Atorvastatin rapidly improves eNOS coupling in human mammary arteries by stimulating GTP-cyclohydrolase I expression and improving vascular tetrahydrobiopterin bioavailability

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No Disclosures to declare
Statins and clinical outcome

Kulik A. et al., Circulation 2008

Mechanism?

HMG-coA reductase

Statins

Mevalonate

Farnesyl-PP

Geranylgeranyl-PP

Cholesterol

RhoA, Rac, etc

eNOS expression

[EXPERIMENTAL STUDIES]

HMG-CoA reductase

Antoniades C et al; Circulation 2010


Statin - Statin

Ezetimibe - Ezetimibe

NADPH-oxidase


Antoniades C et al; Circulation 2010

Statins

-
The “good” and the “bad” face of eNOS

Data from Cell Cultures/Animal Models

Concept based on preliminary data from experimental studies

Limited data in humans

↑GTPCH

RhoA

↑eNOS mRNA

±

eNOS “uncoupling”

Vasques-Vivar et al, PNAS 1998;95
Förstermann & Münzel, Circulation. 2006;113
Aim of the study

Define the direct effect of short-term statin treatment on vascular redox state and NO bioavailability in the human arterial endothelium.

Explore the effects of statin treatment on vascular BH4 levels and eNOS coupling in human vessels.
Study 1: Design and methods

Screened: 332 patients undergoing elective CABG

42 patients not under statin treatment agreed to participate

Baseline investigations

Randomized to

Atorvastatin 40mg/d 3 days

Placebo 3 days

Preoperative investigations

CABG

Segments of IMA

Blood sampling

brachial artery FMD

Blood sampling

brachial artery FMD

Vascular biopterins (HPLC)

Vascular O$_2^-$, eNOS coupling (Lucigenin chemiluminescence and DHE staining)
# Study population

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Atorvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (M/F)</td>
<td>21 (20/1)</td>
<td>21(18/3)</td>
</tr>
<tr>
<td>Age in years</td>
<td>67.4±1.9</td>
<td>66.1±2</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hypertension</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Smokers Curr/Ex</td>
<td>7/10</td>
<td>8/9</td>
</tr>
<tr>
<td>Total Cholesterol mg/dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Baseline</em></td>
<td>205(157-244)</td>
<td>225(184-243)</td>
</tr>
<tr>
<td><em>Post-treatment</em></td>
<td>191(165-251)</td>
<td>171(144-204)</td>
</tr>
<tr>
<td>Triglycerides mg/dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Baseline</em></td>
<td>206(102-264)</td>
<td>132(94-191)</td>
</tr>
<tr>
<td><em>Post-treatment</em></td>
<td>146(105-185)</td>
<td>113(89-166)</td>
</tr>
<tr>
<td>HDL mg/dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Baseline</em></td>
<td>35(31-47)</td>
<td>39(35-43)</td>
</tr>
<tr>
<td><em>Post-treatment</em></td>
<td>39(32-42)</td>
<td>37(33-45)</td>
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<tr>
<td>Medication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin/clopidogrel</td>
<td>12/8</td>
<td>13/6</td>
</tr>
<tr>
<td>B-blockers/ CCBs</td>
<td>13/5</td>
<td>13/ 5</td>
</tr>
<tr>
<td>ACEi/ARB (%)</td>
<td>8/4</td>
<td>11/6</td>
</tr>
<tr>
<td>Diuretics</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>
Effects of short-term atorvastatin treatment on endothelial function

**LDL (mg/dl)**
- Pre: Placebo, Post: Placebo
- Pre: Atorvastatin, Post: Atorvastatin

**FMD (%)**
- Pre: Placebo, Post: Placebo
- Pre: Atorvastatin, Post: Atorvastatin

**EID (%)**
- Pre: Placebo, Post: Placebo
- Pre: Atorvastatin, Post: Atorvastatin

*P=NS*

*P<0.01*
Effects of short-term statin treatment on vascular redox state

Human Internal Mammary Arteries (IMA) n=38

**Arterial $O_2^-$**

**Placebo**

**Atorvastatin**

*P*<0.05

**L-NAME delta $O_2^-$**

**Placebo**

**Atorvastatin**

*P*<0.05

Uncoupled ‘eNOS’ → Produces superoxide

Atorvastatin

Coupled ‘eNOS’ → Produces NO

L-NAME → superoxide

L-NAME ↔ superoxide
Effects of short-term atorvastatin treatment on vascular tetrahydrobiopterin

Human Internal Mammary Arteries (IMA) n=38

BH4 (pmol/g tissue) vs Placebo and Atorvastatin

P<0.05

BH2+B

Endothelial nitric oxide synthase (eNOS)

O2-

BH4

IMAI

Atorvastatin

0

10

20

30

40

50

100

150

Placebo

Atorvastatin
In patients with advanced atherosclerosis

Mechanism?

Statins

Oxygenase

Reductase

Statins

BH4

Fe

O2

L-arginine

L-citrulline

eNOS “uncoupling”

Vasques-Vivar et al, PNAS 1998;95
Förstermann & Münzel, Circulation. 2006;113
Is the effect of atorvastatin on eNOS coupling independent of LDL lowering?
Screened: 54 patients undergoing elective CABG

26 patients agreed to participate

CABG

Segments of IMA

Ex vivo experiments to explore the direct impact of atorvastatin on eNOS coupling
Description of study protocol

Paired Segments of IMA

- Determination of vascular $O_2^-$ production
- Estimation of endothelium derived $O_2^-$ with fluorescence microtopography and DHE staining

Atorvastatin 5μM

Atorvastatin 5μM + mevalonate 200μM

Control

Incubated for 6h in an LDL-free environment
Ex vivo effect of atorvastatin on vascular $O_2^-$

- Atorvastatin
  - Vascular $O_2^-$ (RLU/sec/mg tissue)
    - Control: -15 to 5
    - Atorvastatin: -10 to 0
  - LNAME delta ($O_2^-$) (RLU/sec/mg tissue)
    - Control: -15 to -10
    - Atorvastatin: -10 to 0

P < 0.01
Mevalonate reversed the effect of atorvastatin

Effect due to direct inhibition of mevalonate pathway in the vascular wall, even in an LDL-free environment
Fluorescence microtopography - DHE staining

Control  Atorvastatin  Atorvastatin + Mevalonate

A  C  E

B  +LNAME  D  +LNAME  F  +LNAME
Is the direct effect of atorvastatin on eNOS coupling through improvement of vascular BH4 bioavailability?
Description of study protocol

Paired Segments of IMA

- Measurement of vascular biopterins
- GTPCH gene expression studies

Atorvastatin 5μM
Atorvastatin 5μM + DAHP 1mM
Control

Incubated for 6h in an LDL-free environment
Ex-vivo effect of Atorvastatin on vascular tetrahydrobiopterin

Atorvastatin

Arterial BH4 (pmol/g tissue)

Arterial tBio (pmol/g tissue)

P<0.01

P<0.01
Ex-vivo effect of Atorvastatin GCH1 expression

P<0.05
Atorvastatin increased vascular tetrahydrobiopterin

<table>
<thead>
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<th>DAHP</th>
<th>-</th>
<th>+</th>
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Conclusions

In human arteries

Vasques-Vivar et al, PNAS 1998;95
Förstermann & Münzel, Circulation. 2006;113
Conclusions

This is the first study demonstrating a direct effect of statins on eNOS coupling in human arterial endothelium.

This effect is due to direct inhibition of vascular HMG-CoA reductase, leading to up-regulation of GCH1 gene that results into an increase of GTPCH enzymatic activity and elevation of vascular BH4 in the human arterial wall.

These novel findings document for the first time in humans, a direct pleiotropic effect of statins on endothelial eNOS function in human atherosclerosis.
Acknowledgments

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- Prof. Keith Channon

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- Prof. Christodoulos Stefanadis

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1. The European Commission (MC-IEF, RIG)
2. The Hellenic Society of Cardiology
3. The British Heart Foundation
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Effects of treatment on systemic oxidative stress and low-grade inflammation
Exclusion Criteria

1. ACS < 3 months
2. Renal / Liver disease
3. Any inflammatory disease
4. Malignancy
5. Treatment with NSAIDs / antioxidants
Atorvastatin or Vitamin C (µM)

Superoxide change (% control)

* *p<0.05 vs 0µM, †p<0.05 vs vitamin C