Does Ageing need Chemistry?

Lizzy Ostler
Why Chemistry?

• Synthesis - more, better, faster
  - Synthesis gives mg to g scale
  - Analogues give different / improved activity
  - Ability to add to media or food directly

• Analysis - from titration to Mass Spectrometry
  - What is it and how much is there?
What can Chemistry do?

• Is resveratrol an anti-ageing compound?
  - Synthesis
    *In vitro* activity of Resveratrol and metabolites
  - Analysis
    Resveratrol oxidation and isomerisation

• Why do fruit flies die?
  - Analysis
    What molecular damage occurs as the animals age?
Resveratrol

- Extends lifespan?
- SIRT1 activator
- DNA intercalator
- Topoisomerase inhibitor
- Antioxidant
- Pro-oxidant
- Antiviral
- Antiproliferative
Is cytostasis anti-ageing?
Resveratrol & primary cells

MRC5 fibroblasts, 10-100μM Resveratrol for 48h

• Effect on growth
  - Cells fixed and stained for pKi67
  - Determine growth fraction

• Effect on phenotype
  - Induction of senescence by SA-β-galactosidase staining

S
Results

Ki67

DAPI

SA-β-Gal
**Resveratrol in vitro**

Resveratrol induces cytostasis
Resveratrol in vitro

Resveratrol triggers senescence
Is it really Resveratrol?

trans-Resveratrol

Oxidation

ε-Viniferin

Isomerisation

cis-Resveratrol
Resveratrol $^1$H NMR
Dihydroresveratrol *in vitro*

**Ki 67 staining**
Resveratrol - Conclusions

• Resveratrol induces p38 MAP kinase dependent senescence

  Phenocopy of Werner’s Syndrome!

• Dihydroresveratrol is inactive

• Resveratrol must be acting through alternative mechanisms
“Green” theory of Ageing

Undamaged molecules, healthy tissue

INCREASED TEMPERATURE

A B C D E F etc

Damaged molecules, dysfunctional tissue

REPAIR PROCESSES

DIETARY RESTRICTION

REPAIR PROCESSES

DIETARY RESTRICTION

AGEING

Do flies recycle?
Predictions from green theory

**Predicted trends in AGE fluorescence**

- FF 25°C
- DR 18°C
- DR 25°C

*Graph showing predicted trends in AGE fluorescence*
Do fruit flies recycle?

• Chemical analysis of whole flies
• Variant of metabolomics
• Methods selected for AGE detection
  - Longitudinal data (7/14 day sampling)
  - Live flies collected, frozen & digested
  - Spectrophotometric, NMR and MS analysis
• Age-related signals identified
• Effect of lifespan modifications assessed
Fluorimetry: AGE wavelengths

Graph of relative emission intensities versus wavelength for excitation at 360nm

Graph of Fluorescence intensity vs Age

- 60 Days
- 56 Days
- 39 Days
- 21 Days
- 7 Days

- 360/440
- 330/395
Fluorimetry : Effect of T and DR

Fluorescence intensity at $\lambda_{ex} = 360$nm, $\lambda_{em} = 430$nm vs Age

- Significant increases in AGE-associated fluorescence over lifespan for: a) fully fed b) male (even when DR)
- No increase in DR flies, although $25^\circ C$ scattered
$^1$H NMR (1)

Aromatic Region

Benzylic Region
1H NMR (2)

- Male DR, 25°C upward trend all clusters (p<0.05)

- δ=2.3-2.48ppm

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Female FF, 25°C</th>
<th>Female DR, 25°C</th>
<th>Female DR, 18°C</th>
<th>Male DR, 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>-0.07 ± 0.022</td>
<td>-0.018 ± 0.039</td>
<td>-0.024 ± 0.0077</td>
<td>0.125 ± 0.025</td>
</tr>
<tr>
<td>Y-intercept</td>
<td>9.4 ± 1.0</td>
<td>6.1 ± 1.7</td>
<td>9.5 ± 0.71</td>
<td>2.1 ± 1.0</td>
</tr>
</tbody>
</table>
Do flies recycle? - Conclusions

Analytical signals from acid-stable compounds show:

• Slower rate of change for DR than FF
• More scatter for 25°C than 18°C - treadmilling
• DR males behave like FF females, correlating with male lifespan unchanged by DR
Acknowledgements

Glycation in Drosophila
Leonie Norris
Aamira Iqbal
Joyce Boakye-Acheampong
Angela Merges

Resveratrol
Angela Sheerin
Patricia Majecha
Noel See-Yau Fong

Funders

National Institute on Aging
SPARC
BBSRC
Mass spectrometry

Typical mass spectrum of digest of young (7 days) flies

Typical mass spectrum of digest of old (133 days) flies