Paromomycin liposomes – an Alternative Strategy for treatment of Infectious Diseases

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Leading Causes of Death Worldwide

<table>
<thead>
<tr>
<th>Disease Type</th>
<th>Number of Deaths</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular diseases</td>
<td>16,733,000</td>
<td>29%</td>
</tr>
<tr>
<td>Infectious and parasitic diseases</td>
<td>14,867,000</td>
<td>26%</td>
</tr>
<tr>
<td>Malignant neoplasms</td>
<td>7,121,000</td>
<td>12%</td>
</tr>
<tr>
<td>Violence/injuries/accidents/suicides</td>
<td>5,168,000</td>
<td>9%</td>
</tr>
<tr>
<td>Chronic lung diseases</td>
<td>3,702,000</td>
<td>6%</td>
</tr>
<tr>
<td>Pregnancy-related deaths</td>
<td>2,972,000</td>
<td>5%</td>
</tr>
<tr>
<td>Other</td>
<td>2,388,000</td>
<td>4%</td>
</tr>
<tr>
<td>Digestive diseases</td>
<td>1,968,000</td>
<td>3%</td>
</tr>
<tr>
<td>Neuropsychiatric disorders</td>
<td>1,112,000</td>
<td>2%</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>988,000</td>
<td>2%</td>
</tr>
</tbody>
</table>

Nearly 15 million (>25%) of the total deaths worldwide (57,029,000) are caused by infectious and parasitic diseases.

Visceral Leishmaniasis - Epidemiology

An infectious disease prevalent in Asia, East Africa, South America, and the Mediterranean region.

- Affects 350 million people in 98 countries around the world (Bangladesh, Brazil, India, Ethiopia, Kenya, Nepal, and Sudan represent over 90% of new cases)
- 200,000 – 400,000 new cases of visceral leishmaniasis each year
- VL responsible for 48,000 deaths annually (2012)
- Leishmania-HIV co-infection – growing problem in Southern Europe, Brazil and Africa

Endemic status of VL worldwide, World Health Organization, October 2012
Current Treatments against Visceral Leishmaniasis

Pentavalent antimonials (Stb and meglumine antimoniate) (i.m. /i.v.) – first-line therapy for VL since 1940. Prolonged treatment, toxicity, low patient compliance, less effective, drug resistances constitute major concerns

Pentamidine (i.m. /i.v.) - abandoned as first line-treatment, due to high toxic effects, high cost, development of resistances;

Miltefosine (oral) – gastrointestinal problems, nephrotoxic, hepatotoxic, teratogenic, prolonged treatment, low patient compliance, expensive;

Amphotericin-B /Amphotericin-B deoxycholate (i.v.) – effective, toxicity, nephrotoxicity (monitorization of patients);

Liposomal Amphotericin-B (Ambisome) (i.v.) – minor nephrotoxicity, the highest effective of all VL drugs, very expensive;

Paromomycin (i.m.) – cheapest VL treatment, painful, hepatotoxicity, nephrotoxicity may occur. In 2006 Indian government approved its use against VL.

new strategies / new drugs for the treatment of parasitic diseases are needed
Therapies against Visceral Leishmaniasis

**Ambisome** (liposomal amphotericin-B)

- Highly effective, reduced toxicity, nephrotoxicity,

**Amphotericin-B**

**Paromomycin intramuscular injection**

**OUR STRATEGY**

Improve the therapeutic performance of PRM

http://www.path.org/annual-report/2012/
Requirements for a successful liposomal formulation

**PRM liposomes**

Stability in suspension

Stability in lyophilized form

**Cellular association studies of liposomes (THP-1 cells)**

Fluorescence microscopy revealing internalization of labeled liposomes (Rho) in THP-1 cells, nucleus of THP-1 cells (DAPI) and the conjugation of both labels

**Intracellular activity in macrophages**

<table>
<thead>
<tr>
<th>IC_{50} (µM)</th>
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</thead>
<tbody>
<tr>
<td>Free PRM</td>
<td>100</td>
</tr>
<tr>
<td>Lip PRM</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Gaspar MM et al., Nanomedicine:NBM, 2015
Therapeutic effect of PRM formulations

Murine mycobacterial \textit{avium} model of infection,

PRM liposomes vs Free PRM

\textbf{M. avium} 2447: i.v. Infection (2x10^5 CFU/mouse)

Infected organs (spleen, liver, lung)

Tissue homogenization

Control Free PRM Lip 1 Lip 2 Lip 3

Therapeutic Effect of PRM formulations on growth index

\textbf{Growth index} – difference between the log\textsubscript{10} CFU at the end of treatment and the log\textsubscript{10} CFU at the beginning of treatment

Experimental conditions:

- Control (infected and untreated mice)
- Free PRM
- Lip 1 - DMPC:DMPG:DSPE-PEG
- Lip 2 - DPPC:DPPG:DSPE-PEG
- Lip 3 - DPPC:DPPG

Treatment dose: 12 mg/kg body weight (i.v.)
3 times a week for 3 weeks (2 weeks after infection induction)

Gaspar MM et al., Nanomedicine:NBM, 2015
**Therapeutic effect of PRM formulations**

**Murine visceral leishmaniasis model of infection,**

*L. infantum* (MHOM/MA/67/ITMAP-263) promastigotes: i.v. infection

- **Negative Control** – infected non-treated
- **Positive Control** – Pentavalent antimonial (Glucantime)
  Dose: 45 mg/kg (s.c.)
- **Free PRM** – Free Paromomycin / Dose: 15 mg/kg (i.v.)
- **Lip PRM** - Liposomal Paromomycin DPPC:DPPG
  Mean size: 0.14 µm / Dose: 15 mg/kg (i.v.)

**Experimental Conditions**
- Infection – 2x10^7 promastigotes (i.v.)
- Beginning of treatment – 1 week after infection
- Treatment Schedule – daily injections for 5 days

**Parasite burden of Balb/c mice infected with *L. Infantum***

<table>
<thead>
<tr>
<th></th>
<th>Spleen</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10^12</td>
<td>10^12</td>
</tr>
<tr>
<td>Empty LIP</td>
<td>10^10</td>
<td>10^10</td>
</tr>
<tr>
<td>Glucantime</td>
<td>10^8</td>
<td>10^8</td>
</tr>
<tr>
<td>Free PRM</td>
<td>10^6</td>
<td>10^6</td>
</tr>
<tr>
<td>LIP PRM</td>
<td>10^4</td>
<td>10^4</td>
</tr>
</tbody>
</table>

**Gaspar MM et al., Nanomedicine:NBM, 2015**
Therapeutic effect of PRM liposomes early vs delayed treatment
Murine visceral leishmaniasis model of infection

Parasite burden of Balb/c mice infected with *L. infantum* (MHOM/MA/67/ITMAP-263)

**Experimental Conditions**
Infection – 1x10⁶ promastigotes (i.v.)
**Early treatment** - 1 week after infection
**Delayed treatment** - 83 days after infection
Treatment Schedule - daily injections for 5 days

**Negative Control** – infected non-treated
**PRM Free** – Free PPRM / Dose: 15 mg/kg (i.v.)
**PRM Lip** - LIP PRM (DPPC:DPPG) Mean size: 0.14 µm / Dose: 15 mg/kg (i.v.)

*Gaspar MM et al., Nanomedicine:NBM, 2015*
Therapeutic effect of PRM liposomes vs Ambisome

Parasite burden reduction of Balb/c mice infected with *L. infantum* (MHOM/MA/67/ITMAP-263)

**Spleen**

<table>
<thead>
<tr>
<th>AMB</th>
<th>LIP PRM</th>
<th>Free PRM</th>
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</table>

**Liver**

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<th>AMB</th>
<th>LIP PRM</th>
<th>Free PRM</th>
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**Parasite burden reduction (%)**

**Administered dose (mg/kg body weight)**

**Experimental Conditions**

Infection – 1x10^6 promastigotes (i.v.)

Beginning of treatment – 1 week after infection

Treatment Schedule – daily injections for 5 days
Conclusions

• PRM was efficiently encapsulated in liposomes, PRM formulations showed high stability in suspension, in the lyophilized form liposomes were able to retain more than 90% of encapsulated antibiotic;

• The encapsulation of PRM in liposomes resulted in higher half-life in bloodstream and higher accumulation in liver, spleen in comparison with free PRM;

• In murine model of infection (*M. avium* and VL *leishmania*), PRM encapsulated in liposomes was able to reduce the bacterial load and parasite burden in a very high extent.

• The comparative *in vivo* evaluation of PRM liposomes and Ambisome® suggests that PRM liposomal formulations may be an alternative to Ambisome®.
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