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Reactive oxygen species induced neurodegeneration...

Oxidative damage and free radical production are one of the earliest pathological changes of Alzheimer [1, 2, 3, 4, 5], Parkinson and Huntington diseases, atherosclerosis, diabetes mellitus type 1 [6], psoriatic arthritis [7]. Oxidative stress in Alzheimer’s disease: [8, 9, 10, 11, 12, 13, 14] is a result from an imbalance between the prooxidant and antioxidant systems [9], caused from an overproduction of reactive oxygen species (ROS) and a reduction in antioxidant defense systems [4, 5, 11, 15].

Increased oxidative damage selectively occurs within the brain regions involved in regulation of cognitive performance [16]. The brain neurons are especially sensitive to oxidative inducers: Glutamate and amyloid – beta (Aβ) peptides and are more vulnerable to the toxic effects of free radicals due to: increased oxygen consumption, high content of easily oxidized polyunsaturated fatty acids in neuronal membrane [8, 10], weakened cellular antioxidant defense systems [13], low glutathione content [10] and high rate of catecholamine oxidative metabolic activity [1].

In neuropathology of Alzheimer’s disease the important markers of oxidative stress in brain [17] are: 1) the accumulation in the form of diffuse or neuritic senile plaques [18] of extracellular Aβ1–42 peptides [19, 20] and intracellular Aβ peptides [21]; 2) intracellular neurofibrillary tangles, neuropil threads and dystrophic neuritis, which are: a) accumulated in neuronal perikarya [18]; b) composed of abnormal and hyperphosphorylated microtubule – associated tau protein [22, 23, 24, 25]; c) aggregated into paired helical filaments [13, 14, 19]; d) accompanied by astrogliosis and microglial cell activation; Hirano bodies and granulovascular degeneration of cytoplasm of hippocampal pyramidal neurons [26], losses of neurons, neuropil, and synaptic elements [27]; 3) advanced glycation endproducts [10, 11, 28]; 4) protein oxidation [9, 10, 11, 16, 29]; 5) nucleic acid oxidation [11; 30]: nuclear DNA [10, 11, 31, 32] and ribosomal RNA [33, 34]; 6) lipid peroxidation [10; 11, 29, 35, 36] products: acrolein [37, 38], 4-hydroxy-2-nonenal [38, 39], peroxynitrite [40]; 7) mitochondrial abnormalities [10, 41].

In senile plaques and neurofibrillary tangles are accumulated advanced glycation end products (oxidatively – modified proteins), which: 1) accelerate aggregation of soluble nonfibrillar Aβ peptides; 2) generate ROS, which: induce expression of macrophage colony stimulating factor, enhance proliferation of microglia, increase the production of superoxide radical and nitric oxide [28], cause glycation of

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**REACTIVE OXYGEN SPECIES INDUCED NEURODEGENERATION IN ALZHEIMER’S DISEASE**

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**Abstract.** Oxidative stress is caused by an imbalance between the prooxidant and antioxidant systems. In neuropathology of Alzheimer’s disease the important markers of oxidative stress in brain are senile plaques, neurofibrillary tangles, protein and nucleic acid oxidation, lipid peroxidation and mitochondrial abnormalities. Amyloid – beta aggregates deposition in brain induces oxidative changes and generation of an oxidative microenvironment. The increased levels of soluble amyloid – β1–42 oligomers lead to neurodegeneration, memory deficits and loss of connectivity by causing aberrations in synapse composition, synapse and dendritic loss and abnormalities. Amyloid – beta proteins and mitochondrial oxidative stress cause hyperphosphorylation of tau protein. Soluble hyperphosphorylated tau oligomers disrupt synaptic function and are involved in synapse loss. For pharmacological treatment of Alzheimer’s disease the most important drug discovery strategies include application of antioxidants and inhibitors of acetylcholinesterase, γ – secretase and aggregation of tau protein.

**Key words:** oxidative stress, neurodegeneration, Alzheimer’s disease, antioxidants.
tau, which induces lipid peroxidation [28] and potentiates free radical autoxidation [40].

In mitochondria in frontal, parietal and temporal lobes higher levels of oxidative DNA are observed [32]. The resulting from oxidative stress neuronal DNA damage can cause accumulated synaptic protein alterations [42]. Oxidation of DNA produces DNA – protein crosslinking, strand breaks and sister chromatid exchange [43].

Lipid peroxidation generates: 1) reactive acrolein, which by incorporation into proteins causes the formation of carbonyl derivatives [37]; 2) 4-hydroxy-2-nonenal, which by altering the phospholipid symmetry of the membrane lipid bilayer, leads to apoptotic neuronal loss [44] and promotes Aβ peptides aggregation and fibril formation via covalent modifications of the protein [39]; 3) products, reacting with mitochondrial enzymes, disrupting mitochondrial energetics, increasing free radical release and therefore leading to further oxidative stress [45, 46]. Hydroxyl radicals cause cellular oxidative damage to proteins, lipids and nucleic acids and react with Aβ peptides, leading to the formation of dityrosine cross – linking between Aβ peptides, and enhanced oligomerization and aggregation [47].

By peroxidation of membrane polyunsaturated fatty acids, ROS generate [4] acrolein [11, 37, 38], which is included in formation of neurofibrillary tangles [4]. The increased levels of acrolein and 4-hydroxy-2-nonenal [38, 48, 49] initiate synaptic dysfunction and apoptotic neuronal death by increasing of ROS release into the cytoplasm [4].

Aβ peptide containing senile plaques and neurofibrillary tangles with hyperphosphorylated tau protein are the most important hallmarks in pathology of Alzheimer’s disease and altered in ways characteristic of oxidative damage including: acyl, carbonyl and glycation endproduct modifications, protein cross – linking. The products of lipid peroxidation (acrolein) and Aβ peptides disrupt lipid asymmetry of membe- ne bilayer, which is an early event in apoptosis [13].

In Alzheimer’s disease the formation of the plaques occurs primarily before the onset of cognitive deficits [27] and is a result from the overproduction of Aβ peptides [50], which play a critical role in the cascade leading behavioral deficits [51]. The neurodegenerative process in Alzheimer’s disease is associated with progressive accumulation of intraneuronal Aβ peptides and extraneuronal preplaque Aβ oligomers [52, 53, 54].

Extracellular senile plaques contain a core of Aβ peptides, generated in vivo by specific, proteolytic cleavage of the β - amyloid precursor protein (APP) through sequential cleavages by intramembranous enzymes [55, 56, 57]. The N – terminal cleavage of APP is β - secretase mediated and leads to producing of a membrane – associated C – terminal fragment, which is further cleaved by γ – secretase within the single transmembrane region to obtain Aβ peptides [18, 58]. The phosphorylation of APP at specific residues play an important role in production and accumulation of intraneuronal neurotoxic Aβ peptides [53, 55, 59].

In the process of amyloidogenesis (production and accumulation of the toxic extracellular aggregates of Aβ peptides) are involved complementary factors: 1) mutations of: a) APP gene, located on chromosome 21 [10]; b) presenilin 1 gene in chromosome 14 and presenilin 2 gene in chromosome 1 [60]; c) apolipoprotein E (apo E) gene in chromosome 19 [61]; 2) cytokines, transforming growth factor β1 and interleukin 1 [10].

In Alzheimer’s disease mutations of the APP gene influence APP metabolism [52, 56]. E693 δ mutation in the APP increases the formation of toxic forms of Aβ oligomers. Mutations in APP and presenilin genes lead to increased levels of the Aβ peptides [62], which induce the formation of mitochondrial ROS activation of N-methyl-D-aspartate receptors in hippocampal neurons [63].

The neurodegenerative process in Alzheimer’s disease initiates with axonal and synaptic damage and is associated with progressive accumulation of toxic Aβ oligomers in the intracellular and extracel- lular space [53].

The increased levels of soluble Aβ1-42 oligomers lead to neurodegeneration [64], memory deficits [65], loss of connectivity in Alzheimer’s disease [66], by causing neuronal dysfunction [64], aberrations in synapse composition [66], excitatory synapse loss [67] near senile plaques [68], dendritic abnormalities [69] and dendritic spine loss [70, 71].

Soluble oligomeric proteins are recognized as the main cytotoxic species [72] and cause cellular damage by the following mechanisms: 1) permeabilization of the cellular membranes and lipid bilayers [73]; 2) inserting into membranes and disrupting normal ion gradients; 3) inactivation of functional proteins by sequestering or localizing transcription factors to the wrong cellular compartment; 4) binding to and inactivation of proteasomes [74, 75].

Aβ peptides possess the ability to convert under specific conditions from its soluble form into highly ordered fibrillar aggregates [76]. Oxidative
processes transform nonaggregated β-amyloid into aggregated β-amyloid and produces more free radicals in the presence of free radicals [10]. Different aggregate sizes can induce different degeneration pathways [77].

The extracellular senile plaques in the brain predominantly consist of aggregates of Aβ – a peptide of 39-42 aminoacids [55]. Aβ peptide aggregates are ordered oligomers and oligomerization occurs via distinct intermediates, including oligomers of 3-50 Aβ peptides, annular oligomers and plaques. The most toxic species appear to be small Aβ oligomers [76].

The extracellular accumulation of Aβ peptides in senile plaques impairs acetylcholine synthesis and release, disturbs neuronal signalling, mediated by acetylcholine receptors, decreases the number of cholinergic neurons and reduces the expression of α7 – subtype of nicotinic acetylcholinergic receptors, which are more involved in Aβ peptides neurotoxicity than αβ2 – subtype. This facts suggests that a close relation between Aβ peptides production and cholinergic impairment in Alzheimer’s disease exist. [78].

Aβ1-42 Peptide aggregates play a more critical role in the pathogenesis of Alzheimer’s disease because are more toxic than Aβ40 peptides [79, 80, 81]. Methionine located in residue 35 is critical for the oxidative stress and neurotoxic properties of Aβ1-42 peptides [82].

In Alzheimer’s disease Aβ peptide aggregates deposition in brain induces oxidative changes and generation of an oxidative micro-environment. [8], formation of senile plaques and neurofibrillary tangles, neuronal loss and dementia [50].

Copper (II) modifies APP via the oxidation of cysteines 144 and 158, which leads to the formation of cystine and copper (I), causing production of hydroxyl radicals [10, 83]. In Alzheimer’s disease the e4 allele Apolipoprotein E genotype [61, 84, 85, 86] increases oxidative damage by elevation the (Aβ1-42) induced ROS formation and protein [87] and lipid oxidation[10, 86, 87]. ROS mediates apolipoprotein E peroxidation [10].

Aβ1-40 peptides by metal catalyzed reaction induce ROS production, which mediates disturbing of neuronal cell function and cell death through the oxidation of membrane lipids, proteins [8, 82, 88] and nucleic acids [89].

The Aβ oligomers cause neuronal oxidative stress through an N-methyl-D-aspartate receptor dependent mechanism [63] and induces apoptosis related events in synapses and dendrites [70, 71]. Recent research suggests that neurotoxicity of the Aβ peptides occurs via their interaction with the α7 - subtype of nicotinic acetylcholine receptors [90]. The Aβ1-40 peptide aggregation reduces copper (II) to copper (I), leading to: 1) formation of cell – free hydrogen peroxide [9], which reaction with copper again generates ROS [13, 89, 91]; 2) crosslinking of Aβ peptide, increasing the production of Aβ fibrils [89].

As an inducer of oxidative stress, hydrogen peroxide contribute to the loss of synaptic function and modifications of proteins, lipids and DNA the apoptosis [92] by the following mechanisms: 1) stimulation of the formation of toxic ROS; 2) activation of P2X7 receptors [93]; 3) interaction with iron and copper [92].

The exogenous synthetic Aβ1-42 peptides increase protein oxidation and neurotoxicity in cultured hippocampal neurons. By interacting with vascular endothelial cells [10] and neuronal membranes [8], Aβ peptides form free superoxide radicals, which causes lipid peroxidation [8, 10] and 4-hydroxy-2-nonenal formation [19].

The crosslinked oligomers of reactive Aβ induces generation of ROS and microglial activation [89]. In microglial cells Aβ-amyloid promotes an oxidative stress by generation of ROS superoxide production [10] and nitrogen species (nitric oxide) [94].

Aβ1-42 peptides improve the binding between 4-hydroxy-2-nonenal and glutamate transporters. The deficient functioning of glutamate transporters elevates the concentration of extracellular glutamate, which increases: 1) intracellular ROS; 2) activation of ionotropic glutamate receptors. The result is intracellular calcium overload and excitotoxicity - oxidative stress and cell death [13].

Nitric acid and peroxynitrite appear mediates the neurotoxicity of β-amyloid and to play a crucial role in the excitotoxicity [10, 95]. As a result of local activation of N-methyl-D-aspartate glutamate-receptors activation [10], postsynaptic regions are subjected to high levels of calcium influx and oxidative stress, leading to loss of synaptic proteins, which contributes to synaptic degeneration [4].

In Alzheimer’s disease Aβ peptides induce the oxidative stress by increasing the formation of mitochondrial ROS production [96, 97] via activation N-methyl-D-aspartate glutamate-receptors activation of hippocampal neurons [63]. Aβ peptides and ROS impair mitochondrial function [98, 99], leading to neuronal apoptosis [100] and from an opposite mitochondrial dysfunction promotes brain amyloidosis [101].
Harmful trio “aging, Aβ and tau protein” triggers mitochondrial dysfunction through a number of pathways, such as impairment of oxidative phosphorylation, elevation of ROS production and interaction with mitochondrial proteins, contributing to the development and progression of the Alzheimer’s disease [102,103,104]. Mitochondrial degeneration in dystrophic neurites of senile plaques may lead to extracellular deposition of filament [105].

Mitochondrial dysfunction leads to neuron degeneration in AD through enhances Aβ toxicity [102] by: 1) initiating the depletion of adenosine triphosphate levels [106]; 2) increasing of ROS generation [97,106]; 3) altered calcium homeostasis; 4) activation of the mitochondrial permeability transition, and excitotoxicity [106], which induces apoptosis in synapses and dendrites [42,71].

Aβ and mitochondrial oxidative stress [107] causes hyperphosphorylation of tau [108,109] leading to axonal transport deficits [110]. The tau phosphorylation sites have been characterized as serines and threonines and tyrosine 394 residues [111,112]. Protein kinases that phosphorylate APP are also able to phosphorylate the neuronal protein tau [111].

The intracellular neurofibrillary tangles and neurofil threads are composed of neuronal paired helical filaments and straight filaments [113] of the modified microtubule associated abnormal phosphorylation tau protein [114].

In Alzheimer’s disease the formation of neurofibrillary tangles is associated with a collapse of the microtubule network, disturbances of axoplasmic transports, neuritic atrophy [18]. Neurofibrillary tangles formation leads to the progression of cognitive decline [27] and brain dysfunction, because of synapse and neuronal loss [115], neuronal death [18,115].

In the process of neurofibrillary tangles formation, tau formed different aggregation species: soluble tau oligomers, granular tau, and fibrilar tau. Intracellular aggregates of tau are in soluble or insoluble form [116].

The posttranslational modifications of the microtubule associated tau protein include hyperphosphorylations, glycosylations, enzyme-mediated truncation [23], conformational modifications and the subsequent aggregation [116] in oligomers and paired insoluble helical filaments [117], that deposit as neurofibrillary tangles in the brain [118]. Ther neuroinflammation could play a role for the formation of paired helical filaments [24].

Aggregates of neurofibrillary tangles are toxic to neurons by causing neurotoxic signalling defects and by obstructing the cell function. Factors contributing to accumulation of tau aggregates include the increased rate of protein misfolding, generation of amyloidogenic oligomers, underactivity of repair systems such as ubiquitin-proteasome system or a failure of energy supply and antioxidant defense mechanisms [114].

Soluble hyperphosphorylated tau oligomers disrupt synaptic function and are involved in synapse loss [116]. Insoluble granular tau aggregates lead to neuronal death [115]. In Alzheimer’s disease neurofibrillary tangles formation in the entorhinal cortex is correlated with memory loss in brain and tangles in the limbic and neocortex causes dementia.[115]. The neurofibrillary degeneration leads to dementia even in the absence of amyloid plaques [22].

Normal tau promotes assembly and stabilizes microtubules [115]. The abnormally hyperphosphorylated tau sequesters normal tau and disrupts microtubules. The phosphorylation of tau is regulated by glycogen synthase kinase-3, cyclin dependent protein kinase-5 and protein phosphatase-2A [22].

The oxidation of peptidyl - prolyl cis - trans isomerase decreases its ability for dephosphorylation of tau protein by the activation of cyclic dependent kinase-5 [49], causing neurodegenerative tauopathy [119]. Hyperphosphorylation of tau protein makes it more resistant to proteolytic degradation [120] and leads to oxidative stress by: 1) postoxidation of tau [4]; 2) carbonyl related posttranslational [121] and glutation [122] modification of neurofilament protein. In neurofibrillary tangles presents protein carbonyls, nitrotyrosine, 4-hydroxy-2-nonenal, acrolein, advanced glycation end products and hemeoxyge nase-1 [11].

Stimulation of α4β2 nicotinic acetylcholine receptors inhibits β - amyloid toxicity [123]. γ-secretase inhibitors and vaccination against amyloid protein [124], reduce Aβ40-42 peptides production in brain and are one of the most promising therapeutics for Alzheimer’s disease [125].


Alzheimer’s disease is linked with damaging oxidation reactions and can be prevented by antioxi-
Reactive oxygen species induced neurodegeneration...

Acetylcholinesterase inhibitor Galantamine, determined by TLC – densitometry [131], improves memory and cognitive functions [132] and possesses antioxidant activity [133].

Coumarins which are acetylcholinesterase inhibitors [134] with antioxidant properties [135]. Venlafaxine [136] protects against stress induced oxidative cellular and DNA damage in hippocampus [137].

The new trend in therapy of Alzheimer’s disease pathology is the searching of an effective compounds, possessing both acetylcholinesterase and \( \gamma \)-secretase inhibitory activity, like the synthetized [138] peptide esters of Galantamine: 6-O-N\( \text{N}(3,4\text{-dichlorophenyl})\text{-D,L-Alanil-L-Leucil-L-Glycil}\text{-Galantamine and 6-O-N(N-3,4-dichlorophenyl)-D-L-Alanil-L-Valil-L-Glycil-Galantamine} [139]. This esters possess antioxidant [140] properties and improve dynamics of learning and memory [141].

For pharmacological treatment of Alzheimer’s disease [142, 143] the most important drug discovery strategies [144] include therapy with inhibitors of acetylcholinesterase, \( \gamma \)-secretase, [125] and aggregation of tau protein [114] and antioxidants.

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