8th International Conference and Exhibition

on Pharmaceutics & Novel Drug Delivery Systems

Paromomycin liposomes – an Alternative Strategy for treatment of Infectious Diseases

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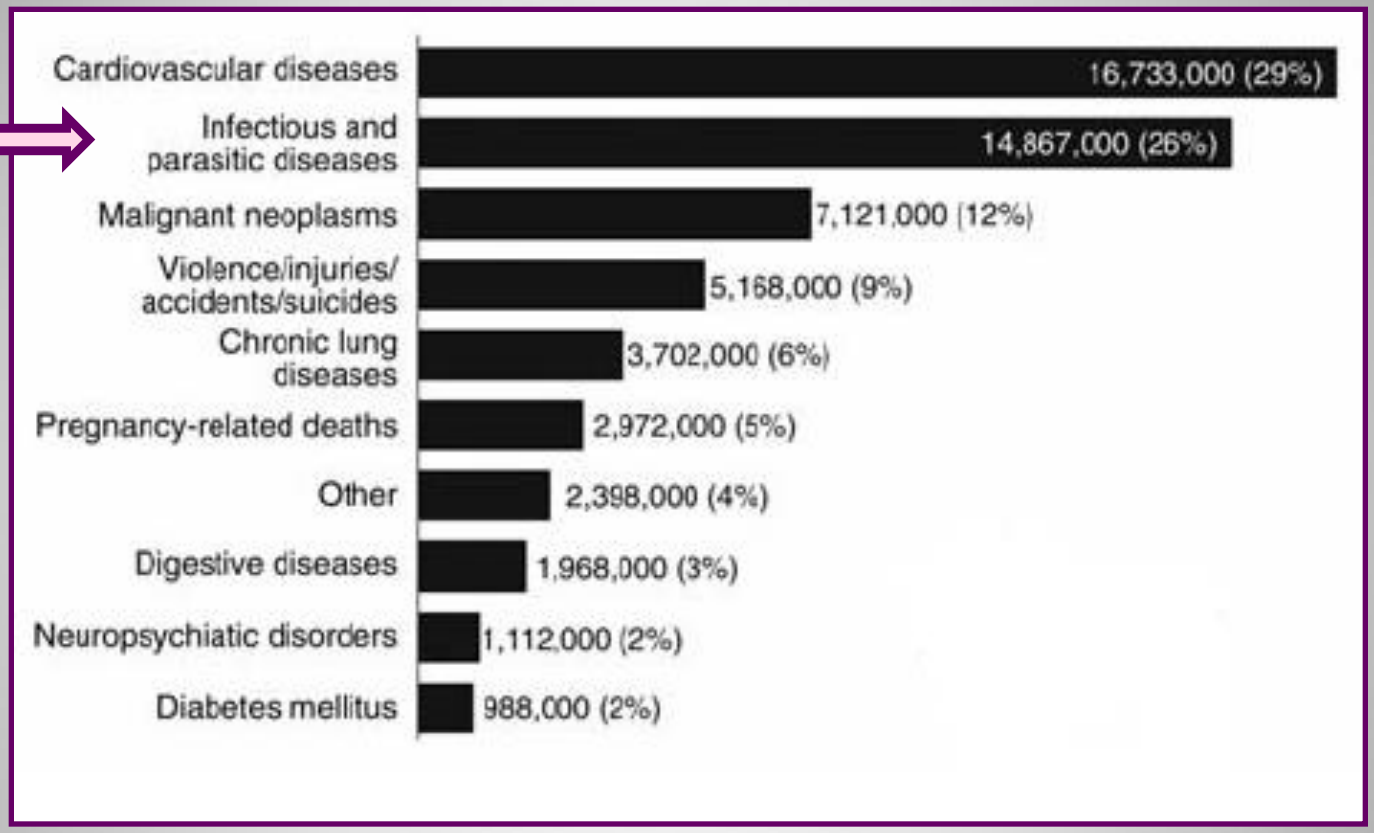
Leading Causes of Death Worldwide



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

World Health Organization. The world health report 2004-changing history. Geneva: The Organization; 2004

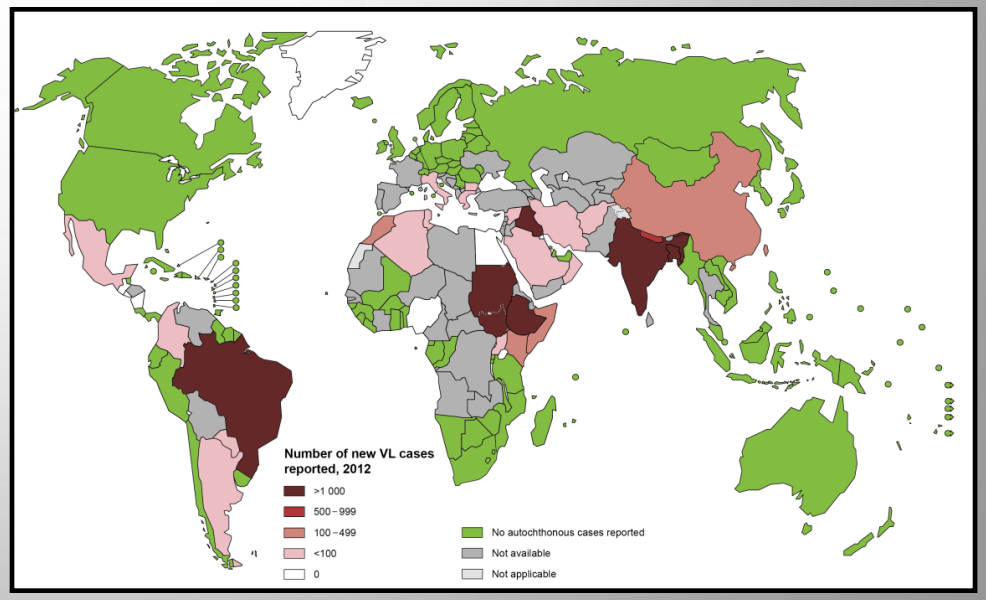
Visceral Leishmaniasis - Epidemiology

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

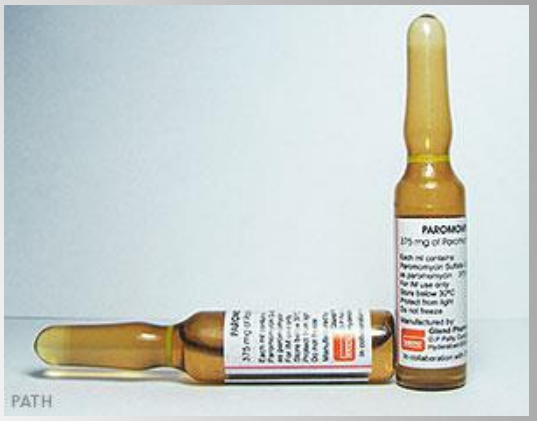
Therapies against Visceral Leishmaniasis

Ambisome (liposomal amphotericin-B)

Highly effective, reduced toxicity, nephrotoxicity,

Amphotericin- B

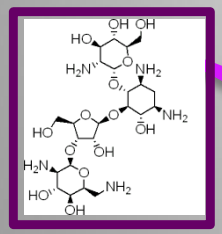
Paromomycin intramuscular injection

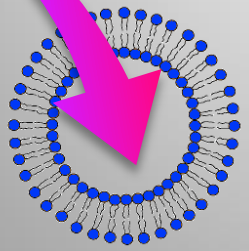


http://www.path.org/annual-report/2012/

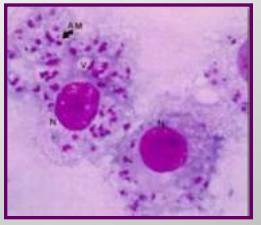
OUR STRATEGY

Improve the therapeutic performance of PRM





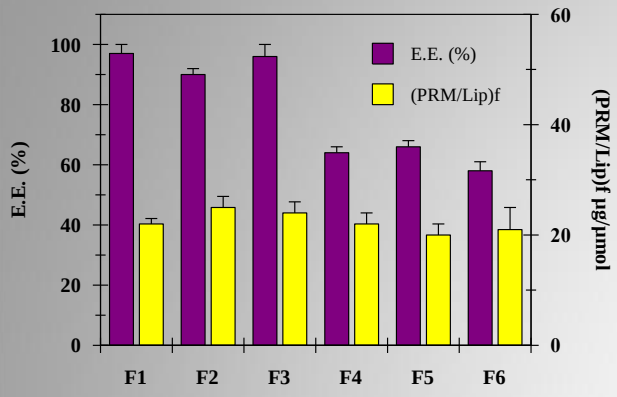
Liposomes



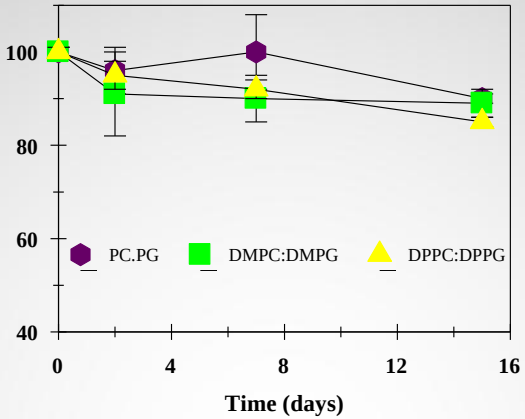
Liver
Spleen
Bone Marrow
Lymph nodes

Requirements for a sucessful liposomal formulation

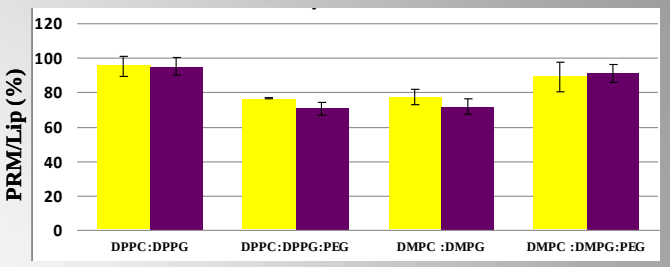
PRM liposomes



Stability in suspension

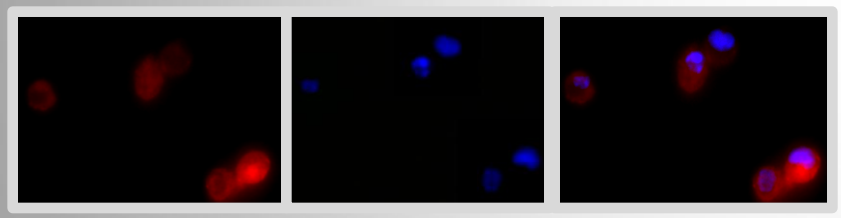


Stability in lyophilized form



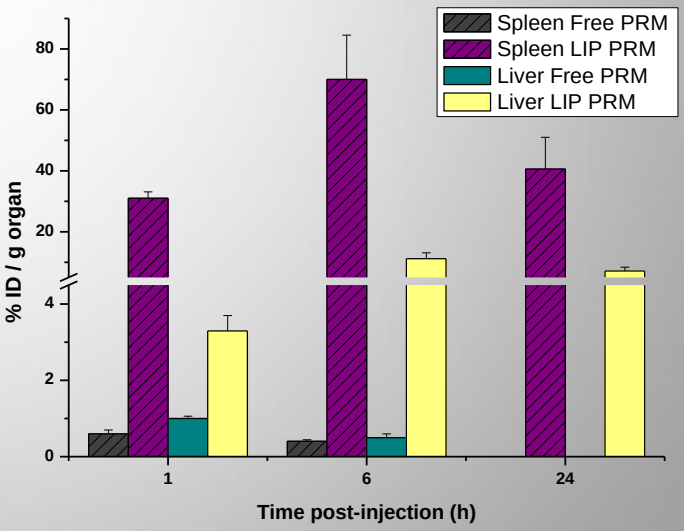
With Threalose / Without Threalose

Cellular association studies of liposomes (THP-1 cells)



Fluorescence microscopy revealing internalization of labeled liposomes (Rho) in THP-1 cells, nucleus of THP1-cells (DAPI) and the conjugation of both labels

Biodistribution profile PRM Free *vs* LIP



Intracellular activity in macrophages

IC_{50} (µM)	
Free PRM	100
Lip PRM	2.5

Gaspar MM et al., Nanomedicine:NBM, 2015

Lip PRM - lipid composition DPPC:DPPG
Mean size: 0.11 µm

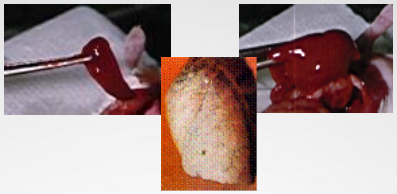
Therapeutic effect of PRM formulations

Murine mycobacterial *avium* model of infection,

PRM liposomes vs Free PRM

Male BALB/c mice

***M. avium* 2447: i.v. Infection** ($2x10^5$ CFU/ mouse)

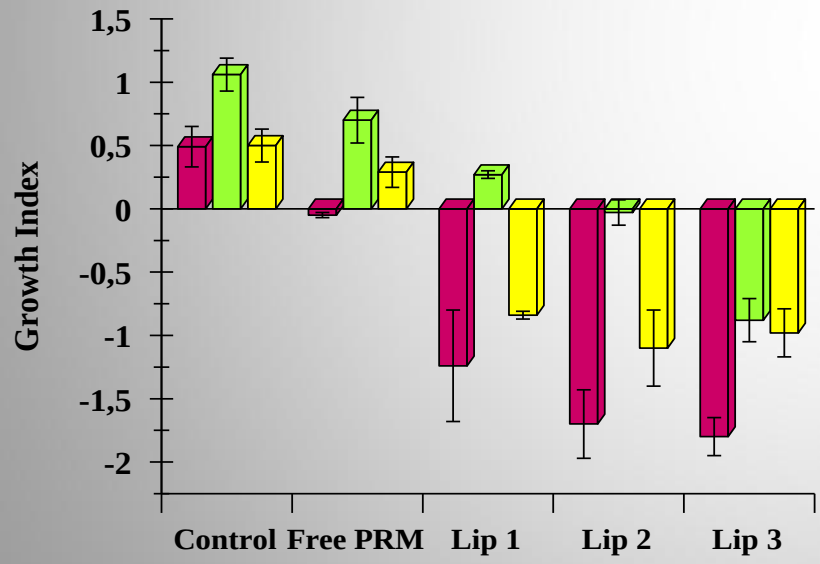


Infected organs (spleen, liver, lung)

Tissue homogenization

CFU countings

Therapeutic Effect of PRM formulations on growth index



Liver / Spleen / Lung

Experimental conditions:

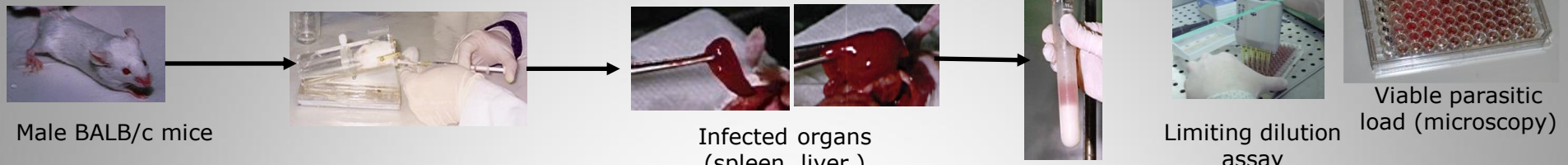
Control (infected and untreated mice)
Free PRM
Lip 1 - DMPC:DMPG:DSPE-PEG
Lip 2 - DPPC:DPPG:DSPE-PEG
Lip 3 - DPPC:DPPG
Treatment dose: 12 mg/kg body weight (i.v.) 3 times a week for 3 weeks (2 weeks after infection induction)

Growth index – difference between the $\log_{10}$ CFU at the end of treatment and the $\log_{10}$ CFU at the beginning of treatment

Gaspar MM et al., Nanomedicine:NBM, 2015

Therapeutic effect of PRM formulations

Murine visceral leishmaniasis model of infection,



Male BALB/c mice

Infected organs (spleen, liver)

Tissue homogenization

Limiting dilution assay Schneider's medium

Viable parasitic load (microscopy)

***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.)

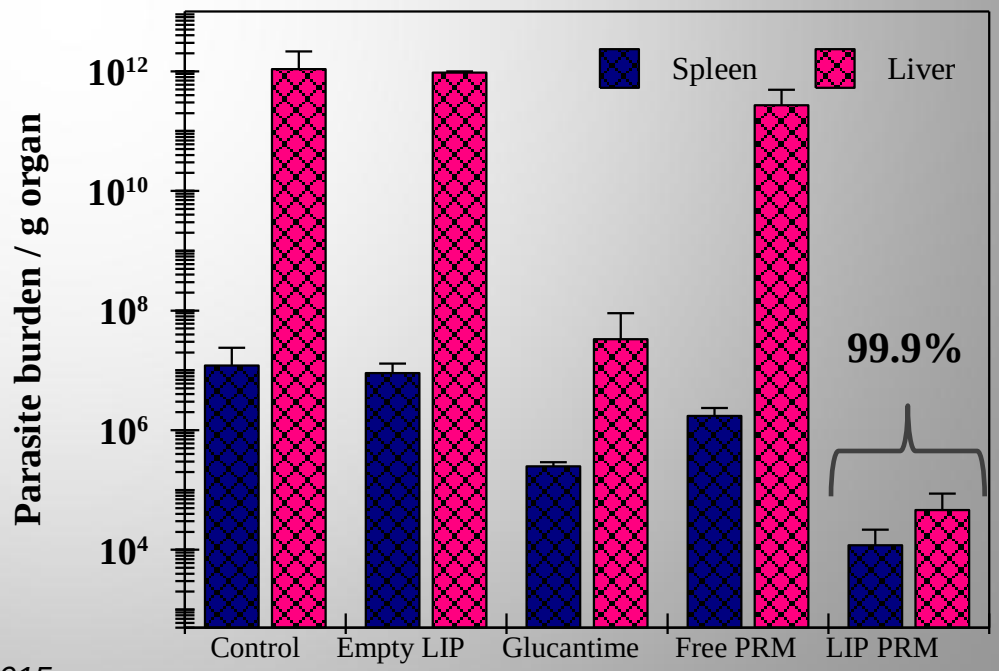
Experimental Conditions

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*

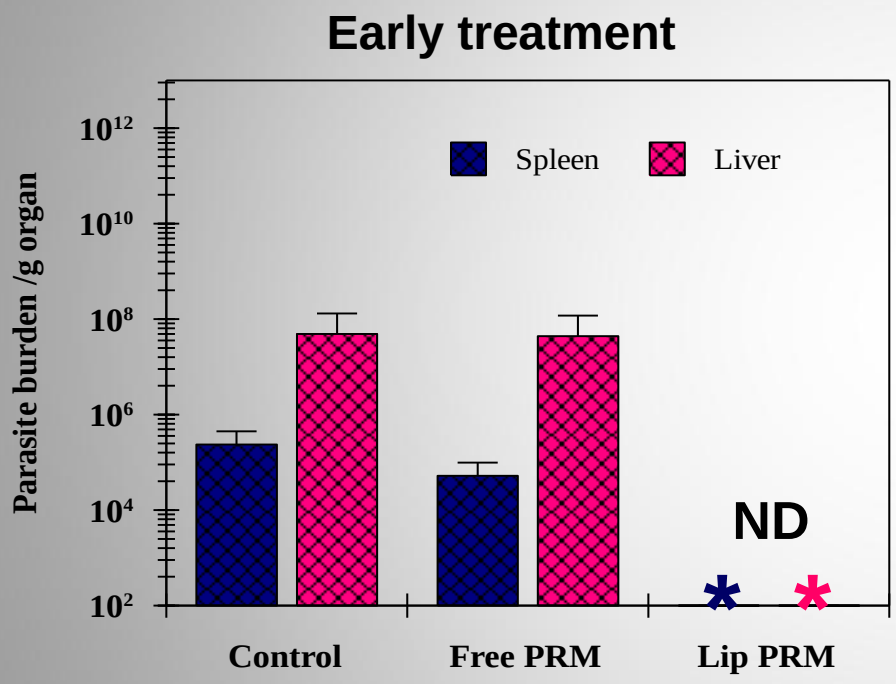


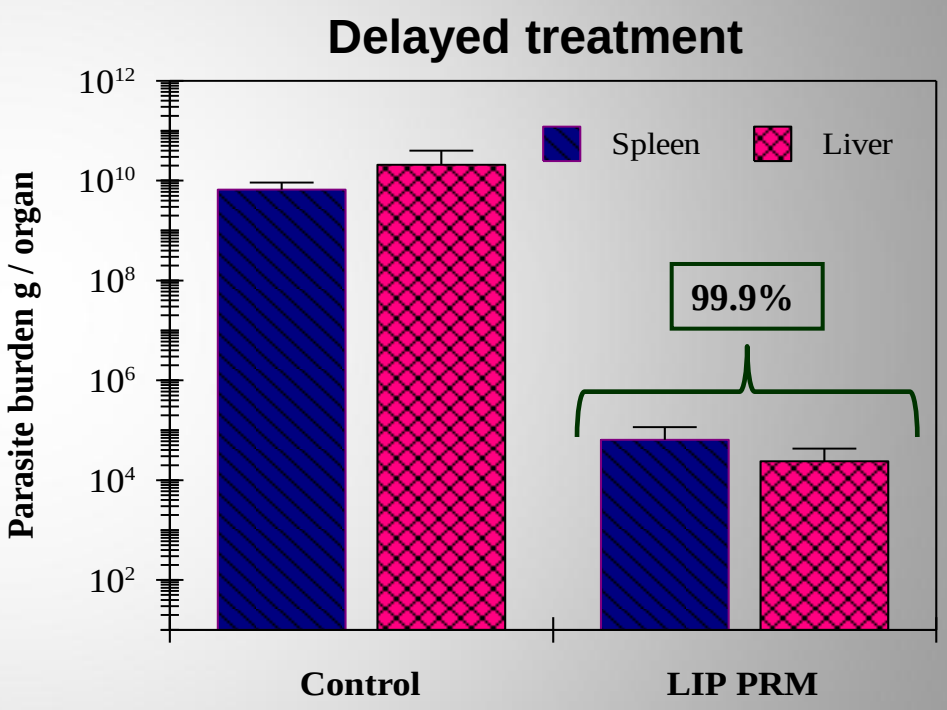
Gaspar MM et al., Nanomedicine:NBM, 2015

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 – $1x10^6$ 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.)

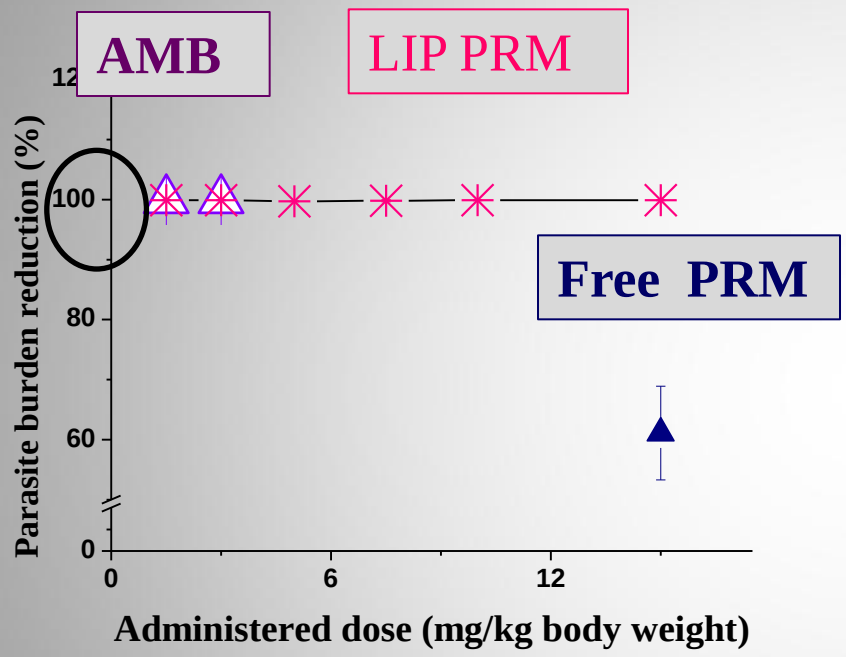
Gaspar MM et al., Nanomedicine:NBM, 2015

Therapeutic effect of PRM liposomes *vs* Ambisome

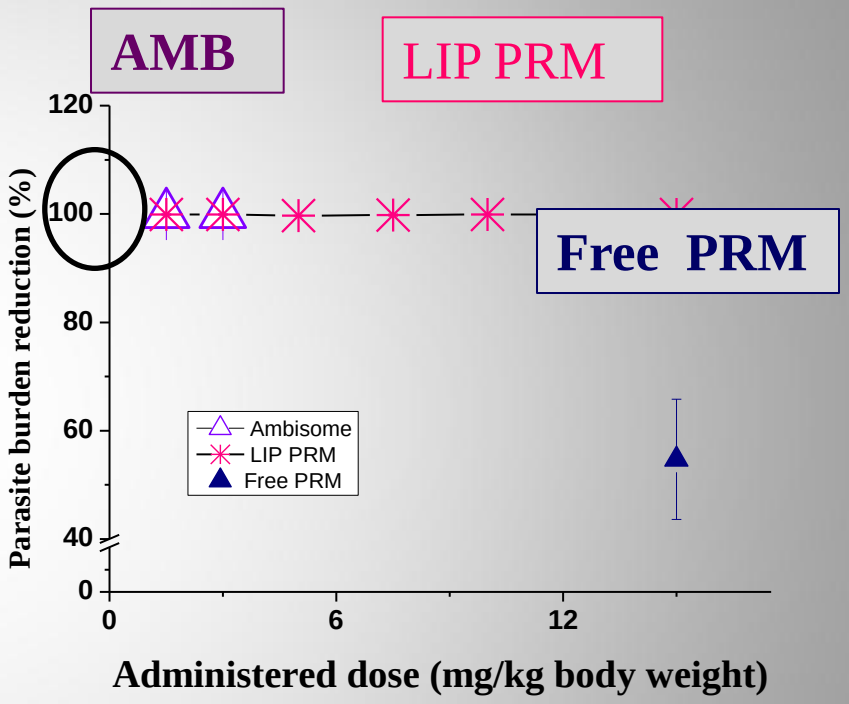
Parasite burden reduction of Balb/c mice infected with *L. infantum*

(MHOM/MA/67/ITMAP-263)

Spleen



Liver



■ Ambisome ■ LIP PRM ■ Free PRM

Experimental Conditions

Infection – $1x0^6$ 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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