Providing an address for delivery of nanoencapsulated TB drugs

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Objective

Reduce the dose frequency of anti-tb drugs and simultaneously shorten duration of treatment by: nanoencapsulation of drugs and adding a targeting ligand to address persistent *M.tb* infection.

Adapted from: Comstock 1999
Background

**Nanoparticles:**
Refer to: Dr. H.S. Swai poster no. 83
Mr. L. Kalambo presentation: Track 1 session 4 (03/06/10)

**History:**
- First drug delivery systems described 1960’s – liposomes
- First controlled release polymer 1976
- Followed by other drug delivery & targeting ligands

**Advantages**
- Hydrophobic & hydrophilic drugs
- Targeted delivery in cells & tissue (small size)
- Controlled release
- Improvement of bioavailability

Background

Targeting ligand: Mycolic acids
Refer to: Prof. J.A. Verschoor presentation: track1 session 2 (02/06/10)

The wax coat:
Mainly consists of mycolic acids (MA)

Properties of MA

- Convert macrophages into foam cells (Korf J.E. et al., 2005)
- Assumes cholesteroloid nature and attracts cholesterol (Benadie Y. et al., 2008).
- Present in high concentrations in the extracellular matrix of *M tb*. biofilms, contributing to drug tolerance (Ojha A. et al., 2008).

Thus, MA may possibly target the cholesterol enriched infected areas
Methods and Results

The cholesteroid nature of native MA

- Mycolic acids actually attract cholesterol!
- Thus, use MA as targeting agent in anti-TB NP drug delivery

SPR biosensor – measures mass accumulation on immobilized ligand

(Benadie Y. et al., 2008)
Methods and Results

The cholesteroloid nature of native MA

- Struct relationship & attraction between free MA and cholesterol (Benadie Y. et al., 2008).
- Principle confirmed using the ESPRIT biosensor
  - Cholesteroloid nature attenuated when MA structure altered

(Lemmer Y et al., 2009)
Methods and Results

Nano encapsulation of mycolic acids

Poly lactide-co-glycolide (PLGA)
MA (with or without fluorophore)
INH

Polymer and / or Targeting ligand and / or Anti-TB drug

Double emulsion evaporation technique (W/O/W).

~ 500nm

(Penco, M. et al.,1998; Tsung, M.J. et al., 2001)
**Methods and Results**

**Nanoparticle uptake in U937 and THP-1 macrophages**

- Live cell images of fluorescently labeled MA PLGA NP uptake after 3 hours in macrophage cell lines.

a) THP-1

b) U937 macrophages.
Methods and Results

Assess antibacterial effect of isoniazid (INH) containing nanoparticles vs free drug

1) Does NP release INH? Yes, but slower
2) Does MA influence efficiency of Mtb inhib? No clear indication at this point

Graph: [INH] = 0.2 µg/ml (2 µg/ml = out of range) ★ ★ = P<0.01

Kisich K. et al., 2007
**Background**

**Targeting ligand: Aptamer against Mannose receptor**

Refer to: Dr. B. Semete-Makokotlela email: Bsemete@csir.co.za

**Aptamers:**
- Ab like molecules made up from nucleic acids
- Directed against ligand
- Used as targeting molecule on surface of NP

**Mannose receptor:**
- Transmembrane receptor protein
- Over expressed in infected macrophages (acute stage only?)
Methods and Results

Synthesis of aptamers:

SELEX process:

Partitioning with NanoSep 100kDa MWCO,
Selection of aptamers against target receptor using size exclusion

Challenges:

Could not enrich beyond 52% recovery
100 bp primer dimers
Methods and Results

Testing for binding affinity on Biacore biosensor:

- Mannose receptor bound to surface on chip
- Test clones for binding affinity

Binding kinetics (10 clones)

KD: 1.3uM±2.5  
KD: 60 nM±0.15
Methods and Results

Conjugate aptamers to nanoparticles:

- Succinamide coupling of PEG to NP
- Derivatise aptamer to form NH2 – couple to NP
Conclusion and future perspective

✓ MA (chol) and Aptamers (against the mannose receptor) could be used as targeting tools

✓ MA:
  ✓ Attract cholesterol
  ✓ Successful production of MA NP ave size ~ 500nm
  ✓ In vitro uptake into macrophages
  ✓ In vitro localization possibly cytoplasm?
  ✓ In vitro drug testing via BACTEC indicated:
    ✓ INH released from NP
    ✓ MA does not show advantage in early replication phase as expected

✓ Aptamers:
  ✓ Against mannose receptor
  ✓ Coupling to NP
  ? In vitro drug testing via BACTEC
  ? Confocal imaging of THP-1 cells
  ? FACS to obtain quantitative data
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