Development of Novel Oral Lipid-Based Amphotericin B Formulations for the Treatment of Systemic Fungal Infections and Visceral Leishmaniasis

Principal Investigator:
Dr. Kishor M. Wasan, Ph.D.
Professor & Distinguished University Scholar
CIHR/iCo Therapeutics Research Chair in Drug Delivery for Neglected Global Diseases
Faculty of Pharmaceutical Sciences
University of British Columbia

Team Members:
Dr. Ellen K. Wasan
Dr. Sheila J. Thornton
Dr. Karen Bartlett
Mr. Ian Bell
Dr. John Clement

2010 McGill Annual Global Health Conference
April 26th 2010
Acknowledgments

Research Faculty and Staff

- Dr. Kristina Sachs-Barrable
- Dr. Sheila Thornton
- Dr. Carlos Leon
- Dr. Pavel Gershkovich
- Ms. Olena Sivak
- Dr. Cheri Barta
- Dr. Robin Stoodley
- Mr. Mike Rosland
- Ms. Verica Risovic

Research Funding

- Canadian Institutes of Health Research (CIHR) Operating Grants
- AAPS Lipid-Based Drug Delivery Award/Grant
- iCo Therapeutics Inc.
- CPDD/Gates Foundation
- Gattefosse Canada

Current Graduate Students

- Jennifer Locke
- Stephen Lee
- Jackie Fleischer
- Alexis Twiddy

Website

- [http://www.wasanlab.ubc.ca](http://www.wasanlab.ubc.ca)

Numerous Undergraduate Students

Dr. Tom Kanyok (Gates Foundation)
Dr. Richard Tidwell (CPDD)
Expert Opinion

The reformulation of Amphotericin B for oral administration to treat systemic fungal infections and visceral leishmaniasis

Sheila J Thornton & Kishor M Wasan†
University of British Columbia, Division of Pharmaceutics and Biopharmaceutics, Vancouver, BC V6T 1Z3, Canada

Amphotericin B (AmB) is a parenterally administered broad-spectrum antifungal and leishmanicidal drug that has been on the market for over sixty years.
Amphotericin B

Polyene antifungal drug discovered in 1955
Mainstay of antifungal therapy for systemic mycoses
Available in 4 formulations
Demonstrated Efficacy

Fungal infections

Cryptococcosis
Candidiasis
Aspergillosis
Hastings Street's misery stuns and frightens Irish visitors

I recently visited Vancouver with my wife and two-year-old son from Ireland and, while we found the hospitality welcoming, we were shocked at what we saw.

Having wandered (as tourists do) into Chinatown, we ended up by accident on Hastings Street. The site of scores of shuffling homeless, vacant and rambling mentally ill and drug addicts reminded me of a scene from a George A. Romero zombie film.

My wife was immediately fearful and we fled the area.

For a city boasting the Winter Olympics, might I suggest that you take a few million out of the kitty and use it to assist these poor unfortunates? I have lived and worked in India and South Africa, the仅对 of both have such problems.
Candidiasis

Oral cavity of an AIDS patient covered by white curdlike exudate containing numerous fungal organisms.
Overcoming Barriers to Treatment

Oral route of administration
- Decreased toxicity
- Efficacious
- Thermal stability at tropical temperatures
- Stability – pH
- Affordability
Rat Model of *Aspergillus Fumigatus*

- *Aspergillus fumigatus* collected from a pool of clinical isolates of patients with disseminated aspergillosis (BC Centre for Disease Control)

- Rats were inoculated 48 hours before the beginning of treatment to allow for aspergillosis to develop.

- Treatment groups
  - iCo-009 – 10 mg/kg PO, bid
  - Abelcet (ABLC) 5mg/kg IV, qd

- Organ colony forming units (CFU) as an indicator of antifungal activity

- Renal toxicity was indirectly assessed by determining creatinine concentration in plasma
# Data Overview - Rat model of Aspergillus Fumigatus

- **Reduction in CFU by iCo-009 comparable to ABLC**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Infected tissues (CFU*/ml of homogenized tissues)</th>
<th>Brain</th>
<th>Lungs</th>
<th>Heart</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-treated control (n=9)</td>
<td></td>
<td>3538**</td>
<td>74</td>
<td>101</td>
<td>308</td>
<td>1163</td>
<td>364</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+/- 1810</td>
<td>+/- 30</td>
<td>+/- 63</td>
<td>+/- 114</td>
<td>+/- 772</td>
<td>+/- 119</td>
</tr>
<tr>
<td>ABLC (5 mg/kg, IV, qd) (n=4)</td>
<td></td>
<td>550</td>
<td>10</td>
<td>15</td>
<td>18</td>
<td>88</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+/- 445</td>
<td>+/- 4</td>
<td>+/- 3</td>
<td>+/- 5</td>
<td>+/- 44</td>
<td>+/- 0</td>
</tr>
<tr>
<td>iCo-009 (10 mg/kg, PO, bid) (n=7)</td>
<td></td>
<td>736</td>
<td>51</td>
<td>20</td>
<td>180</td>
<td>107</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+/- 186</td>
<td>+/- 18</td>
<td>+/- 4</td>
<td>+/- 48</td>
<td>+/- 32</td>
<td>+/- 10</td>
</tr>
</tbody>
</table>

*CFU = colony forming units  
** mean +/-SEM

- *plasma galactomannan levels (an indicator of fungal load) were significantly reduced 80% by 48 hr*
Data Overview - Plasma creatinine levels

- iCo-009 vs ABLC - no kidney toxicity at doses showing anti-fungal activity

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Creatinine (mg/dl)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blank**</td>
<td>0 hr</td>
<td>48 hr</td>
</tr>
<tr>
<td>Control (infected – non treated)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=9)</td>
<td>0.4* +/- 0.1</td>
<td>0.5 +/- 0.1</td>
<td>0.9 +/- 0.2</td>
</tr>
<tr>
<td>ABLC (5 mg/kg, IV)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=4)</td>
<td>0.3 +/- 0.2</td>
<td>0.4 +/- 0.1</td>
<td>0.5 +/- 0.1</td>
</tr>
<tr>
<td>iCo-009 (10 mg/kg, PO)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=7)</td>
<td>0.6 +/- 0.2</td>
<td>0.6 +/- 0.2</td>
<td>0.5 +/- 0.1</td>
</tr>
</tbody>
</table>

*mean +/- SEM  **Blank: plasma sample before fungal infection and before treatment
"0" : plasma sample after 48 hrs of fungal infection and before treatment
"48" : plasma sample after 96 hrs of fungal infection and after 48 hrs of treatment
Data Overview - Rat Model of Candida Albicans

- *Candida Albicans* (1-1.35 x 10^6 colony forming units (CFU)) was injected via the jugular vein & 48h later male rats (350-400 g) were treated

- Treatment groups
  - iCo-009, 5 or 10 mg/kg, PO bid
  - Abelcet (ABLC) 5 mg/kg IV qd
  - Physiological saline, IV qd

- Organs were harvested at sacrifice (day 3)

- Blood was drawn before inoculation (Blank), pre-dose (0 hour) and 48 hours after treatment for plasma creatinine analysis

- Efficacy determined by decrease in CFU

- Renal toxicity was assessed using plasma creatinine
Data Overview - Rat model of C. albicans

Kidney [CFU/ml]
Mean +/- SEM

CFU = colony forming unit
* p<0.05

Control (n=11)

iCo-009 [5mg/kg, PO] (n=5)

iCo-009 [10mg/kg, PO] (n=7)

ABLC [5mg/kg, IV] (n=5)
# Plasma creatinine levels in animals infected with C. albicans

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Creatinine (mg/dl)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blank**</td>
<td>0 hr</td>
<td>48 hr</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (infected – non treated)</td>
<td>0.5* +/- 0.3</td>
<td>0.5 +/- 0.3</td>
<td>1.0 +/- 0.5</td>
</tr>
<tr>
<td>(n=9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iCo-009 (10 mg/kg, PO)</td>
<td>0.6 +/- 0.5</td>
<td>0.5 +/- 0.5</td>
<td>0.5 +/- 0.2</td>
</tr>
<tr>
<td>(n=6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABLC (5 mg/kg, IV)</td>
<td>0.3 +/- 0.3</td>
<td>0.4 +/- 0.2</td>
<td>0.5 +/- 0.2</td>
</tr>
<tr>
<td>(n=4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*mean +/- SEM

**Blank:** plasma sample before fungal infection and before treatment

"0": plasma sample after 48 hrs of fungal infection and before treatment

"48": plasma sample after 96 hrs of fungal infection and after 48 hrs of treatment
Candidiasis Infection of Kidney - Gross Morphology of a Treated & Untreated Kidney
How can the poorest of the poor share in University Discoveries?

- Need mechanisms for encouraging and funding expensive research for Neglected Diseases.
- Must creatively protect early discoveries.
- Delivery!!!!
COMMENTARY

The Global Access Initiative at The University of British Columbia (UBC): Availability of UBC Discoveries and Technologies to the Developing World

KISHOR M. WASAN, SHEILA J. THORNTON, IAN BELL, REBECCA E. GOULDING, MICHAEL GRETES, ANDREW P. GRAY, ROBERT E.W. HANCOCK, BARBARA CAMPBELL

1The University of British Columbia, Faculty of Pharmaceutical Sciences, Division of Pharmaceutics and Biopharmaceutics, 2146 East Mall, Vancouver, B.C., Canada V6T 123
2The University of British Columbia, University Industrial Liaison Office, Vancouver, Canada V6T 123
3The University of British Columbia Universities Allied for Essential Medicines Chapter, Vancouver, Canada V6T 123
4The University of British Columbia, Department of Microbiology and Immunology, Vancouver, Canada V6T 123
5Dalhousie University, Halifax, NS, Canada

Received 5 June 2008; revised 6 June 2008; accepted 9 June 2008

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.21495
Mission Statement
To develop drugs for Neglected Global Diseases and to ensure delivery to those in need

Working Group Members
- Dr. Charles Larson
- Jennifer Choi
- Dr. Brett Finlay
- Dr. Jennifer Love
- Dr. Rebecca Goulding
- Dr. Mike Gretes
- Dr. Robert Hancock
- VP Dr. John Hepburn
- Kevin Hooi
- Dr. Jerry Spiegel
- Mr. Terry Kellam
- Mr. Angus Livingstone
- Dr. James Tansey
- Dr. Kishor Wasan

www.ngdi-ubc.ca
Spectrum of disease which affects approximately 12 million people in 88 countries

- About 2 million new cases annually
- 75% involve cutaneous leishmaniasis, with the remainder being visceral leishmaniasis (VL)

Mortality rate for VL is close to 100% in the absence of treatment

Source: WHO/TDR/Marsden
“Real World” Efficacy

2 million new cases reported every year (WHO)
Visceral leishmaniasis causes ~59 000 deaths annually
New antileishmanial candidates and lead compounds
Julian V Richard and Karl A Werbovetz

Although miltefosine and paromomycin were registered as clinical agents against visceral leishmaniasis in the last decade, the antileishmanial drug arsenal still requires improvement, particularly in the area of oral antileishmanial drugs for both visceral and cutaneous diseases. Several new compounds and formulations have displayed promising efficacy in animal models of leishmaniasis, including the 8-aminoquinoline NPC1161, a series of bis-quinolines, DB766, rhodacyanine dyes, amiodarone, and an oral formulation of amphotericin B. Herein we provide a review of those molecules whose antileishmanial properties have been described over the past few years and a brief assessment of the studies required to identify new preclinical antileishmanial candidates.

Address
Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA

Both VL and CL for many years, but are no longer useful against Indian VL because of the development of resistance. Amphotericin B deoxycholate (Fungizone) is effective against VL but is nephrotoxic, and its use requires 30 days of hospitalization [2]. Ambisome, a liposomal formulation of amphotericin B, is highly effective against VL and is less toxic than Fungizone, but is more expensive than the other current antileishmanial drugs [2]. The phospholipid analog miltefosine was registered in India as an oral treatment for VL in 2002. Although miltefosine is the first oral antileishmanial drug, its limitations include gastrointestinal tract toxicity, teratogenicity, and relatively high cost [2,3]. Evaluation of the in vitro susceptibilities of Indian L. donovani patient isolates to sodium antimony gluconate, amphotericin B, and miltefosine indicates that cross-resistance may be emerging among these three drugs [4]. However, combination therapy employing a single dose of Ambisome (3.75–5 mg/kg)
# Current Treatments of VL

<table>
<thead>
<tr>
<th></th>
<th>Liposomal amphotericin</th>
<th>Miltefosine</th>
<th>Paromomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficacy in VL</td>
<td>~99%</td>
<td>~97%</td>
<td>~95%</td>
</tr>
<tr>
<td>Safety</td>
<td>Safe</td>
<td>G-I intolerance</td>
<td>Reversible ototoxicity 2%</td>
</tr>
<tr>
<td>Administration</td>
<td>Intravenous infusion</td>
<td>Oral tablet. Contraception in child-bearing -age women</td>
<td>Intramuscular injection</td>
</tr>
<tr>
<td>Price for 35 kg Indian VL adult</td>
<td>~US $140 –220</td>
<td>~US$ 61 – 75</td>
<td>~US$ 15</td>
</tr>
</tbody>
</table>

Wasan & Thornton 2009
Implications for Developing Countries

Parenteral administration results in:

- Loss of income
- Increased cost of administration
- Increased risk of side effects
- Decreased availability of treatment
Single-Dose Liposomal Amphotericin B for Visceral Leishmaniasis in India

Shyam Sundar, M.D., Jaya Chakravarty, M.D., Dipti Agarwal, M.D., Madhukar Rai, M.D., and Henry W. Murray, M.D.

ABSTRACT

BACKGROUND

Some 50% of patients with visceral leishmaniasis (kala-azar) worldwide live in the Indian state of Bihar. Liposomal amphotericin B is an effective treatment when administered in short courses. We wanted to determine whether the efficacy of a single infusion of liposomal amphotericin B was inferior to conventional parenteral therapy, consisting of 15 alternate-day infusions of amphotericin B deoxycholate.
Highly Effective Oral Amphotericin B Formulation against Murine Visceral Leishmaniasis

Kishor M. Wasan,¹ Ellen K. Wasan,¹³ Pavel Gershkovich,¹ Xiaohua Zhu,⁴ Richard R. Tidwell,⁵ Karl A. Werbovetz,⁴ John G. Clement,² and Sheila J. Thornton¹

¹Faculty of Pharmaceutical Sciences, University of British Columbia, and ²iCo Therapeutics, Vancouver, and ³School of Health Sciences, British Columbia Institute of Technology, Burnaby, British Columbia, Canada; ⁴Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus; and ⁵Department of Pathology and Lab Medicine, Consortium for Parasitic Drug Development, University of North Carolina at Chapel Hill, Chapel Hill
Data Overview - Visceral Leishmaniasis (VL)

- Mice were infected i.v. with $1 \times 10^7$ *Leishmania donovani*

- Treatment begins on Day 7 post infection

- Oral Amp B administered bid for 5 consecutive days

- Mice were sacrificed Day 14 post infection

- Livers were then weighed and impression smears prepared

- The number of *Leishmania* amastigotes per liver cell nuclei was determined microscopically

- Studies performed in independent laboratory
  - part of the Consortium for Parasitic Drug Development, a Gates Foundation funded organization
Data Overview - Antiparasitic Activity

Miltefosine - 3mg/kg PO, qd x 5 d
Ambisome - 2 mg/kg iv, once
iCo-009 - 10 & 20 mg/kg PO, bid x 5 d
Efficacy of iCo-009 in Murine VL Model

Vehicle, PO BID  
iCo-009, 2.5 mg/kg BID  
iCo-009, 5 mg/kg BID  
iCo-009, 10 mg/kg BID  
AmBisome, 2 mg/kg IV bolus

Untreated control

macrophage nuclei

amastigotes

AmB 10 mg/kg po

amastigote
VL-infected Hamster Study (Acute Results)

Liver [LDU]  Mean +/- SD

- Untreated Controls (n=3)  **
- AmBisome 2 mg/kg iv (n=4)  *
- Miltefosine 30 mg/kg PO QD (n=4)  *
- iCo-009 10 mg/kg PO bid (n=4)  *

* P<0.05 vs. Untreated Controls
Kayser et al., IJP 2003
Mechanisms of Enhanced Drug Absorption

- Solubility Issues
- Dissolution Rate Limited Issues
- Passive Diffusion/Active Transport
- Drug Efflux Transporters
- pH stability
- Lymphatic Transport
- Intestinal Wall Macrophages
- Peyer’s Patches
Oral Amphotericin B Formulation Technology

- Proprietary blend of mono- and di-glycerides (FDA GRAS approved)
- Solubilized AmpB Formulations
- Nanosuspensions/dispersions
- Affordable lipid excipients
- Ease of formulation scale-up
- Formulation Stability over 7 days
- Drug Stability at 37°C over 21 days
Advantages of Oral Amphotericin B Formulation

- Affordable
- Easy to store
- Easy to administer
- Lack of kidney toxicity
- Lack of Infusion-related side effects (i.e. fever, chills etc.)
- Lack of liver and GI toxicity
Advantages of Oral Amphotericin B Formulation

• Treating patients with drug-resistant strains (decrease hospitalization and eliminate IV AmpB Therapy)
• First available Oral Fungicidal Agent (only Fungistatic Agents, Diflucan® from Pfizer)
Acknowledgments

Research Faculty and Staff
- Dr. Kristina Sachs-Barrable
- Dr. Sheila Thornton
- Dr. Carlos Leon
- Dr. Pavel Gershkovich
- Ms. Olena Sivak
- Dr. Cheri Barta
- Dr. Robin Stoodley
- Mr. Mike Rosland
- Ms. Verica Risovic

Research Funding
- Canadian Institutes of Health Research (CIHR) Operating Grants
- AAPS Lipid-Based Drug Delivery Award/Grant
- iCo Therapeutics Inc.
- CPDD/Gates Foundation
- Gattefossese Canada

Current Graduate Students
- Jennifer Locke
- Stephen Lee
- Jackie Fleischer
- Alexis Twiddy

Website
- http://www.wasanlab.ubc.ca

Numerous Undergraduate Students
Dr. Tom Kanyok (Gates Foundation)
Dr. Richard Tidwell (CPDD)