Targeting Antioxidants and Redox Probes to Mitochondria

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Mitochondria and Oxidative Stress

Overview

First, the generally recognised consensus on mitochondrial ROS production and the critical questions to be addressed when considering Mitochondria and Oxidative Stress
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First, a *generally recognised* consensus on mitochondrial ROS production and the critical questions to be addressed when considering Mitochondria and Oxidative Stress

“*generally recognised*” = “*I think, but I haven’t bothered to look up the references*”
Critical questions

• ROS
  – Which?
  – Where?
  – Effects?
Critical questions

• Antioxidants
  – Which ROS?
  – Effective?
  – Other effects?
  – Measurable endpoints in vivo/patients?
Mitochondrial ROS metabolism

Proton cycling coupled to ATP synthesis

Futile/uncoupled proton cycling

Mitochondrial inner membrane

Intermembrane space

Mitochondrial ROS metabolism

Protein catalysed leak

H⁺ non-protein catalysed leak

Superoxide

Aconitase damage

Oxidative damage

MnSOD

PRX III

GPX

GR

GSSG

NADPH

NADH

H₂O₂

Fe²⁺

OH⁻

ONOO⁻
Uptake of MitoQ by Mitochondria

```
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>MitoQ uptake (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
</tr>
</tbody>
</table>
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+FCPP

_JBC_ (2001) 276 4588-4596
Reduction of MitoQ by the Respiratory Chain

Absorbance = 0.1

Wavelength (nm)

Time
Prevention of Lipid Peroxidation by Reduced MitoQ

![Graph showing MDA (nmol/mg protein) levels with varying concentrations of MitoQ and Mitoquinone. The x-axis represents [MitoQ] (µM) and [Mitoquinone] (µM), while the y-axis represents MDA (nmol/mg protein). The graph shows a decrease in MDA levels with increasing concentrations of MitoQ.]
Turnover of MitoQ by mitochondria
MitoQ variants

Partition coefficient

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Partition Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPMP</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td>MitoQ&lt;sub&gt;3&lt;/sub&gt;</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>MitoQ&lt;sub&gt;5&lt;/sub&gt;</td>
<td>13.9 ± 1.1</td>
</tr>
<tr>
<td>MitoQ&lt;sub&gt;10&lt;/sub&gt;</td>
<td>2,760 ± 220</td>
</tr>
<tr>
<td>MitoQ&lt;sub&gt;15&lt;/sub&gt;</td>
<td>20,000 ± 4,900</td>
</tr>
</tbody>
</table>

Phospholipid

FEBS Letts (2004) 571 9-16
Coenzyme Q
Distribution of MitoQ
Complex III with MitoQ
Could steric hindrance explain the low reactivity of \( \text{MitoQ}_{10} \) with Complex III?

JBC (2007) in press
Steric inhibition is not involved in Complex III
Complex II with MitoQ$_{10}$, MitoQ$_3$ and MitoQ$_5$
Mitochondrial Membranes

SV Constant (% of max)

Pyrene (CH₂ groups)

MitoQ₁₀
DecylTPP
Idebenone

JBC (2007) in press
Distribution of MitoQ
Potency of MitoQ in a Friedreich’s Ataxia model

Concentration [nM]

MitoQ
MitoQ + FCCP
Decylubiquinone
Idebenone

Distribution of TPMP in Mice

TPMP (nmol/g wet weight)

Days of ingestion

 PNAS (2003) 100, 5407-5412
MitoQ decreases tissue damage during I/R injury
Protection of mitochondrial function

NAD^+ Linked respiration

$\text{Respiratory Control Index}$

$\text{pre-ischaemia}$ $\text{control}$ $\text{TPMP}$ $\text{Q}_{3}\text{OH}$ $\text{mQ}_{10}$

$\text{post-ischaemia}$ $***$ $**$

FASEB J (2005) 19 1088-1095
Nitroglycerin tolerance due to damage to mitochondria

In vivo treatment
Rat Aorta

% of initial tension

Control
MitoQ
Nitroglycerin
Nitroglycerin + MitoQ

Nitroglycerin (log M)

Pharmaceutical development of MitoQ

• Change counter-ion, complex to β-cyclodextrin
• Toxicity
  – No Observable Effect Level (NOEL) 2.4 mg/kg
  – No Observable Adverse Effect Level (NOAEL) 10.6 mg/kg
• Bioavailability ~ 10 %
• Minimal excretion in urine of unmodified MitoQ.
• Major metabolites in urine - glucuronides on Q ring
• 10 mg MitoQ tablets
Plasma levels of MitoQ in humans following oral administration

MitoQ (1 mg/kg oral)
$C_{\text{max}} = 33.15 \text{ ng/ml}$
$T_{\text{max}} = 1 \text{ hour}$
Mean ± SD, $N = 6$
Human Phase II trials with MitoQ
(Antipodean Pharmaceuticals Inc
www.antipodeanpharma.com)

• **Parkinson’s Disease “Protect Trial”**
  – A double-blind, prospective, randomized comparison of 2 doses of MitoQ (40 and 80 mg) and placebo for the treatment of patients with Parkinson’s Disease
  – Primary outcome: Unified Parkinson's Disease Rating Scale (UPDRS) score at the final study visit compared to baseline
  – Multi centre (New Zealand and Australia), fully recruited (128 patients), outcome due March 2008.

• **Hepatitis C**
  – Phase II clinical trial of MitoQ to investigate the drug’s efficacy to reduce liver damage in patients with raised liver enzymes associated with the Hepatitis C virus (HCV).
  – Auckland, NZ. Started recruiting February 2007