Dose-dependent effects of myeloperoxidase inhibition on endothelial function and atherosclerotic lesion formation in apolipoprotein E deficient mice

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Conflict of Interest

None.
Background

- Inflammation has been implicated in all stages of atherosclerotic plaque development.
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- Myeloperoxidase (MPO), a member of the heme peroxidase-cyclooxygenase superfamily, is an enzyme linked to both inflammation and oxidative stress.

Lau et al., Pharm Ther 2006
Background

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- Myeloperoxidase (MPO), a member of the heme peroxidase-cyclooxygenase superfamily, is an enzyme linked to both inflammation and oxidative stress.

- MPO is abundantly expressed in the azurophilic granules of most leukocyte subspecies, including polymorphonuclear neutrophils, monocytes and macrophages.

Lau et al., Pharm Ther 2006
Chemical reactions of MPO

MPO + H\textsubscript{2}O\textsubscript{2} → primary MPO-products

- HOX
- HOSCN
- NO\textsubscript{2}•
- Tyr

MPO + HOCl → secondary MPO-products

- HOCI

Cl\textsuperscript{-} + X\textsuperscript{-} → Host defense, bacterial/viral killing

+ pathogens

+ unsaturated lipids

+ DNA

+ proteins

+ εNH\textsubscript{2}

+ ROOH

Physiology

Pathophysiology

- Cardiac dysfunction
- Atherosclerosis
- Glomerulosclerosis
- Ischemia/reperfusion

- 5-chlorouracil
- 3-chloro-Tyr
- Haloamines
- Radical products

- Initiation of new lipid radicals able to promote lipid peroxidation

Malle E, British J Pharm 2007;152:838

Munich ESC Congress 2012

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Pro-atherogenic effects of MPO

Liberation of oxygen reactive species

Increase of vascular reactivity
Unstable plaque
Facilitates platelet adhesion
Metalloproteinase activation of matrix
Endothelial cell apoptosis

LDL Modification pro-atherogenic form
Mediates LDL-C uptake by macrophages
Reduction in cholesterol efflux
HDL dysfunction
Catalytic consumption of nitric oxide

Endothelial dysfunction
Serum marker MPO

Serum Myeloperoxidase Levels Independently Predict Endothelial Dysfunction in Humans

Myeloperoxidase Serum Levels Predict Risk in Patients With Acute Coronary Syndromes

Vita JA, Circulation 2004;110:1134
Baldus S, Circulation 2003;108:1440
MPO and plaque tissue

Macrophage Myeloperoxidase Regulation by Granulocyte Macrophage Colony-Stimulating Factor in Human Atherosclerosis and Implications in Acute Coronary Syndromes

American Journal of Pathology 2001;158
Expression of Human Myeloperoxidase by Macrophages Promotes Atherosclerosis in Mice
Aim of the study

Impact of specific myeloperoxidase inhibition on ROS, endothelial function and atherosclerotic lesion formation in apolipoprotein E deficient mice
8 weeks old homozygous apolipoprotein E deficient mice (ApoE-/-; genetic background C57BL/6J)

high-fat, cholesterol rich diet for 8 weeks

4-aminobenzoic acid hydrazide (4-ABAH)/vehicle i. p. every second day for 8 weeks

Study protocol

Vehicle (10 % DMSO)

n=5

12.5 µg/g 4-ABAH (10 % DMSO)

Low-dose

n=6

25 µg/g 4-ABAH (10 % DMSO)

High-dose

n=6
MPO inhibition by 4-ABAH

- Specific irreversible inhibition of MPO and production of hypochlouros acid
- MPO oxidizes ABAH to a radical that reduces the enzyme to its ferrous intermediate
- Ferrous MPO reacts either with oxygen to allow enzyme turnover, or with hydrogen peroxide to give irreversible inactivation

\[
\begin{align*}
\text{MP}^{3+} + \text{ABAH}^* & \rightarrow \text{MP}^{2+} + \text{ABAH}_{\text{ox}} \\
\text{MP}^{2+} + \text{H}_2\text{O}_2 + \text{ABAH} & \rightarrow \text{MP}_{\text{inact}}
\end{align*}
\]
Results – Blood pressure

Systolic blood pressure

- Vehicle
- Low-dose 4-ABAH
- High-dose 4-ABAH

Diastolic blood pressure

- Vehicle
- Low-dose 4-ABAH
- High-dose 4-ABAH
Results – Heart rate and body weight

Heart rate

Body weight

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Results – Cholesterol levels

- **Vehicle**
- **Low-dose 4-ABAH**
- **High-dose 4-ABAH**
Results – Vascular oxidative stress

L-012 chemiluminescence

Vascular oxidative stress (% of control)

Vehicle
Low-dose 4-ABAH
High-dose 4-ABAH

p<0.05

p<0.05
Results – Endothelial function

Developed tension (% of max. phenylephrine-induced)

Carbachol (log/mol)

Nitroglycerin (log/mol)

- vehicle
- low-dose 4-ABAH
- high-dose 4-ABAH

p<0.05
Results – Atherosclerotic lesion formation

- Vehicle
- Low-dose 4-ABAH
- High-dose 4-ABAH

Plaque area (% total area)

Vehicle: 20%
Low-dose 4-ABAH: 15%
High-dose 4-ABAH: 10%

p < 0.05
Summary

Our data demonstrate dose-dependent effects of specific MPO inhibition by 4-ABAH on ROS, endothelial function and atherosclerotic lesion formation in a mouse model of atherosclerosis. MPO inhibition could possibly be beneficial in the prophylaxis and treatment of atherosclerosis.