Role of Alpha-lipoic acid (ALA) and statin in vascular smooth muscle cell proliferation

In-kyu Lee, M.D., Ph. D.

Dept. of Internal Med, Keimyung Univ.,
School of Med. Taegu, Korea
Oxidative Stress and Atherosclerosis

- Hyperlipidemia
- Diabetes
- Obesity
- Hypertension

Oxidative Stress

High

Low

Redox-Sensitive Signaling Pathway, Transcriptional Factors

Atherogenic Genes

Inflammation

Vascular Dysfunction

Atheroprotective Genes

Anti-inflammatory Vascular Protection

Kunsch C, Circulation Research. 1999
Mode of action

Alpha-lipoic acid (ALA) is a metabolic cofactor of the pyruvate dehydrogenase complex and a strong antioxidant at the same time.
Alpha lipoic acid has the potential to impact on CV risk

↓ Reduce body weight - obesity

↓ Blood pressure

↓ Adhesion Molecule

↓ Insulin resistance

↓ CVD Risk

Improve endothelial function, endothelial cell survival

↓ PAI-1

↓ MMP, Migration and proliferation of VSMC

↓ Atherosclerosis, cardiovascular disease
1. Effects of Alpha-Lipoic Acid on VSMC PAI-1 expression
Effect of α-Lipoic Acid on PAI-1 expression on high glucose condition in VSMC

1. Normal glucose (NG)
2. High glucose (HG, 22 mM)
3. High glucose containing 250 µM α-Lipoic acid
4. High glucose containing 500 µM α-Lipoic acid
5. High glucose containing 1 mM α-Lipoic acid
6. High glucose containing 2 mM α-Lipoic acid
7. High glucose containing 4 mM α-Lipoic acid
2. Effects of Alpha-Lipoic Acid on VSMC migration and MMP expression in Vitro and in Vivo
Inhibitory effect of ALA on migration of VSMCs stimulated by high glucose and TNF-α
Effect of ALA on MMP-9 activity

A

<table>
<thead>
<tr>
<th>Glucose</th>
<th>N</th>
<th>H</th>
<th>H</th>
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<tbody>
<tr>
<td>TNF-α</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>ALA</td>
<td>-</td>
<td>-</td>
<td>+</td>
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Phospho Image Units (folds over N control)

B

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<th>Glucose</th>
<th>N</th>
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<td>TNF-α</td>
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<td>10</td>
<td>10</td>
<td>10</td>
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<td>ALA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
<td>1.0</td>
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Phospho Image Units (folds over N control)
Effect of ALA on MMP-9 promotor activity
Effects of α-Lipoic Acid on NF-κB Activity

• ALA decreases AGE induced NFκB activation in cultured endothelial cells
  \textit{Bierhaus A et al, Diabetes, 1997}

• ALA decreases ROS generation and NFκB activity induced by glucose
  \textit{Du X et al, Free Radic Biol Med, 1999}
Effects of ALA on the NF-κB activity in VSMC Cells
Effects of ALA on the activities of NF-κB and AP-1

Schematic structure of wild type and mutant MMP-9 promoter constructs used for promotor assay
Summary and Conclusion

• α-Lipoic acid suppress PAI-1 expression, MMP-9 expression, VSMC migration.  

• This effect would be related with blocking NFκB pathway which increase expression of genes associated with atherosclerosis including TNF-α, IL-1, IL-6, MCP-1, VCAM-1, ICAM-1, E-selectin, endothelin-1, tissue factor and ets.
3. Effects of Alpha Lipoic Acid in Cell Cycle Regulation and Apoptosis
# Cell cycle arrest in ALA treated HASMC

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<th>Time (h)</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>36</th>
<th>48</th>
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<td>2mM ALA</td>
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- CycD1
- PCNA
- CycA1
- CycB1
- p27
- PTEN
- Cdk2
- CycE
Apoptotic Effects of ALA in RASMC

NG
HG
HG + ALA 2mM
Induce apoptosis by ALA treatment

Smooth muscle cells were treated with alpha lipoic acid. After 20 hours, cells were harvested, fixed, processed and analysed for TUNEL staining. The apoptotic effect by alpha lipoic acid was shown.
Induce of apoptosis by ALA treatment

FACS analysis was performed smooth muscle cells after alpha lipoic acid. Ten thousand cells were sorted. The percentages of cells in G1, S and G2-M phases of the diploid cell cycle are shown. Alpha lipoic acid induced apoptosis.
Adenovirus-mediated expression of a variant of TR3(Nur77), decreases DNA synthesis and increases p27Kip1 protein expression in cultured SMCs.

Protective Function of Transcription Factor TR3 Orphan Receptor(Nur77) in Atherogenesis Decreased Lesion Formation in Carotid Artery Ligation Model in TR3 Transgenic Mice.

_Circulation_. 2002;106:1530-1535
Carotid artery ligation in TR3 and TA transgenic mice.
A, ligated carotid arteries from wild-type and TA-F mice 2.5 weeks after ligation. Neointima/media ratios of ligated left carotid arteries are 3-fold larger in TA-D and TA-F transgenic mice compared with wild-type mice (n4 to 6) (*P<0.05).

B, left carotid arteries from wild type and TR3-A mice 4 weeks after ligation. Morphometric analyses disclosed that TR3 overexpression results in a 5-fold reduction of neointima/media ratio compared with ratio in wild-type mice (n5 to 8) (**P<0.01). Bar 100 m.

*Circulation. 2002;106:1530-1535*
Nur77 Family

- Steroid/thyroid hormone receptor superfamily
- Orphan nuclear receptor / Immediate early gene

<table>
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<tr>
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<th>Transactivation Domain (TAD)</th>
<th>DNA binding Domain (DBD)</th>
<th>Ligand binding Domain (LBD)</th>
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<tr>
<td>Nur77</td>
<td>Zn</td>
<td>Zn</td>
<td>A</td>
</tr>
<tr>
<td>Nurr1</td>
<td>27%</td>
<td>92%</td>
<td>67%</td>
</tr>
<tr>
<td>Nor-1</td>
<td>21%</td>
<td>91%</td>
<td>54%</td>
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</table>
Major targets for activation of Nur77 transcription.

Several critical secondary messengers including cAMP, Ca++, and the activation of ERK, are associated with the activation of Nur77.
Does ALA treatment can induce Nur77 Expression?
Expression of Nur77 and Nurr1 in SMC by alpha lipoic acid

The rat aortic smooth muscle cells were treated with alpha lipoic acid for indicated time and dose.
Expression of Nur77 in SMC by alpha lipoic acid

Protein Expression of Nur77
Effect of Alpha lipoic acid on Nur77 promoter activity

Effects of ALA on the promoter activity of Nur77-luc promoter in VSMCs. VSMCs were transfected with the Nur77-luciferase reporter plasmid, the cells were treated with 2mM of alpha lipoic acid for 16hrs. The promoter activity was increased by dose dependent manner.
Evaluate Effects of $\alpha$-lipoic acid on Neointimal Formation in Balloon Injury Model

-3day

0

Weeks

ALA intraperitoneal injection

2 Weeks

Balloon injury

Sacrify

Study (dose dependent)

1) Normal control
2) Balloon injury (BI)
3) ALA – BI – ALA (25mg/kg)
4) ALA – BI – ALA (50mg/kg)
5) ALA – BI – ALA (100mg/kg)
Cross-section of the common carotid artery of control rat and ALA treated rat 14 days after balloon injury
Summary and Conclusion II

- \( \alpha \)-Lipoic acid suppress PAI-1 expression, MMP-9 expression, RASMC proliferation, migration and neointimal formation.

- This effect might be related with blocking NF\( \kappa \)B pathway which increase expression of genes associated with atherosclerosis including TNF-\( \alpha \), IL-1, IL-6, MCP-1, VCAM-1, ICAM-1, E-selectin, endothelin-1, tissue factor.

- Tx with ALA in VSMC induced apoptosis and inhibition of VSMC proliferation, possibly through the increased Nur 77 expression.
Alpha-Lipoic acid

- Prevent or improve metabolic syndrome
- and premature cardiovascular disease

- Insulin Resistance
- Obesity
- Beta Cell Failure

- Oxidative stress↓
  - Increased AMPK

- NFκB ↓
  - Nur 77↑

- Hypertension

- Endothelial Dysfunction

- Vascular Smooth Muscle cell
  - Proliferation and Migration
Pleiotropic effects of statins

Inkyu Lee, M.D., Ph.D.

Dept of Endocrinology
School of medicine, Keimyung University
Fig. 2. Cholesterol biosynthesis pathway. Inhibition of hydroxy methylglutaryl coenzyme A (HMG-CoA) reductase by statins reduces the synthesis of mevalonate, cholesterol and isoprenoids. Reducing these metabolites with statins leads to subsequent changes in bioactive proteins, including the activation of the GTP-binding proteins Rho and Rac.
Our study, to clarify whether

In *in vivo* Balloon injury Model.

1. Atorvastatin can suppress neointimal Formation and *ICAM-1 Expression* in *in vivo* Balloon injury Model.

In *in vitro* EC and SMC

2. Atorvastatin can suppress IFN-γ induced expression of *ICAM-1*.

3. Atorvastatin can modulates MAP kinase activity which suppresses IFN-γ-induced phosphorylation of serine 727 in STAT1.

Lee et al, EMM, 34(6), 451-461, 2002
ICAM 1- Expression in neointimal after Balloon Injury 2 weeks

Balloon Injury Model-Control

Balloon Injury Model-Atorvastatin 10 mg/Kg

Balloon Injury Model-Atorvastatin 20 mg/Kg
Figure 1. Flow cytometric analysis of statin effect on ICAM-1 upregulation in response to IFN-γ and TNF-α.

Lee et al, EMM, 34(6), 451-461, 2002
Figure 2. Effects of statin, IFN-γ and TNF-α on ICAM-1 gene expression in EC and vascular smooth muscle cells.

Lee et al, EMM, 34(6), 451-461, 2002
This study demonstrates that atorvastatin inhibits the ability of IFN-\(\gamma\) to induce ICAM-1 in endothelial and vascular SMC.

Thus, this finding suggests that statins may be useful as therapeutic agents for atherosclerosis, because they inhibit the action of IFN-\(\gamma\) and they lower serum lipids.

Lee et al, EMM, 34(6), 451-461, 2002
Figure 3. Effects of PD98059, IFN-γ and TNF-α on ICAM-1 gene expression in EC and SMC.

Lee et al, EMM, 34(6), 451-461, 2002
Summary & Conclusion

In cells treated with atorvastatin and IFN-γ, ICAM-1 was expressed at a lower level than in cells treated with IFN-γ alone. However, atorvastatin does not reduce TNF-α induced expression of ICAM-1. A similar result was observed in cells treated with the MEKK inhibitor PD98059 and IFN-γ.
Acknowledgements

Keimyung University, School of Medicine
Endocrinology and Metabolism
Institute for Medical Sciences

Mi-Jung Kim
Hye-Soon Kim
Jong Deok Ahn
Hyo-Jeong Lee
Hye-Jin Kim
Yong-Deuk Kim
Seo-Youn Jung

University of Ulsan, College of Med.
Endocrinology and Metabolism
Asan Institute for Life Sciences

Ki-Up Lee
Joong-Yeol Park M.D
Min Sun Kim

University of Chunnam.
Hormone Research Center

Heung-sik Choi

Osaka University

Y. Kaneda
R. Morishita