single- or double-strand breaks in the DNA chain, which are toxic to the cell and can cause its death [30]. As a result of DNA oxidation, nitrogenous bases and deoxyribose are damaged, and DNA-protein cross-links are formed. Oxidatively modified products of nitrogen bases: 8-hydroxyguanine, dipyrimidine adducts of adenine and guanine, and thymine glycol are responsible for point mutations in DNA, e.g. G-C-> T-A or C-> T [25, 31]. Mutations of this type affect the growth, division, differentiation and maturation of cells as well as intercellular adhesion, and constitute one of the possible mechanisms for the initiation of malignant transformation. DNA oxidative damage is therefore considered one of the most serious damage caused by oxidative stress. An alternative mechanism of the formation of oxidative DNA damage is the formation of bulky adducts with oxidized proteins or lipids [24]. ROS increase the influx of Ca^{2+} ions to the cell, entailing the activation of DNA-degrading endonucleases dependent on these ions. The increase in the concentration of calcium ions also activates Ca2+-dependent protein kinases which are responsible for the phosphorylation of transcription factors and thereby affect the transcription process [14,25]. Mitochondrial DNA (mtDNA) is particularly sensitive to ROS. It has been found that the amount of mtDNA damage exceeds the level of nuclear DNA damage of the same cell 10 times [24]. This is due to the presence of the respiratory chain in the mitochondria and the lack of histones – proteins that protect DNA from damage. At the same time, it has been observed that the ability to repair mtDNA and proteins related to oxidative phosphorylation and damaged due to replication errors is limited [14,24,25]. Deletion or duplication of a larger gene fragment in mtDNA impairs the energy efficiency of mitochondria, which is typical of aging cells. It has been shown that an increased level of oxidative DNA damage leads to a decrease in synthesis as well as in the activity of enzymes removing these lesions, which is associated with an increase of cancer cases [27,30,32].

REPAIR SYSTEMS OF OXIDATIVELY MODIFIED PROTEINS

Cellular homeostasis depends on the spatial structure of proteins consistent with the genetic make-up, particularly on the folding of the polypeptide chain. Only proteins that are correctly folded and have a stable conformation are fully functional. As previously mentioned, oxidative stress interferes with the folding process of the primary protein structure, resulting in the formation of biologically inactive polypeptide chains. Damaged protein cellular components are repaired or removed with post-translational system of protein quality control formed by chaperone proteins and proteases (ubiquitin-proteasome pathway) and via autophagy by autophagosomes and lysosomes [33-35].

Under physiological conditions, the aim of chaperone proteins is to provide protection against aggregation of newly emerging polypeptides and to mediate their correct folding. Under the conditions of oxidative stress, chaperones protect other proteins against denaturation, inactivation, aggregation of protein complexes, and lead to the degradation of irreversibly damaged proteins.

It is worth mentioning about a group of chaperone proteins referred to as heat shock proteins (HSP). Regarding molecular weight, HSP are divided into families. The HSP100 family includes the so-called ATPases AAA+ (ATPases associated with various activities) responsible for dissociation of protein aggregates and protein reactivation. The HSP90 family and the cooperating complexes HSP70/HSP40 and HSP60/HSP10 act as folder chaperones mediating the folding and restoration of the correct conformation of polypeptide chains inactivated by ROS. ATP-independent chaperones are the HSP25/HSP27 complex called holder chaperones, which binds to the improperly folded proteins into stable complexes to prevent aggregation of these proteins.

The degradation process occurs on the ubiquitin-proteasome pathway and in lysosomes.

Proteasomes are macromolecular enzyme complexes. Each cell is equipped with about 30,000 proteasomes, although their number changes depending on the current cellular needs. These structures are located in the cytoplasm and cell nucleus. The site in which the degradation of cytoplasmic proteins occurs is the 26S proteasome complex consisting of the 20S catalytic subunit responsible for the proteolytic activity, and the 19S complex. The 19S complexes are comprised of subunits involved in unfolding protein substrates using the energy derived from ATP, and subunits

that recognize and bind ubiquitin-labelled protein substrates. It should be noted that the oxidatively modified protein is labelled with an 8.5 kDa polypeptide called ubiquitin in the mono- and poliubiquitination processes (Figure 1). The specific enzymes responsible for finding proteins intended for biodegradation and enzymes helping to label these proteins (and thus enabling the proteasome to recognize the polypeptide destined for destruction) have been isolated. Within the channel formed by the proteasome subunits, a protein substrate molecule lacking spatial structure as a result of unfolding is proteolyzed into short oligopeptides that are degraded to amino acids by cell oligopeptidases [33,34,36,37]. Within the mitochondrial proteome, the removal of unfolded and oxidized proteins is mediated by the ATP-dependent mitochondrial proteases included in the AAA+ proteins.

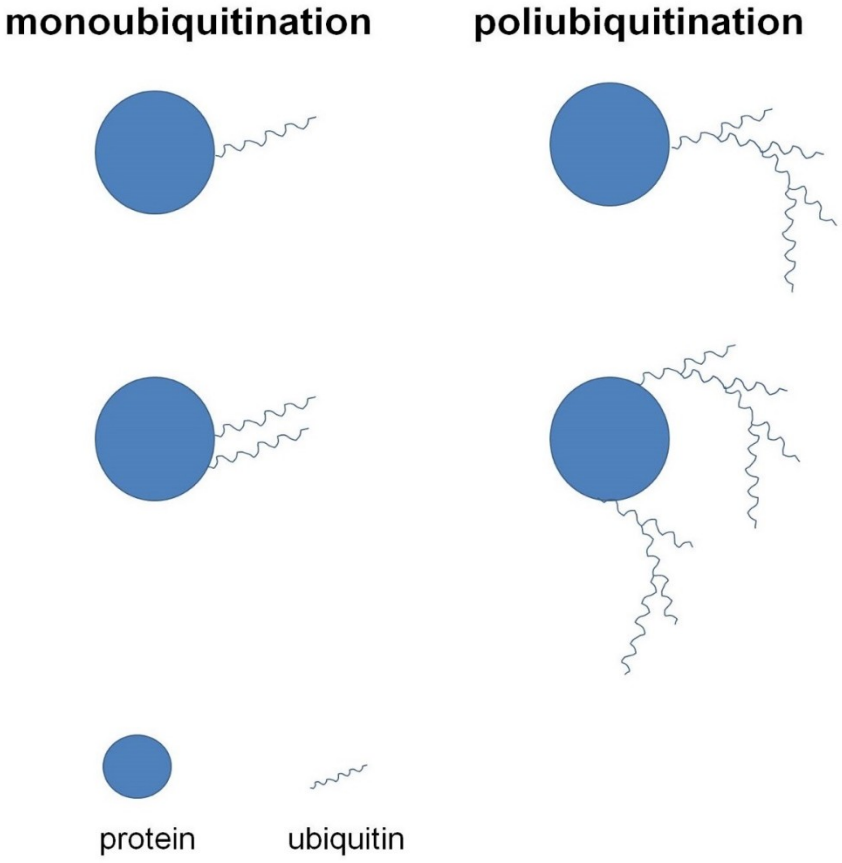

Figure 1. Ubiquitination

In the mitochondrial matrix the Lon and Clp proteases (Cleaves peptides serine proteases) are located, whereas the FTSH protease (Filamentous temperature sensitive H, ATP-dependent zinc metalloprotease) is associated with the inner mitochondrial membrane. These enzymes prevent the formation of protein aggregates by catalysing a hydrolysis reaction of oxidatively damaged proteins to peptides that are broken down to amino acids by mitochondrial oligopeptidases [36,38,39]. It is suggested that the proteasome-mediated protein degradation mechanism is coupled with the autophagy process by means of a molecular linker: HDAC6 protein (histone deacethylase) – a protein responsible for the degradation of misfolded polypeptide chains. In the situation of blocking the activity of proteasomes, their function was performed by autophagosomes activated as a result of overexpression of the HDAC6 gene [29,33,40].

Another important mechanism of degradation of oxidized proteins is their proteolysis in lysosomes containing cathepsins that catalyze the hydrolysis reaction of oxidatively damaged polypeptide chains of proteins. The combined action of numerous cathepsins causes complete degradation of the damaged protein. Several enzymes of this group have been described and marked with letters of the Latin alphabet. The most numerous are cysteine cathepsins (B, H, L), and there are also aspartyl cathepsins (D, E) but these are less numerous. Only cathepsin A is a serine

protease. Based on the manner of delivering the substrate to the lysosome, we can distinguish three basic types of autophagy: macroautophagy occurring with the participation of autophagosomes; microautophagy which is the endocytosis of a cytoplasmic fragment via penetrating inside the lysosome; and chaperone-mediated autophagy (CMA) which involves the transportation of protein substrates containing the KFERQ motif (Lys-Phe-Glu-Arg-Gln) inside the lysosome. The protein with amino acid sequence containing the KFERQ motif, serving as a sequence targeting the lysosome in the cytoplasm, is recognized by the family of HSP70 chaperones. The substrate-chaperone complex is delivered to the surface of the lysosomal membrane where it is bound by the receptor protein LAMP-2A (lysosome-associated membrane protein type 2A) which recognizes the substrate and transports it to the lysosome interior [33,34,41,42].

The formation of cross-links and protein aggregates is very unfavourable for the utilization of damaged proteins. Aggregates of oxidatively modified proteins are weakly susceptible to proteolytic enzymes and inhibit the activity of the catalytic subunit of 20S proteasome, which hinders their degradation. The accumulation of protein aggregates in the cell results in a loss of its biological properties and may cause cell disintegration in the apoptosis or necrosis processes.

Oxidized forms of sulphur-containing amino acids: methionine and cysteine demonstrate the ability to repair oxidative damage. ROS action leads to oxidation of these amino acids, resulting in the formation of methionine sulphoxide, and oxidation of cysteine thiol group results in the formation of disulfide bonds. Due to the glutathione reductase and methionine sulphoxide reductase present in the cells, the aforementioned modifications are directly converted to the reduced forms [29].

Although the body has systems that differentiate oxidatively modified proteins from their native forms, damaged proteins may not be recognized by repair systems. Such situations occur when oxidized amino acids are transformed into other amino acids (e.g. proline into hydroxyproline). It has been demonstrated that this type of transformation of oxidized amino acids favours processes leading to cancer mutations.

REPAIR SYSTEMS OF OXIDATIVELY MODIFIED FATS

Repairing damaged lipids involves removing their damaged fragments. Several groups of enzymes, cooperating with one another, are involved in this process.

Peroxidase and glutathione S-transferase catalyze the coupling of lipid peroxides with glutathione, resulting in the reduction of peroxide to the corresponding alcohol. The reduction of peroxide to alcohol precludes the possibility of reinitiating the chain reaction, i.e. inhibits the lipid peroxidation process. Furthermore, glutathione S-transferase catalyzes the coupling of aldehyde products of lipid peroxidation (e.g. 4-HNE) with glutathione. A certain obstruction in the operation of the abovementioned enzymes is the location of lipid peroxides in the phospholipids inside the lipid layer. In this situation, another enzymatic mechanism of lipid peroxidation repair, involving phospholipase A2 and acyltransferase, is helpful. Phospholipase A2 catalyzes the hydrolysis of glycerophosphates by cleaving to the free fatty acid, preferably the one that is bond to the middle carbon atom of the glycerol residue. This is the very location of peroxidation residues of unsaturated fatty acids. Phospholipase A2 preferentially releases fatty acid peroxides as these are good substrates for peroxidase and glutathione S-transferase. The oxidized lipid peroxides may also be released from membrane phospholipids by the acylhydrolase of the platelet activating factor. Appropriate acyltransferases cooperate with the phospholipase by incorporating new fatty acids in place of the oxidatively modified lipid.

There is also another form of glutathione peroxidase – phospholipid hydroperoxide glutathione peroxidase (PHGPx, GPx4) which is capable of independent reduction of phospholipid peroxides located in cell membranes.

REPAIR SYSTEMS OF OXIDATIVELY MODIFIED NUCLEIC ACIDS

The effectiveness of nDNA (nuclear DNA) and mtDNA (mitochondrial DNA) repair depends on individual activity of approximately 130 proteins belonging to specialized systems [43]. A number of DNA repair mechanisms are distinguished, including: base excision repair (BER), nucleotide incision repair (NIR), nucleotide excision repair (NER), homologous recombination (HR), and mismatch repair (MMR).

1. Base Excision Repair, BER

This path is the basic method of repairing oxidative damage associated with a change in the chemical structure of bases or sugar residues. It enables the removal of etheno adducts of DNA: 1,N6-ethenoadenine, 3,N4-ethenocytosine, N2,3-ethenoguanine, 1,N2-ethenoguanine, as well as propane adducts, e.g. 1,N2(3-hydroxypropane)-2'-deoxyguanosine [29]. In the case of repair of 8-oxo-2'-deoxyguanosine, the process involves the use of specific enzymes and is performed through a mechanism called the ‘GO’ system. In its first phase, phosphohydrolase hMTH1 (human 8-oxo-

dGTPase – a homologue of the bacterial enzymatic protein MutT) removes the defective nucleotide, thus preventing its incorporation into DNA, and then glycosidase hydrolyzes the N-glycosidic bond between the modified base and 2'-deoxyribose. The next stage involves replacing the damaged base with a single nucleotide – it is the so-called 'short DNA repair path'. When a break is formed in the DNA chain, it has to be filled with a few nucleotides – then it is a 'long DNA repair path'[29,30,43]. The BER repair system allows for the elimination of alkylated purines, hypoxanthine, uracil, formamidopyrimidine, thymine glycol and oxidized 2'-deoxyribose. The BER mechanism is also active in case of mtDNA repair. Changes caused by the oxidation of guanine to 8-oxoguanine or deamination of cytosine to uracil are removed by means of glycosidases [29,44,45].

2. Nucleotide Incision Repair, NIR

The NIR mechanism consists in removing oxidatively damaged DNA by means of endonucleases. These enzymes catalyze the reaction of nucleic acid phosphodiester bond hydrolysis at the 5 'end of the oxidized nitrogen base, while the 3'-hydroxyl and 5'-phosphate ends remain free. Delta and epsilon DNA polymerases of 3' to 5' exonuclease activity remove the nucleotide that does not match the template, and then, with the participation of the polymerase, complementary nucleotides are added to the template at the 3' end. The final step is the formation of a phosphodiester bond between the 3' end and the 5' end with the participation of DNA ligase III (Figure 2) [29,45, 46].

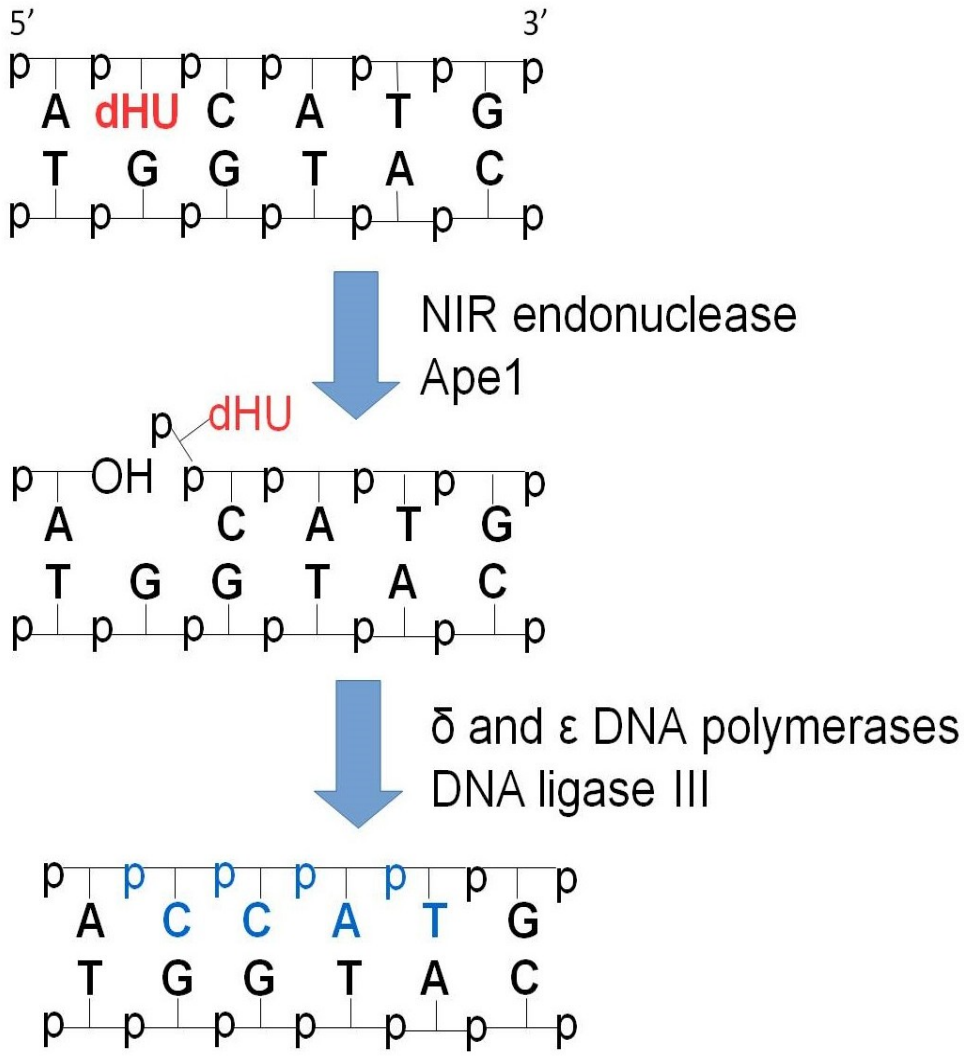

Figure 2. Nucleotide Incision Repair (NIR).
Abbreviations: Ape1, AP endonuclease.

3. Nucleotide Excision Repair, NER

It enables the removal of numerous types of damage, also more complex than those removed via the BER process. The damage types include photoproducts – pyrimidine dimers, intra-strand bonds, large adducts resulting from exposure to aflatoxin, benzopyrene, psoralens, or polycyclic aromatic hydrocarbons. The repair occurs upon identification of a distorted double helix of the damaged nucleotide, nucleotide excision using nucleases, and synthesis of a new DNA strand on a complementary strand matrix. 30 different proteins are engaged in a nucleotide-cut repair. This system is supported by a repair apparatus that is either coupled to transcription or transcription-independent. The NER mechanism does not occur in case of damage within mtDNA. Specific phosphatases are responsible for the elimination of modified free nucleotides in mtDNA as they prevent the incorporation of damaged nucleotides into the DNA chain [29,37,43].

4. Homologous Recombination, HR

It allows for the removal of lesions within large parts of the DNA strand, mainly double-strand breaks, which may cause the loss of some chromosomes or translocation of genetic material between them. There are two main pathways for repairing double-strand breaks: homologous recombination (HR) and non-homologous end joining (NHEJ). There is also a system combining HR and NHEJ features: the single-strand annealing (SSA). Homologous recombination repair ensures the damage removal with simultaneous exact reproduction of the original sequence of the modified DNA. Errors are eliminated as a result of exchange of sister chromatids or homologous chromosomes. The path of DNA repair by non-homologous recombination enables the connection of broken strands but does not require the existence of homology between them. Although NHEJ is much less accurate and generates errors, it is the dominant system in mammals [29,43].

5. Mismatch Repair, MMR

This system of repairing incorrectly paired nitrogen bases removes errors that emerged during DNA replication, or improper base pairs formed as a result of DNA recombination. MMR corrects errors caused by spontaneous or induced deamination, oxidation or methylation of nitrogenous bases. Erroneously paired bases are recognized by protein complexes capable of binding to the damage [24, 29, 30, 43].

The efficient operation of the DNA damage repair systems described above leads to the reconstruction of the correct structure of the damaged genetic material. However, there are situations in which the abovementioned repair mechanisms are not capable of repairing the exiting damage. The so-called SOS (save our souls) system is then activated, making fast, but inaccurate repair of the DNA strand. The SOS system recreates the structure of the DNA strand to the extent enabling DNA replication, but unfortunately the reconstruction of the original nucleotide sequence is extremely rare. The result of repairing the DNA strand by the SOS system is usually a mutation.

CONCLUSIONS

Oxidative stress leads to oxidative damage to DNA, proteins and lipids, which disturbs the course of numerous physiological processes by intensifying inflammatory reactions and affecting the growth, differentiation, proliferation and apoptosis of cells. Oxidative damage to cell components includes DNA rupture, fragmentation of the polypeptide chain, formation of cross-links between proteins, or the formation of oxidized lipid-protein or oxidized lipid-DNA adducts. However, changes in nucleic acids are particularly dangerous as they may lead to mutation of genetic material and thus initiate tumour formation or cause aging of cells. Under physiological conditions, the body is capable of removing oxidative damage due to specialized repair systems. These include both the operation of reparative enzymes (e.g. proteases, phospholipases, nucleases, peroxidases) and removal of cell components via apoptosis. However, in aging bodies and during the decrease in enzymatic and non-enzymatic antioxidant capacity, the activity of the said repair mechanisms may decrease. This state is responsible for the accumulation of oxidative damage in cells and may cause numerous pathologies, the pathogenesis of which results from the participation of oxidative stress.

Conflicts of interest

Authors declare no conflicts of interest.

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