Atorvastatin and Pravastatin Differentially Regulate Akt/mTOR Activation in Cardiac Myocytes and Differentially Alter Mortality in Mice With Inherited Cardiomyopathy

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Ten Minute Overview

- Brief background on heart failure and cholesterol-lowering statin drugs

- Rationale for our basic science research

- Novel research data on statin effects on cardiac myocytes 1) in vitro, and 2) in vivo
1 in every 33 will have HF by 2030
# Heart Failure: Common Cause of Death in Older Adults

<table>
<thead>
<tr>
<th>Disease or Condition</th>
<th>Prop. contribution, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart failure</td>
<td>20.0 (17.9–22.2)</td>
</tr>
<tr>
<td>Dementia</td>
<td>13.6 (12.1–15.2)</td>
</tr>
<tr>
<td>Chronic lower respiratory disease</td>
<td>12.4 (10.4–14.9)</td>
</tr>
<tr>
<td>Pneumonia, influenza</td>
<td>5.3 (4.5–6.3)</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>4.1 (3.3–5.2)</td>
</tr>
<tr>
<td>Acute kidney injury</td>
<td>3.3 (2.7–4.1)</td>
</tr>
<tr>
<td>Stroke</td>
<td>2.4 (2.0–2.9)</td>
</tr>
<tr>
<td>Septicemia</td>
<td>1.4 (1.1–1.9)</td>
</tr>
<tr>
<td>Liver disease, hepatitis</td>
<td>1.2 (0.5–2.5)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>1.1 (0.7–1.4)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>0.6 (0.2–1.1)</td>
</tr>
<tr>
<td>Unintentional injury</td>
<td>0.6 (0.3–0.9)</td>
</tr>
<tr>
<td>Acute intestinal event (obstr., peritonitis, abscess)</td>
<td>0.6 (0.3–0.9)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>0.5 (0.2–0.7)</td>
</tr>
<tr>
<td>Head and neck cancer</td>
<td>0.4 (0.1–0.7)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>67.6 (66.0–69.4)</strong></td>
</tr>
</tbody>
</table>

Atherosclerosis causes CVD
CVD Risk factors (4 Framingham)
More CVD Risk factors
Pharmacotherapy to reduce LDL- Cholesterol
## Statin Classes

<table>
<thead>
<tr>
<th>Chemical Nature</th>
<th>Derivation/Class</th>
<th>logP</th>
<th>Active Ingredient</th>
<th>Company &amp; Brand Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>lipophilic</td>
<td>Synthetic/class II</td>
<td>1.69</td>
<td>Cerivastatin</td>
<td>Bayer Baycol ® (withdrawn 2001)</td>
</tr>
<tr>
<td>lipophilic</td>
<td>Fermentation-derived/class I</td>
<td>1.70</td>
<td>Lovastatin</td>
<td>Merck Mevacor ®</td>
</tr>
<tr>
<td>lipophilic</td>
<td>Fermentation-derived/class I</td>
<td>1.60</td>
<td>Simvastatin</td>
<td>Merck Zocor®</td>
</tr>
<tr>
<td>lipophilic</td>
<td>Synthetic/class II</td>
<td>1.49</td>
<td>Pitavastatin</td>
<td>Kowa Pharmac. Livalo®</td>
</tr>
<tr>
<td>lipophilic</td>
<td>Synthetic/class II</td>
<td>1.27</td>
<td>Fluvastatin</td>
<td>Novartis Lescol ®</td>
</tr>
<tr>
<td>lipophilic</td>
<td>Synthetic/class II</td>
<td>1.11</td>
<td>Atorvastatin</td>
<td>Pfizer Lipitor ®</td>
</tr>
<tr>
<td>hydrophilic</td>
<td>Synthetic/class II</td>
<td>-0.33</td>
<td>Rosuvastatin</td>
<td>Astra Zeneca Crestor ®</td>
</tr>
<tr>
<td>hydrophilic</td>
<td>Fermentation-derived/class I</td>
<td>-0.84</td>
<td>Pravastatin</td>
<td>Bristol-Meyer-Squibb Pravachol ®</td>
</tr>
</tbody>
</table>
HMG-CoA reductase is anchored in the ER membrane

(Roitelman et al, J Cell Biol, 1992)
Statins inhibit mevalonate synthesis

(adapted from Abd and Jacobson, Exp Opin Drug Safety, 2011)
HMG-CoA Reductase

Dolichol

Cholesterol

Prenylated Proteins

Squalene

Geranyl-Geranyl

Geranyl-
Prenylated

Farnesyl PP

Dolichol-
Prenylated

PP

Hem A

CoQ10

Iso-
Pentyl

PP

Mevalonic Acid

HMG-CoA
Statin Side Effects

The FDA adds warnings in 2012

1) Muscle weakness/pain = statin-myopathy
2) Increase in type II diabetes
3) Cognitive side effects
Since statins may induce skeletal myopathy, we aimed to investigate effects of statin therapy on cardiac muscle.
Statin effects in cardiac myocytes

Zemljic-Harpf, J Cell Sci., 2014
Lipophilic Statin Drugs induced LDH Release in Cardiac Myocytes
Atorvastatin induced ER stress and apoptosis
Pravastatin increases and atorvastatin decreases mTOR activity.
Atorvastatin decreased integral protein expression in CMC
Statin effects in cardiac muscle
Atorvastatin increased creatine kinase serum levels
Preserved systolic function after long-term statin treatment
Atorvastatin altered cardiac ultrastructure
Protein aggregate formation after atorvastatin treatment
Statins did not alter the decline of heart function in cVclKO mice

* = Ator: Co vs cVclKO, p< 0.0001
# = Vehicle as well as Prava: Co vs cVclKO, p< 0.001
Atorvastatin increased mortality in cardiomyopathic (cVclKO) mice
Summary of *in vitro* and *in vivo* studies

Atorvastatin, but not pravastatin;

1) ↑ ER stress

2) ↓ mTOR signaling and Akt activation

3) ↑ Apoptotic signaling

4) Atorvastatin induced mitochondrial disarray & protein aggregate formation

5) Atorvastatin increased the mortality of cardiomyopathic mice
Conclusion

Our studies suggest that lipophilic statins penetrate cellular membranes (including the ER and mitochondria) easier than hydrophilic statins, thereby affecting ER function, mitochondrial integrity and cell survival signaling.
Acknowledgements

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Atorvastatin treatment reduced respiratory reserve capacity in CMC
Decreased LDL-C after statin treatment

A. LDL-C (direct) showing a decrease with statin treatment compared to vehicle.
   - Atorva: p<0.03
   - Prav: p<0.0008
   - Prav: p<0.02

B. HDL-C showing an increase with statin treatment compared to vehicle.
   - Atorva: p<0.008

C. Total Cholesterol showing a decrease with statin treatment compared to vehicle.
   - Atorva: p<0.008
   - Prav: p<0.002

D. Triglyceride showing no significant change with statin treatment compared to vehicle.

E. Creatine Kinase showing a significant increase with Atorva treatment.
   - Atorva: p<0.0004
   - Prav: p<0.005

F. Albumin showing no significant change with statin treatment compared to vehicle.
Genetics of statin-myopathy

✧ **Statin transporter proteins:** SLCO1B1, and ABCB1

✧ **Statin metabolism genes:** CYP2D6

✧ **Known muscle disease mutations:** PYGM (McArdle’s disease), RYR1, COQ2, LPIN1, ATP2B1 (Ca\(^{2+}\) transporting ATPase), mitochondriopathies

✧ **Pain perception:** HTR3B, HTR7

✧ **Vascular receptors:** AGTR1 (angiotensin II Type 1 rec.), NOS3

Risk factors for statin-myopathy

✩ Age (especially >80 years) (Pasternak, Stroke, 2002)
✩ Female gender (Voora, J Am Coll Cardiol 2009)
✩ Low vitamin D levels (Ahmed, Transl Res, 2009)
✩ Renal Insufficiency (Pasternak, Stroke, 2002)
✩ Surgery (Pasternak, Stroke, 2002)
✩ Physical exercise (Meador, Muscle Nerve 2010; Thompson, Metabolism 1997; Frudakis, Pharmacogenetic Genomics, 2007)
✩ History of statin-myopathy (Bruckert, 2005)

Needham et Mastaglia, Neuromuscul Disord, 2014
Statins and drug-drug interactions

- Cytochrome p450 (CYP3A4) enzyme inhibitors (simva, atorva, lova) (grapefruit juice, diltiazem, erytromycin, fluconazol, prot. inhibitors)

- CYP2C9 enzyme inhibitors (rosuva, fluva) (warfarin)

- Fibrates/Gemfibrozil (simva, atorva, lova)

- Amiodarone (increases simva toxicity)

- Alcohol Abuse

Needham et Mastaglia, Neuromuscul Disord, 2014
Statin Side Effect #2: Risk of type 2 diabetes mellitus


✓ Statins interfere with lifestyle intervention in the prevention of diabetes (Rautio, BMJ, August 2012).


✓ 43 genetic analysis of 223 463 individuals T2DM partially due to HMG-CoA reductase inhibition (Swerdlow et al, Lancet 2014).
Statin AE on cognitive Function


Statins Reduce CV Mortality


Future Plans and Research Opportunities on Different Statin Class Effects on:

- Gender differences in statin drug toxicity
- Mitochondrial function
- Cardiac hypertrophy
- I/R tolerance
- T2DM

Contact Info: azemljicharpf@ucsd.edu
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(adapted. Needham et Mastaglia, Neuromuscul Disord, 2014)
Decreased LDL-C after statin treatment