Defining the Role(s) of Nox2-containing NADPH Oxidase in the Cerebral Circulation

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Overview

The role of cerebral artery Nox2-NADPH oxidase in:

- Endothelial dysfunction associated with hypercholesterolemia
- Gender-dependent differences in responses to angiotensin II
Hypercholesterolemia and the cerebral circulation

Hypercholesterolemia

↓

Endothelium

↓ NO

Impaired Relaxation

Vascular Smooth Muscle
Hypercholesterolemia and the cerebral circulation

**HYPERTROPHIC PULMONIC STENOSIS**

**OXIDATIVE STRESS?**

\[ \overset{\uparrow}{\cdot}O_2^- \quad \rightarrow \quad \overset{\downarrow}{NO}\cdot \]

Endothelium

**IMPAIRED RELAXATION**

Vascular Smooth Muscle
Hypercholesterolemia and the cerebral circulation

HYPERCHOLESTEROLEMIA

OXIDATIVE STRESS?

SOURCE?

Endothelium

$\uparrow \cdot O_2^- \rightarrow \downarrow NO^-$

IMPAIRED RELAXATION

Vascular Smooth Muscle
Cerebrovascular NADPH oxidases

Adapted from Miller et al. (2006) Pharmacology & Therapeutics
NADPH oxidase activity and expression are higher in the rat cerebral circulation under physiological conditions

Superoxide production

NADPH oxidase activity and expression are higher in the rat cerebral circulation under physiological conditions

Superoxide production

Nox2 protein expression


Miller, De Silva et al., (2009) AJP: Heart Circ Physiol
Enhanced superoxide production by Nox2-NADPH oxidase leads to impaired endothelial function in cerebral arteries of hypercholesterolemic mice
Total cholesterol levels are elevated in ApoE\(^{-/-}\) and Nox2\(^{-/-}/\text{ApoE}^{-/-}\) mice

*\(P<0.05\) vs. wild-type (n=12-23)

Representative of n=3
Cerebral artery basal superoxide production is substantially elevated in hypercholesterolemic mice

Superoxide Production

*P<0.05 vs. wild-type (n=14 per group)

Cerebral artery: pooled basilar and middle cerebral
Nox2 deficiency decreases cerebral artery basal superoxide in hypercholesterolemic mice

*P<0.05 vs. wild-type, ApoE+/−/Nox2+/− (n=14-17)

Cerebral artery: pooled basilar and middle cerebral
Nox2 deficiency decreases cerebral artery basal superoxide in hypercholesterolemic mice

*P<0.05 vs. wild-type, ApoE<sup>-/-</sup>/Nox2<sup>-/-</sup> (n=14-17)

Cerebral artery: pooled basilar and middle cerebral
NO• bioavailability is decreased in hypercholesterolemic mice

*P<0.05 vs. wild-type (n=6 per group)
Nox2 deficiency restores NO• bioavailability in hypercholesterolemic mice

*P<0.05 vs. wild-type (n=6 per group)
Acute scavenging of superoxide restores NO• bioavailability in hypercholesterolemic mice

In the presence of tempol (1 mmol/L)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Diameter (% Change)</th>
<th>Wild-type</th>
<th>ApoE⁻/⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-NAME (100 μmol/L)</td>
<td></td>
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<tr>
<td>K⁺ (124 mmol/L)</td>
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</tbody>
</table>

n=6 per group
HYPERCHOLESTEROLEMIA

MOLECULAR BASIS?

\[ \text{Nox2} \]
\[ \text{p22} \]
\[ \text{p47phox} \]
\[ \text{p67phox} \]

\[ \cdot \text{O}_2^- \rightarrow \text{NO}^- \rightarrow \text{Decreased NO}^- \text{ bioavailability} \]
HYPERCHOLESTEROLEMIA

MOLECULAR BASIS?

Increased Nox2

\[ \cdot O_2^- \rightarrow NO^\cdot \rightarrow \text{Decreased NO}^\cdot \text{ bioavailability} \]
Cerebral artery Nox2 protein expression is NOT elevated in hypercholesterolemic mice

Cerebral artery: pooled basilar and middle cerebral; n=8 per group
Cerebral artery $p47^{\text{phox}}$ protein expression is elevated in hypercholesterolemic mice

Cerebral artery: pooled basilar and middle cerebral; $^* P<0.05$ vs. wild-type, n=5 per group

MONASH University
Medicine, Nursing and Health Sciences
Summary 1

In the cerebral circulation of hypercholesterolemic mice:

• Excessive superoxide production and endothelial dysfunction occur in the absence of atherosclerotic lesions

• Such changes are due to increased activity of Nox2-NADPH oxidase
Overview

The role of cerebral artery Nox2-NADPH oxidase in:

- Endothelial dysfunction associated with hypercholesterolemia
- Gender-dependent differences in responses to angiotensin II
NADPH oxidase activity is lower in cerebral arteries from female animals

NADPH (100 μM)-stimulated superoxide production

Rat

Mouse

* $P < 0.05$ vs. male

Adapted from Miller et al., (2007) Stroke
Angiotensin II-stimulated NADPH oxidase activity is lower in cerebral arteries from female animals

*P<0.05 vs. male

De Silva et al., (2009) Stroke
Angiotensin II and Nox2-containing NADPH oxidases

Gender influences responses to angiotensin II through Nox2-NADPH oxidase
Angiotensin II (0.1µM)-stimulated $O_2^-$ production is lower in cerebral arteries from female mice

$O_2^-$ production (L-012)

n=7-10, *P<0.05 vs WT males

De Silva et al., (2009) Stroke
Nox2 deletion significantly attenuates angiotensin II (0.1µM)-stimulated $O_2^-$ production in males

$O_2^-$ production (L-012)

n=7-10, *P<0.05 vs WT males

WT: Wild type
Nox2<sup>−/−</sup>: Nox2 deficient mice

De Silva et al., (2009) Stroke
Nox2 deletion significantly attenuates angiotensin II (0.1µM)-stimulated $\text{O}_2^-$ production in males

$\text{O}_2^-$ production (L-012)

n=7-10, *$P<0.05$ vs WT males

WT: Wild type
Nox2$^{-/-}$: Nox2 deficient mice

De Silva et al., (2009) Stroke
Contractions of middle cerebral arteries to angiotensin II are smaller in female mice

Angiotensin II

Artery Diameter

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>109.5 ± 1.6 µm</td>
<td>101.3 ± 4.9 µm</td>
</tr>
</tbody>
</table>

n=5-6, *P<0.05 vs. WT females

De Silva et al., (2009) Stroke
Nox2 deletion significantly attenuates angiotensin II induced contractions in males

Angiotensin II

<table>
<thead>
<tr>
<th>Angiotensin II (log mol/L)</th>
<th>Diameter (% change)</th>
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<tbody>
<tr>
<td></td>
<td>Male WT</td>
</tr>
<tr>
<td></td>
<td>Female WT</td>
</tr>
<tr>
<td></td>
<td>Male Nox2⁻/⁻</td>
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<tr>
<td>Male WT</td>
<td>109.5 ± 1.6 µm</td>
<td>101.3 ± 4.9 µm</td>
</tr>
<tr>
<td>Male Nox2⁻/⁻</td>
<td>121.7 ± 3.7 µm</td>
<td></td>
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</tbody>
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n=5-6, *P<0.05 vs. WT females and Nox2⁻/⁻ males

De Silva et al., (2009) Stroke
Nox2 deletion significantly attenuates angiotensin II induced contractions in males

*P<0.05 vs. WT females, Nox2<sup>−/−</sup> males and Nox2<sup>−/−</sup> females

De Silva et al., (2009) *Stroke*
Summary 2

The female gender is associated with:

- Lower levels of vascular superoxide in response to angiotensin II
- Smaller contractions to angiotensin II

Gender differences appear to be:

- Exclusively dependent on Nox2-NADPH oxidase
Perspectives

In the cerebral circulation, Nox2-NADPH oxidase plays a pivotal role in:

- Endothelial dysfunction during hypercholesterolemia
- Responses to angiotensin II

Nox2 = therapeutic target?
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