Potential for treating tuberculosis with nano drug delivery system

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Global incidence is rising at 1% largely due to the African epidemic

Worldwide Statistics
- 8 million develop active TB annually
- 3 million die annually from TB
- 2 billion – infected with TB (75% of sub-Sahara)
- Once infected for life

Africa - high HIV
Africa - low HIV
World
E Europe
World (exc Afr EEur)
Highest incidence rates per 100 000 in Africa…
How small?

- Ant head - 1mm
- Human hair - 100um, 100 000nm
- Red blood cell - 10um, 10 000nm
- DNA - 4nm wide
- H₂O Molecule - 0.2nm
Worldwide statistics

- SA 125,000 (1997) 256,000 (2004) – doubled
- TB biggest cause of death in SA – 67,000 deaths in 2003 compared to 22,000 in 1997
- Male deaths increase by 60% between 1997 and 2003 and female death by 93%
- Co-infection of HIV and TB in 85% of cases
- Multi-drug resistant TB (MDR)
- XDR-TB

[WHO report: 2000]
XDR-TB – extensive and 'extreme' TB drug resistance

Of 17,690 isolates during 2000-2004: 20% were MDR and 2% were XDR

XDR = MDR-TB plus resistance to at least 3 of the 6 available classes of second line drugs

Of 544 TB cases in SA during 2006: 40.6% were MDR and 24% of MDR were XDR.
All XDR tested positive for HIV

XDR found in:
- USA: 4% of MDR
- Latvia: 19% of MDR
- S Korea: 15% of MDR
TB drugs / recommended treatment

Isoniazid

Rifampicin

Ethambutol

Pyrazinamide
TB: shortfalls of existing treatment

Shortfalls of current therapy

• Extended treatment time
• Degradation of the drugs before reaching their target
• Low permeability and Poor bioavailability / poor bio distribution

Consequences:

• Large doses can cause toxic side effects
• Excretion of native drug
• Patient non compliance due to long treatment period
• Emergence of MDR-TB

DOTs programme:

• Logistics are impractical and expensive-cure rate is 53%
• Research to improve treatment is in progress, but nothing changed in the last 40 years

Solution:

• Polymeric nanoparticles loaded with anti-TB drugs for sustained release
Nano encapsulated TB drugs

Smallest human cell is 2um
Nanoparticles diameter = 2% of a human hair diameter

Polymeric shell

Anti-TB drugs

INH-loaded PLGA nanoparticles
Objectives

Improve the bioavailability of ADTs
- Minimise degradation of drugs in the stomach
- Steady and controlled release

Reduce the dosage and dosage frequency
- Treatment 4 drugs/day – 4 drugs/week
- Improve patient compliance
- Minimise the toxicity of drugs
- Reduce the cost of TB treatment

Targeting TB in infected Macrophages
Dosage and pharmacokinetics of anti-TB drugs for 60 kg body weight

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Adult dose per day</th>
<th>Peak serum conc. (µg/ml)</th>
<th>Usual range MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>0.3 g</td>
<td>3 - 5</td>
<td>0.01 - 1.25</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.45 – 0.6g</td>
<td>8 – 20</td>
<td>0.06 – 0.25</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>1.5g</td>
<td>20 - 60</td>
<td>6.2 – 50</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>1.2g</td>
<td>3-5</td>
<td>0.5 – 2.0</td>
</tr>
</tbody>
</table>
Weekly required dose for unencapsulated and nano-encapsulated drugs (Khuller et al. 2004)

<table>
<thead>
<tr>
<th>Essential ADT</th>
<th>Total weekly dose taken daily of unencapsulated drug (average body weight 60 kg)</th>
<th>Weekly dose nano-encapsulated (average body weight 60kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>2.1g</td>
<td>0.6g</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>4.2g</td>
<td>0.72g</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>10.5g</td>
<td>1.5g</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>8.4g</td>
<td>0.84g</td>
</tr>
</tbody>
</table>
Advantages of nano drug delivery

- Nano DDS have made it possible to extend the residence time in the GIT to weeks
- High encapsulation efficiency
- Good bioavailability and reduce dose frequency
- Ability to target the drug
  - Smallest capillaries in the body are 5-6µm
  - Typical human cell is 2µm hence, nanoparticles can move easily within the body
- Once Optimised for TB - NDDS can be applied for treatment of:
  - Anti-Malaria drugs
  - Anti-Cancer drugs
  - Anti-Retrovirus
  - Antibiotics
  - Long term pain killers etc
Lung delivery
Size and deposition of inhaled particles

Upper airways:
Particles >10µm impact in the upper ways

Tracheobronchial (TB):
Particles in the size range of 2-5µm deposit by sedimentation in the TB

Alveoli:
Particles < 3µm deposit in the alveolar regions

• Large surface area - 140cm²
• Reduce systemic toxicity-high drug concentration
Biocirculation

Nano particles uptake from the gut

Structure of the GIT

Internal structure of the intestine

- Para-cellular via M cell
- Intracellular via epithelial cell-intestine mucosa
- Peyer's patches

Drug Targeting

Active targeting-surface modification/ functionalisation
Routes and mechanisms of particle transport across epithelia

- **PARACELLULAR**
- **ENDOCYTOSIS** by ordinary enterocytes
- **UPTAKE via MALT**

Particle diameter (< 1µm, preferably nanometer)
Surface charge, hydrophobicity, ligands
Anti-TB drug carrying nanovehicles that target TB infected macrophages

Mannose
Mannose receptor
Mycolic acid
Cholesterol
Research in progress: preparation of nanoparticles

Polymers used

- Poly(lactide-glycolic acid) PLGA(50:50)
  - Biocompatible and biodegradable polymer
  - Hydrolytically degraded to lactic and glycolic acid
- Alginate-Chitosan
- Block copolymers
  - PEG/Pluronic-PPS (Micelles, Vesicles and solid nanoparticles)

Encapsulation techniques

- Double emulsion-solvent evaporation
- Double emulsion spray drying technique
- Nanoprecipitation
- Ionotropic gelation of Alginate-Chitosan
- Supercritical CO₂
Results of different techniques of encapsulation

1. Supercritical CO₂
2. Sonication
H.S.
Homogenisation

EE of INH: 11%
EE of INH: 55%
EE of RIF: 60%

Spray drying of emulsion
Chitosan-TPP+PVA
EE- INH:65%

Chitosan-TPP+PVA
FTIR-ATR results
In vitro RIF release assays

Release assays: PBS pH 7.37 at 37 degrees celsius

Release assays: PBS pH 1.2 and 5.0 at 37 degrees celsius
Transport/uptake study: confocal microscopy

Z-stack 30 min incubation

60 min incubation

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Oral nano-encapsulated ATD delivery system
(Khuller et al. 2003)

Plasma Isoniazid (mg/L)

Time (hrs)

Unencapsulated drugs
Encapsulated drugs
Tissue levels of ATDs at day 10 following oral admin of NP formulation to mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (µg/ml)</th>
<th>Lung (µg/ml)</th>
<th>Liver (µg/ml)</th>
<th>Spleen (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIF</td>
<td>0.25</td>
<td>0.6</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td>INH</td>
<td>0.1 – 0.2</td>
<td>3</td>
<td>5.5</td>
<td>1</td>
</tr>
<tr>
<td>PYZ</td>
<td>8 - 20</td>
<td>15</td>
<td>25</td>
<td>23</td>
</tr>
</tbody>
</table>
Toxicology studies

- Performed over 28 days in mice
  - Three drug formulation
  - Four drug formulation
- Drugs administered orally
- No side effects detected in lung and spleen
Toxicology studies

- Performed in guinea pigs
  - Similar pathophysiology to humans
  - Drugs administered orally
  - Analyzed in lung, spleen and liver

- ATD loaded PLGA detectable up to 11 days

- Free ATD cleared in 1-2 days

- No toxic side effects
Primate studies to clearly establish the uptake mechanism

- MRC, US (Prof Seier)

- Objectives
  - Tissue distribution
  - Degradation profile
  - Bioavailability and bioequivalence
  - Determine dose level to be administered per kg/bwt
  - Pharmacokinetic (UCT) and histopathology
The TB project involves the development of delivery systems for tuberculosis treatment. The project focuses on two main approaches: PLGA or natural polymers, and nanoparticles or polysome.

- **PLGA or Natural Polymers**
  - Targeting
  - Pulmonary slow release
  - Oral route
  - Pulmonary
    - Cardiff University
  - Oral
    - Nottingham University
  - CSIR Jewish National Aktiv dry Pharmaceutical
    - Colorado University will develop this

- **Nanoparticles or Polysome**
  - Oral route
  - PLGA
  - PolySome
  - CSIR-PGIMER India / Univ of London
  - Animal trial which will be done in EPFL
  - These will decide between the 2 delivery systems
Project organisational overview
National collaborators

CSIR

MRC
Dr Jürgen Seier
Assist in the non-human primate studies. Analyzing how non-human primates react to the nanoparticles as well as determine the tissue distribution of these particles with the animals.

University of Stellenbosch
Prof Van der Bijl
One of South Africa's leading experts in tissue diffusion / permeability to various therapeutic agents. Will analyse the permeability of nanoparticles in various types of tissues.

UCT
Prof Smith
Experience in the field of TB, particularly research involving Anti-TB drugs. Based on this, his role in the project would be to assist in the pharmacokinetic studies of the nanoparticle encapsulated anti-TB drugs.

North West University, Potch
Prof Kotze and Dr. Anne Glober
State of the art confocal microscope which will be used for fluorescent mechanism to elucidate the mode of transport of the nanoparticles. MRC-Primate studies

Tshwane University of Technology
Prof Hamman
Expertise in the in vivo cell models for drug transport assay. Working closely with the CSIR (Biosciences) on elucidating the mode of transport of the Nonparticles.

MRC
Dr Kobus Venter, Dr Karen Weyer
Accredited research on mice challenged with TB. Pharmacokinetic, tissue distribution as well as toxicity studies of the nanoparticle encapsulated anti-TB drugs in mice.

National collaborators

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International collaborators

International nano drug delivery research collaborators

Natural polymers
Prof Khuller
Research collaboration on natural polymers

Double emulsion, spray drying & nanoprecipitation
Prof Oya Alpar
HR capacity building through Exchange Program

Drug targeting
Cardiff and Nottingham University
Possible Funding through the Wellcome Trust and drug targeting

Self-assembling & solid polymeric particles
EPFL Jeffrey Hubbell
HR capacity building through exchange program

Jewish Medical Univ, AKTIV-dry, Colorado, USA
MDR-TB
Possible funding through Gates Foundation/research collaboration
The project team
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  • Dr Cingo (TUT)
  • Dr Hille (CSIR, NML)
  • Prof Vershoor (UP)
  • Prof Donald, Prof Van Helden (US, MRC)
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• International collaborators
  • EPFL
    • Prof Hubbell
  • Nottingham University
    • Dr Stolnik
    • Dr Alexander
    • Prof Shakesheff
  • University of London
    • Prof Alpar
  • Cardiff University
    • Dr Jones
    • Prof Duncan
Thank you
Questions?
What is nano?

- A **nanometre** (nm) is a unit of measurement equal to a billionth of a metre, tens of thousands of times smaller than the width of a human hair. The prefix “nano” comes from the Greek word meaning “dwarf”.

- A **micrometre** (µm) is a unit of length equal to one thousandth (10^-3) of a millimetre or one millionth (10^-6) of a metre.

- **Nanoscience** is the study of the fundamental principles of molecules and structures with at least one dimension roughly between 1 and 100 nm. It is concerned with materials and systems of which the structures and components exhibit novel and significantly improved physical, chemical and biological properties, phenomena and processes, due to their nanoscale size.

- **Nanotechnology** is the application of nanoscience in technology devices. The essence of nanotechnology is the ability to work at the molecular level, atom by atom, to create large structures with fundamentally new molecular organisation.
Preparation of IHN-PLGA nanoparticles via the double emulsion solvent evaporation

1. **Solvent, polymer adsorption**
2. **Sonication/homogenisation/magnetic stirring**
3. **Hydrophobic interface, shear force**
4. **Hardening, solvent removal**
5. **Lyophilisation via desiccation/freeze drying**
6. **Dehydration**
7. **Harvest**
   - 1. Centrifugation
   - 2. Spray drying

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**Organic solvent/polymer** (DCM, ethylacetate, ethanol)

**Aqueous drug solution**

**Nanoparticles**

Second aqueous phase, Emulsifier