Βιοδείκτες οξειδωτικού στρες

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Generation of superoxide (\(\cdot O_2\)) and \(H_2O_2\) from \(O_2\) in vascular cells

Schematic diagram of the structure of the active NADPH oxidase complex
Upstream regulators of NAD(P)H oxidase in vascular cells

Interplay between NADPH oxidase and other sources of ROS

Sources of ROS in vascular cells

Endothelial cell
- Cyto P450 oxidases
- Lipooxygenase
- NAD(P)H oxidases
- eNOS

Smooth muscle cell
- Lipooxygenase
- NAD(P)H oxidases
- Heme oxygenase

Adventitial cell
- Lipooxygenase
- NAD(P)H oxidases

Macrophage
- MPO

Reactions:
- NO$^-$ → OONO$^-$
- O$_2$$^-$ → H$_2$O$_2$
- H$_2$O$_2$ → HClO$^-$
- SOD
Functions of ROS in the immunological response against environmental pathogens

Regulation of the transcription factor hypoxia-inducible factor 1 (HIF-1)
Role of ROS in epidermal growth factor (EGF) receptor-mediated signaling
Role of ROS in insulin receptor kinase activation
Regulatory events and their dysregulation depend on the magnitude and duration of the change in ROS or reactive nitrogen species (RNS) concentration.
ROS production in vascular injury

Papaharalambus CA, et al.
*Trends Cardiovasc Med* 2007;17:48–54
In situ detection of ROS and glutaredoxin in human coronary arteries
Macrophage

Endothelial Cells

NAD(P)H oxidases
Xanthine oxidase
Cyclooxygenases

Macrophage

O$_2^-$

NADPH oxidase
H$_2$O$_2$

MPO $\rightarrow$ HOCI

ONOO$^-$

NO$^-$

iNOS

Antioxidant depletion
ApoB modification
Lipid (per)oxidation

LDL $\rightarrow$ oxLDL
Συστατικά της OxLDL με προαθηρογόνο δράση

- Οξειδωμένη apoB
- Οξειδωμένα φωσφολιπίδια
- Λυσο-φωσφατιδιδυλοχολίνη
- Οξειδωμένα παράγωγα λιπαρών οξέων (πχ: ισοπροστάνια)
- Οξυστερόλες
- Αλδεϋδες
- Ολόκληρο το σωματίδιο της OxLDL
Ρόλος της OxLDL στην αθηροσκλήρωση
Macrophage

NAD(P)H oxidases
Xanthine oxidase
Cyclooxygenases

Endothelial Cells

eNOS

Macrophage

NADPH oxidase

H₂O₂

O₂⁻

MPO

Cl⁻ → HOCl

NO^•⁻

ONOO^•⁻

NO₂^•⁻

O₂⁻

H₂O₂

Antioxidant depletion
ApoB modification
Lipid (per)oxidation

HDL

oxHDL (HOCl-HDL)

HOCl
Colocalization of HOCl-modified epitopes with apoAI or MPO is present on the endothelial layer as well as in the connective tissue.
Oxidative stress induces dysfunctional HDL

Redox-dependent signaling pathways in vascular cells

Role of ROS in the response to injury

ROS mediate many of the responses to vascular injury

Mechanical factors
- Oscillatory shear stress
- PTCA

Biological factors
- Hypercholesterolemia
- Excess free radicals
- Diabetes
- Homocysteine
- Infectious agents

Vessel Tone
- Ang II
- Con

Migration

Apoptosis

Hyperplasia

Inflammation

Vascular effects of ROS

Oxidative phosphorylation, superoxide production, and scavenging pathways in Mitochondria

Madamanchi NR, et al. Circ. Res. 2007;100;460-473
Atherogenic mechanisms
of mitochondrial dysfunction

Madamanchi NR, et al.
*Circ. Res.* 2007;100;460-473
Insulin resistance and hyperglycemia-induced increased mitochondrial superoxide production activates atherogenic signaling pathways

Madamanchi NR, et al. Circ. Res. 2007;100;460-473
Biochemistry of oxidant stress biomarkers
Serum Levels of TBARS Predict Cardiovascular Events in Patients With Stable CAD (PREVENT Study)
Elevated plasma 8-isoprostane and reduced antioxidant capacity are associated with the extent and the severity of CAD.

Myeloperoxidase Predicts Progression of Carotid Stenosis in States of Low High-Density Lipoprotein Cholesterol

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Vienna, Austria
Assays for in-vivo detection of oxidative stress in uremia

- Lipids
  - Malondialdehyde and other aldehydes
  - Lipid peroxidation
  - Oxidized LDL
  - Exhaled ethane
  - Advanced lipoxidation endproducts
- Arachidonic acid derivatives
  - \( \mathrm{F}_2 \)-isoprostanes
  - Isolevuglandins
- Carbohydrates
  - Reactive aldehydes
  - Advanced glycosylation endproducts
- Amino acids
  - Cysteine/cystine
  - Homocysteine/homocystine
  - Isocaspartate
- Proteins
  - Thiol oxidation
  - Carbonyl formation
  - Advanced oxidation protein products
- DNA
  - 8 hydroxy 2' deoxyguanine
- Other
  - Spin traps (electron paramagnetic resonance)
Elevated plasma protein 3-chlorotyrosine a specific biomarker of MPO-catalyzed oxidation
Effect of antioxidants on the risk of CAD

Cohort studies – high versus low intake
- β-Carotene (eight studies, n=38,768) 0.88 (0.77–1.01)
- α-Tocopherol (nine studies, n=82,379) 0.74 (0.66–0.83)
- Ascorbic acid (eleven studies, n=50,000) 0.89 (0.79–0.99)

Cohort studies – high versus low plasma/serum levels
- β-Carotene (four studies, n=5061) 0.46 (0.37–0.58)
- α-Tocopherol (one study, n=286) 1.61 (0.78–3.33)
- Ascorbic acid (five studies, n=13,018) 0.58 (0.47–0.72)

Randomized controlled trials of food supplements
- β-Carotene (six studies, n=86,056) 1.02 (0.96–1.08)
- α-Tocopherol (four studies, n=48,346) 0.96 (0.88–1.04)
- Ascorbic acid (two studies, n=16,700) 0.98 (0.75–1.26)
SPACE Trial profile

598 records reviewed to identify eligible patients

243 patients identified as eligible

196 patients randomised

99 assigned placebo
34 primary endpoints

97 assigned vitamin E
18 primary endpoints

HD ασθενείς με CAD
800 IU/day

Lancet 2000; 356: 1213–18
SPACE Trial
Kaplan-Meier survival curves from primary cardiovascular-disease endpoints

![Graph showing Kaplan-Meier survival curves for SPACE Trial with placebo and vitamin E groups, showing p=0.014 significance.]

Lancet 2000; 356: 1213
Randomized Prospective Clinical Trials of Vitamin E in the Prevention of CAD

<table>
<thead>
<tr>
<th>Trial</th>
<th>Vit E, IU/dl</th>
<th>Subjects, n</th>
<th>Follow-up, years</th>
<th>Prevention</th>
<th>Cardiovascular risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATBC</td>
<td>50</td>
<td>29,133</td>
<td>6.1</td>
<td>Primary</td>
<td>No effect</td>
</tr>
<tr>
<td>GISSI</td>
<td>300</td>
<td>8,488</td>
<td>3.5</td>
<td>Secondary</td>
<td>No effect</td>
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<tr>
<td>HOPE</td>
<td>400</td>
<td>9,541</td>
<td>4.5</td>
<td>Secondary</td>
<td>No effect</td>
</tr>
<tr>
<td>CHAOS</td>
<td>400-800</td>
<td>2,002</td>
<td>1.4</td>
<td>Secondary</td>
<td>Decrease, 47%</td>
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<tr>
<td>SPACE</td>
<td>800</td>
<td>196</td>
<td>1.4</td>
<td>Secondary</td>
<td>Decrease, 54%</td>
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</table>
Summary of clinical trials for the effect of vitamin E supplementation in CAD

<table>
<thead>
<tr>
<th>Trial</th>
<th>Subjects’ description</th>
<th>Number of subjects</th>
<th>Dose and type of vitamin E</th>
<th>Follow-up (years)</th>
<th>Parameters</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary prevention</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATBC</td>
<td>Male smokers; 50 - 69 years</td>
<td>29133</td>
<td>50 mg <em>all rac</em>-tocopheryl-acetate</td>
<td>6.1</td>
<td>MI, stroke deaths</td>
<td>0.95</td>
</tr>
<tr>
<td>PPP</td>
<td>At least one major risk factor for CVD; mean age 64.4 years</td>
<td>4495</td>
<td>300 mg <em>all rac-α</em>-tocopherol</td>
<td>3.6</td>
<td>CVD mortality, MI Peripheral-artery disease</td>
<td>0.87</td>
</tr>
<tr>
<td>ASAP</td>
<td>Plasma cholesterol &gt; 5 mM; age 45 - 69 years</td>
<td>458</td>
<td>136 IUx2/day RRR-α-tocopheryl-acetate</td>
<td>3.0</td>
<td>IMT progression in common carotid artery</td>
<td>0.56</td>
</tr>
<tr>
<td><strong>Secondary prevention</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHAOS</td>
<td>Clinical and angiographic evidence of CAD; mean age 62 years</td>
<td>2002</td>
<td>400-800 IU RRR-α-tocopherol</td>
<td>1.4</td>
<td>CVD and total mortality Non-fatal MI</td>
<td>1.18</td>
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<tr>
<td>SPACE</td>
<td>Hemodialysis and known CVD; age 40 - 75 years</td>
<td>196</td>
<td>800 IU RRR-α-tocopherol</td>
<td>1.4</td>
<td>MI, CVD mortality</td>
<td>0.46</td>
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<tr>
<td>GISSI</td>
<td>Recent MI (&lt; 3 months); age &gt; 50 to &lt; 80 years</td>
<td>11324</td>
<td>300 mg <em>all rac-α</em>-tocopherol</td>
<td>3.5</td>
<td>CVD mortality, non-fatal MI</td>
<td>0.88</td>
</tr>
<tr>
<td>HOPE</td>
<td>Known CVD or diabetes; mean age 66 years</td>
<td>9541</td>
<td>400 IU RRR-α-tocopheryl-acetate</td>
<td>4.5</td>
<td>CVD mortality</td>
<td>1.05</td>
</tr>
</tbody>
</table>
Strategies for the targeted delivery of antioxidants to mitochondria

Targeting based on biophysical properties of mitochondria: high negative internal potential

Targeting based on unique mitochondrial localization of enzymes that catalyze the release of drugs from prodrugs

Targeting based on transporter-dependent delivery of prodrugs