Path501
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Dietary Restriction Mimetics

CR mimetics

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Why mimetics?

1) There is some evidence that CR in humans might be beneficial.
   - Epidemiological studies correlate incidence of disease with caloric intake.
     E.g., diabetes, neurodegeneration, cardiovascular disease.
   - Accidental CR in Biosphere 2 improved some physiological parameters.
   - Primates have a physiological response to CR that is similar to that of rodents.
   - CR has increased longevity in dogs and cows.

2) The implementation of CR in humans is not practical.
   - People get hungry without food.
   - Food tastes good.
   - Even short-term diets usually fail.
The goal of CRM is to produce some or all of these responses without actually reducing caloric intake.

### Known physiological effects of CR

<table>
<thead>
<tr>
<th>Decreased body temperature</th>
<th>Decreased adipose tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased fasting insulin</td>
<td>Decreased triglycerides*</td>
</tr>
<tr>
<td>Decreased fasting glucose</td>
<td>Decreased LDL cholesterol</td>
</tr>
<tr>
<td>Increased insulin sensitivity</td>
<td>Increased HDL cholesterol</td>
</tr>
<tr>
<td>Decreased accumulation of oxidative damage</td>
<td>Increased mitochondrial biogenesis</td>
</tr>
<tr>
<td>Increased stress resistance</td>
<td>Decreased tumor growth</td>
</tr>
<tr>
<td>Decreased incidence of chronic disease</td>
<td>...and long life</td>
</tr>
</tbody>
</table>
What would we like the mimetics to accomplish?

Mutations in many genes affect aging.
→ pharmacological intervention in function of these genes should have the same effect

1) activation of stress response pathways and thus protection against stressors
   • Mitohormesis
   • Reduced oxidative stress and damage
   • Reduced DNA damage, enhanced repair
   • Reduced damage to lysosomes and peroxisomes

2) Improvement of insulin sensitivity

3) Improvement of metabolism and reduction of age-related disease, and maintenance of organismal function
Types of mimetics

Should stimulate specific pathways, inhibit DNA modification, or act as inhibitors

- Antioxidants
- Hormonal replacement
- Metabolic enhancers
- Kinase inhibitors
Currently proposed CR mimetics

<table>
<thead>
<tr>
<th>2DG</th>
<th>↓ glycolysis by inhibiting phosphohexose isomerase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biguindes</td>
<td>Type 2 diabetes drugs, inhibit gluconeogenesis and output of glucose into bloodstream</td>
</tr>
<tr>
<td>STACs</td>
<td>Sirtuin activators</td>
</tr>
<tr>
<td>Retinoids Soy isoflavones Somatostatin analogs</td>
<td>IGF1 pathway inhibitors</td>
</tr>
<tr>
<td>Thiazolidinediones (TZDs)</td>
<td>PPARγ antagonists, insulin sensitizers</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain derived neurotrophic factor</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>TOR inhibitor</td>
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</table>
Sirolimus/Rapamycin

- Originally developed as an antifungal agent
- Currently used as an immunosuppressant drug used to prevent rejection in organ transplantation
- Has potent antiproliferative properties
  - Restenosis
  - Cancer treatment

- inhibits the response to interleukin-2 (IL-2) and thereby blocks activation of T- and B-cells.
- binds the cytosolic protein *FK-binding protein 12* (FKBP12)
- the sirolimus-FKBP12 complex inhibits the *mammalian target of rapamycin* (mTOR) pathway
TOR pathway

- PPARγ
- Ribosome biogenesis
- Secretion of mitogens and VEGF
- Changes in cell morphology
- Protein synthesis
- Cell growth

Cell growth → ⋯ → senescence

P-21 and damage-induced senescence
Inhibition of TOR pathway

Upstream (effectors):  
- Akt
- insulin
- PTEN

Antagonists and downstream targets of TOR:
- Hsp70
- SIRT1
- S6K
- TOR

Insulin sensitivity has been linked to extreme longevity.
- Marker of genetically downregulated TOR activity
Resveratrol

- Polyphenolic phytoalexin produced in response to bacterial or fungal infection
- “Therapeutic properties”:
  - Cancer
  - Antiviral
  - Neuroprotective
  - Anti-aging
  - Anti-inflammatory
• Isolated in 1940 from the roots of white hellebore (Takaoka)

• In 1963 – and currently, best natural source is japanese knotweed root

• In 1992 -- presence in wine considered an explanation for “French Paradox” and cardioprotection
Anti-aging properties

• Resveratrol extends lifespan of *S.cerevisiae* (Horowitz and Sinclair, 2003)

• Also in *C.elegans* and *D.melanogaster* (Sinclair and Wood, 2004) through a Sir2 dependent manner
  
  – 2007 – repeated in *C. elegans* (Gruber, 2007) but could not be consistently replicated in *C. elegans or D. melanogaster* (Bass, 2007)
  
  – 2006 – Mice on a high fat diet (60% energy from fat, hydrogenated coconut oil) and 30% more calories than standard chow (Baur, 2006)
    
    • high-fat diet plus 22 mg/kg resveratrol -- 30% lower risk of death than mice on the high-fat diet.
    
    • Gene expression analysis -- addition of resveratrol opposed the alteration of 144 out of 155 gene pathways changed by the high-fat diet.
    
    • Insulin and glucose levels in mice on the high-fat+resveratrol diet were closer to the mice on standard diet.
    
    • Addition of resveratrol to the high-fat diet did not change the levels of free fatty acids and cholesterol
Anti-aging properties

- 2006 -- *Notobranchius furzeri* (avg. 9 week lifespan) resveratrol increased the median lifespan by 56% (Valenzano, 2006)
  - higher general swimming activity
  - better learning to avoid an unpleasant stimulus
  - slight increase of mortality in young fish caused by resveratrol (hormesis)

- Epidemiologic studies show that resveratrol lowers the risk of age-related diseases (Sinclair, 2005)

- *In vitro* – protects against: (Sinclair, 2005)
  - Oxidative stress
  - Radiation
  - ischemia
Molecular Targets

• The mechanisms of action are not fully understood
  – appear to mimic several of the biochemical effects of calorie restriction (Cell 2006)
    • Inhibit lipase
    • Reduced fat absorption
    • activates SIRT1 and PGC-1α
    • improve mitochondria function
Molecular Targets

• Thought to be one of 18 phenolic activators of human SIRT1 (Sinclair, 2005)
  – Sirtuin-activating compound (STACs)
• Increases lifespan in nematodes and
• Thought to be one of 18 phenolic activators of human SIRT1 (Sinclair, 2005)
  – Sirtuin-activating compound (STACs)
Molecular Targets

• Increase MnSOD activity by 14-fold (Ellen, 2008)
  
  – MnSOD reduces superoxide to H2O2 (Zsolt, 2000)
  – Superoxide O2- is a byproduct of respiration in complex 1 and 3 of the electron transport chain (Zsolt, 2000)
  – extracts an electron from biological membrane and other cell components, causing free radical chain reactions (Zsolt, 2000)

– MnSOD reduces superoxide and thereby confers resistance to: (MacMillan-Crow, 2001)
  • mitochondrial dysfunction
  • permeability transition
  • apoptotic death
Molecular Targets

- RESV --> SIRT1 / NAD+ --> FOXO3a --> MnSOD (Ellen, 2008)
  - SIRT1 dependent migration of FOXO transcription factors to the nucleus and stimulates FOXO3a transcriptional activity, enhancing deacetylation (activity) of FOXO3a (Stefani, 2007 and Brunet, 2004)
- Important in:
  - lifespan extension (Sun, 2002)
  - inhibits pancreatic cancer (Kanmar, 2003)
  - resistance to reperfusion injury (Wong, 1995)
  - irradiation damage (Hu, 2007)

- AMP-activated kinase (AMPK) - - senses cellular energy levels and is activated by increases in the cellular AMP:ATP ratio (Dasgupta, 2007)
- regulation of food intake by hypothalamic neurons
- resveratrol activates AMPK in primary neurons in vitro as well as in the brain and promoted neurite outgrowth
  - stimulates mitochondrial biogenesis through AMPK
Wine and Resveratrol

- Resveratrol is found in the skin of grapes and some seeds.
  - Quantity is variable:
    - grape cultivar
    - geographic origin
    - exposure to fungal infection
    - fermentation time
## Natural abundance of Resveratrol

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>TOTAL RESVERATROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscadine Wines</td>
<td>14.1 – 40 mg/L</td>
</tr>
<tr>
<td>Red Wines</td>
<td>1.98 – 7.13 mg/L</td>
</tr>
<tr>
<td>White Wines</td>
<td>0.05 – 1.80 mg/L</td>
</tr>
<tr>
<td>Red Grape Juice</td>
<td>1.14 – 8.69 mg/L</td>
</tr>
<tr>
<td>Red Grapes</td>
<td>1.50 – 7.81 mg/kg</td>
</tr>
<tr>
<td>Peanuts (Raw)</td>
<td>0.07 – 1.78 mg/kg</td>
</tr>
<tr>
<td>Peanuts (Boiled)</td>
<td>1.78 – 7.11 mg/kg</td>
</tr>
<tr>
<td>Blueberries</td>
<td>0.03 – 0.04 mg/kg</td>
</tr>
<tr>
<td>Bilberries</td>
<td>0.01 – 0.02 mg/kg</td>
</tr>
</tbody>
</table>
• 150-lb person would have to drink 382-1648 liters of red wine per day to get the same amount as the mice in the nature paper.
Pharmacokinetics

• Resveratrol rapidly undergoes conjugation resulting in less than 5% of the oral dose being observed as free resveratrol in blood plasma.
  – 70% absorbed and 99% is quickly metabolized to conjugates using 25mg doses (Walle)
  – Half life = 9 hours

• To calculate human dose, do not use mg/kg
  – larger quantities due to slower metabolic rate therefore, mg/kcal
  – \((\text{human dose/kg}) = (\text{animal dose mg/kg}) \times (\text{animal kg/human kg})^{(1-P)}\) where P=2/3 is used by convention to give a larger margin of safety for FDA pharmaceutical and EPA toxicology uses, but P=3/4 is more accurate
Resveratrol in a pill

- Companies:
  - Biomarker Pharmaceuticals
  - Chronogen
  - GeroNova Research
  - Irazu Biodiscovery
  - Juvenon
  - Longenity

- Resveratrol nutritional supplements – became popular in 2006
  - ground dried grape skins and seeds (expensive!)
  - Japanese knotweed
    - 187 mg/kg
Other benefits of resveratrol

- **Obesity**
  - induces apoptosis in human fat cells
  - inhibits production of cytokines involved with obesity-related disorders

- **Performance enhancement**
  - 15 week resveratrol into mouse chow increased treadmill endurance (Auwerx, 2005) and supports Sinclair (target is SIRT1)

- **Anti-cancer**
  - Topical resveratrol prevent skin cancer in mice (Jang, 1997)
  - cancer chemopreventive agent - reduces colon carcinogenesis in rats and mice (Saiko and Delmas and Sale, 2003)
Other benefits of resveratrol

- Increases potency of HIV antiretrovirals (Heredia, 2000)
- HSV activates NF-κB (Faith, 2006)
  - Inhibited by resveratrol
  - multiple viral protein products reduced or blocked
  - reduced viral DNA production

- Also blocks influenza virus from transporting viral proteins to the viral assembly site, hence restricting its ability to replicate (Palamara, 2005)
Add it to the public water supply?

• Resveratrol may stimulate the growth of breast cancer cells because it is similar to a phytoestrogen
  – other studies have found that resveratrol prevents breast cancer
• resveratrol is estrogenic - interfere with oral contraceptives and that women who are pregnant or intending to become pregnant should not use the product
  – not be taken by children or under 18 as no studies have shown how it affects development
• single dose of up to 5 g = no side effects

• Exacerbate West Nile virus
  – Mediated by p53 and virus worsened by increased apoptosis
How are potential CR mimetics identified?
Evaluating CRM candidates

• The number of possible drugs and targets is large.

• The development of useful screens will be very important for the field of CRM.

• In general, actual CR must be avoided while evaluating a drug.
  – Otherwise, it is difficult to assess whether an effect is due to the drug or to CR.
Evaluating CRM candidates

- **In vitro screens**
  - Directly expose cells to the drug.
  - Measure activation of a specific target.
    - E.g., sirtuins, AMPK
  - Measure a more general response.
    - E.g., stress response from heat or free radicals
  - Evaluate drug based on post-stress survival.
  - Measure the gene expression profile (microarray).
Spindler, 2006
Evaluating CRM candidates

- *In vitro* - - (de Cabo, 2003)
  - Candidate CRM administered to mice or rats for 4 weeks
    - Draw blood and isolate serum
    - Add serum to cell culture system
    - Subject cells to a stressor and evaluate the response of target molecule or other molecules
      - Western blot
      - Real-time PCR
      - Microarray
Evaluating CRM candidates

• In vivo screens
  – Evaluate mortality.
    • This is usually the most expensive approach.
    • Interventions Testing Program is useful for this step.
  – Measure the incidence of disease.
  – Measure physiological parameters.
    • glucose, temperature, hormone profile, etc.
  – Measure the effect on tumor growth.
  – Measure the gene expression profile (microarray).
Evaluating CRM candidates

- Human systems
  - Decreased incidence of age-related diseases
  - Decreased body temperature
  - Decreased plasma insulin
  - Glucocorticoids
  - Thyroid hormones
  - Adipokines
    - Leptin and adiponectin