Molecular Interventions for Cardiovascular Disease: It Isn't That Simple!

A Cautionary Tale
Causes of death, 2001:

USA

6. Infectious and parasitic diseases: 14.9 million

1. Heart diseases: 11.1 million

2. Cancers: 7.3 million

3. Stroke: 5.5 million

4. Respiratory diseases: 3.6 million

5. Accidents, fires, drowning, etc.: 3.5 million

7. Maternal and perinatal: 3.0 million

8. Violence (war, homicide, suicide): 1.6 million

World Health Organization
World Health Report 2002
Atherosclerosis

Risk increases with higher [LDL]
Risk decreases with higher [HDL]
Familial Hypercholesterolemia (FH)

Heterozygotes (1:500)
- 300-500 mg/dl plasma cholesterol
- Xanthomas in third decade
- Coronary heart disease in fourth decade
- Treat w/ statins and bile acid binding resins

Homozygotes (1:10^6)
- 500-1200 mg/dl plasma cholesterol
- Xanthomas at birth
- Death by MI before age 30
- Treat w/ plasma LDL apheresis

Xanthoma
Cholesterol

1) Acetyl-CoA $\rightarrow$ HMG-CoA $\rightarrow$ Mevalonate

2) Mevalonate ($C_6$) + 3ATP $\rightarrow$ Isopentenyl-PP$_i$ ($C_5$ “isoprene”) + CO$_2$ + 3ADP + P$_i$

3) 6 Isoprene units ($C_5$) $\rightarrow$ Squalene ($C_{30}$)

4) Squalene ($C_{30}$) $\rightarrow$ Cholesterol ($C_{27}$)
Isopentylpyrophosphate (MEV Pathway)
The Statins

**Fermentation-Derived Statins**
- **Mevastatin**
- **Lovastatin**
- **Simvastatin**
- **Pravastatin**

**Synthetic Statins**
- **Atorvastatin**
- **Fluvastatin**
- **Cerivastatin**

**SHORT HISTORY**
- 1976 Mevastatin from *Penicillium citrinum*
- 1980 Mevinolin from *Aspergillus terreus*
- 1987 FDA approves Lovastatin
- 1988 Lovastatin not effective in FH homozygotes
- 1995 Pravastatin decreases heart transplant rejection and mortality independently of lowering cholesterol levels

STATINS REDUCE [CIRCULATING CHOLESTEROL] BY UP TO 50%
FAIRLY SAFE (rhabdomyolysis)
Lipid Trafficking Enzymes

Lipoprotein Lipase, LPL
(located on endothelium of muscle, adipose tissue)

Lecithin:Cholesterol Acyltransferase (LCAT)
(located on HDL)

Also CETP
# Lipoprotein Particles

<table>
<thead>
<tr>
<th>Apolipoprotein</th>
<th>M.W.</th>
<th>gL⁻¹</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoA-I</td>
<td>28 kD</td>
<td>1.0-1.2</td>
<td>CMC, HDL; activates LCAT</td>
</tr>
<tr>
<td>ApoA-II</td>
<td>17 kD</td>
<td>0.3-0.5</td>
<td>CMC, HDL</td>
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<tr>
<td>ApoA-IV</td>
<td>46 kD</td>
<td>0.15-0.16</td>
<td>CMC, HDL</td>
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<tr>
<td>ApoB-48</td>
<td>264 kD</td>
<td>0.03-0.05</td>
<td>CMC; (intestine only)</td>
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<tr>
<td>ApoB-100</td>
<td>512 kD</td>
<td>0.7-1.0</td>
<td>VLDL, IDL, LDL; binds receptor</td>
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<tr>
<td>ApoC-I</td>
<td>7 kD</td>
<td>0.04-0.06</td>
<td>CMC, VLDL, HDL</td>
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<tr>
<td>ApoC-II</td>
<td>9 kD</td>
<td>0.03-0.05</td>
<td>CMC, VLDL, HDL; activates LPL</td>
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<td>ApoC-III</td>
<td>9 kD</td>
<td>0.12-0.14</td>
<td>CMC, VLDL, HDL</td>
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<tr>
<td>ApoD</td>
<td>33 kD</td>
<td>0.06-0.07</td>
<td>HDL</td>
</tr>
<tr>
<td>ApoE</td>
<td>38 kD</td>
<td>0.03-0.05</td>
<td>CMC, VLDL, IDL, HDL; binds receptor</td>
</tr>
</tbody>
</table>

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ApoA-I Milano Clinical Trial in Humans, JAMA 290(17):2292 2003
ApoB RNAi in Mice, Nature 432:173 2004
Lipoprotein Transport

Key:
- Blue: Hydrophilic layer (protein, phospholipids, etc.)
- Yellow: Triacylglycerols
- Orange: Cholesterol

1. Dietary fat and cholesterol enter the intestine.
2. Chylomicrons are released in the lymph.
3. Chylomicrons and cholesterol are hydrolyzed in capillaries.
4. LDL receptor binds LDL.
5. LDL binds to LDL receptor on peripheral tissues.
6. Remnant chylomicrons and cholesterol return to the liver.
7. Lipoprotein lipase hydrolyzes triacylglycerols in capillaries.
8. LDL is transported by serum albumin.
9. β-Oxidation occurs in peripheral tissues.
10. Resynthesis and storage mainly in adipose tissue.

To endocrine glands for steroid hormone synthesis.
IDEA:

Statins act by inhibiting cholesterol synthesis in patients.
Clinical Observation

Lovastatin therapy in receptor-negative homozygous familial hypercholesterolemia: Lack of effect on low-density lipoprotein concentrations or turnover

Ricardo Uauy, MD, PhD, Gloria Lena Vega, PhD, Scott M. Grundy, MD, PhD, and David M. Bilheimer, MD

From the Departments of Pediatrics, Internal Medicine, and Clinical Nutrition, and Center for Human Nutrition, University of Texas Southwestern Medical Center at Dallas

To determine whether at least part of the fall in low density lipoprotein (LDL) levels during lovastatin therapy might be the result of a reduced secretion of lipoproteins by the liver, three children 6 to 9 years of age with receptor-negative homozygous familial hypercholesterolemia underwent treatment with lovastatin. These patients have no capacity to synthesize LDL receptors. During lovastatin therapy, at a dose of 2 mg/kg/day, there was no decrease in LDL-cholesterol levels, nor was the turnover rate of LDL affected by the drug. The only significant change was a 74% drop in very low-density lipoprotein during treatment. We conclude that lovastatin is not effective in treatment of receptor-negative homozygous familial hypercholesterolemia. The most likely mechanism of action for this drug is to increase LDL receptor activity. (J Pediatr 1988; 113:387-92)

Sterol Regulation of Transcription by SREBP

SREBP Transcription Factor Localization

SCAP/Insig proteins also (HMG CoA R)

ER Retention

Proteases
IDEA:

Statins act by activating SREBP, resulting in increased LDL receptor and more efficient recruitment of LDL out of plasma.
Clinical Observations:  
The Pleiotrophic Effects of Statins

- Statins reduce organ rejection and mortality after cardiac transplant. Randomized, placebo-controlled  

- Statins reduce inflammation in patients with chronic rheumatoid arthritis. Randomized, placebo-controlled  

- Statins reduce incidence of MS lesions. Small open-label clinical trial.  

- Statins reduce proteinuria in systemic lupus erythematosus. Small open-label clinical trial.  
Multiple Sclerosis

- Effects up to one million worldwide
- 16,000 deaths in 2002 (0.03 %)
- Autoimmune attack of myelin in brain and spinal cord
- Progressive physical disability
- Current therapies include injected Ifn-β and copaxone (basic peptides)

- Statins reverse disease pathology in the EAE mouse model of MS!


Lesions on cerebellum and spinal cord of an MS patient; Jean Cruvelhier circa 1860
Immune System Overview

Inate

NK cells

Adaptive

APC

B cells

T cells

γδ helper

CD8 cytotoxic

CD4 professional

IFN-γ induced

IL12 from activated macrophages and dendritic cells

T_H1

T_H2

IL2

IFN-γ

TNFα

IL4, 10

TGFβ

IFN-β
Cell Mediated Adaptive Immune Response
Tc/MHC1/CD8 \textit{versus} Th/MHC2/CD4
MHC Antigen Presentation

Expressed in ALL CELLS

INF-γ can induce expression in non-APC during acute immune response; this is also a hallmark of auto-immune diseases.
The MHC Molecules

Class I

Class II
<table>
<thead>
<tr>
<th>Typical pathogens</th>
<th>Cell-mediated immunity</th>
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<th>Humoral immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinia virus</td>
<td>Mycobacterium tuberculosis</td>
<td>Clostridium tetani</td>
<td></td>
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<tr>
<td>Influenza virus</td>
<td>Mycobacterium leprae</td>
<td>Staphylococcus aureus</td>
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<tr>
<td>Rabies virus</td>
<td>Leishmania donovani</td>
<td>Streptococcus pneumoniae</td>
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<tr>
<td>Listeria</td>
<td>Pneumocystis carinii</td>
<td>Polio virus</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pneumocystis carinii</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Trichinella spiralis</td>
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<tr>
<td>Location</td>
<td>Cytosol</td>
<td>Macrophage vesicles</td>
<td>Extracellular fluid</td>
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<tr>
<td>Effector T cell</td>
<td>Cytotoxic CD8 T cell</td>
<td>T&lt;sub&gt;H&lt;/sub&gt;1 cell</td>
<td>T&lt;sub&gt;H&lt;/sub&gt;1 and T&lt;sub&gt;H&lt;/sub&gt;2 cells</td>
</tr>
<tr>
<td>Antigen recognition</td>
<td>Peptide:MHC class I complex on infected cell</td>
<td>Peptide:MHC class II complex on infected macrophage</td>
<td>Peptide:MHC class II complex on antigen-specific B cell</td>
</tr>
<tr>
<td>Effector action</td>
<td>Killing of infected cell</td>
<td>Activation of infected macrophages</td>
<td>Activation of specific B cell to make antibody</td>
</tr>
</tbody>
</table>
Th1 vs. Th2 Helper Cells

e.g. helminths
also oral antigens

extracellular pathogens

microbe (pathogen A)

Th1

Th2 Helper Cells

SITE OF INFECTION

immature dendritic cells

pathogen A in phagosome

peptide from pathogen in groove of class II MHC protein

endocytosed antigen from pathogen B

anti-parasite antibodies

parasite (pathogen B)

expulsion of parasite

LFA-1!

LYMPHOID ORGAN

mature dendritic cell

costimulatory molecules (B7)

IL-12

effector Th1 cell

cytokine X

effector Th2 cell

naïve or memory B cell

B

B

deactivator Th2 cell

Th1 CELL ACTIVATION

Th2 CELL ACTIVATION
Th1 Helper Cells (macrophage/inflammatory)

**IN PERIPHERAL LYMPHOID ORGAN**
- Mature dendritic cell with ingested bacteria
- B7
- Class II MHC protein
- T cell receptor
- IL-12
- IL-12 receptor

**Naïve helper T cell**

**AT SITE OF INFECTION**
- Effector TH1 cell
- CD40 ligand
- CD40
- Interferon-γ receptor
- Interferon-γ

**Infected macrophage**

**INFECTED DENDRITIC CELL STIMULATES NAÏVE HELPER T CELL TO DIFFERENTIATE INTO EFFECOR TH1 CELL**

**EFFECOR TH1 CELL ACTIVATES INFECTED MACROPHAGE TO KILL THE INTRACELLULAR BACTERIA**
Th2 Helper Cells (B cell response)

also mast cell degranulation and eosinophil activation
high [antigen], nonprofessional
Th1 vs. Th2 Antagonism

STATINS ALTER THIS BALANCE IN FAVOR OF TH2 HELPER CELLS. BUT HOW?
Statins Bind to LFA-1 Directly

- T cell initially bind APC through low-affinity LFA-1:ICAM-1 interactions
- Subsequent binding of T-cell receptors signals LFA-1
- Conformational change in LFA-1 increases affinity and prolongs cell–cell contact
Isoprenoids in Humans

1. Steroids Hormones
2. Metabolites (Vit. A, E, K; co-Q; 25,000 terpenoids)

3. Isopentyl adenosine (tRNA)
4. Dolichol (N-linked glycosylation)
5. Protein Prenylation
Isoprenoids in G Protein Signaling

Heterotrimeric (GPCR’s)
How Do Statins Work?

Reported to:

• Decrease occurrence of AD by 70% in retrospective study
• Suppress MS (clinical trials)
• Block SMC proliferation/migration in vitro
• Modulate NF-κB Function in vitro
• Block IFN-γ induced MHCII expression in vitro
• Suppress expression/secretion of immunoinflammatory molecules in vitro
• Direct inhibition of LFA-1

Most in vitro effects reversed by mevalonate, transF, transGG, but not cisF, cisGG, squalene, isopentyl adenosine or ubiquinone; prenylation inhibitors have many of the same immunomodulatory effects that are observed for statins.
The Next Cardiovascular Blockbuster?: Torcetrapib

- Rationally designed inhibitor of CETP.
- Reduces \textit{in vivo} \([\text{LDL}]/[\text{HDL}] < 1\!\)!
- Decreases atherogenesis in the rabbit model.
- Additive effects w.r.t. statins.
The Next Cardiovascular Blockbuster?: Ezetimibe

- Cholesterol absorption inhibitor derived from ACAT inhibitors.
- Discovered using *in vivo* hypocholesterolemic hamster assay!
- The target was *unknown* during the discovery process.
- Reduces cholesterol absorption by 75% in mice.
- Reduces plasma cholesterol by 20% in humans.
- Additive effects w.r.t. statins.
How do Torcetrapib and Ezetimibe work?
