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

- Oxidative stress is important in their etiology (association)
- **Aging** has been established as the most important risk factor (AD & PD)
- **Aging:** cumulative oxidative stress leads to mitochondrial mutations, mitochondrial dysfunction, & oxidative damage
- Is oxidative stress a result 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 will the use of antioxidant therapy be successful?
OUTLINE

- 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 (**O₂⁻²**), hydrogen peroxide (**H₂O₂**), & 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
Oxygen, Brain & Oxidative Stress

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

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

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

PROTEINS
- SH groups
GSH/GSSG
3-nitrotyrosine
3-chlorotyrosine
dityrosine
carbonylated proteins

LIPIDS
malondialdehyde
8-isoprostaglandin
F₂-isoprostane
TBARS
conjugated dienes
4-hydroxy-2-nonenal

DNA
2,6-diamino-4-hydroxy-5-formamidopyrimidine
4,6-diamino-5-formamidopyrimidine
8-hydroxyadenine
8-hydroxydeoxyguanosine
8-hydroxyguanosine
5-hydroxycytosine
ROS PRODUCERS IN MAMMALIAN BRAIN
**NADPH Oxidase**

- A multi-subunit enzyme complex
- Is a member of the NOX gene family
- Also called phagocytic oxidase (PHOX)
- Seven NOX genes have been identified
- The most expressed of the NOX enzymes in the brain is NOX$_2$
- The enzyme transfers the proton across the membrane, & the end product of the enzyme is superoxide
Xanthine Oxidase

- It is a molybdo-flavo enzyme complex
- 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*
  - *Aconitase*
  - *α-ketoglutarate dehydrogenase complex*
- The production of superoxide is dependent on the value of mitochondrial membrane potential.
Mitochondria... Cont.

- 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 un-couplers (how?)
- 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 $\text{H}_2\text{O}_2$ to water using reduced glutathione (GSH) as an electron donor
  
  \[
  (\text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow \text{GS}-\text{SG} + 2\text{H}_2\text{O})
  \]
- 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 10-fold higher than in neurons
Catalase

- Catalase is a ferriheme-containing enzyme
- Converts hydrogen peroxide to water
- It is localized in peroxisomes, cytoplasm & mitochondria
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**.

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*Memories of You make me look forward to Alzheimers*
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 (mitophagy)
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
- 1-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, & rotenone-like increased production of ROS in mitochondria.
- Abnormal aggregation of 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 & induces 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**
Promising but!

- 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, CoQ, & glutathione** clinical 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)