Review Article
Mechanism of Oxidative Stress in Neurodegeneration
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OXIDATIVE STRESS & NEURODEGENERATION
OUTLINE

- Oxidative Stress & Neurodegeneration
- Oxygen, Brain & Oxidative Stress
- ROS Producers in Mammalian Brain
- The Antioxidant System
- Oxidative Stress in Neurodegenerative Diseases (AD & PK)
- Mechanisms of Oxidative Stress: ROS Production by Mitochondrial Dysfunction
- Mechanisms of Oxidative Stress: ROS Production via NADPH Oxidase
- Use of Antioxidant Therapy in Neurodegenerative Disease
Oxidative Stress & Neurodegeneration

- Oxidative stress is important in the etiology of a variety of late onset neurodegenerative diseases
- **Aging** has been established as the most important risk factor (Alzheimer’s disease (AD), & Parkinson’s disease (PD))
- **Aging:** cumulative oxidative stress leads to mitochondrial mutations, mitochondrial dysfunction, & oxidative damage
- Is oxidative stress a phenomenon of dysfunctional & dying neurons, or does oxidative stress itself cause the dysfunctionality/death of neurons?
- How does a global event such as oxidative stress result in the selective neuronal vulnerability seen in most neurodegenerative diseases?
- & finally, if oxidative stress is truly fundamental to pathogenesis, then why has the use of antioxidant therapy been thus far largely unsuccessful in such diseases?
In order to address these questions:
- Definition of oxidative stress
- Show how ROS is generated in the human brain
- The antioxidant defense mechanisms
- Is there an evidence that oxidative stress can be found in neurodegenerative disease?
- Is oxidative stress truly pathogenic in disease models?
- What treatment experimental studies have been performed?
Oxygen, Brain & Oxidative Stress

- **Oxygen** is essential for the normal function (respiration, high redox potential, excellent oxidizing agent)
- Neurons & astrocytes, are responsible for the massive consumption of O₂ (~2% vs. >20%)
- The state of **hyperoxia** produces toxicity (including neurotoxicity)
- Partially reduced forms of oxygen are highly active (**ROS**)
- Varieties of (ROS): superoxide (**O•−2**), hydrogen peroxide (**H₂O₂**), & the hydroxyl radical (**OH•**) (the most reactive)
- The modern use: radicals & non-radicals (**O₃, O₂, OH−**)

**What do they do?** Chemically interact with biological molecules

- Aerobic organisms survive its presence only because they contain **antioxidant defenses**
Oxygen, Brain & Oxidative Stress

- Brain cells require **more effective** antioxidant protection:
  - *They exhibit higher* (10-fold) oxygen consumption
  - *Non-dividing* cells (long life duration)
  - *Nitric oxide has a prominent role in the brain* (*RNS*)

- **Oxidative stress**: is a condition in which the balance between production of ROS & level of antioxidants is significantly disturbed & results in damage to cells by excessive ROS

- ROS may target several different substrates in the cell, causing protein, DNA, RNA oxidation, or lipid peroxidation
Oxygen, Brain & Oxidative Stress

**Reactive oxygen species (ROS)**

- Oxygen ($O_2$) + 1e → Superoxide ($O_2^-$) → Phagocyte oxidase (phox) → Superoxide dismutase → Hydrogen peroxide ($H_2O_2$) + 1e → Hydroxyl radical ($OH^•$) → Water ($H_2O$)

**Reactive nitrogen species (RNS)**

- Guanidino nitrogen of l-arginine → Nitric oxide synthase (iNOS) → Nitric oxide (•NO) → RSH + $O_2$ → NO$_2^-$ → NO$_2^-$ → Nitrite → •NO$_2$ → Nitrogen dioxide → NO$_3^-$ → Nitrate

- RSH sulphydryl → H$^+$ + e → RSNO → Nitrosothiol
Oxygen, Brain & Oxidative Stress

- The peroxidation products of **polyunsaturated fatty acids**, especially **arachidonic acid & docosahexanoic acid** which are abundant in brain, are **malondialdehyde & 4-hydroxynonenal** (markers)

- ROS attacks **protein**, oxidizing both the backbone & the side chain, which in turn reacts with amino acid side chains to form **carbonyl functions** (oxidation of **lysine, proline, arginine, & threonine** can yield aldehydes and ketones)

- ROS attacks **nucleic acids** in a number of ways, causing DNA-protein crosslinks, breaks in the strand, & **modifies purine & pyridine bases** resulting in DNA mutations
# Oxygen, Brain & Oxidative Stress

<table>
<thead>
<tr>
<th>PROTEINS</th>
<th>LIPIDS</th>
<th>DNA</th>
</tr>
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<tbody>
<tr>
<td>-SH groups</td>
<td>malondialdehyde</td>
<td>2,6-diamino-4-hydroxy-formamidopyrimidine</td>
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<tr>
<td>GSH/GSSG</td>
<td>8-isoprostaglandin</td>
<td>4,6-diamino-5-formamidopyrimidine</td>
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<tr>
<td>3-nitrotyrosine</td>
<td>F&lt;sub&gt;2&lt;/sub&gt;-isopropane</td>
<td>8-hydroxyadenine</td>
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<tr>
<td>3-chlorotyrosine</td>
<td>TBARS</td>
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<tr>
<td>dityrosine</td>
<td>conjugated dienes</td>
<td>8-hydroxyguanosine</td>
</tr>
<tr>
<td>carbonylated proteins</td>
<td>4-hydroxy-2-nonenal</td>
<td>5-hydroxycytosine</td>
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ROS PRODUCERS IN MAMMALIAN BRAIN
NADPH Oxidase

- A multi-subunit enzyme complex
- Is a member of the NOX gene family
- Also called NOX\textsubscript{2} & phagocytic oxidase (PHOX)
- Seven NOX genes have been identified
- The most expressed of the NOX enzymes in the brain is NOX\textsubscript{2}
- The enzyme transfers the proton across the membrane, & the end product of the enzyme is superoxide
**Xanthine Oxidase**

- It is a complex **molybdo-flavo-enzyme**
- A key enzyme of **purine catabolism**
- XO catalyses the oxidation of a wide range of substrates & pass electrons to molecular oxygen to produce uric acid, superoxide, & hydrogen peroxide
Mitochondria

- Mitochondria (electron transport chain-ETC), in contrast to other cellular producers of ROS, **generate free radicals all the time**
- Mitochondria, which harbor the bulk of oxidative pathways, **leak single electrons to oxygen**
- Depending on the metabolic conditions, isolated mitochondria **produces superoxide** in e.x.;
  - **Respiratory complex I**
  - **Complex III**
  - **α-ketoglutarate dehydrogenase**
- The production of **superoxide is dependent** on the value of mitochondrial membrane potential
Inhibition of neuronal respiration leads to a significant increase in ROS in mitochondria

Overproduction of ROS in mitochondria leads to imbalance & induce oxidative stress & neurodegeneration

This effect can be reduced by mitochondrial uncouplers

Significant neuroprotection by mild uncoupling with UCP2 in cerebral stroke

Mutations in mitochondrial complexes I–IV leads to activation of ROS production & neuronal cell death
Monoamine Oxidase

- Flavoenzymes
- Mitochondrially located (outer membrane)
- Monoamine oxidase A & B (MAO A & B); ~70% identical
- Their role in oxidative catabolism of important amine neurotransmitters (serotonin, dopamine, & epinephrine)

- Expressed in neurons (MAO-A) & glial cells (MAO A & B)
- MAO breaks down monoamines using FAD & results in the production of aldehydes. The FAD-FADH2 cycle generates hydrogen peroxide
THE ANTIOXIDANT SYSTEM - ENZYMES
Superoxide Dismutases

- Play a crucial role in scavenging $O_{2}^{-2}$
- Specialized in eliminating superoxide anion radicals
- Three distinct isoforms:
  - Copper-zinc superoxide dismutase (Cu/Zn SOD)
  - Manganese superoxide dismutase (Mn SOD)
  - Extracellular superoxide dismutase (EC SOD)
Glutathione Peroxidases

- A family of multiple isozymes
- Catalyze the reduction of $H_2O_2$ to water using reduced glutathione (GSH) as an electron donor
  
  \[ (H_2O_2 + 2GSH \rightarrow GS-SG + 2H_2O) \]
- In mammalian tissues, there are four major selenium-dependent glutathione peroxidases
- GPX1 is known to localize primarily in glial cells, in which GPX activity is tenfold higher than in neurons
Catalase

- Catalase is a **ferriheme**-containing enzyme
- Converts **hydrogen peroxide to water**
- It is localised in **peroxisomes** & may also be found in cytoplasm & mitochondria

\[
2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2
\]
THE ANTIOXIDANT SYSTEM -
NON-ENZYMATIC ANTIOXIDANTS
**GSH**

- The main antioxidant in CNS
- The most abundant small molecule, non-protein thiol in cells
- Consists of a tripeptide
- Reduced GSH can **non-enzymatically act directly with free radicals**, notably superoxide radicals, hydroxyl radicals, nitric oxide, & carbon radicals for their removal
- GSH peroxidase & GSH reductase can act **enzymatically** to remove H₂O₂ & maintain GSH in a reduced state
Vitamin E

- A lipid soluble molecule with **antioxidant function** (mainly)
- It appears to neutralize the effect of peroxide & prevent lipid peroxidation in membranes
OXIDATIVE STRESS OCCURS IN NEURODEGENERATIVE DISEASES
Alzheimer’s disease

- The most common neurodegenerative disease, affecting approximately 16 million people worldwide
- Characterized by progressive neuronal loss associated with aggregation of protein as extracellular amyloid (βA) plaques, & intracellular tau tangles
- AD brains also show evidence of ROS mediated-injury;
  - Increase in levels of malondyaldehyde & 4-hydroxynonenal in brain & cerebrospinal fluid
  - Protein carbonyl moieties are increased in the frontal & parietal cortices, & hippocampus with sparing of the cerebellum
  - Increase in hydroxylated guanosine
Parkinson’s disease

- The second most common
- Characterized by progressive loss of dopaminergic neurons in the substantia nigra, & aggregation of the protein α-synuclein
- Concentration of PUFAs in the substantia nigra is reduced, while the levels of lipid peroxidation markers (malondialdehyde & 4-hydroxynonenal) are increased
- Protein oxidative damage in the form of protein carbonyls is also evident
- Increased levels of 8-hydroxydeoxyguanosine
**Mechanisms of Oxidative Stress: ROS Production by Mitochondrial Dysfunction**

- Mitochondrial pathology is evident in many neurodegenerative diseases including AD & PD
- The spectrum of **mitochondrial dysfunction** is vast;
  - *Respiratory chain dysfunction*
  - *Oxidative stress*
  - *Reduced ATP production*
  - *Calcium dysregulation*
  - *Mitochondrial permeability transition pore opening*
  - *Deregulated mitochondrial clearance*
ROS Production by Mitochondrial Dysfunction; PD

- A reduction in complex I activity in the substantia nigra
- The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been shown to produce parkinsonian symptoms
- L-methyl-4-phenylpyridinium (MPP+), the active metabolite of MPTP, can block ETC (same site as rotenone)
- Rotenone or MPP+ also produces superoxide anions in sub-mitochondrial particles
- Mild uncoupling of mitochondria with UCP2 overexpression reduces ROS production (MPP+, rotenone)
- The identification of a number of PD-related genes that are strongly associated with mitochondrial function (PINK1, DJ-1, & Parkin)
Oxidative stress is a primary event in PD pathogenesis.

- Mutations in PINK1 (mitochondrial kinase) cause a recessive form of PD.
- PINK1 deficiency results in inhibition of complex I, reduced substrate availability, and rotenone-like increased production of ROS in mitochondria.
- Abnormal aggregation of protein is a characteristic feature of neurodegenerative disease; aggregation of the protein, α-synuclein, which accumulates in all PD brain.
- Mutations in α-synuclein gene cause a familial form of autosomal dominant PD.
- Expression of mutant α-synuclein in neurons results in increased ROS production.
- α-synuclein binds mitochondria and induce mitochondrial fragmentation.
ROS Production by Mitochondrial Dysfunction; AD

- A reduction in complex IV activity in mitochondria from the hippocampus
- Deregulation of calcium homeostasis;
  - βA causes increased cytoplasmic calcium levels & mitochondrial calcium overload, resulting in increase in ROS production & opening of the PTP
- βA directly interact with cyclophilin D (a PTP component) forming a complex in the mitochondria that has reduced threshold for opening
- Fragmented mitochondria are seen in AD hippocampus
Use of Antioxidant Therapy in Neurodegenerative Disease

- The rationale for the use of antioxidants as therapies is clear.
- The benefits of antioxidants in animal & cell models of disease was promising.
  - Vitamin E
  - Vitamin C
  - Coenzyme Q
Promising!

- **Vitamin E** supplementation in AD mouse model resulted in improved cognition & reduced βA deposition

- **AD**: Daily injections of **vitamin C** in mouse model significantly reduced memory deficits

- **PD**: **Coenzyme Q** has been shown to have multiple protective effects within the mitochondria

- **PD**: **CoQ protects** MPTP-treated mice from dopaminergic neuronal loss & also attenuated α-synuclein aggregation
There has been no proven benefit for the use of vitamin E &/or vitamin C in either AD or PD from large randomised controlled clinical trials.

Vitamin E clinical trials, CoQ10 trials, & glutathione trials in PD concluded that there were only minor treatment benefits in the CoQ trials that may have been due to improvement in the respiratory chain deficit rather than a direct antioxidant action.

None of the trials have shown significant benefit to warrant recommendation for use in the clinical setting!!!
Promising but!

- All animal models are limited in recreating the human disease
  - long-time frame
  - Gradual accumulation of age-related changes
- Antioxidants must be administered at an early stage where the process influences pathogenesis most
- The bioavailability of reducing molecules in the human brain in the doses used in animal models
- The effective targeting of such molecules to the mitochondria in human brain
- Several different producers of oxidative stress in each disease (need to be targeted separately but simultaneously)