Oxidative stress and aging: is intervention possible?

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Buck Institute for Age Research
The Buck Institute for Age Research
Aging research approaches and opinions of the past

- Grind & Find
- Correlations, correlations, correlations…..
- Too complex
- Programmed death
- Thousands of genes involved
- Dogma
1990’s - THE decade of invertebrate aging research

Trivial to extend lifespan in invertebrates

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Demonstrable success in aging research

- Known mechanisms of action are key (bias)
- Worm, fly, mouse
- New mutants are no longer novel (caveats)
- Linkage of gene action to physiology
- Inferred therapeutics
What are the effects of oxidative stress?

Exogenous studies
- toxicological studies, dose response etc.
- correlative

Endogenous studies
- ROS produced as a result of normal metabolism

Genetics: transgenetic as well as Knockouts
Efficacy of EUK antioxidants in biology

- Autoimmune disease
- Stroke
- Alzheimers disease
- Parkinsons disease
- ALS
- Apoptosis
- Mitochondrial dysfunction
- Radiation damage
- Aging
Catalytic antioxidants tested in \textit{Sod2}\(-/-\) mice, and in aging paradigms

Euk-8

Euk-134

Euk-189
EUK-134 protects dopaminergic neurons from toxicity by MPP⁺

K. Pong et al., Brain Res. (2000)
Caenorhabditis elegans

• Advantages as an aging model
  – Small size, complete genome, AGE mutants (e.g. age-1), short lifespan
  – Pharmacological screening
    • (disadvantage - pharmacological screening)

• Advantages as a mitochondrial model

  Metabolic mutants
  respiratory chain
    increased lifespan
  mev-1
    Oxidative damage,
    shortened lifespan
Extension of lifespan in *C. elegans* through antioxidant treatment.
EUK-134 inhibits nitration of tyrosine hydroxylase in DA neurons

1. Control
2. EUK-134
3. MPP⁺
4. MPP⁺ and EUK-134

K. Pong et al., Brain Res. (2000)
Reactive Oxygen species and the Respiratory Chain

H$_2$O$_2$ → O$_2^-$ → O$_2$ → H$_2$O$_2$ → Fe$^{2+}$ → IMS

SOD1

SDH

Succinate

H$_2$O$_2$ → Fe$^{2+}$ → MATRIX

SOD2

NADH

Complex I

Complex II

Complex III

Complex IV

FeS

N-2

H$^+$
Oxygen free radicals generated as a function of metabolic rate cause cumulative oxidative damage, resulting in structural degeneration, functional decline, and age-related diseases.

**WEAK FORM**

Oxygen free radicals generated as a function of metabolic rate cause cumulative oxidative damage, resulting in structural degeneration, functional decline, and age-related diseases. Oxidative stress is the predominant cause of age-associated degenerative change, and thus the determinant of MLSP.

**MODERATE FORM**

Oxygen free radicals generated as a function of metabolic rate cause cumulative oxidative damage, resulting in structural degeneration, functional decline, and age-related diseases. Oxidative stress is the predominant cause of age-associated degenerative change.

**STRONG FORM**

Oxygen free radicals generated as a function of metabolic rate cause cumulative oxidative damage, resulting in structural degeneration, functional decline, and age-related diseases. Oxidative stress is the predominant cause of age-associated degenerative change, and thus the determinant of MLSP.
Brief History

Rate-of-Living Hypothesis (1906, 1928)
Rubner Pearl

Stochastic (“wear and tear”) Theories (1930s-1950s)
Cell Biology

Analytical Biochemistry

Free Radical Theory (1956)
Fridovich & McCord

Superoxide Dismutase (1969)
SOD

O$_2^-$ → H$_2$O$_2$

Mitochondrial Superoxide (1979)
Sies & Boveris

H$_2$O$_2$

Radiation Biology: Morphology

EPR Spectroscopy: •OH, O$_2^-$

Denham Harman

Central Dogma (mid-1980s)

Stochastic (“wear and tear”) Theories (1930s-1950s)
The Mechanistic Abyss (circa mid-1980s)

Oxidative Stress

???

Neurodegeneration
Vascular Tone
Cardiac Output
Sensory Acuity
Skin Thickness
Bone Density
Endocrine Function
Immune Function
Cancer
A Simplifying Hypothesis

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<th>Oxidative DNA Damage</th>
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The Mechanistic Canyon (circa 1995)

Oxidative Stress

DNA Damage

Oncogenesis

Neurodegeneration

Vascular Tone

Cardiac Output

Sensory Acuity

Skin Thickness

Bone Density

Endocrine Function

Immune Function

Cancer
The Mechanistic Canyon (circa 1995)

Oxidative Stress → Cell Death

Cell Death →
- Neurodegeneration
- Vascular Tone
- Cardiac Output
- Sensory Acuity
- Skin Thickness
- Bone Density
- Endocrine Function
- Immune Function
- Cancer
The Mechanistic Canyon (circa 1995)

- Oxidative Stress
- Mitochondrial Damage
- Energy Crisis
  - Neurodegeneration
  - Vascular Tone
  - Cardiac Output
  - Sensory Acuity
  - Skin Thickness
  - Bone Density
  - Endocrine Function
  - Immune Function
  - Cancer
\( O_2 \xrightarrow{e^-} O_2^- \xrightarrow{e^-} H_2O_2 \xrightarrow{e^-} \cdot OH \xrightarrow{e^-} H_2O \)

4 e\(^{-}\) reduction to water

Unreactive at STP, but a great electron acceptor
Biological activation via radicals, transition metals
Generally, radical intermediates are enzyme-bound

Reacts with virtually any molecule at diffusion-limited rates
The molecule that makes ionizing radiation toxic

Actually a chemical \textit{reductant}
Not so terribly reactive with most biomolecules
Mitochondrial superoxide the major source of active oxygen
Maintained at very low concentration
Superoxide dismutases

Not so terribly reactive with most biomolecules
Maintained at very low concentration
Catalases, peroxidases, GSH, etc…
The diagram illustrates the redox reactions involving oxygen and water. It shows the reduction of oxygen to water by electrons, mediated by enzymes such as SODs and CAT + POXs. The reaction can be summarized as:

$\text{O}_2 + 4e^- \rightarrow \text{H}_2\text{O}_2$

$\text{H}_2\text{O}_2 + 2e^- \rightarrow \text{H}_2\text{O}$

The diagram also highlights the involvement of iron-sulfur clusters (Fe-S) and ferric ions ($\text{Fe}^{3+}$) in the process. The reaction is spontaneous under certain conditions and is catalyzed by various enzymes.
Types of Evidence

1) Oxidative Phenomenology
2) Dietary Restriction
3) Rate-of-Living/Oxygen Tension
4) Dietary Supplementation
5) Pharmacological Intervention
6) Comparative Biology
7) Classical and Population Genetics
8) Transgenic Models
9) Human Degenerative Disease
Oxidative Biomarkers

DNA
- 8-oxoguanine
- thymine glycol
- thymidine glycol
- dsDNA breaks

PROTEIN
- carbonylation
- mixed disulfides
- loss of Fe•S (aconitase, glutamine synthetase)

MIXED
- lipofuscin
- P-S-S-H
- glycooxidation products
- etheno adducts

GSH

SUGARS
- aldehydes
- TBARS
- exhaled ethanes
- isprostanes

LIPIDS

Oxidative Phenomenology
The Problem With Biomarkers

1. Increased damage or decreased repair?

2. Cause of consequence of aging?

3. False negatives?

4. What does it all mean?

Endocrine function

Oxidative Phenomenology
Evidence in favor of the FRTA:

1) “Expected” changes in 100s of studies.
2) Many tissues, many species.
3) Specific repair systems for many end-products characterized.

BUT…

1) Methodological problems with most such work.
2) Negative studies buried?
3) Specific repair systems for many end-products characterized.
4) Absolute and relative magnitude of increases underwhelming.

HOWEVER…

1) Grind-and-find studies necessary to establish baselines.
2) Biomarkers most useful in comparative and intervention studies.
Dietary Restriction

Note: dietary restriction does not generally decrease metabolic rate or activity in mammals.

Evidence in favor of the FRTA:

1) Generally: decreased age-specific accumulation of biomarkers.
2) Generally: decreased sensitivity to oxidative stress.
3) Sometimes: increased antioxidant activities.

BUT…

1) Almost all age-related alterations are slowed by DR.
2) Hence: cause and effect are hopelessly entangled.

HOWEVER…

1) DR is a litmus test, and the FRTA has “passed” it.
Rate-of-Living/Oxygen Tension

Evidence in favor of the FRTA:

1) Models:
   A. Physical restraint of insects.
   B. Thermal manipulation of poikilotherms.
   C. Increased/decreased oxygen tension of invertebrates.

2) Results largely supportive of the FRTA.

   BUT…

3) Applicability of models to other phyla?
4) Decreased life span is not a powerful phenotype.

HOWEVER…

• Negative results would have been robust, so positive results are important.
Dietary Supplementation

Evidence in favor of the FRTA:

1) Some amelioration of age-related degenerative change:
   1) ALCAR/lipoic acid in rat.
   2) Phenolic antioxidants in rodents (blueberries).

2) Data appear to support the weak form of FRTA.

BUT…

1) Most experiments have been negative.
2) Extension of life span: virtually no evidence.

HOWEVER…

Dietary supplementation is a flawed approach -- physiology restricts degrees of experimental freedom and potency. Falsification is problematic.
Pharmacological Intervention

Evidence in favor of the FRTA:

1) Some amelioration of age-related degenerative change:
   1) PBN in gerbils

2) Extension of life span:
   1) Euk-134 in nematodes
   2) Efficacy in Mammals
   3) Euk-189 in mice?

BUT…

1) Many experiments have been negative.

HOWEVER…

Falsification of the FRTA with drugs will be difficult.
Oxidative DNA damage rates correlate with metabolic rate
Mitochondrial Lipid Content

Comparative Biology
Evidence in favor of the FRTA:

1) Many long-lived mutants demonstrate increased antioxidant defenses and better tolerance of oxidative stress.
2) Population selection for increased life span sometimes (not always) associated with increased SOD activity in long-lived strains.
3) Short-lived mutants often associated with decreased antioxidant defenses, increased ROS generation.

BUT…

4) Long-lived mutants possess generally better resistance against many stressors.

HOWEVER…

Many stressors may act via oxidative mechanisms.
Transgenic Models

Evidence in favor of the FRTA:

1) Life span extensions with transgenic SOD *Drosophila*.

   BUT…

2) Negative results with various Tg KO and heterozygous organisms.

HOWEVER…

1. Many stressors may act via oxidative mechanisms.
2. Oxidative defenses are both redundant and interconnected -- crude genetic engineering is likely to be often compromised.
3. Overexpression of SOD prohibited *in vitro*. 
## Value of Evidence

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**Functional and Comparative Genomics**

VERY HIGH
Amplification Mechanisms?

$O_2 \xrightarrow{e^-} O_2^- \xrightarrow{e^-} H_2O_2 \xrightarrow{e^-} \cdot OH \xrightarrow{e^-} H_2O$

- NO•
- Fe•S
- Lipofuscin/Lysosomes
- Cellular Redox
- Telomeres
- Chromosomal Architecture
- Protein S-Nitrosation
- Signal Transduction
- Gene Expression
- Apoptosis
Homeostasis: Is Oxidative Stress Special?

**Stresses/Damage**
- Oxidative DNA damage
- DNA polymerase errors
- Depurination/deamination
- Lipid oxidation
- Non-enzymatic glycation
- Protein denaturation
- Protein oxidation
- Chromosomal demethylation
- Cellular garbage accumulation
- Mitochondrial oxidant generation
- Lysosomal fragility
- Etc...

**Defenses/Repair**
- Base-excision repair
- Nucleotide-excision repair
- DNA proof-reading
- Lipid repair/turnover
- Catalytic antioxidants
- Radical scavenging systems
- Protein chaperones
- Protein repair/turnover
- Lysosomal turnover
- Mitochondrial maintenance
- Cellular proteolysis/turnover
- Etc...

**Degenerative Change**
- Mutagenesis (cancer)
- Endothelial dysfunction (heart disease)
- Immune dysfunction (rheumatoid arthritis)
- Connective tissue dysfunction (osteoarthritis)
- Neuroendocrine dysfunction (muscle loss, wasting)
- Amyloidosis (Alzheimer’s disease)
- Myopathies (weakness)
- Etc...

**Evolutionary Pressure**
Questions/Answers

• WEAK: Are oxygen free radicals important in aging?  Yes.
• STRONG: Do oxygen free radicals determine MLSP?  No.
• MODERATE: Are oxygen free radicals predominant in aging?  ???

• energetics
• cell division
• cell arrest
• cell death
• chromosomal stability
• gene expression
• signal transduction
Questions/Answers

Does CO$_2$ determine plant growth? No…

Does transcription determine embryogenesis? No…

CO$_2$ has “nothing to do with” the determination of plant growth.

Transcription has “nothing to do with” embryogenesis.

Mechanisms — not measurement.
The FRTA is no longer theoretical in the “weak” form.

The FRTA is unintelligible in the “strong” form.

The Free Radical “Perspective” on Aging has been productive, and is an object lesson for homeostasis.

Oxidative stress is ubiquitous, and may be the single most significant category of cellular stress. This is clearly something which can be therapeutically targeted.

It’s not a question of whether, but rather of when, how, and how much.
Catalytic antioxidants tested in Sod2-/- mice, and in aging paradigms
Potential sites of intervention

Oxidative Stress

Mitochondrial Damage

DNA Damage

Oncogenesis

Energy Crisis

Cell Death

Neurodegeneration

Vascular Tone

Cardiac Output

Sensory Acuity

Skin Thickness

Bone Density

Endocrine Function

Immune Function

Cancer