Synthetic Peroxides: A Viable Alternative to Artemisinins for the Treatment of Uncomplicated Malaria?

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Susan A. Charman
Monash University, Australia
Why do we need a new class of peroxides?

- The clinical utility of peroxide antimalarials is well established; recent alarming reports of ACT treatment failures
- Fully synthetic peroxides would provide a substantial benefit in relation to cost and availability relative to artemisinins
- Design of new peroxides, with improved properties, could:
  (i) provide more convenient dosing regimens
  (ii) help to limit the ‘fallout’ if artemisinin resistance develops
  (iii) possibly, remove the current contraindication in pregnancy
Synthetic Peroxides

1,2,4-trioxolanes (secondary ozonides)

- Potent inhibitors of *P. falciparum* *in vitro* (IC$_{50} < 2$ nM) and *P. berghei* *in vivo* (single dose ED$_{90} < 5$ mg/kg)

- Effective against all blood stages of the parasite with rapid onset of action

- Peroxide bond is required for biological activity

- Fe(II) reactivity is a necessary, but insufficient, requirement for antimalarial activity

- 100-fold less potent than artemisinin in inhibiting PfATP6
Objectives of the Synthetic Peroxide Project

• First Generation OZ project aimed to:
  – identify a new class of fully synthetic peroxides
  – more potent than the available semi-synthetic artemisinins
  – low cost when used in combination
  – 3-day treatment regimen when used in combination
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  – identify a new class of fully synthetic peroxides
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• Next Generation OZ project aimed to extend these goals:
  – provide a single-dose oral cure (in combination) for patients with uncomplicated *P. falciparum* malaria
  – potential for prophylactic treatment and intermittent preventative treatment in pregnant women and infants (IPTp and IPTi)
To Achieve Next Generation Goals…

• First generation OZ:
  – significantly improved biopharmaceutical properties compared to artemisinins, but overall exposure still limited

• Single dose oral cure and prophylaxis would require:
  – dramatic increase in the exposure profile following oral administration…but without sacrificing potency
  – understand the mechanisms of in vivo clearance
  – structural design modifications to reduce clearance and increase the half life
Suspected Clearance Mechanisms

First Generation OZ

CYP450-mediated clearance
- *in vitro* studies in hepatic microsomes
- *in vivo* PK studies in rats

Blood-mediated clearance
- *in vitro* studies in blood
- inherent instability of peroxide bond required for activity

Relative contribution of these processes was unknown
Clearance Mechanisms of First Generation OZ

- determine contribution of CYP450 metabolism
- administer an irreversible CYP inhibitor (ABT)

1-Aminobenzotriazole

- irreversible inhibitor of Cytochrome P450 enzymes
- safe in animals at doses up to 100 mg/kg
- for compounds highly metabolised by CYP enzymes, there is a substantial reduction (80-90%) in \textit{in vivo} clearance following pre-dosing with ABT
Clearance Mechanisms of First Generation OZ

Oral administration of first generation OZ (30 mg/kg PO) ± pre-dose with ABT (100 mg/kg PO) in rats

- without ABT: CL 147 mL/min/kg
- with ABT: CL 72 mL/min/kg
Relatively rapid degradation of first generation OZ and Artemisinins in rat whole blood *in vitro* (37°C)
Structural Modifications to Reduce Clearance

- CYP450 metabolism
  - blocking known site of metabolism adversely affected activity

- Blood instability
  - cleavage of peroxide, and subsequent formation of free radicals required for activity
  - could we reduce blood instability without sacrificing potency?
Structural changes led to a significant increase in rat blood stability *in vitro* (37°C).
**In Vivo** Plasma Profiles in Rats after Oral Dosing

Substantial decrease in clearance and increase in exposure profile in rats (each dosed at 10 mg/kg PO)
But what about antimalarial activity?

<table>
<thead>
<tr>
<th>Compound (mg/kg)</th>
<th>Survival (d), Cure (%)</th>
</tr>
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<tbody>
<tr>
<td>Artesunate</td>
<td>9 d, 0%</td>
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<td>10 d, 0%</td>
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<td>Mefloquine</td>
<td>22 d, 0%</td>
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<td>First Generation OZ</td>
<td>11 d, 0%</td>
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Limited survival; no cures with artemisinin derivatives, conventional drugs or First Generation OZ.
But what about antimalarial activity?

Single 30 mg/kg oral dose to mice (P. berghei)

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<td>25 d, 64%</td>
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<td>&gt;30 d, 100%</td>
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Survival and 100% cure for Next Generation OZ
..And prophylaxis?

Single 30 mg/kg oral dose 24 h prior to *P. berghei* infection (mice)

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Only mefloquine has prophylactic properties
..And prophylaxis?

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Three next generation OZ are 100% prophylactic and all are equivalent to mefloquine.
Characteristics of Next Generation OZ

• Specific structural design concepts of the next generation OZ have resulted in:

  – improved stability in blood...without compromising biological activity
  – reduced *in vivo* clearance, prolongation of half-life and increased exposure in rats (as well as mice and dogs)...and better oral bioavailability
  – enhanced biological activity in a well-established mouse model of malaria
  – excellent prophylactic activity in mice – exceeds that of the benchmark chemoprophylactic mefloquine
Steps Forward…

• Chemistry: straightforward synthesis, low COG confirmed characterisation of different salt forms on-going metabolite synthesis planned expertise in process chemistry/scale up planned

• Efficacy: *Aotus P. falciparum* monkey model on-going synergism/antagonism studies on-going cross resistance studies on-going

• ADME: PK properties in mice, rats, dogs completed *in vitro* metabolism, CYP inhibition near completion PK studies with potential partner drugs planned

• Toxicology: exploratory toxicity studies in rats completed safety pharmacology studies on-going exploratory embryotoxicity studies on-going

• Selection of development candidate planned for 1H 2008
The Synthetic Peroxide Discovery Team

Project Management
Fulcrum Pharma
London
(S. Arbe-Barnes)

Medicinal/Synthetic Chemistry
University of Nebraska
Omaha
(J. Vennerstrom, Y)

Activity/Efficacy/Safety Assessment
Swiss Tropical Institute, Roche, Basilea
Basel
(S. Wittlin, J. Chollet, H. Matile, H. Urwyler)

ADME, Compound Profiling
Monash University
Melbourne
(S. Charman, B. Charman)

Medicines for Malaria Venture

MMV, ESAC
Dr Carl Craft
Dr Win Gutteridge