Reverse Cholesterol Transport and Atherosclerosis

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Topics

★ HDL-mediated reverse cholesterol transport and importance of HDL-C management

★ Novel functions of HDL and dysfunctional HDL

★ Anti-atherogenic effects of probucol and their mechanisms

★ Anti-atherogenic effects of probucol from human clinical studies
REVERSE CHOLESTEROL TRANSPORT

Liver

ABCA1
ApoA-Ⅰ
Discoidal HDL

SR-BI

LDL receptor
ApoB-containing lipoprotein (VLDL, IDL, LDL)

Intestine

ApoA-Ⅰ

ApoB-containing lipoprotein

Large HDL

SR-BI

ABCA1

Atheroma (Foam cell)

ABCG1

LCAT

TG

CE

CETP

CE

TG

LCAT

Small HDL

Disoidal HDL

abc

abc

ABC1

abc

abc

abc
Familial HDL Deficiency

- Tangier disease (Deficiency of ABCA1)
- Familial LCAT deficiency / Fish eye disease
- Familial apo A-I/C-III deficiency
- Familial HDL deficiency with planar xanthoma
- Familial apo A-I deficiency
- Familial hypoalphalipoproteinemia

Often associated with corneal opacity & premature coronary artery disease
Reduction of Serum HDL-cholesterol Alone Accelerates Atherosclerosis
## Two Cases of Marked Hyperalphalipoproteinemia with Premature Corneal Opacity

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>CHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>58 y.o. Male</td>
<td></td>
<td>261</td>
<td>68</td>
<td>154</td>
<td>(-)</td>
</tr>
<tr>
<td>61 y.o. Male</td>
<td></td>
<td>238</td>
<td>64</td>
<td>138</td>
<td>(+)</td>
</tr>
</tbody>
</table>

CETP Deficiency

Liver

ApoA-I, ABCA1

Intestine

SR-BI, ABCG1

ApoB-containing lipoprotein (VLDL, IDL, LDL)

LDL receptor

ApoB-containing lipoprotein

CE, TG, CETP

LDL

Small HDL

Large HDL

Discoidal HDL

LCAT

Atheroma (Foam cell)
Lipoprotein Abnormalities of CETP Deficiency

LDL in CETP deficiency
- Polydisperse LDL poor in CE
- Low affinity to LDL receptors

HDL in CETP deficiency
- Marked increase in HDL2
- Large HDL particles enriched with CE & apo E
- Reduced capacity of cholesterol efflux

Yamashita S et al, Atherosclerosis 1988
Yamashita S et al, J Clin Invest 1990
Ishigami M et al, J Biochem 1994

Hyperalphalipoproteinemia is a disorder of reverse cholesterol transport
Epidemiological Study of Hyper-HDL-cholesterolemia in Omagari Area of Japan

Large Population-based study in Omagari, Japan
Subjects: Male=39567, Female=64938

Prevalence of intron 14 splicing defect ---------------------
Marked hyperalphalipoproteinemia ---
( HDL-C > 100 mg/dl )

CETP Gene Mutation

<table>
<thead>
<tr>
<th>City</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omagari</td>
<td>29.7%</td>
</tr>
<tr>
<td>Osaka</td>
<td>1.0%</td>
</tr>
<tr>
<td>Tokyo</td>
<td>1.5%</td>
</tr>
<tr>
<td>Shizuoka</td>
<td>1.3%</td>
</tr>
</tbody>
</table>
Relationship between HDL-C and Ischemic ECG Changes – Omagari Study –

Plaque score is increased in CETP deficiency
(Fushimi E and Yamashita S, unpublished)

Hyper-HDL-C by CETP deficiency is atherogenic! Atherogenic Hyperalpha-lipoproteinemia
(Yamashita S: Atherosclerosis 2000)
Prevalence of a Marked HALP (HDL-C ≥100 mg/dL) and Intron 14 Splice Donor Site Mutation in Subgroups Divided by Every 10 Years of Age (Omagari Study)

Subjects with low CETP activity had a higher risk for CV events than those with high CETP activity

Framingham Study

Vasan, R. S. et al. Circulation 2009;120:2414-2420
Lipid-lowering Effects of CETP Inhibitors/Modulators

% Change from Baseline

<table>
<thead>
<tr>
<th>CETP Inhibitor</th>
<th>Torcetrapib</th>
<th>Dalcetrapib</th>
<th>Anacetrapib</th>
<th>Evacetrapib</th>
</tr>
</thead>
<tbody>
<tr>
<td>dose (mg/day)</td>
<td>60</td>
<td>600</td>
<td>100</td>
<td>500</td>
</tr>
<tr>
<td>HDL-C (%)</td>
<td>+61</td>
<td>+31</td>
<td>+38</td>
<td>+29</td>
</tr>
<tr>
<td>LDL-C (%)</td>
<td>-24</td>
<td>-2</td>
<td>-40</td>
<td>-36</td>
</tr>
<tr>
<td>TG (%)</td>
<td>-9</td>
<td>-3</td>
<td>-7</td>
<td>-11</td>
</tr>
</tbody>
</table>

Adapted from Cannon C et al. JAMA. 2011;306:2153-2155.
Effects of Torcetrapib in Patients at High Risk for Coronary Events

**Death from Any Cause**

- Patients without Event (%) over time.
- HR = 1.58 (95% CI: 1.14-2.19) for Atorvastatin + Torcetrapib vs Atorvastatin,
  - p = 0.006

**Primary composite outcome (Major Cardiovascular Events)**

- Patients without Event (%) over time.
- HR = 1.25 (95% CI: 1.09-1.44) for Atorvastatin + Torcetrapib vs Atorvastatin,
  - p = 0.001

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Failure of CETP Inhibitor Torcetrapib

Torcetrapib reduces LDL-C by 20% and increases HDL by more than 60%, however:

**ILLUMINATE Study**: Combination of atorvastatin and torcetrapib increased total mortality, including cardiovascular mortality.

**ILLUSTRATE Study**: Torcetrapib had no effect on plaque volume.

**RADIANCE 1 & 2**: Torcetrapib had no effect on IMT in FH heterozygotes and mixed hyperlipidemia.

Torcetrapib elevated blood pressure due to increase of aldosterone.
Rationale and design of the dal-OUTCOMES trial: efficacy and safety of dalcetrapib in patients with recent acute coronary syndrome. 


DESIGN: The study will randomize approximately 15,600 patients to receive daily doses of dalcetrapib 600 mg or matching placebo, beginning 4 to 12 weeks after an index ACS event. There are no prespecified boundaries for HDL cholesterol levels at entry. Other elements of care, including management of low-density lipoprotein cholesterol, are to follow best evidence-based practice. The primary efficacy measure is time to first occurrence of coronary heart disease death, nonfatal acute myocardial infarction, unstable angina requiring hospital admission, resuscitated cardiac arrest, or atherothrombotic stroke. The trial will continue until 1,600 primary end point events have occurred, all evaluable subjects have been followed for at least 2 years, and 80% of evaluable subjects have been followed for at least 2.5 years.

Roche stops dalcetrapib trial for lack of benefit

MAY 7, 2012 Reed Miller

Basel, Switzerland—Roche has stopped the phase 3 dal-OUTCOMES trial of the cholesteryl ester transfer protein (CETP) inhibitor dalcetrapib after interim analysis of the study showed the HDL-cholesterol-boosting drug was not significantly reducing cardiovascular adverse events [1].

As reported by heartwire, the earlier dal-PLAQUE study showed that dalcetrapib reduced inflammation in the carotid artery and that there was an inverse relationship between HDL-cholesterol levels and markers of arterial inflammation in patients treated with the drug. Dal-OUTCOMES was a major morbidity and mortality study currently planned for about 16,000 stable coronary heart disease patients with recent acute coronary syndrome (ACS). Patients in the study were randomized to either 600 mg daily of dalcetrapib and standard medical therapy or placebo and standard medical therapy.

Roche provides update on Phase III study of dalcetrapib

Roche (SIX: RO, ROG; OTCQX: RHHBY) today announced that following the results of the second interim analysis of the dalcetrapib dal-OUTCOMES Phase III trial, the independent Data and Safety Monitoring Board (DSMB) has recommended stopping the trial due to a lack of clinically meaningful efficacy. The dal-OUTCOMES trial evaluated the efficacy and safety profile of dalcetrapib when added to existing standard of care in patients with stable coronary heart disease (CHD) following an acute coronary syndrome (ACS). No safety signals relating to the dal-OUTCOMES trial were reported from the DSMB.
Risk Reduction for CHD Events
As a Function of Changes in TC, LDL-C, and HDL-C

<table>
<thead>
<tr>
<th>PERCENT CHANGE</th>
<th>CHD EVENT RATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C</td>
<td>1% ↓</td>
</tr>
<tr>
<td>TC</td>
<td>1% ↓</td>
</tr>
<tr>
<td><strong>HDL-C</strong></td>
<td>1% ↑</td>
</tr>
</tbody>
</table>

*4S, CARE, LIPID, WOSCOPS
**HELSINKI, VA-HIT, AFCAPS/TexCAPS
Torcetrapib: Markedly increases serum HDL-C, and reduces LDL-C

Dalcetrapib: Increases serum HDL-C, but does not reduce LDL-C

→ Elevation of HDL-C by CETP inhibition may not affect CVD or rather increase CVD. HDL functions and the efficiency of reverse cholesterol transport are more important for prevention and regression of atherosclerosis
Forest Plot Showing Effect of CETP Inhibitors on Risk of All Cause Mortality Stratified by CETP Inhibitors

**Anacetrapib**
- Define 2010: 11/811 vs 8/812
- Subtotal: 11/811 vs 8/812
- Test for heterogeneity: Not applicable
- Test for overall effect: z=0.69, P=0.49

**Dalcetrapib**
- Dal-Vessel 2012: 0/239 vs 1/237
- Dal-Plaque 2011: 1/64 vs 2/66
- Dal-Outcomes 2012: 226/7938 vs 229/7933
- Subtotal: 227/8241 vs 232/8236
- Test for heterogeneity: $\chi^2=0.00$, $\chi^2=0.73$, df=2, P=0.69, $I^2=0\%$
- Test for overall effect: $z=0.23$, P=0.82

**Torcetrapib**
- Radiance 1 2007: 0/450 vs 1/454
- Radiance 2 2007: 1/377 vs 1/375
- Illuminate 2007: 93/7533 vs 59/7534
- Illustrate 2007: 8/591 vs 6/597
- Subtotal: 102/8951 vs 67/8960
- Test for heterogeneity: $\chi^2=0.00$, $\chi^2=1.05$, df=3, P=0.79, $I^2=0\%$
- Test for overall effect: $z=2.69$, P=0.007

**Total (95% CI)**: 340/18 003 vs 307/18 008
- Test for heterogeneity: $\chi^2=0.01$, $\chi^2=7.91$, df=7, P=0.34, $I^2=12\%$
- Test for overall effect: $z=1.31$, P=0.19
- Test for subgroup difference: $\chi^2=6.12$, df=2, P=0.05, $I^2=67.4\%$

Keene D et al. BMJ 2014;349:bmj.g4379
Without Background Statin Treatment, Fibrates and Niacin, But Not CETP Inhibitors Were Found to Reduce Non-fatal Myocardial Infarction

Keene D et al. BMJ 2014;349:bmj.g4379
Why CETP Inhibitors Do Not Work in Humans

Liver

ApoA-Ⅰ

ABCA1

SR-BI

Discoidal HDL

LDL receptor

ApoB-containing lipoprotein (VLDL, IDL, LDL)

Intestine

ApoA-Ⅰ

ABCA1

SR-BI

Discoidal HDL

Small HDL

LCAT

Obese very large HDL = Dysfunctional LDL

Atheroma (Foam cell)

CETP

Small dense LDL

LDL receptor

TG

CE

CETP
Anti-atherogenic Actions of HDL

- Cellular Cholesterol Efflux & Reverse Cholesterol Transport
- Anti-infectious activity
- Anti-thrombotic activity
- Endothelial Repair
- Anti-inflammatory activity
- Anti-oxidative activity
- Anti-apoptotic activity
- Anti-diabetic
- Vasodilatory Activity

Functions of HDL-Associated Proteins

Lipid Metabolism
- CETP
- LCAT
- ApoC-I
- ApoC-II
- ApoC-III
- ApoC-IV
- PON3
- SAA4
- SAA2
- SAA1
- ApoA-IV
- ApoA-I
- ApoH
- PON1
- Clusterin
- ApoA-II
- ApoL-1
- ApoD
- ApoE
- ApoF
- ApoM

Proteinase Inhibitor
- AGT
- SERF2
- SERF1
- AHSG
- HRP
- SERA1
- AMP
- KNG
- RBP4
- TF
- FGA
- HPX
- ITIH4
- ORM2
- TTR
- VTN

Complement Regulation
- C3
- C4A
- C4B
- C9

Acute Phase Response
- C3
- C4A
- C4B
- C9
- VTN
When good cholesterol goes bad?
(Fogelman AM et al, Nat Med 2004; 10: 902-903)

**HDL: is it always atheroprotective?**
(Ansell BJ et al, Curr Atheroscler Res 2006; 8: 405-411)

“Functional HDL”

“Dysfunctional HDL”

Myeloperoxidase

(Ansell et al, JACC 2005; 10: 1792-1798)

Quality is more important than Quantity?
Composition of HDL is important for playing its proper role?
Effects of HDL Obtained from Healthy Subjects, Patients with CAD or Acute Coronary Syndrome on NO Release from Human Aortic Endothelial Cells

HDL Infusion Improves Endothelial Function in Humans

Flow-mediated Dilation (%)

Before HDL

After HDL

Systemic inflammation / Oxidative stress
- Infection
- Coronary disease
- Diabetes mellitus
- Metabolic syndrome
- Smoking
- Rheumatologic conditions
- Chronic kidney disease
- Surgery
- Obstructive sleep apnea
- Saturated fat diet

Chronic acute phase response

Proinflammatory HDL

- LDL oxidation
- Vascular inflammation

Anti-inflammatory HDL

Odds Ratios for Coronary Artery Disease According to Cholesterol Efflux Capacity

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of Patients</th>
<th>Odds Ratio for Coronary Artery Disease (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adjusted for Cardiovascular Risk Factors</td>
</tr>
<tr>
<td>Quartile 1</td>
<td>198</td>
<td>1.00</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>198</td>
<td>0.75 (0.48–1.16)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>198</td>
<td>0.58 (0.37–0.89)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>199</td>
<td>0.40 (0.25–0.63)</td>
</tr>
<tr>
<td>P value for trend</td>
<td></td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Cardiovascular risk factors included in the logistic-regression model were age, sex, smoking status, presence or absence of diabetes, presence or absence of hypertension, and low-density lipoprotein cholesterol. HDL denotes high-density lipoprotein.
Cholesterol efflux capacity is more important for reduction of coronary artery disease than serum HDL-C levels.
# HDL Cholesterol Efflux Capacity and Incident Cardiovascular Events

<table>
<thead>
<tr>
<th>Models</th>
<th>No. of Participants with Event/Total No. of Participants</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL cholesterol</td>
<td>132/2416</td>
<td>0.64 (0.40–1.03)</td>
</tr>
<tr>
<td></td>
<td>Unadjusted analysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Analysis adjusted</td>
<td></td>
</tr>
<tr>
<td></td>
<td>For traditional risk factors</td>
<td>0.80 (0.47–1.37)</td>
</tr>
<tr>
<td></td>
<td>For traditional risk factors and HDL particle concentration</td>
<td>1.08 (0.59–1.99)</td>
</tr>
<tr>
<td>Cholesterol efflux capacity</td>
<td>132/2416</td>
<td>0.44 (0.27–0.73)</td>
</tr>
<tr>
<td></td>
<td>Unadjusted analysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Analysis adjusted</td>
<td></td>
</tr>
<tr>
<td></td>
<td>For traditional risk factors</td>
<td>0.30 (0.18–0.50)</td>
</tr>
<tr>
<td></td>
<td>For traditional risk factors and HDL cholesterol</td>
<td>0.31 (0.18–0.52)</td>
</tr>
<tr>
<td></td>
<td>For traditional risk factors and HDL particle concentration</td>
<td>0.34 (0.20–0.56)</td>
</tr>
<tr>
<td></td>
<td>For traditional risk factors, HDL cholesterol, and HDL particle concentration</td>
<td>0.33 (0.19–0.55)</td>
</tr>
</tbody>
</table>
HDL Cholesterol Efflux Capacity and Incident Cardiovascular Events

Atherosclerotic Cardiovascular Disease

Total Cardiovascular Disease

Unique Characteristics of Probucol

● Lowers HDL-C as well as LDL-C
● HDL is small and poor in CE
● Enhances HDL-mediated cholesterol efflux from mφ
● Accelerates HDL-mediated reverse cholesterol transport *in vivo*
  by enhancement of CETP and SR-BI
● Strong anti-oxidative effect
● Reduces xanthomas (Achilles tendon, xanthelasma, etc)
Effects of Probucol on HDL

- Probucol reduces HDL-C
- HDL of probucol-treated patients is small and poor in cholesteryl ester
- HDL of probucol-treated patients has more capacity for cholesterol efflux
- HDL of probucol-treated patients has a strong anti-oxidative activity
Typical Absorption Profiles (Conjugated Diene Formation) Produced during Oxidation of LDL by AAPH
Influence of HDL Derived from FH Patients on LDL Oxidation by AAPH

**p<0.01. ***p<0.001
Anti-atherogenic Functions of Probucol

<table>
<thead>
<tr>
<th>Trial</th>
<th>Location</th>
<th>Intervention</th>
<th>Outcome</th>
<th>Effect</th>
<th>p-value</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>PQRST</td>
<td>FA</td>
<td>P vs Placebo</td>
<td>lumen volume</td>
<td>n.s.</td>
<td></td>
<td>Walldius 1994</td>
</tr>
<tr>
<td>PART</td>
<td>CoroA</td>
<td>P vs Con</td>
<td>restenosis rate</td>
<td>23% vs 58%</td>
<td>(p&lt;0.001)</td>
<td>Yokoi 1997</td>
</tr>
<tr>
<td>MVP</td>
<td>CoroA</td>
<td>P vs MV</td>
<td>repeated PTCA</td>
<td>13% vs 26%</td>
<td>(p&lt;0.009)</td>
<td>Tardif 1997</td>
</tr>
<tr>
<td>FAST</td>
<td>CarotA</td>
<td>P vs Pra</td>
<td>CV event</td>
<td>2.4% vs 4.8% vs 13.6% P vs Diet</td>
<td>(p&lt;0.001)</td>
<td>Sawayama 2002</td>
</tr>
<tr>
<td>PAB</td>
<td>FemorA</td>
<td>P vs Con</td>
<td>restenosis rate</td>
<td>23% vs 58%</td>
<td>(p&lt;0.001)</td>
<td>Gallino 2004</td>
</tr>
<tr>
<td>SAKURA</td>
<td>DiabNeph</td>
<td>P vs Con</td>
<td>interval to HD</td>
<td>27mo vs 11mo</td>
<td>(p&lt;0.02)</td>
<td>Endo 2006</td>
</tr>
<tr>
<td>POSITIVE FH</td>
<td>P vs Con</td>
<td>CV event</td>
<td>CV event</td>
<td>27% vs 64%</td>
<td>(p&lt;0.001)</td>
<td>Yamashita 2008</td>
</tr>
</tbody>
</table>
**POSITIVE:**

Kaplan-Meier Survival Curve (Secondary prevention)

- **Group of non-probucol treatment (N)**
- **Group of probucol treatment (P)**

- Cumulative survival rate
- Censored cases

*P* < 0.001 (2-sided) by log rank test

Probucol therapy improves long-term (>10-year) survival after complete revascularization

Hazard ratio of probucol use mortality

No-probucol (n=225) vs Probucol (n=225)

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>(95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-case</td>
<td>0.45</td>
<td>(0.27-0.75)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

All-cause Death in the Matched Dataset

Cumulative survival (%)

Years after revascularization

Probucol group
No-probucol group

Probucol group  225  213  123  1
No-probucol group  225  200  119  16

P<0.001

Atherosclerosis 220:463-469, 2012
Cardiac Death in the Matched Dataset

Cumulative survival (%)

- Probucol group
- No-probucol group

Years after revascularization

<table>
<thead>
<tr>
<th></th>
<th>Probucol group</th>
<th>No-probucol group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>225</td>
<td>225</td>
</tr>
<tr>
<td>Follow-up Period</td>
<td>213 123 1</td>
<td>200 119 16</td>
</tr>
</tbody>
</table>

NS (P=0.057)

Atherosclerosis 220:463-469, 2012
Non-cardiac Death in the Matched Dataset

Atherosclerosis 220:463-469, 2012

P=0.004
Content of secondary prevention study of probucol

TRI (Translational Research Informatics Center)

Secondary prevention study
(Number of 860) Japan
Entry period: 2 years, Follow-up period: 3 years
Open, Prospective, Random, Multiple

Meta analysis of three countries of cardiovascular

Effect confirmation of secondary prevention of three countries

Japan (Number of patients: 860)
Primary endpoint: Cardiovascular events,
Secondary endpoint: IMT

Korea (Number of patients: 150)

China (Number of patients: 192)
Primary endpoint: IMT,
Secondary endpoint: Cardiovascular events

Three countries Meta analysis
Take Home Messages

- HDL-cholesterol level is important, but marked hyper-HDL-cholesterolemia is not always protected from atherosclerosis.
- The functions of HDL such as cholesterol efflux capacity, anti-oxidant activity and anti-inflammatory property need to be tested.
- Enhancement of reverse cholesterol transport (RCT) by CETP may protect CV events rather than inhibition of CETP.
- Probucol reduces HDL-C by enhancing CETP and RCT, preventing atherosclerotic cardiovascular diseases and coronary restenosis after PCI.