Biochemical reactions implicated in ageing – how radical can you get?

Paul Winyard
31 August 2007
New facilities - Exeter
Age-dependent changes in some anatomical and physiological factors in humans
“AGEING-RELATED DISEASES” WITH AN INFLAMMATORY COMPONENT:
CARDIOVASCULAR DISEASE
NEUROMUSCULAR
NEURODEGENERATIVE CANCER
Gene-environment interactions in healthy ageing

From: Mary Eubanks (1994) Environmental Health Perspectives
Bertha Remience of Belgium celebrates her 100th birthday.
National Geographic 2001
Free radical theory of ageing

• Dr Denham Harman, 1956
What is a free radical?

*Any atom or molecule with one or more unpaired electrons*

*e.g. OXYGEN!*
Oxidative damage

Protein

DNA

Lipid

J.A. Littlechild, Exeter

Wikimedia

Wikimedia
Rancidity is due to free radical reactions
Cumulative oxidative damage resulting in ageing and death

Henrik Poulsen,
Clinical Pharmacology Network Copenhagen
(www.cpnc.dk)
Radical generation is associated with inflammation.
Autoantigenicity of oxidatively modified proteins

(A. Nissim, P.G. Winyard et al, Arthritis & Rheumatism 2005)

1 = $^\cdot$OH
2 = glycation
3 = HOCl
4 = ONOO-
5 = native
ANTIOXIDANT ENZYMES

- SOD ($2\text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$)
- Catalase ($2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$)
- Glutathione peroxidase
- Glutathione transferase
- Peroxiredoxin
- Others
Fruit flies
Percent unable to walk 15 cm. in 15 seconds

OXIDATIVE STRESS (EXCESS RADICALS) IN CHRONIC INFLAMMATORY DISEASES

\[ \text{NO}^\cdot, \text{ONOO}^-, \text{\'OH}, \text{HOCI}, \text{H}_2\text{O}_2, \text{O}_2^\cdot \]

\[ \text{GP}_x, \text{Cat}, \text{SOD}, \text{Vit. E} \]
• Episodes of inflammation in early life may alter the “redox set point” of an individual in the direction of a subtle increase in oxidative stress?

• This shift of redox set point may affect the ageing process?
OXIDATIVE STRESS (EXCESS RADICALS) IN CHRONIC INFLAMMATORY DISEASES
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Anti-oxidant Face Gel

Time to moisturise.
Rejuvenating Day Moisturiser SP

time delay

Skin looks younger, day by day.
Inverse correlation between plasma vitamin E and ischaemic heart disease

Healthy ageing and fresh fruit/vegetables

From: www.oralcancerfoundation.org
In clinical trials to date, antioxidant vitamins did not prevent ischaemic heart disease

• BUT …

• Most studies have not asserted if the vitamins are doing anything to do DNA damage, protein damage or lipid damage
Panel of assays for oxidative stress
How to measure oxidative stress?

- Many assays available
- BUT
- No single assay, alone, is adequate
- Mostly laborious or need large volumes – therefore not suitable for high throughput
Criteria for assay selection

- Already validated – reproducible
- Published applications of the assay
- Small sample volume, preferably plasma or serum
- Rapid, and amenable to automation
- Cost effective
PRODUCTS OF NITRIC OXIDE REACTIONS

\[
\text{NO}^- + \text{O}_2^- \rightarrow \text{ONOO}^- \rightarrow \text{NO}_2^-\text{-Tyr} \rightarrow 3\text{-nitrotyrosine}
\]
High-throughput nitrotyrosine assay

Commercial ELISA kit
Sandwich type
96-well plate
Plasma
Gene-environment interactions in healthy ageing

From: Mary Eubanks (1994) Environmental Health Perspectives
Less than 30,000 genes

\[\text{DNA} \rightarrow \text{RNA} \rightarrow \text{Protein}\]
Mechanisms of effects of polymorphisms / mutations

- Most common changes are single nucleotide polymorphisms (SNPs) – 5m common ones!
  - Amino acid substitution (SNP), deletion, insertion etc
    - change in structure / activity of protein product
    - changes in breakdown and excretion?
  - Change in regulation
  - Other?
“Oxigenes” and cancer

Association between manganese superoxide dismutase promoter gene polymorphism and breast cancer survival

RCG Martin, J Ahn, SA Nowell, DW Hein, MA Doll, BD Martini and CB Ambrosone

Breast Cancer Research 2006, 8:R45
• Advent of “genome-wide” association studies
A post-genomic view of the free radical theory of ageing:

Polymorphisms in oxidative stress genes → Oxidative stress → Ageing
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Hypothesis:

• Variation in genes encoding radical-generating enzymes and/or antioxidant enzymes contributes to oxidative stress and thence to ageing