IV Symposium of Tropical Health/COST Action CM 1307 (WG3 and WG4) Joint Meeting

Priorities in Tropical Health and parasite-borne disease: new drugs with new targets, and how to deliver them

Pamplona-Spain, 4-5 May 2017
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INVITED SPEAKERS

José Luis Arias Mediano. University of Granada, Spain
Josaphat Matasyoh. University of Egerton, Kenya
Xavier Fernández-Busquets. Institute for Global Health (ISGlobal). University of Barcelona, Spain
Alfons Renz. University of Tübingen, Germany
Ericsson Coy-Barrera. Universidad Militar Nueva Granada, Colombia
Ignacio Moriyon. Institute of Tropical Health, Department of Microbiology and Parasitology, University of Navarra, Spain
On behalf of the Organising Committee, it is our great pleasure to invite you to the IV Symposium of Tropical Health/COST Action CM 1307 (WG3 and WG4) Joint Meeting Priorities in Tropical Health and parasite-borne disease: new drugs with new targets, and how to deliver them, which will be held on 4-5 May 2017 in Pamplona, Spain.

The Symposium has been declared of sanitary interest by the Department of Health of the Government of Navarra.

Six plenary lectures will be presented and up to twenty talks from proffered abstracts will be selected and integrated into plenary sessions. In addition, one session of Poster Flash Presentations will take place.

Pamplona, the capital of Navarra, is a modern and welcoming city that invites you to its many pleasures: enjoy its parks, stroll through the streets of its Old Quarter, walk around century-old walls and rest on its scenic terraces. Discover all of these particularities of Pamplona and do not forget to taste its gastronomy and its popular tapas (or pinchos as they are known locally).

We are looking forward to seeing you enjoying the Symposium and all that Pamplona has to offer.

Local Organizers
VENUE

The 4th Symposium will take place at the Instituto Cultura y Sociedad (Edificio Biblioteca de Humanidades), of University of Navarra.

Participants will have access to free parking. For more information, please visit: http://www.simposiosaludtropical.com/venue-and-accommodation/
INFORMATION

REGISTRATION DESK
The registration desk will be open at 9:00 on 4\textsuperscript{th} and 5\textsuperscript{th} May.

NAME BADGES
For identification and security purposes, name badges are mandatory for all participants when at venue (including coffee breaks and lunches).

INTERNET ACCESS
Wireless internet is freely available at the venue.

PARKING
Participants will be have access to free parking. For more information visit http://www.simposiosaludtropical.com/venue-and-accommodation/

PRESENTATIONS GUIDELINES
Presentations must be handed in by the speakers on the presentation day, in the meeting room “Aula ICS”, before 9 am.

The use of PowerPoint presentations is strongly encouraged. A Windows computer with USB ports, a projector and a laser pointer will be available. The computer will have Microsoft PowerPoint and PDF viewer software.

Please arrive ahead of your scheduled time so as to have time to assure that your presentation will work with the equipment.

\textbf{Plenary speakers}: 45 minutes presentation, including 5 minutes for questions.

\textbf{Oral communications}: 20 minutes per communication, including 5 minutes for questions.

\textbf{Poster Flash Presentations}: 5 minutes per presentation, including 1 minute for questions.

\textbf{Poster}: The maximum allowed dimensions for posters are as follows: 174 cm (height) by 94 cm (width) and will be presented on the designated poster areas. Authors should remain next to their poster during the poster session.

Posters may be hung up the day before (May 3\textsuperscript{rd}, in the afternoon) the event.
PROGRAM

MAY 4th

9:00 Registration

9:15 Welcome and Opening Remarks

A. MEDICINAL CHEMISTRY

Chairpersons: Philippe M. Loiseau and Thomas J. Schmidt

9:30-10:15 Plenary Lecture A: New drugs against Onchocerca filarial parasites: Lessons from the bovine model in Cameroon. Alfons Renz. University of Tübingen, Germany

10:15-10:45 Coffee break-Poster viewing

10:45- 11:45 Oral communications (OC), 15+5 min/ communication

   OC 1A: Looking for hits and leads through library screening in the NMTRYPI platform in the field of trypanosomatidic infections. Maria Paola Costi, Università Degli Studi di Modena e Reggio Emilia

   OC 2A: Antileishmanial activity of methylselenocarbamates. Mikel Etxebeste. Instituto de Salud Tropical University of Navarra (ISTUN), Spain

   OC 3A: Generating evidence for a single drug combination dose (ivermectin+albendazole) to improve mass drug administration programmes to control soil transmitted helminths, strongyloides stercoralis included, in Bahir Dar, Amhara region, Ethiopia. Juan José de los Santos Sanz-Bustillo. Mundo Sano Foundation

11:45-12:45 Poster Flash Presentation (PF), 4+1 min/ poster

   PF 1 On the road to functional understanding the divergent actin 2, a new target for malaria transmission blocking. Maria Andreadaki, Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, Greece

   PF 2: Leishmania major nucleus-located Yinp protein is a genotoxic drugs target. Miriam Algarabel. Universidad de Navarra, Instituto de Salud Tropical (ISTUN), Departamento de Microbiología y Parasitología, Spain
PF 3: Involvement of the serine/threonine kinase – Jean3 – in leishmania infectivity. Celia Fernández Rubio, Universidad de Navarra, Instituto de Salud Tropical (ISTUN), Departamento de Microbiología y Parasitología, Spain

PF 4: Lactococcus lactis HSP65 producer as an alternative therapy for cutaneous leishmaniasis. Juliana Rebouças, Gonçalo Moniz Institute, Oswaldo Cruz Foundation (FIOCRUZ), Salvador, Brazil

PF 5: Leishmania vaccination using microneedles and nucleosomal histones. Esther Moreno, Universidad de Navarra, Instituto de Salud Tropical (ISTUN), Departamento de Farmacia y Tecnología Farmacéutica, Spain

PF 6: Histone fold domain dimerization of oocyst rupture proteins (ORPs) as target for antimalarial drugs development. Chiara Currà, Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, Greece

PF 7: Study and characterization of a newly discovered oncogenic domain in leishmania spp. José Peña, Universidad de Navarra, Instituto de Salud Tropical (ISTUN), Departamento de Microbiología y Parasitología, Spain

PF 8: Exploring the scope of new arylamino alcohol derivatives: synthesis, antimalarial evaluation, toxicological studies, and target exploration. Miguel Quililiano, Instituto de Salud Tropical (ISTUN), Departamento de Quimica Orgánica y Farmacéutica, Spain


12:45-13:45 Lunch

B. NATURAL PRODUCTS AS ANTIPARASITIC AGENTS

Chairpersons: Harry P. de Koning and Alfons Renz

13:45-14:30 Plenary Lecture B: Antischistosomal and mosquitocidal secondary metabolites from medicinal African plants. Josphat Matasyoh, University of Egerton, Kenya

14:30-15:50 Oral communications

OC 1B: Drug targeting of natural products: the example of antileishmanial quinolines. Philippe M. Loiseau. Université Paris-Sud, France
OC 2B: Steroidal alkaloids with anti-trypanosomal activity from Holarrhena africana (Apocynaceae). Thomas J. Schmidt. Institute of Pharmaceutical Biology and Phytochemistry (IPBP), University of Münster, Germany

OC 3B: Natural products are closer to drugs than non-drugs and can find use in antiparasitic treatment. Alfonso T. García-Sosa. Institute of Chemistry, University of Tartu, Estonia

OC 4B: Anti-trypanosomalelemanolide sesquiterpene lactones from Vernonialasiopus O. Hoffm. Mark Kimani. Institute of Pharmaceutical Biology and Phytochemistry (IPBP), University of Münster, Germany


16:35-16:45 Coffee break-Poster viewing

16:45 – 17:15 Working session

17:30 Guided Tour

MAY 5th

C. BIOLOGICAL TARGETS FOR CHEMOTHERAPY

Chairpersons: Juan M. Irache and Josphat Matasyoh

9:00-9:45 Plenary Lecture C: Development of nanocarriers for innovative antimalarial combination strategies. Xavier Fernández-Busquets, Institute for Global Health (ISGlobal). University of Barcelona, Spain

9:45-11:05 Oral communications

OC 1C: Transmission blocking targets in Plasmodium berghei mosquito midgut stages. Inga Siden-Kiamos. Foundation for Research and Technology-Hellas, Institute of Molecular Biology and Biotechnology, Heraklion, Greece.

OC 2C: Strategies to identify the genes encoding pyrimidine-specific transporters in protozoa. Khalid Jamaan Alzahrani. Institute of Infection, Immunity and Inflammation, University of Glasgow, United Kingdom; Department of Clinical Laboratory, College of Applied Medical Sciences, Taif University, Saudi Arabia.
OC 3C: *Trypanothione reductase and superoxide dismutase as current drug targets for Trypanosoma cruzi: an overview of compounds with activity against Chagas disease*. Iván Beltrán-Hortelano. Universidad de Navarra, Instituto de Salud Tropical (ISTUN), Departamento de Química Orgánica y Farmacéutica, Spain.

OC 4C: *Recent neuroimmunological findings in eosinophilic meningoencephalitis due to Angiostrongylus cantonensis*. Alberto Juan Dorta-Contreras. Laboratorio Central de Líquido Cefalorraquídeo (LABCEL). Facultad de Ciencias Médicas “Miguel Enríquez”, Universidad de Ciencias Médicas de La Habana, Cuba.

**11:05-11:30** Coffee break-Poster viewing

**D. DRUG TARGETING AND DRUG RESISTANCE**

Chairpersons: Francisco J. Otero Espinar and Alfonso T. García-Sosa

**11:30-12:15** Plenary Lecture D: *A cell targeting nanostrategy to bypass drug resistances in African trypanosomiasis*. José Luis Arias Mediano. University of Granada, Spain

**12:15-13:00** Oral communications

OC 1D: *A decrease in mitochondrial membrane potential is associated with diminazene resistance in Trypanosoma congolense*. Harry P. de Koning. Institute of Infection, Immunity and Inflammation, University of Glasgow, United Kingdom.

OC 2D: *An ex vivo phenotypic screening for antileishmanial drugs using infrared-transgenic cells*. Rosa Mª Reguera. Dpt. Biomedical Sciences; University of León, Spain

**13:00-14:30** Lunch-Poster viewing

Chairpersons: Socorro Espuelas and Fabio Rocha Formiga

**14:30-15:30** Oral Communications

OC 3D: *Developing nanoparticles for 17-AAG delivery against Leishmania infection*. Fabio Rocha Formiga. Gonçalo Moniz Institute, Oswaldo Cruz Foundation (FIOCRUZ/BA), Salvador, Brazil
OC 4D: **Lipid-based EmulsomeNanoformulations for Targeted Delivery of Antiparasites.** Mehmet Hikmet Ucisik. Department of Biomedical Engineering, School of Engineering and Natural Sciences, Istanbul Medipol University; Medipol Regenerative and Restorative Medicine Research Center (REMER), Istanbul, Turkey.

OC 5D: **The trypanosomatid serine/threonine protein kinase “Jean3” may confer resistance to drugs such as paromomycin.** Andrés Vacas-Oleas. Instituto de Salud Tropical University of Navarra (ISTUN), Spain

15:30-16:15 Plenary lecture E: **The quest for new vaccines against brucellosis.** Ignacio Moriyón. Universidad de Navarra, Instituto de Salud Tropical (ISTUN), Department of Microbiology and Parasitology, Spain.

16:15–18:00 Meeting of the Working Groups 3 and 4
Oral Communications
NEW DRUGS AGAINST FILARIAL PARASITES: LESSONS FROM THE BOVINE *Onchocerca ochengi* MODEL IN CAMEROON

Alfons Renz¹, Albert Eisenbarth¹, Daniel Achukwi², Kingsley Manchang², Carlos Chaccour³

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Testing of new antifilarial compounds against nodule-forming *Onchocerca* parasites was hampered by the lack of a suitable animal model. During the past 25 years, A. Renz established, together with colleagues from the Liverpool School of Tropical Medicine, the ‘*Onchocerca ochengi*’ model in Zebu cattle in Northern Cameroon. This filarial parasite is phylogenetically closest to the human parasite *O. volvulus*, with which it shares the *Simulium damnosum s.l.* vector flies.

Cattle in Northern Cameroon harbor at least 4 *Onchocerca* species, plus *Setaria* and *Dipetalonema* filariae.

Nodules of *O. ochengi* are located in the ventral skin and can easily be removed by a minor surgical intervention.

Major results came from the discovery of the adulticidal action of Doxycycline by killing the Wolbachia endosymbionts, the prophylactic and long-time sterilizing action of high doses of avermectins, and the advantages of slow-release depots of ivermectin.

Presently we are testing the use of slow-release depots of ivermectin, implanted subcutaneously for maintaining serum levels high enough to prevent the re-appearance of microfilariae in the skin and to effect blood-sucking vectors.

References


Acknowledgements

These studies received financial support from the CEC, WHO/OCT and DFG
In search of antiparasitic agents, arylmethylamino steroids\(^1\) were identified as potent antiparasitic compounds. The lead hybrid steroid-ortho-aminocresol derivative \(1o\) is a fast acting and highly active against intraerythrocytic stages of chloroquine-sensitive and resistant *Plasmodium falciparum* parasites (IC\(50\) 1-5 nM) as well as against gametocytes. In *P. berghei*-infected mice oral administration of \(1o\) drastically reduces parasitaemia and cures the animals. Furthermore, three doses of \(1o\) were sufficient to fully block parasite transmission from mice to mosquitoes. The steroid compounds show low cytotoxicity in mammalian cells and do not induce acute toxicity symptoms in mice. Moreover, the steroid compounds were found to have remarkable physiological and morphological effects on adult *Schistosoma mansoni*, resulting in the death of this trematode parasite in vitro. The lipophilic steroid carrier of these antiparasitic lead compounds is likely to facilitate membrane permeation and bioavailability whereas the essential hydroxyarylmethylamino moiety points to a chelate-based quinone methide mechanism involving metal or haem bioactivation. A detailed physicochemical study evidenced drug-heme adducts, by the mass spectrometry (ESI-MS) collision-induced dissociation method\(^2\), using dissociation of the heme-binding compound complexes. The covalent character of the haem adducts observed with \(1o\) was further substantiated by co-incubation of the compounds and haem at pH<1 followed by ESI-MS. Based on our studies we propose a novel approach to drug development for fighting malaria, schistosomiasis and potentially other parasitic diseases.


LOOKING FOR HITS AND LEADS THROUGH LIBRARY IN THE NMTRYPI PLATFORM IN THE FIELD OF TRYPANOSOMATIDIC INFECTIONS

Maria Paola Costi¹, Alberto Venturelli, Sheraz Gul³, Anabela Cordeiro⁴, Carolina Morales⁵, Matteo Santucci¹, Antonio Quotadamo¹-², Pasquale Linciano¹, Wolfgang Muller⁶, Ulrike Wittig⁶, Maria Laura Bolognesi⁷, Sean Ekins⁸

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Research on Trypanosomatidic diseases is limited and fragmented, funding initiatives are generally weak or lacking critical mass. Problems associated with existing drugs include inefficient delivery and efficacy, excessive toxicity and increasing resistance. New drugs are urgently needed now and in the near future. The New Medicines for Trypanosomatidic Infections - NMTrypl project¹ aims at obtaining new candidate drugs against Trypanosomatidic infections from the lead phase to the final preclinical phase that are more accessible to patients. One of the intermediate objective is to understand the biological profile of the best compounds on the folate dependent proteins as targets and on the Trypanosomatidic parasites. This includes high throughput screening (HTS) approaches to enzyme inhibition, anti-parasitic activity and early tox studies for compound selections. As an early approach we have focused on the identification novel scaffolds that can promise innovative compounds and targets discoveries. In doing so, our platform has screened the TYBox library including 730 in house Tydock² compounds against 5 cell lines (Trypanosoma brucei, Leishmania infantum, Trypanosoma cruzi, A549 human cells) and 5 early tox assays (hERG, 5 Cytochrome P450). Data analysis was performed adopting a Bayesian method and others. Then structure/anti-parasitic activity relationships evaluations were derived. We were able to recognize specific fragments responsible for anti-parasitic activity and positive early-tox properties separated from fragments responsible for toxicity to human cells. We crossed the information from the phenotypic screening analysis with the target-based enzyme inhibition assay results. The next step will use these information to guide drug lead identification and optimization using different strategies. The exploitation of discoveries will contribute to a reduction in the high socio-economic impacts of Leishmaniasis, Human African Trypanosomiasis and Chagas disease: it will improve the chances to identify new chemical entities for the development of innovative drugs. The NMTrypl project participate to the Data Sharing principle³ through the SEEK database⁴.


Acknowledgment. FP7 EUPROGRAM grant agreement no. 603240 (NMTRYPI) and from MIUR project PRIN2012 N° 2012 74BNKN_003.
Leishmaniasis encompasses a number of poverty-associated diseases caused by different species of flagellate protozoa parasites of the genus *Leishmania*. The disease affects both animals as well as humans, producing a series of clinical manifestations. Even though exact statistical data are lacking within the 350 million people that live in areas where leishmaniasis is endemic approximately 12 million people are infected. The current chemotherapy is far from being satisfactory and present several problems including toxicity, many adverse side effects, high costs and resistances. That's why drug development against parasitic diseases is needed.(1,2)

In this work, 11 aliphatic, aromatic, and heteroaromatic carbamate derivatives containing a methylselenol moiety have been synthesized.

The new compounds have been evaluated in vitro for their cytotoxicity activity against *Leishmania infantum* axenic amastigotes. In order to establish their selectivity indexes (SI) the cytotoxic effect of each compound was also assayed in THP-1 cell line. Some of the compounds presented a better activity than the reference drug Miltefosine, and similar or better SI.

References


Acknowledgements

ISTUN
GENERATING EVIDENCE FOR A SINGLE DRUG COMBINATION DOSE (IVERMECTIN+ALBENDAZOLE) TO IMPROVE MASS DRUG ADMINISTRATION PROGRAMMES TO CONTROL SOIL TRANSMITED HELMINTHS, STRONGYLOIDES STERCORALIS INCLUDED, IN BAHIR DAR, AMHARA REGION, ETHIOPIA.

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Soil transmitted helminths (STH), (Ascaris lumbricoides, Trichuris trichiura and hookworm), are among most common infections worldwide and widely distributed in Ethiopia. STH are included in the neglected tropical diseases (NTDs) program of WHO. Strategies for controlling STH are based on periodic mass drug administration (MDA) of school-aged children (SAC) with albendazole. S. stercoralis is not included in NTDs group, but there is a growing awareness of its underestimation. It needs a non-standard diagnosis and the choice of treatment is ivermectin.

Our project is located in Bahirdar, in the North West Ethiopia, aimed at improving the current approach for STH by 1) extending coverage to all the community, including adults, and 2) while including S. stercoralis in STH and MDA programs, by generating scientific evidence of the underestimation.

This project is executed in the frame of a bilateral agreement between the Institute of Health Carlos III and Mundo Sano Foundation, from Spain, and the Amhara National Regional State Health Bureau and the Bureau of Finance and Economic Development, from Ethiopia. The project protocol includes a specific diagnosis for S. stercoralis. Parasitological techniques test are implemented in Bahir Dar; molecular techniques in Madrid, Spain, being the combination of all techniques the approach to get the prevalence.

The phase one, was accomplished in 2013 in the rural area of Bahir Dar, focusing on 396 SAC, being the prevalence of STH 80%, 21% of S. stercoralis, the highest prevalence ever found in the country. In 2016, we implemented a second phase in a specific community from the same geographic area, where 792 people were included, age range 5-85 (mean 24.4). The overall prevalence of STH was 86.37%. The prevalence of S. stercoralis in the sample was 56%, being significantly higher in adults (p=0.002).

In both phases, albendazole was provided, combined with ivermectin for treating S. stercoralis, only to infected people, without side effects. Ivermectin is the drug of choice for onchocercasis and other filariasis; we work in the idea that a single dose combination of ivermectine+albendazole will be of much help to achieve more cost-efficient and effective MDA programs and, eventually, for the control and elimination of these parasitosis.

References
The Burden of Neglected Tropical Diseases Ethiopia, and opportunities for integrated control and elimination. K. Deribe et all. Parasites & Vectors, 2012, 5:240


ANTISCHISTOSOMAL AND MOSQUITOCIDAL SECONDARY METABOLITES FROM MEDICINAL AFRICAN PLANTS

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The helminthosis burden, in terms of prevalence, is equivalent to 50% of that of Malaria and 25% of that of HIV/AIDS with approximately 2.9 billion people being affected worldwide [1]. Shistosomiasis, which is caused by the helminth *Shistosoma mansoni*, is one of the neglected tropical diseases and is a public health problem in sub-Saharan Africa. There is an estimated 200 million people living with shistosomiasis and most of them in Africa [1, 2]. One of the strategies in combating shistosomiasis is through the interruption of the life cycle by the control of snails, miracidia, cercaria and adult worms. This research study focused on the control of shistosomiasis by interruption of the life cycle at the miracidia stage using secondary metabolites from the plants *Teclea nobilis* and *Rapanae melanophloes* that are used in African traditional herbal medicine to treat helminthosis. The bioactivity of these metabolites against *S.mansoni* miracidia will be discussed. Interest in the control of *Anopheles gambiae* lies in the fact that it acts as a vector of malaria which is the most severe tropical diseases and is caused by mainly *Plasmodium falciparum* [3]. There is no effective vaccine for malaria and therefore, the best approach of minimizing the disease incidences is the application of larvicides to larval habitats. The plant-derived natural products as larvicides have the advantage of being harmless to beneficial non-target organisms and environment when compared to synthetic ones. As part of our continued search for natural mosquito larvicides, we assayed the larvicidal activity towards third instar larvae of *A. gambiae* of compounds from *Piper capense*, *Zanthoxylum lemairei*, *Zanthoxylum leprieurii*, *Zanthoxylum gilletii*. The essential oil of *P. Capense* showed good larvicidal activity with LC\(_{50}\) and LC\(_{90}\) values of 34.9 and 85.0 µg/ml, respectively. Alkaloids isolated from *Z. lemairei* showed high potency against the larvae with mortality rates of over 95% at a concentration of 250 µg/ml. Acridone alkaloids isolated from *Z. leprieurii* had high larvicidal activity. The most active one 1-hydroxy-3-methoxy-9-acridone had LC\(_{50}\) and LC\(_{90}\) values of 39.6 and 77.5 µg/ml respectively. Secofuroquinoline alkaloids from *Z. gilletii* showed LC\(_{50}\) of 110.3 µg/ml and LC\(_{90}\) of 216.3 µg/ml.

References

Quinolines of natural origin have shown interesting antileishmanial activities on several experimental leishmaniasis models. A classical daily treatment with 2-n-propylquinoline (2-n-PQ) on five consecutive days in mice model is not sufficient to cure the mice infected with *Leishmania donovani* and the activity requires a 10-day treatment duration whatever the route (oral, parenteral) because of a short half-life elimination of the drug.

Therefore, 2-n-PQ derivatives were bound to soluble polysaccharides to improve their solubility, delay their elimination half-life and therefore enhance the activity. *In vitro* release at 37°C in phosphate buffer was performed in various conditions and showed that around 65% of the compound was released in 24 h. *In vitro*, the most active conjugate was the dextran-2PQA conjugate exhibiting an IC₅₀ value at 12 µg/mL on *Leishmania donovani* intramacrophage amastigotes. However, this system did not allow a sufficient release of the active principle explaining the lack of *in vivo* activity.

Another approach consisted in administering 2-n-PQ intravenously. Two systems were successful both *in vitro* and *in vivo*: a liposomal formulation named 2-n-PQ-LIP and a hydroxypropyl beta-cyclodextrin inclusion complex designated as 2-n-PQ-HPC. The most interesting one was the liposomal formulation, active on the *L. donovani* Balb/c mouse model, by reducing the parasite burden by more than 80% after an intravenous treatment regimen of 3 mg/kg/day given on five consecutive days. No synergistic activity between 2-n-PQ and Amphotericin B was monitored either *in vitro* or *in vivo*.

These formulations should be studied further on other leishmaniasis models and for toxicological considerations.

**Acknowledgements**

This work was supported by a grant from the Indo-French Centre of Advanced Research, New Delhi (CEFIPRA) (No. 4803-04) and Kaluvu Balaraman was recipient of a CEFIPRA postdoctoral fellowship.
In continuation on our work on alkaloids with anti-protozoal activity [1, 2] we have studied the constituents of leaves and stem bark of West African Holarrhena africana (Apocynaceae). An extract of the leaves of this plant had previously been reported to possess in vitro and in vivo activity against Trypanosoma brucei, causative agent of human African Trypanosomiasis, without characterization of active constituents [3]. Bioactivity-guided isolation yielded six steroidal alkaloids from the leaves [2], and eleven steroidal alkaloids as well as one nitrogen-free steroid from the bark. All compounds were tested in vitro against T. brucei rhodesiense and for cytotoxicity to L6 rat cells. The most active compound has an IC₅₀ value of only 0.08 µM. Comparison of all compounds revealed structure-activity relationships (see Figure). Most importantly, the presence of a basic amino group at C-3 of the pregnane skeleton was found to be an essential requirement for anti-trypanosomal activity. The configuration at C-3 was found of importance: Derivatives with a β-oriented amino group are significantly more active than the α-amino analogues. Monomethylation at the 3-amino group represents an optimum: Methylamino derivatives are more active than dimethylamino or unmethylated congeners. The antitrypanosomal activity of such steroid alkaloids has not been reported before. QSAR studies are currently in progress and will represent the basis for eventual lead optimization.

References

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NATURAL PRODUCTS ARE CLOSER TO DRUGS THAN NON-DRUGS
AND CAN FIND USE IN ANTI-PARASITIC TREATMENT

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Natural compounds have been extensively used to develop drugs. However, a new avenue of research can design compounds to become more natural product-like in order to approach drug profiles. Chemical compounds can be assigned into regions of the vast Chemical Space based on their molecular properties. Mapping these atlases of chemical and biological properties shows that natural products are closely related to approved drugs in their properties, more so than similar-potency, biologically-active non-drugs. This has been shown by PCA, probability distribution functions, logistic regression, and Bayesian classification. In addition, this work has helped in identifying new compounds that can be inhibitors of Aspergillus, Plasmodium, and Leishmania N-methyl transferase (sometimes simultaneously, similar to 2), Leishmania arginase, as well as for other parasites such as Cystoisospora suis. Different sources, suppliers, and databases for natural products have their own advantages and disadvantages, which will also be discussed. New sources of natural products can have unique diversity, such as secondary metabolites from fungi and microbes.

References


Acknowledgements

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Estonian Ministry of Science and Education, Grant Number: IUT34-14
EU COST Action CM1307 Targeted chemotherapy towards diseases caused by endoparasites EU COST Action CA15135 Multi-target paradigm for innovative ligand identification in the drug discovery process (MuTaLig)
As part of our continuous search for new antiprotozoal agents in plants of the Asteraceae family [1], we have investigated Vernonia lasiopus O. Hoffm., an indigenous African plant. This plant has extensively been reported to be used ethno-medicinally as a treatment for malaria [2] and was therefore chosen by us to search for possible antiprotozoal compounds. V. lasiopus crude extracts were screened for anti-protozoal activity and the dichloromethane extract was found to be most active against Trypanosoma brucei rhodesiense (Tbr; IC<sub>50</sub> = 0.17 µg/ml). Bioassay guided chromatographic fractionation and isolation from the dichloromethane extract led to identification of six elemanolide type sesquiterpene lactones (STLs): 8-Desacylvernolide (1), vernolepin (2), vernomenin (3), Vernodalol (4), vernodalin (5) and 11,13-Dihydrovernodalin (6). The compounds were identified by HR-MS and 1D and 2D NMR in comparison with literature data [3,4]. Vernolepin (2) was the main component of the extract. All these elemanolide STLs showed in vitro anti-trypanosomal activity. They were also tested for cytotoxicity against mammalian cells (L6 cell line). Vernolepin (2) was the most potent with an IC<sub>50</sub> value of 0.051 µg/ml (0.185 µM) against Tbr trypomastigotes with a selectivity index of 14.5. The mixture of compounds 5 and 6 displayed an interesting activity of 0.069 µg/ml IC<sub>50</sub> value but a lower selectivity index of 7.7. Vernodalol (4) showed interesting activity with IC<sub>50</sub> value of 0.1 µg/ml (0.255 µM) and a selectivity index of 14.4. Compounds 1 and 3 had IC<sub>50</sub> values of 0.779 (2.529 µM) and 0.14 µg/ml (0.507 µM) and selectivity indices of 13.7 and 4.6 respectively. Vernolepin (2), due to its high activity and yield, was chosen for in vivo anti-trypanosomal activity tests and determination of its mechanism of action which are currently in progress. To the best of our knowledge, elemanolides have not previously been reported to possess anti-Tbr activity. The bioactivity data complement previous data obtained in our lab and give more insights into structure- anti-trypanosomal activity relationships of STLs [1].

References

Acknowledgements
The authors gratefully acknowledge support from DAAD, NACOSTI and ResNetNPND.
Neotropical biodiversity is a colossal source of bioactive agents for a wide-range of purposes. Several studies have been conducted in order to discover novel chemical entities with antileishmanial activity based on neotropical biodiversity [1]. The results have demonstrated the enormous potential of this kind of sources. However, the lack of suitable research programs oriented towards chemoprospecting and lead finding from neotropical organisms has hindered the progress on this topic, despite the potential of the biodiversity within the group plants initially called Plantæ columbianæ has been discovered to be enormous. In Colombia, cutaneous Leishmaniasis (CL) is a public health problem in some tropical regions. The therapeutics is based on traditional drugs (1st and 2nd line), thus the searching for novel treatments is an urgent requirement. Therefore, in order to contribute to the discovery for therapeutic alternatives as proof-of-concepts for the CL control, several studies have been aimed in our group to the chemical and biological characterization of extracts and compounds using modern approaches within biodirected and non-biodirected initiatives, in-silico strategies and in-vitro protocols against amastigotes and promastigotes of Leishmania panamensis and L. amazonensis. These studies let to the discovery of some biologically important compounds and leads such as limonoids, aryltetralin lignans, kaurane-related diterpenes and 8-O-4'-neolignans. The safety of these compounds was also estimated through evaluation against murine and human macrophages, which allowed determining selectivity indices. In addition, some of the most active compounds (e.g., limonoids) have been evaluated using an in vivo model for CL, with good injury healing results. A detailed description for each case will be described within lecture.

References

Acknowledgements
MU Nueva Granada finances this work. Gratitude is extended to Immunotoxicology group at Univesidad Nacional de Colombia for supporting some biological assays
The concept of antimalarial therapy has been locked for over 100 years on the administration of drugs against which \textit{Plasmodium} has evolved resistance shortly after their deployment. More often than not, economy-related issues have been hampering the progress of nanotechnology-based medicines against malaria with the dubious argument that they are too expensive to be used in developing areas. Unfortunately, it is true that the application of nanoscience to infectious disease has been traditionally neglected, with most research resources overwhelmingly biased towards other pathologies more prominent in developed regions. Thus, extra ingenuity is demanded from us: malaria-oriented nanomedicines not only need to work spotless; they have to do so in a cost-efficient way because they will be deployed in low-income countries. In this regard, the use of molecular elements combining several antimalarial activities, whether drug, targeting, carrier, or booster of immune reactions, will contribute to reduce the cost of their development. The implementation of a new delivery method is usually cheaper than the process leading to the discovery of a new drug, and it has the additional advantage that, if well designed, these strategies can be adapted to several drugs. As an example, the direct delivery of drugs to the mosquito vector would allow a simplification of preclinical assays, thus contributing to a reduction in both the budget of product development and the bench-to-bed time of future antimalarial medicines.

Rather than focusing all efforts on identifying new drugs whose efficacy is rapidly diminished by the parasite’s evolution of resistance, an important and often disregarded battlefront is the implementation of targeted delivery methods capable of increasing the doses reaching the pathogen up to local levels sufficiently high to minimize this resistance emergence. Regrettably, the search for this long sought-after \textit{magic bullet} against malaria has not taken off in earnest yet. However, recent data outline the feasibility of some such potential novel approaches, among which we can count new types of combination therapies where one of the activities does not act on individual \textit{Plasmodium} gene products.

\textbf{Reference}


\textbf{Acknowledgements}

This research was supported by grants BIO2014-52872-R (Ministerio de Economía y Competitividad, Spain), which included FEDER funds, and 2014-SGR-938 (Generalitat de Catalunya, Spain).
Strategies for blocking transmission of malaria parasites through the mosquito have achieved interest as an important part of elimination and eradication strategies. Development of such strategies are challenging due to the fact that the mosquito stages of the malaria parasite are complex and still not well understood. The mosquito stages are initiated when gametocytes, which have formed in the mammalian host, are taken up by the mosquito. Immediately gametogenesis takes place resulting in the formation of the motile zygote called the ookinete. The ookinete traverses the midgut epithelium and transforms into the oocyst. Here during the next ten days the infectious sporozoites develop.

Drug repositioning is the identification of new or alternative functions for existing drugs and is an attractive approach to provide new therapeutic interventions against parasitic diseases. Within this framework we have studied the effect of HIV protease inhibitors (HIVPIs), which target the HIV aspartyl protease, on early mosquito midgut stages of the malaria parasite. There is now a growing body of evidence that HIVPIs interfere at many stages of the malaria parasite life cycle. We specifically investigated whether HIVPIs have an effect on zygote to ookinete transition. Using cell based assays we determined their inhibitory action on this parasite stage and furthermore using comparative proteomics we gained insight into the pathways affected by this class of drugs in the parasite.

Other approaches to discover putative drug targets in the mosquito midgut stages will also be discussed highlighting the challenges and pitfalls in this research area.
Most protozoa are capable of both salvaging preformed pyrimidines and *de novo* pyrimidine biosynthesis. This study seeks to identify the gene family encoding the protozoan pyrimidine transporters using next-generation RNA-sequencing (RNA-seq) and RNA interference target sequencing (RIT-seq). The De Koning laboratory created 5-FU-resistant parasite lines of *L. mexicana* (promastigotes) and of *T. b. brucei* (bloodstream forms) by *in vitro* exposure to increasing concentrations of 5-FU. We carried out a comparative RNA-Seq analysis of the parental wild-type strains and the 5-FU resistant lines of *T. b. brucei* and *L. mexicana* in order to identify differences in gene expression. We were particularly interested in any genes that encode for proteins with at least 3 transmembrane domains that are significantly down-regulated in the resistant lines Tbb-5FURes and Lmex-5FURes. In addition, genome-wide RNAi library screens were performed in both pyrimidine auxotrophic and prototrophic 2T1 cells exposed to 5-FU. High-throughput RIT-seq implicated several strong hits from the 5-FU screens, which apparently confer resistance to this pyrimidine analogue when their expression is knocked down. We then compared the hits generated by RIT-seq with the down-regulated genes in *L. mexicana* and *T. brucei*. Several candidate pyrimidine transporters genes were identified. These results provide a valuable resource for further exploration to identify the gene (family) encoding the protozoan pyrimidine transporters that we know are expressed in *Trypanosoma, Leishmania, Trichomonas* and other species. Functional expression, targeted RNAi knockdown and reverse genetics of these candidate pyrimidine transporters genes are in progress and will establish whether any of the pyrimidine transport activities that we have previously identified in the various protozoa is encoded by the genes under study. In conclusion, the strategies proposed here are likely to lead to the identification of the protozoan pyrimidine transporter genes. Identification of pyrimidine transporter genes in protozoa will significantly improve our understanding of the evolution of nutrient transporters.
It has been over a century since Carlos Chagas discovered the *Trypanosoma cruzi* (*T. cruzi*) as the causative agent of Chagas disease (CD), a neglected tropical disease with several socioeconomic, epidemiological and human health repercussions. Currently, there are only two commercialized drugs to treat CD in acute phase, nifurtimox and benznidazol, with several adverse side effects. Thus, new orally available and safe drugs for this parasitic infection are urgently required.\(^1\)

Despite of efforts, actions and strategies by WHO and several organizations, the research of new potential treatments against CD, continues being a challenge for drug discovery programs. Nowadays, one of the strategies is based on the search for molecules that can interfere with enzymes involved in *T. cruzi* metabolism, which have an important role in the survival of the parasite. Thus, many enzymes have been studied and reported as potential targets for the discovery and design of new compounds for the treatment of CD.\(^2,3\)

We will focus on two of the most promising targets for the therapy of CD: trypanothione reductase (TR) and the iron-containing superoxide dismutase (Fe-SOD), which protect the parasite against oxidative damage by reactive oxygen species. A brief comparison of the function, mechanism of action and the active sites between *T. cruzi* TR and Fe-SOD with their analogues enzymes in human, glutathione reductase (GR) and the corresponding SODs, will be discussed.

We will also summarize the recent development of novel compounds reported for their ability to selectively inhibit these targets, aiming to define molecular bases in the search for new effective treatment of CD.\(^3\)


Acknowledgements

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Angiostrongylus cantonensis was first reported in Cuba in 1981. Now it can be found in all the Caribbean area and in Ecuador and Brazil. Eosinophilic meningoencephalitis was the main disease produced by the helminthes.

Material and methods: 35 patients from Cuba and Ecuador were studied. CSF and serum samples were obtained. Albumin, major immunoglobulins, IgE and complement components from different pathways like C3c, C4, C5, MBL, MASP2, H and M ficolins were measured in both biological fluids.

Results: During the acute phase IgE and C3c, C5, MBL were intrathecally synthesized according to their respective reibergrams. MASP2, and H and M ficolins were also synthesized in central nervous system.

In a second lumbar puncture, at least one week after the beginning of the symptoms, intrathecal synthesis of three or two major immunoglobulins patterns were observed. According with the intrathecal activation due to the corresponding complement pathway included lectin pathway different patterns were identified.

Conclusions: A complex intrathecal activation of the different complement pathways join to the intrathecal immunoglobulin synthesis can be consider as an auxiliary tool in the diagnosis and following of this parasite-borne disease.

References


Exponential grown of the biomedical applications of nanoparticles has taken place recently. To optimize the therapeutic outcome of these biocompatible drug-loaded nanoplatforms, special attention has been given to the revolutionary introduction of both passive and active drug targeting strategies in their engineering, i.e. involving the formulation of long-circulating and surface functionalized (and/or stimuli-sensitive) particles, respectively. More specifically, surface functionalization of the nanoplatform to assure ligand-receptor interactions reporting an endocytotic uptake by the parasite has shown promising results in the management of infectious diseases, e.g. improved pharmacotherapy [1] and/or facilitated disease diagnosis [2].

African trypanosomiasis (AT) is a severe infectious disease caused by Trypanosoma brucei. Conventional drug therapies against the disease are characterized by severe toxicity and by the development of resistances, principally related to mutations in drug transporters. To meet the challenge, specific targeting of drugs facilitated by surface-functionalized nanoparticles is probably becoming a cost-effective approach for disease treatment compared with the discovery of novel drug molecules. Such nanostrategy against AT has been reported to reduce systemic drug toxicity and to circumvent resistances acquired through impaired compound uptake [3-5].

This contribution will provide an insightful vision on these nanotechnology-based strategies being designed for the treatment of AT, approaching them from a biology-driven perspective.

References


Acknowledgements

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A DECREASE IN MITOCHONDRIAL MEMBRANE POTENTIAL IS ASSOCIATED WITH DIMINAZENE RESISTANCE IN TRYPANOSOMA CONGOLENSIS

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Animal trypanosomiasis is a parasitic disease of livestock that is predominantly caused by infection with Trypanosoma congolesense. It causes economic hardship in much of sub-Saharan Africa due to illness and death of infected domestic animals, impacting on food security and economic development of the afflicted regions. Although treatment is available, mostly with diminazene, there are many field reports of Trypanosoma congolesense resistant to this drug. The cause of this resistance has not been definitively determined; the preliminary identification of the TcoAT1 adenosine transporter as a conduit for diminazene uptake in this parasite was shown to be incorrect (Munday et al., 2015). This project therefore aimed to determine the mechanism by which T. congolesense can develop resistance to diminazene to aid future drug administration, drug discovery and improve resistance reporting. T. congolesense IL3000 were cultured in vitro and resistant lines were acquired by in vitro exposure to diminazene. No cross-resistance was observed to other trypanocides except the experimental diamidine DB75. Uptake of [3H]-diminazene was low affinity and slow in wild-type T. congolesense and partially inhibited by pentamidine and folate. It was not affected in the resistant clones. However, growth of all the resistant clones was somewhat slower than wild-type T. congolesense and we are investigating the fitness cost. However, all the resistant strains displayed a lower mitochondrial membrane potential than the control, linked to a slower accumulation of the drug into the mitochondrion. We propose that this is at least partially responsible for the resistance phenotype and are currently undertaking whole genome sequencing and RNA-seq of the wild-type and resistant clones in order to identify genetic adaptations.

References.

Acknowledgements
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AN EX VIVO PHENOTYPIC SCREENING FOR ANTILEISHMANIAL DRUGS USING INFRARED-TRANSGENIC CELLS

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The massive screening of compound libraries by high throughput techniques (HTS) is a powerful methodology to identify new molecules against parasite-borne diseases. Current target-based (biochemical) and non-target (phenotypic) approaches are valid in vitro methods to test thousands of compounds but often they are based on hard-to-conciliate paradigms. On the one hand, target-based screenings require a consolidated druggable protein involved in essential biochemical pathways to perform in silico studies of the compounds that best interact with the target. Nevertheless, it often occurs that the best-designed compounds are poorly transported, demolished by the xenobiotic-metabolizing enzymes, or have more than one non-identified target. On the other hand, phenotypic screenings are cell-based assays that allow us to assess the effect of compounds on killing or preventing the proliferation of the pathogen, but they provide no evidence about its mechanism of action. Furthermore, it is mandatory that the parasite form responsible of clinical symptoms in the host is culturable. The use of ex vivo explants obtained from organs dissected from experimentally infected rodents is a promising method of drug discovery for visceral leishmaniasis. This approach involves real amastigotes infecting spleen macrophages surrounded of the full repertoire of immune cells to test a battery of compounds. This methodology requires of genetically modified parasites to confer a rapid optical readout. Validated examples of this new technology are the hamster-derived spleen explants used to screen a library of several thousands of drugs using a firefly luciferase transfected strain of L. donovani or the murine splenic explants from BALB/c mice infected with iRFP-L. infantum strain that emits infrared fluorescence. Remarkably, by using this same tool, it has been possible to study parasite dissemination in an in vivo chronic model of visceral leishmaniasis based on infrared fluorescence signal. In this communication, we show our recent results with an iRFP-L. infantum amastigote-infecting splenocytes platform ready to perform phenotypic screenings of small molecules and to monitor the progress of in vivo visceral leishmaniasis at preclinical levels.

References

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DEVELOPING NANOPARTICLES FOR 17-AAG DELIVERY AGAINST LEISHMANIA INFECTION

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**Background:** 17-N-allylamino-17-demethoxygeldanamycin (17-AAG, tanespimycin) is an inhibitor of heat shock protein 90 (HSP90), which may represent a promising therapeutic agent for the elimination of intracellular Leishmania [1]. However, the delivery of 17-AAG is difficult due to its poor aqueous solubility, requiring the use of some organic excipients, such as DMSO or Cremophor EL which are toxic to a certain level. One promising strategy is to formulate 17-AAG into solid lipid nanoparticles (SLN), which are delivery systems with potential for improving the performance of pharmaceuticals [2]. We have hypothesized that SLN could provide 17-AAG solubilization and toxicity elimination observed in conventional vehicles, e.g. Cremophor EL, used in clinical trials. In addition, this novel SLN-based 17-AAG formulation could offer several pharmacokinetic advantages, such as specific drug delivery, high metabolic stability, improved bioavailability, and long duration of action. **Methods:** SLN containing 17-AAG were prepared by the water-in-oil-in-water (W/O/W) double emulsion method [3]. In addition to SLN characterization, long-term stability was assessed by monitoring pH, conductivity and turbidity of lipid nanosuspensions during 120 days. Furthermore, fluorescent SLN were prepared with FITC green dye as fluorescent marker for nanoparticle uptake assay. Briefly, peritoneal macrophages from CBA mice were co-incubated with FITC-loaded SLN and examined by confocal microscopy.

**Results:** SLN formulations exhibited a small size (~100 nm), a low PDI (<0.2) and good colloidal stability. SLN were morphologically spherical in shape with negligible aggregation. An efficient 17-AAG entrapment into the lipid matrix was reached, approximately 80% by UV-Visible spectroscopy. Of note, blank and 17AAG-loaded SLN maintained their physical stability during 120 storage days at 25°C. On the other hand, a separate set of experiments with FITC-loaded SLN showed a remarkable macrophage uptake, peaking within 2 hours of incubation as observed under confocal microscopy.

**Conclusions:** This investigation led to an optimized SLN-based 17-AAG formulation, which exhibited high 17-AAG loading, stability and ability to be taken up by macrophages. Collectively, these results indicate the feasibility of SLN as potential delivery systems for 17-AAG in leishmaniasis chemotherapy. Currently, SLN uptake assays using macrophages infected with L. amazonensis are in progress.


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LIPID-BASED EMULSOME NANOFORMULATIONS FOR TARGETED DELIVERY OF ANTIPARASITES

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Chemotherapy is the most efficient strategy in fight against many parasitic diseases including leishmaniasis, whereas high toxicity of many anti-parasitic compounds restricts their utility, and the emergence of drug resistant strains often impairs the lifespan of a given drug. Hence, discovery of new anti-parasitic compounds and establishment of new therapies with targeting features are essential.

Recent advances in drug targeting clearly demonstrate that the association of an active molecule with a carrier system modifies its distribution within the host and can therefore increase its concentration at the site of action thus reducing the amount that reaches sensitive sites where the drug is toxic. For instance, several successful examples have achieved already to be on the market: AmBisome® and Fungizone®, commercial liposome and micelle formulations of Amphotericin B (AmB), respectively, are currently in medical used for treatment of leishmaniasis. Likewise, our study focuses on discovery of new anti-leishmanial nanoformulations based on emulsomes. Emulsomes are lipoidal vesicular systems composed of an internal solid fat core surrounded by phospholipid (PL) multilayers. [1] Emulsomes are preferred mainly because of its four major features. Firstly, owing a solid lipid core like the solid lipid nanoparticles, emulsome may offer high loading capacities for hydrophobic substances [1,2]. Secondly, composed of only lipids and in the absence of any surfactants, emulsome is highly biocompatible [2]. Thirdly, the solid character of the nanocarrier provides a prolonged drug release profile, which can be controlled, or tuned, by the selection of the lipid composition as well as by surface modifications [3]. Lastly, but most importantly, the natural feature of lipids allows emulsome to accumulate in the organs of the reticuloendothelial system (RES) instead of the kidney, which will not only largely reduce toxicity, but will also improve the anti-leishmaniasis efficacy of the loaded drug, as parasites are also located in the organs of RES.

The development of emulsome-based antiparasitic nanoformulations facilitating the targeted delivery to the macrophages is expected to substantially contribute to the improvements in treating parasitic diseases such as Leishmaniasis in European region as well as worldwide.

References

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**ORAL COMMUNICATION**

**OC 5D. THE TRYPANOSOMATID SERINE/THREONINE PROTEIN KINASE “JEAN3” MAY CONFER RESISTANCE TO DRUGS SUCH AS PAROMOMYCIN**

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Protein kinases (PKs) are known crucial mediators of the transduction of environmental signals and the coordination of intracellular processes. They are potential drug targets to treat diseases such as cancer and Alzheimer's. In *Leishmania* and *Trypanosoma*, several PKs have been described to be essential during the proliferation and/or the viability of parasites in the clinically relevant stages. The toxicity current treatments display and the emergence of resistant strains, urge the correct understanding of the biology of these parasites to therefore overcome those critical issues. Our group has identified in trypanosomatids “Jean3”, a constitutively expressed Ser/Thr protein kinase. We have studied Jean3 expression during the growth cycle of *Leishmania major* and evaluated its implication in drug resistance.

RT-qPCR confirmed the constitutive expression of Jean3 in *L. major*. Using the expression vector pXG-LmJean3 we generated Jean3-overexpressing *L. major* parasites. MTT-based drug screening assays showed Jean3-overexpressing parasites had increased sensitivity towards amphotericin B and miltefosine treatments.

*In silico* docking revealed that Jean3 may be one of paromomycin (PMM) targets. This result was confirmed as Jean3-overexpressing strain showed a higher EC50 value with respect to the control. Assays performed with G418, a structurally related aminoglycoside, exhibited similar results. Therefore, reinforcing the association between Jean3 and PMM resistance.

Understanding the mechanisms of drug resistance is critical to develop novel treatments and improve the current ones. Our results revealed that Jean3 kinase may be involved in this process and particularly in PMM resistance. To date, no clinical resistance has been reported for PMM. However, more research needs to be performed to prevent this phenomenon.

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PLENARY LECTURE E.

THE QUEST FOR NEW VACCINES AGAINST BRUCELLOSIS
Posters
POSTER LIST

POSTER FLASH PRESENTATION

PF1  ON THE ROAD TO FUNCTIONAL UNDERSTANDING THE DIVERGENT ACTIN 2, A NEW TARGET FOR MALARIA TRANSMISSION BLOCKING

   Maria Andreadaki, Elena Deligianni, Rhiannon Morgan, Inga Siden-Kiamos

POSTER FLASH PRESENTATION

PF2  LEISHMANIA MAJOR NUCLEUS-LOCATED YINP PROTEIN IS A GENOTOXIC DRUGS TARGET


POSTER FLASH PRESENTATION

PF3  INVOLVEMENT OF THE SERINE/THREONINE KINASE - JEAN3 - IN LEISHMANIA INFECTIVITY


POSTER FLASH PRESENTATION

PF4  LACTOCOCCUS LACTIS HSP65 PRODUCER AS AN ALTERNATIVE THERAPY FOR CUTANEOUS LEISHMANIASIS

   Priscila Guerra, Rafael Santos, Juliana Rebouças, Daniel Feijó, Ana Faria, Cláudia Brodskyn

POSTER FLASH PRESENTATION

PF5  LEISHMANIA VACCINATION USING MICRONEEDLES AND NUCLEOSOMAL HISTONES

   Esther Moreno, Juana Schwartz, Alba Calvo, Laura Blanco, Esther Larrea, Carmen Sanmartí, Juan M. Irach, James Birchall, Manuel Soto, Socorro Espuelas
**P6** HISTONE FOLD DOMAIN DIMERIZATION OF OOCYST RUPTURE PROTEINS (ORPs) AS TARGET FOR ANTIMALARIAL DRUGS DEVELOPMENT

Chiara Currà, Renate Gessmann, Tomasoio Pace, Leonardo Picci, Giulia Peruzzi, Vasiliki Varamogianni-Mamatsi, Lefteris Spanos, Celia R Garcia, Roberta Spaccapelo, Marta Ponzi, Inga Siden-Kiams

**P7** STUDY AND CHARACTERIZATION OF A NEWLY DISCOVERED ONCOGENIC DOMAIN IN *LEISHMANIA SPP*

José Peña-Guerrero, Miriam Algarabel-Olona, Andrés Vacas-Oleas, Celia Fernández-Rubio, Paul Nguewa

**P8** EXPLORING THE SCOPE OF NEW ARYLAMINO ALCOHOL DERIVATIVES: SYNTHESIS, ANTIMALARIAL EVALUATION, TOXICOLOGICAL STUDIES, AND TARGET EXPLORATION

Miguel Quiliano, Adela Mendoza, Kim Y. Fong, Adriana Pabón, Nathan E. Goldfarb, Isabelle Fabing, Ariane Vettorazzi, Adela López de Cerain, Ben M. Dunn, Giovanny Garavito, David W. Wright, Eric Deharo, Silvia Pérez-Silanes, Ignacio Aldana, Silvia Galiano

**P9** ANGIOSTRONGYLUS CANTONENSIS. EMERGENCIA EN AMÉRICA


**P10** THE TRYPANOSOMATID SERINE/THREONINE PROTEIN KINASE “JEAN3” MAY CONFER RESISTANCE TO DRUGS SUCH AS PAROMOMYCIN

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Malaria is an infectious disease caused by the *Plasmodium* parasites, which are transmitted by mosquitoes. It is a widespread disease in tropical and sub-tropical areas and causes more than half a million deaths yearly. Drug resistance threatens progress against malaria. Consequently, transmission blocking strategies for control of this disease are urgently needed. The long term goal of our studies is to contribute to the development of new intervention strategies targeting the mosquito stages. These need to be based on detailed knowledge of the parasite. We focus on parasite actin 2 due to its essential roles in mosquito parasite stages. Actins are important proteins in all eukaryotic cells, which form filaments and carry out many different functions. *Plasmodium* parasites have two different actin isoforms with divergent structures and functions, named actin 1 and actin 2. In addition they are considerably diverged from actins of higher eukaryotes. Thus detailed analysis of parasite actins to understand the function and the stage-specific role is high priority. Actin 1 is ubiquitous in all life stages. The gene cannot be modified or deleted due to the crucial function of the protein in asexual blood stages. Actin 2 is found only in male gametocytes and the mosquito parasite stages (gametes, zygotes and ookinete). The deletion of actin 2 gene results in a block of the transmission through the mosquito vector. Genetic crosses and different actin 2 mutant parasite lines have been generated to understand the molecular function during parasite development in the mosquito vector. These studies revealed an important role of actin 2 in male gametogenesis and zygote to ookinete transformation with an essential impact in oocyst development at the mosquito midgut. The function of actins depends on filament dynamics, which is regulated by a plethora of actin binding proteins (ABPs) and one of our next experimental plans is the systematic identification of ABPs in actin 2. While actin in itself may not be an ideal target for malaria drugs the facts that it interacts with many proteins and such interactions are essential validate actin as a focus of investigation in these parasites.

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Protozoan parasites from the genus *Leishmania* cause a diverse set of human pathologies and endanger the life of at least 350 million people worldwide. Current first-line drug treatments are suboptimal due to their high toxicity, cost, the requirement for hospitalization and the emergence of resistant strains. Genes involved in *Leishmania* infectivity and drug-resistance are an important pool of therapeutic target candidates. The discovery of novel therapeutic targets against these parasites is critical to alleviate treatments deficiencies.

Genomic screening allowed our group to identify *YinP* in *Leishmania* spp. By homology-based inference, *YinP* was expected that *YinP* to be involved in ribosomal biogenesis, nucleolar assembly and cell proliferation. Previous *in vitro* studies revealed that *YinP*-overexpressing parasites possess higher infectivity compared to that of the control parasites. To further study the infectivity of these strains, we analysed their sensitivity to the immune system’s first-line defence mechanism by exposing them to various concentrations of human sera. We then observed that overexpressing parasites exhibited the same serum-sensitivity to that of the control.

To assess the implication of *YinP* in drug resistance, several antileishmanicidal compounds were tested against the overexpressing strains. Our results showed that *YinP* is associated with increased sensitivity to genotoxic drugs. Consequently, *YinP* may be a robust therapeutic target candidate.

Finally, the expression plasmid pXG-mCherry34-*YinP* was constructed to determine the localization of *YinP* protein in *L. major*. Fluorescent microscopy disclosed that red fluorescence of mCherry-*YinP* was localized inside the nucleus, which is concurrent with the functions previously attributed to it.

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*L. major* promastigotes (Lv39c5) were kindly provided by Dr. Manuel Soto (Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain). pXG-HYG *Leishmania* vector was a kind gift from Dr. Rosa M. Reguera (Departamento de Ciencias Biomédicas, Universidad de León, León, Spain). This work has been funded by Fundación Roviralta and Fundación Caja Navarra. Miriam Algarabel acknowledges the pre-doctoral fellowship provided by the Institute of Tropical Health of the University of Navarra (ISTUN).
Leishmaniasis is a vector-borne disease caused by intra-cellular parasites from the genus *Leishmania*. The World Health Organization (WHO) estimates that more than 350 million people worldwide are under the risk of contracting this disease and has set, among other objectives, the generation of effective vaccines, the discovery of new drugs. Our group has identified “Jean3”, a Serine/Threonine-protein kinase with no orthologues in the mammalian hosts, which is constitutively expressed and conserved in the trypanosomatid species causative agents of human pathologies.

To evaluate Jean3 implication in *Leishmania major* infectivity, we generated Jean3-overexpressing parasites using the expression vector pXG-LmJean3. The expression of several genes related to infectivity was evaluated in overexpressing strains. *In vitro* assays using murine peritoneal macrophages and *in vivo* infections in BALB/c mice were performed to determine the infectivity index of the overexpressing parasites and cytokines expression of the host, respectively. We also studied *in silico* the immunological potential of the protein.

Our results exposed the altered expression of SHERP, gp63 and QDPR in overexpressing strains compared to controls. Moreover, these strains exhibited lower infectivity *in vitro*. *In vivo* infections revealed that Jean3-overexpression generated an attenuated infectivity profile, associated to a beneficial inhibition of Th2 response in BALB/c mice. Furthermore, *in silico* analysis displayed the high immunogenicity of this protein, with 16 putative antigenic epitopes, three of them predicted to trigger CD4+ and CD8+ mediated cellular response in at least 96.6% of the global population. Finally, these epitopes were highly conserved in most of the *Leishmania* species causative of human pathologies.

**Conclusions**

These data suggested that the infection with live overexpressing parasites may promote the control of the inflammatory Th2 response in BALB/c mice, through the inhibition of immune system molecules implicated in its modulation. The generation of protective Th1 responses appeared unaltered respect to controls, but the global balance of Th1/Th2 seemed to have changed towards Th1 dominance. Infectivity results and the high predicted immunogenicity suggested that Jean3 immunogenic epitopes and overexpressing parasites as suitable candidates for vaccination assays.

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In cutaneous and mucosal leishmaniasis caused by *Leishmania braziliensis*, there is induction of a Th1 response accompanied by a strong inflammatory reaction. The exacerbated inflammation observed in cutaneous lesions might be controlled by anti-inflammatory responses. *Lactococcus lactis* are nonpathogenic gram positive bacteria and *Lactococcus lactis* HSP65 are genetically modified bacteria that produces a heat shock protein (HSP65), which has immunomodulatory activity by inhibiting TNF-α and IFN-γ production and increase IL-10 secretion by T cells. HSP65 also has a strong effect on induction of regulatory T cells. In this study, our main objective is to test oral administration of *Lactococcus lactis* producing HSP65 as potential immunomodulatory pre-treatment in experimental model of cutaneous leishmaniasis. BALB/c mice received by oral route recombinant *Lactococcus lactis* strains, which produces or not HSP65 for 4 consecutive days. Twelve days after administration, animals were infected with *Leishmania braziliensis*. Two days before infection, HSP65 group received PAM3CSK4 (TLR2 agonist) intraperitoneally. Evaluation of ear thickness showed that during inflammation, mice treated with *L. lactis* HSP65-associated Pam showed smaller lesions compared to control groups (Lb and Pam+Lb), being associated with parasite load, which was lower in this group. We also observed a tissue destruction reduction, as well as higher IL-10 and lower IFN-γ production by draining lymph nodes in treated animals. Our data suggest that pre-treatment with *L. lactis* HSP65 may lead to inflammatory response modulation and inflammation reduction, compared with control groups.

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Leishmaniasis is an infectious disease caused by protozoan parasite species of the genus Leishmania. Approximately, one billion people are at risk of infection worldwide and more than 1.3 million new infections occur each year. Current control of the disease is based on chemotherapeutic treatments which are expensive, toxic and associated with high relapse and resistance rates. Due to these reasons, the development of an effective vaccine against this disease is promising. Intradermal DNA vaccination with microneedles has not been explored yet and its use could increase the immunogenicity of DNA vaccines as compared with hypodermic injections or improve dose-sparing. Moreover, intradermal vaccination could find more application by the use of devices easy to disposal, needle-free and painless.

In this work, we proposed to load a microneedle device with DNA encoding nucleosomal histones and to compare its protective immunity with a conventional s.c. or i.d. injection of the plasmid. For this purpose, 30 solid microneedles coated with 60 µg of the DNA cocktail encoding Leishmania nucleosomal histones were applied in the back of mice intradermally and compared with a s.c. or i.d. administration of the same amount of DNA using conventional methods. Mice were immunized three times with three weeks interval between each immunization. Four weeks after the last immunization, spleens and lymph nodes of some mice were collected for analysis. Other mice were challenged with 10^5 infective metacyclic promastigotes of L. major in the base of the tail. Mice immunized with microneedles showed increased expression of IFN-g/IL-10, IFN-g/IL-13 and IFN-g/IL-4 ratio in the spleens compared with mice immunized by s.c. and i.d. routes. Furthermore, CCXCL9, CXCL10 and CCL2 levels were also higher. In lymph nodes, the i.d. vaccination with microneedles enhanced the expression not only of IFN-g/IL-10, IFN-g/IL-4 and IFN-g/TGF-β ratio but also iNOS and TNF-α levels. These results are in synchrony with a predominance of IgG2a antibodies found for the histones H2A and H4 in serum samples, which suggest a Th1 response. However, none strategy was able to control Leishmania major infection, as shown by the increase in lesion size and parasite burden.

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Malaria caused by *Plasmodium* parasites, is still the most deadly parasitic disease. The sporozoite, the stage transmitted from the mosquito to the vertebrate during a mosquito bite, initiates the malaria infection in the new host. Sporozoites are produced in the mosquito inside the oocyst in about 12 days after uptake of a blood meal. Oocyst rupture is an essential step for the release of the sporozoites, which next travel to the salivary glands where they will be transmitted to the new host. In *Plasmodium* two proteins containing a histone fold domain (HFD), similar to subunits of the NF-Y transcription factor complex (Nardone 2016), have been characterized and named ORPs (Oocyst Rupture Proteins). ORP1, also annotated as NF-YB, and ORP2, containing a HFD similar to that of NF-YC are probably not *bona fide* NF-Y subunits as no orthologue of the NF-YA subunit has been identified in *Plasmodium*. In addition, ORPs are much bigger than the NF-Y subunits of higher eukaryotes. ORP1 is expressed in the cytoplasm of all *Plasmodium* stages and it localizes at the oocyst wall. ORP2 is detected only in the cytoplasm of young oocysts and at the oocyst wall after sporozoites are formed. Mutant parasites lacking either one of the *orp* are blocked at the oocyst stage, though motile sporozoites develop normally but they remain trapped in the oocyst, leading to complete block in transmission to mice (Currà, 2016). ORP HFDs are implicated in the mechanism of oocyst rupture, possibly through the formation of the dimer. Our recent data on progressive deletion of ORP2, excluding the HFD, suggest that other portions of ORP2 may play a role in the localization of the protein at the mature oocyst wall, where also ORP1 localizes, thus promoting the interaction of the HFDs and capsule rupture. Taken together our data showed that *Plasmodium* exploited ORPs containing the DNA binding histone-fold domains for a divergent function in the unique process of oocyst wall rupture. ORPs, or their specific domains, could be a possible target for antimalarial strategies development to stop malaria transmission to the vertebrate host.

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STUDY AND CHARACTERIZATION OF A NEWLY DISCOVERED ONCOGENIC DOMAIN IN LEISHMANIA SPP

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Background
Leishmaniasis is a vector-borne disease caused by intracellular parasites that threatens more than 350 million people from 98 countries worldwide. Current treatments carry important drawbacks, such as toxicity and resistances. Thus, the World Health Organization emphasizes the necessity of novel therapies.

The sequencing of Leishmania parasite’s genomes allowed us to identify YinP, the homologue of an oncogene and a novel therapeutic target involved in L. major infectivity and replication. One of our current objectives is the characterization of ONC, a domain of YinP.

Methods
To study evolutionary distance of Leishmania YinP-ONC domain, the phylogenetic reconstruction of ONC was carried. Homology modeling was employed to predict ONC tertiary structure and perform in silico docking studies.

On the other hand, with the aim of generating ONC-overexpressing parasites we constructed and transfected expression vector pXG-ONC in L. major. RT-qPCR was conducted to determine the expression pattern of ONC during the life cycle of WT and overexpressing strains.

MTT-based assays were utilized to determine the EC₅₀ of amphotericin B (AMB), miltefosine (MIL), fluorouracil (5FU) and cisplatin (CIS) from both WT and ONC-overexpressing parasites. PNA-negative selection as well as in vitro infections allowed the quantification of the differentiation capability and the infectivity index of ONC-overexpressing strains.

Results
Phylogeny studies revealed that this domain is highly conserved among Trypanosomatid species. However, a moderate homology was observed with other organisms. Modeling validated the canonical structure described previously. RT-qPCR analysis showed that the highest expression of ONC in L. major was exhibited during the metacyclic (infective) stage. Furthermore, overexpressing strains generated more metacyclic forms than the control and their infectivity index was also higher. ONC-overexpressing parasites displayed lower YinP expression levels and higher EC₅₀ values for CIS.

Conclusions
We have described a new domain and a potential therapeutic target that plays a role in L. major drug resistance, infectivity and gene regulation. We have also characterized its structure and found compounds that may inhibit its function. Nevertheless, more research is needed to fully understand the implications of this domain in Leishmania biology.
**EXPLORING THE SCOPE OF NEW ARYLAMINO ALCOHOL DERIVATIVES: SYNTHESIS, ANTIMALARIAL EVALUATION, TOXICOLOGICAL STUDIES, AND TARGET EXPLORATION**

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Malaria, a major tropical disease, is still a major health problem in developing countries. In 2015 alone, 212 million cases were reported globally resulting in an estimated 429,000 deaths [1]. The emergence of resistance to almost all available treatments leads to an alarming situation in endemic areas. New therapeutic alternatives are thus urgently needed. Synthesis of new 1-aryl-3-substituted propanol derivatives followed by structure-activity relationship, in silico drug-likeness, cytotoxicity, genotoxicity, in silico metabolism, in silico pharmacophore modeling, and in vivo studies led to the identification of compounds 22 and 23 with significant in vitro antiplasmodial activity against drug sensitive (D6 IC₅₀ ≤ 0.19 μM) and multidrug resistant (FCR-3 IC₅₀ ≤ 0.40 μM and C235 IC₅₀ ≤ 0.28 μM) strains of *Plasmodium falciparum* [2]. Adequate selectivity index and absence of genotoxicity was also observed. Notably, compound 22 displays excellent parasitemia reduction (98 ± 1%), and complete cure with all treated mice surviving through the entire period with no signs of toxicity. One important factor is the agreement between in vitro potency and in vivo studies. Target exploration was performed; this chemotype series exhibits an alternative antimalarial mechanism.

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The book has 17 chapters divided into three sections. General topics and one section each devoted to the main research results in Cuba, Ecuador and Brasil. In the first section comprises the chapter of neuroimmunological findings in Eosinophilic meningoencephalitis due to the parasite, the molecular markers of the complement system in the disease and the morphological characterization of the parasite and its live cycle inclusions of the parasite and its live circle includes the terrestrial mollusks like Lissachatina fulica and Rattus norvegicus as definitive host. Also it summarizes the lab diagnosis of the disease and a scientometrics study about the scientific production from authors from the occidental hemisphere. In the Chapter devoted to Cuba there are the chapter related to the disease in human adult as well as the clinical characteristics of the infection by the helmint and the intermediate mollusk in Cuba. The Ecuadorians authors start with historical information about the presence of the parasite in Ecuador, the description of the first outbreak and the first case of Angiostrongyliasis in the country as well as the ocular impact in the Ecuadorians patients suffering from the disease. Brasil presents the disease in the country and its intermediate mollusks found in relation to the infection.

The book is available in Research gate as well as in Google Scholar. In the first three months was downloading from the first site more than 600 times and positive comments. The book is nominated to obtain the Premio Annual de Salud from the Cuban Ministry of Public Health and was already presented in Sao Paulo, Havana and Guayaquil.

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THE TRYPANOSOMATID SERINE/THREONINE PROTEIN KINASE “JEAN3” MAY CONFER RESISTANCE TO DRUGS SUCH AS PAROMOMYCIN

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Protein kinases (PKs) are known crucial mediators of the transduction of environmental signals and the coordination of intracellular processes. They are potential drug targets to treat diseases such as cancer and Alzheimer’s. In Leishmania and Trypanosoma, several PKs have been described to be essential during the proliferation and/or the viability of parasites in the clinically relevant stages. The toxicity current treatments display and the emergence of resistant strains, urge the correct understanding of the biology of these parasites to therefore overcome those critical issues. Our group has identified in trypanosomatids “Jean3”, a constitutively expressed Ser/Thr protein kinase. We have studied Jean3 expression during the growth cycle of Leishmania major and evaluated its implication in drug resistance.

RT-qPCR confirmed the constitutive expression of Jean3 in L. major. Using the expression vector pXG-LmJean3 we generated Jean3-overexpressing L. major parasites. MTT-based drug screening assays showed Jean3-overexpressing parasites had increased sensitivity towards amphotericin B and miltefosine treatments.

In silico docking revealed that Jean3 may be one of paromomycin (PMM) targets. This result was confirmed as Jean3-overexpressing strain showed a higher EC50 value with respect to the control. Assays performed with G418, a structurally related aminoglycoside, exhibited similar results. Therefore, reinforcing the association between Jean3 and PMM resistance.

Understanding the mechanisms of drug resistance is critical to develop novel treatments and improve the current ones. Our results revealed that Jean3 kinase may be involved in this process and particularly in PMM resistance. To date, no clinical resistance has been reported for PMM. However, more research needs to be performed to prevent this phenomenon.

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L. major promastigotes (Lv39c5) were kindly provided by Dr. Manuel Soto (Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain). pXG-HYG Leishmania vector was a kind gift from Dr. Rosa M. Reguera (Departamento de Ciencias Biomédicas, Universidad de León, León, Spain). This work has been funded by Fundación Roviralta (www.roviralta.org) and Fundación Caja Navarra. Andrés Vacas acknowledges the financial support provided by Santander Universities (http://www.becas-santander.com/)
The brucellae are facultative intracellular pathogens of mammals that cause brucellosis, a worldwide-extended zoonosis. As described for Brucella abortus and other classical Brucella species, their pathogenicity relates in part to a cell envelope composition that facilitates resistance to bactericidal peptides and other effectors/receptors of innate immunity. However, B. microti, a recently described species infecting rodent-like mammals, is more readily detected by innate immunity and, consistent with a more ancestral phylogenetic position close to plant symbionts rhizobia and free-living soil α-Proteobacteria, grows under a wider range of conditions. Among other amino lipids, both rhizobia and brucellae display ornithine lipids (OL). Complete OL carry one α-amide linked acyl-oxyacyl residue that results from the sequential action of olsB and olsA. In rhizobia, the hydroxyl-linked acyl residue is C2 hydroxylated by an olsC-encoded hydroxylase, and hydroxylation has been found important in pH adaptation in these bacteria. Since, olsC is mutated in B. abortus and other classical species but not in B. microti, the relevance of this difference was studied by deleting olsB and olsC in B. microti. The mutants did not show changes in growth rates, pH and bactericidal peptide sensitivity, although differences in these properties with B. abortus were consistently observed. Moreover preliminary data suggest that OL or hydroxylated-OL (OH-OL) are not involved in B. microti virulence. Therefore the presence of OH-OL in B. microti is unlikely to account for the differences with B. abortus and the lost of olsC in classical Brucella species might represent a case of genomic reduction in the adaptation of brucellae to intracellular life.

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Antimonials remain one of the most important therapeutic options for the treatment of VL. Their co-administration with other antileishmanial drugs and their selective targeted delivery to infected macrophages could decrease the effective dose and the risk of resistance and therapeutic failures. The aim of this study was to find a drug with synergistic effect in combination with Glucantime and their co-delivery in liposomes with M1-polarization features. The efficacy of antimonials has been associated with the immunological status of the patients (1); thus, carriers able to reverse the macrophage de-activation induced by the parasite could benefit the resolution of the infection. Cholesterol, saturated or cationic lipids have been associated with the induction of pro-inflammatory macrophage polarization and then, suitable for the preparation of liposomes with indirect antileishmanial activities (macrophage-mediated) (2).

Several compounds were combined with Glucantime and their activity was tested in vitro in L. donovani infected macrophages. A combination is defined as synergistic when its fractional inhibitory concentration (FICI) is below 0.5 (3). Liposomes were prepared by the film method, containing Lecithin, Cholesterol and Dimethyldioctadecylammonium bromide (DDAB), Dihexadecyl phosphate (DAP) or D-α-Tocopherol succinate (TS) at 75:40:5 mM. Size and homogeneity were adjusted by sonication. Bone-marrow derived macrophages (BMDM) were incubated with the different liposomes formulations for 24 h. Nitric oxide was measured in the supernatant with Griess Reagent. M1 or M2 polarization markers were also measured by real-time PCR.

The combination of glucantime and berberine chloride showed a synergistic effect against L. donovani amastigotes-infected macrophages (FICI = 0.16). Liposomes had a mean size of 150-200 nm and polydispersity index below 0.3. The loading of each drug was around 10.29 nmol Berberine/µmol lipids and 37.79 nmol Glucantime/µmol lipids. Although the presence of cholesterol enhanced the encapsulation of drugs, M2- instead of M1-polarization effect was observed: the PPAR-γ expression was up-regulation and the liposomes inhibited LPS induced pro-inflammatory effect.

Liposomes can be more than inert carriers for drugs. Their composition should be carefully selected in order to achieve maximal therapeutic benefit. In the context of VL, we are looking for liposomes composition with M1-polarization effect in macrophages.

References

Acknowledgements
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H2A PHOSPHORYLATION AS RESPONSE OF DOUBLE-STRAND DNA DAMAGE IN LEISHMANIA INFANTUM TREATED WITH TOPOISOMERASE POISONS

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Topoisomerases are pointed as targets for design and synthesis of new antileishmanial drugs. TopIB poisons stabilize the TopIB-DNA bond preventing the ligation step, yielding single strand breaks (SSBs) that may evolve to double-strand breaks (DSBs) when the replication fork collides with them, triggering apoptotic processes. Eukaryotic DNA is packaged into nucleosomes, arranged by H2A, H2B, H3 and H4 histones. In Leishmania the induction of DSBs undergoes histone H2A phosphorylation on a threonine residue placed within 1 Mb flanking nucleosome. This process is mediated by members of phosphoinositide 3-kinases (PI3-K) family, like ATM-, ATR-, and DNA-dependent protein kinases, which presence has been pointed in trypanosomatids. The current manuscript describes for the first time how TopIB camptothecins (CPT) and indenoisoquinolines are responsible for DNA damage and time-dependent phosphorylation of Leishmania H2A.

DNA cleavage induced by CPTs and indenoisoquinolines was assessed by agarose gel electrophoresis in the presence of etidium bromide. H2A phosphorylation was evaluated by confocal microscopy. In addition, cell extracts from promastigotes treated with the compounds were resolved in SDS-PAGE gels. Phosphorylated H2A histone was identified by immunoblotting using a specific antibody. DSBs was measured using SDS/K precipitation assay of previously treated promastigotes. Fluorescence microscopy was used to visualize the consequences of exposure to CPT analogues on leishmanial DNA. Anti-γH2A fluorescence microscopy revealed signals in approximately 10% of unperturbed wild-type cells and substantially increased proportion of cells with these signals following exposure to DNA damaging agents. Five-micromolar final concentration of CPT-derivatives and 1 microM indenoisoquinolines were incubated during 30 to 120 min, stepwise 30 min with Leishmania promastigotes. H2A phosphorylation was partially prevented by wortmannin and caffeine thus pointing to the involvement of members of PI3-K.

Leishmania parasites were able to phosphorylate H2A histone after DNA insults produced by the exposure to Top poisons partially via PI3-K. However, further investigation is required to identify any effectors that may interact with these histone modifications.

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SEARCHING FOR NEW TAGGED BRUCELLOSIS VACCINES AND ASSOCIATED DIAGNOSTIC TESTS

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Backgrounds: Brucellosis, a zoonosis caused essentially by *B. melitensis* and *B. abortus*, infects livestock and humans causing heavy economic losses. A complete lipopolysaccharide (LPS) is critical for their virulence. Its O-chain is a homopolymer of N-formyl-perosamine, and carries the immunodominant epitopes.

The only useful vaccines to control brucellosis are smooth (S) live attenuated: *B. melitensis* Rev1 (small ruminants), and *B. abortus* S19 (bovine). However, the differentiation between infected and vaccinated animals (DIVA problem) is difficult, when performed soon after vaccination, since the serological diagnostic tests detect the antibody response against the O-chain that is present in both vaccines and wild-type strains.

Objectives: To develop a *Brucella* tagged vaccine and associated DIVA diagnostic tests by modifying *Brucella* O-chain.

Methods: We tagged wild-type *B. abortus* LPS by inserting into the chromosome *wbdR*, a gene that encodes an acetyltransferase that adds an acetyl group to the perosamine of *E.coli* O157 O-chain (Ba::Tn7wbdR). We also combined insertion of *wbdR* with deletion of gene *wbkC* encoding the *Brucella* perosamine formyltransferase (Ba::Tn7wbdRΔwbkC). Both Ba::Tn7wbdR and Ba::Tn7wbdRΔwbkC carry LPS with O-chains that contain new N-acetyl perosamine-associated epitopes that are not present in *B. abortus* wild-type LPS. The strains were tested in mice. We also tagged *B. melitensis* Rev 1 vaccine strain with *wbdR* (Rev1::wbdR) and tested in sheep. Finally, we developed two associated serological tests (agglutination test and iELISA) for DIVA purposes.

Conclusions: The DIVA tests developed (an agglutination test and an iELISA with S-LPS antigen obtained from Ba::Tn7wbdR) allow the differentiation of mice infected with Ba-parental strain from those infected with Ba::Tn7wbdRΔwbkC or Ba::Tn7wbdR. Moreover these results were confirmed in a preliminary study in the natural host (sheep) using the Rev1::wbdR. Thus, introducing *wbdR* into *Brucella* vaccinal background might represent a suitable strategy to solve the DIVA problem in brucellosis.

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A PEGYLATED DENDRITIC POLYGLYCEROL NANOCARRIER DELIVERY SYSTEM IN Leishmania infantum IN VITRO INFECTIONS

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Drugs against visceral leishmaniasis are costly; plenty of undesirable side effects and most of them have to be administered parenterally. Drug release at the target site is one of the challenges of antileishmanial therapy that can improve the efficacy and toxicity of the compounds in clinical use. Amastigotes are the stage form of the parasite to be targeted by drugs. Amastigotes live and grow inside the parasitophorous vacuole of host resident macrophages in liver, spleen and bone marrow. Antileishmania drugs must accumulate in this compartment at such amount that can kill the parasite but do not produce toxicity to cell host. PEGylated dendritic polyglycerol nanoparticles (PEG-PG) have outstanding characteristics of controlled release of drugs and thanks to PEGylation can reduces immune activation efficiency of the host. A pH cleavable PEG-PG conjugated to doxorubicin (DOX) – an anthracycline antibiotic with antitumor activity due to multiple mechanisms. DOX poisons eukaryotic DNA topoisomerase II stabilizing cleaving complexes with DNA, preventing the replication and transcription of DNA. (PG-DOX(pH)-PEG) has been tested on two murine J744A.1 and RAW 264.7 macrophages and ex vivo infected BALB/c murine splenocytes. The naturally occurring fluorescence of doxorubicin was useful to monitor the progress and fate of the drug inside the infected cells by flow cytometry and confocal microscopy. Our results show that PG-DOX(pH)-PEG slowly released doxorubicin inside the targeted macrophages, protecting host of toxic drug concentrations. In addition, unlike free doxorubicin, PG-DOX(pH)-PEG is actively internalized through the acidic endocytic pathway and co-localize selectively with lysosome dyes, surrounding amastigotes. These results point to PG-(pH)-PEG nanoparticles as promising controlled release vehicles for antileishmanial drugs.

References

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**TOPICAL EFFICACY OF PARAMOMYCIN PLUS ANTI-TNF-α ANTIBODIES IN L. MAJOR INFECTED BALB/C MICE**

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*Leishmania* spp. infection is controlled by the activation of a Th1 response and nitric oxide production by macrophages. Although TNF-alpha is considered one of the most important cytokines involved in this response, the systemic treatment of mice with anti-TNF-α did not significantly interfere with the outcome of infection and the elimination of the parasites were similar in both TNF-alpha receptor deficient and normal mice [1]. On the other hand, TNF-alpha has been involved in the immunopathology of CL and a positive correlation between lesion size and TNF-alpha levels has been observed in CL patients [2]. Thus, in the current study we compared the efficacy of topical paramomycin (PM, the only topical treatment currently in use in CL) with the drug given in combination with anti-TNF-antibodies. Firstly, we demonstrated *in vitro* in *L. major* infected bone marrow derived macrophages that PM plus anti-TNF-α antibodies has similar antileishmanial activity as the drug alone and that the strategy did not interfere with iNOS expression and NO production induced by IFN-γ plus LPS. Next, we demonstrated *in vivo* in a model of imiquimod-induced inflammation that topical anti-TNF-α antibodies were able to inhibit the infiltration of inflammatory cells, confirming the ability of their topical application to exert any local activity irrespective of its penetration into the skin. In infected mice, combination of PM plus anti-TNFα significantly reduced the parasite burden in skin, lymph nodes, liver, and spleen similarly to PM alone. However, in *L. major*-infected BALB/c mice, the combination therapy of PM with anti-TNFα had a stronger anti-inflammatory activity that was confirmed by higher down-regulation of TNF-α, IL-1β, iNOS, IL-17, and CCL3 and a significant decrease of neutrophilic infiltrate. Therefore, topical application of PM plus anti-TNF-α antibodies could be useful to reduce inflammation and scarring in CL. In addition, the local inhibition of TNF-α could avoid the adverse effects (and leishmaniasis reaction) associated with the systemic administration of anti-TNF-α therapies.

**References**


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NEW HYDRAZINE AND HYDRAZIDE QUINOXALINE 1,4-DI-N-OXIDE DERIVATIVES: IN SILICO ADMET, ANTIPLASMODIAL AND ANTILEISHMANIAL ACTIVITY

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Malaria and leishmaniasis are serious tropical diseases with far reaching and important global implications. According to the WHO, an estimated 212 million cases and 429,000 deaths were attributed to malaria in 2015.1 Meanwhile, there have been 1.3 million cases and 20,000–30,000 deaths annually attributed to leishmaniasis.2 To avoid parasite drug resistances as well as to improve pharmacokinetics of the existing drugs in recent years, novel structurally diverse compounds with high potency against these diseases and minimal side effects and cost are urgently needed.

We report the design (in silico ADMET criteria), synthesis, cytotoxicity studies (HepG-2 cells), and biological evaluation of 15 hydrazine/hydrazide quinoxaline 1,4-di-N-oxide derivatives against the 3D7 chloroquine sensitive strain and FCR-3 multidrug resistant strain of Plasmodium falciparum and Leishmania infantum (axenic amastigotes). Compounds 18 (3D7 IC50 = 1.40 μM, FCR-3 IC50 = 2.56 μM) and 19 (3D7 IC50 = 0.24 μM, FCR-3 IC50 = 2.8 μM) were identified as the most active against P. falciparum, and they were the least cytotoxic (CC50-values > 241 μM) and most selective (SI > 86).

References

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A NEW ENZYME WITH DUAL-FUNCTION FRUCTOSE/SEDOHEPTULOSE BISPHOSPHATASE SUSTAINS GLUCONEOGENESIS IN BRUCELLA SUIS BIOVAR 5

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Bacteria of the genus Brucella are facultative intracellular parasites causing brucellosis, a worldwide-extended zoonosis. The pathogenicity of these bacteria resides in their ability to adjust their metabolism to the nutrients available in the intracellular niche. Recently, we showed that a B. suis biovar 5 double mutant in the phosphoenolpyruvate carboxykinase (PckA) and the pyruvate phosphate dikinase (PpdK), two anabolic enzymes bridging the TCA cycle and the gluconeogenic pathway, is attenuated. Unexpectedly, a double mutant in the two genes (fbp, glpX) encoding a fructose-1,6-bisphosphatase (FBPase) was able to grow under gluconeogenic carbon sources.

Objectives

Since FBPases are essential for gluconeogenesis, this observation suggested that B. suis 5 remains gluconeogenically competent in the absence of Fbp and GipX. The aim of this work was to identify the third FBPase or the metabolic bypass that sustains gluconeogenesis when Fbp and GipX are absent.

Methods

Bibliographic and genomic analyses allowed us to identify the phosphatase Gpm. We constructed a triple mutant fbp-glpX-gpm and we tested the capability of the mutant to grow on gluconeogenic substrates. Finally, we studied the infection kinetics of the mutant in mice. Moreover, we expressed, purified and characterized Gpm.

Conclusions

The mutant lacking the three FBPases was not able to grow on gluconeogenic substrates and was attenuated in the mouse model, confirming that gluconeogenesis is essential during infection. Moreover, characterization of Gpm showed that i), it has dual-function fructose-1,6/sedoheptulose-1,7-bisphosphatase; ii), it does not require a metal cofactor and iii), it does not belong to any of the five types of FBPases.
COLOSTRAL IMMUNITY IN PIGLETS FROM SOWS ORALLY VACCINATED WITH NANOPARTICLES CONTAINING *ESCHERICHIA COLI* VIRULENCE FACTORS

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Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of illness and death in neonatal and recently weaned pigs. There are some vaccines in the market but, unfortunately, these are not sufficiently safe and efficient. Following a simple procedure and antigenic complex was obtained from the main strains involved in perinatal mortality in pigs, ETEC F4 and F18. To improve their immunogenic properties, the antigens were encapsulated into a foodborne-protein polymer nanoparticle formulation. Loaded nanoparticles were homogeneous and spherical in a shape, with a size of 220-280 nm. In vitro studies indicated that nanoparticles were efficiently captured and activated RAW cell-macrophages; in addition, antigen loaded nanoparticles diffuse efficiently through pig-mucus in vitro, supporting their oral use. Thus, BALB/c mice were immunized by the oral route with either free or encapsulated ETEC antigens. Results indicated that a single dose of loaded nanoparticles was able to elicit high levels and balanced systemic specific antibody response [IgG1 (Th2-response) and IgG2a (Th1-response)] and higher levels of intestinal secretory IgA, with respect to the free antigens administration. These results merit further studies in the natural host.

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Sleeping sickness or African trypanosomiasis is a serious health problem with an added socio-economic impact in sub-Saharan Africa, due to direct infection in both humans and their domestic livestock. There is no vaccine available against African trypanosomes and the main reason is the ability of the parasite to change the major surface glycoprotein (VSG) avoiding antibody-mediated responses. The current drugs used to treat African trypanosomiasis are effective, but most of them present resistances, toxicity and specificity problems. Therefore, there is a clear need of novel, safe, and affordable treatments.

Natural products are the main source of new drugs as they are structures that have been synthesized, degraded and transformed by enzymatic systems. In a search for new molecules with trypanocidal activity, we have performed a high throughput screening of 2000 microbial extracts from a fungi and actinomycetes natural products library which belong to Medina foundation, centre of excellence for research and development in drug discovery in Andalusia. Initially, 267 extracts (13.5%) showed activity. Liquid chromatography fractionation and mass spectrometry analysis reveals that 185 extracts contained potentially new compounds. From them, 83 presented a good dose-response curve. Several known active molecules were identified, including Cordycepin, Curvicollide A-C, Chaetocin, 11-Deoxyverticillin A & Verticillin A and a new fraction with an unknown molecular formula similar to curvicullide family that was termed Curvicollide D.

In in vitro studies, Curvicollide D showed a dose dependent effect on trypanosome viability with an IC50 of 1uM. At the same concentrations there was no effect in cells derived from human hepatocarcinoma (HEP-G2). Curvicollide D induced an alteration of the cell cycle with an accumulation of parasite in G2/M phases. Changes in cell morphology and mitochondrial membrane potential were also observed. In summary, through a high throughput screening of a natural products library we have identified a new member of curviculloide family with an unreported molecular structure and trypanocidal activity.
Trichomoniasis is the most prevalent non-viral worldwide sexually transmitted disease. It is generally associated with serious public health problems (1). This pathology caused by the protozoan parasite *Trichomonas vaginalis* is usually related to bacterial infections that modify both, the normal cervical-vaginal innate immunity and the inflammatory response, producing increased levels of tumoral necrosis factor α (TNFα), interleukins (IL-1β, IL-8) and vaginal neutrophils (2, 3).

Metronidazole is the current treatment for trichomoniasis despite the number of metronidazole resistant strains has been increasing (2), therefore, new treatments are needed.

Curcumin, a natural polyphenol derived from the rhizomes of turmeric, exhibits several pharmacological properties such as anti-inflammatory and antiparasitic (4, 5), displaying activity against *T. vaginalis* (6). Because of that, curcumin has been proposed as a new agent in trichomoniasis treatment.

Regardless of its efficacy and safety, curcumin has certain limitations as low water solubility, rapid degradation and rapid metabolism which lead to a very low oral bioavailability (7, 8, 9). Biodegradable microparticles are promising novel formulations that allow increasing drug stability via encapsulation, and hence, to improve pharmacokinetics and efficacy in drugs therapeutic activity (10). We propose a biodegradable microparticulate system based on zein and poly (methyl vinyl ether)-co-(maleic anhydride) to enhance curcumin stability and activity in trichomoniasis treatment (11).

In this study, the morphology, size and encapsulation efficacy of the microspheres were evaluated. After characterization, the role of curcumin and curcumin-loaded microspheres on pro-inflammatory responses induced in RAW 264.7 phagocytic cells by LPS or parasite proteinases were also assessed. For this purpose, the effects of curcumin and curcumin-loaded microspheres on pro-inflammatory mediators such as the production of nitric oxide (NO) and expression of TNFα, IL-1β, chaperone heat shock protein 70 and a glucocorticoid receptor were investigated.

Curcumin and curcumin-loaded microspheres inhibited the *in vitro* growth of *T. vaginalis* trophozoites, and it also inhibited NO production and decreased the expression of pro-inflammatory indicators in macrophages. The findings demonstrate the potential usefulness of curcumin and curcumin-loaded microspheres as an antiparasitic and anti-inflammatory treatment for trichomoniasis. Further *in vivo* studies are ongoing to investigate these effects and to obtain an optimal control the disease and mitigate the associated immunopathogenic effects.
References


ARYLAMINE MANNICH BASE DERIVATIVES AS POTENT AGENTS AGAINST TRYpanosoma cruzi

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Chagas disease (CD), caused by the parasite Trypanosoma cruzi, affects about 6-7 million people worldwide according to WHO1. Benznidazole and Nifurtimox remain the only available drugs for CD1. Thus, there is an urgent need for new effective, safe and affordable drugs to fight against this disease2. As a continuation of our efforts to identify new compounds for the treatment of CD, twenty new derivatives were synthetized by simple and cheap synthetically routes and their trypanocidal effects were evaluated considering potency and toxicological studies. Four out of twenty derivatives were included in in vivo model. The in vivo acute model showed that the compounds decreased the parasitemia from the very beginning of the treatment and parasites were not detected since day 25 post-infection with two of the tested compounds. None of the compounds showed reactivation after immunosuppression with the dose used with the reference drug (100 mg/kg) and compound 7 showed no reactivation also at 50 mg/kg. Regarding the curative effect, all compounds showed less target organs infected than the reference drug. Moreover, the eight target organs of mice treated with compound 4 were completely free of parasites. In the case of compound 7, six out of eight organs were not infected and the two other organs presented 83% less parasites than control. From the toxicological point of view all the compounds tested in the genotoxicity screening test were not genotoxic and the lead compounds showed no mutagenicity in the Ames test. Considering the mechanism of action, it seems that this family could be inhibitors of the Fe-SOD exclusive antioxidant defense trypanosomatids and, concerning to metabolite excretion, they affected the glucose metabolism of the parasite being the succinate the most affected metabolite. This data could be related to mitochondria malfunction3.

References

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SYNTHESIS AND IN VITRO ACTIVITY OF NOVEL AMINOKETONES AGAINST TRYPANOSOMA CRUZI AND LEISHMANIA spp.

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The WHO recognizes Chagas disease and Leishmaniasis as the most neglected among neglected tropical diseases. Both diseases, caused by trypanosomatid parasites, affect hundreds of millions of people worldwide.¹ The available therapeutic arsenal remains insufficient and inadequate.¹ For that reason, different organizations have proposed big challenges to combat them being one of the objectives the search of new effective, safe and affordable drugs for the treatment of these diseases.²

During the last 5 years, our research group has been working on the synthesis, structural characterization and antiparasitic evaluation of new arylamine Mannich base-type derivatives. As a result of these studies, we identified some compounds as promising molecules for developing new anti-trypanosomatid agents.³

In an effort to improve the potency and the pharmacological and safety profile of the compounds, twenty-three new derivatives have been synthesized by different, simple and cheap synthetically routes. Their trypanocidal effect has been evaluated in the epimastigote form in three different T. cruzi strains (SN3, Arequipa and Tulahuen) for Chagas disease and in the promastigote form in L. braziliensis, L. donovani and L. infantum. The cytotoxicity has also been determined in order to establish their selectivity index (SI). Subsequently, the activity of the selected compounds is being carried out in their intracellular forms of the parasites. The last results of these studies will be exposed in this Symposium.

References

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WadD, A NEW GLYCOSYLTRANSFERASE ACTING ON BRUCELLA LIPOPOLYSACCHARIDE CORE SYNTHESIS, ITS INTERACTION WITH INNATE IMMUNE SYSTEM AND VIRULENCE.

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Brucellosis is a zoonotic disease caused by Brucella. The lipopolysaccharide (LPS) of Brucella plays a major role in virulence as it impairs normal recognition by the innate immune system, and delays the immune response. The LPS core is involved in the resistance to complement and polycationic peptides. Mutants in glycosyltransferases involved in its synthesis are attenuated and good vaccine candidates against brucellosis. The chemical structure of the Brucella LPS core suggests that, in addition to the already identified WadB and WadC (Conde-Álvarez et al., 2012; Gil-Ramírez et al., 2014), other glycosyltransferases should also be implicated in its biosynthesis.

The main objective of the project is the identification of new genes encoding glycosyltransferases involved in synthesis of Brucella LPS core and analysis of their role in virulence.

We constructed mutants in 7 not yet identified ORFs putatively encoding core glycosyltransferases in B. abortus. We analysed their LPS structure, sensitivity to different components of innate immune system and virulence.

All mutants kept the O-chain in their LPS. Interestingly, mutant in ORF BAB1_0953 (named wadD) lost reactivity against the antibodies that recognize the core section. This suggests that WadD is a new glycosyltransferase adding one or more sugars to the core ramification of Brucella LPS that is not linked to the O-chain. WadD mutants were more sensitive than the parental strain to components of the innate immune system. In vivo studies suggest that WadD plays a role in chronic stages of infection. This opens new perspectives for the design of new Brucella vaccines since it is known that mutants in the core branch protect against brucellosis.

References

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TOWARDS A SUBUNIT VACCINE. INCREASED OUTER MEMBRANE INSTABILITY IN A SHIGELLA FLEXNERI tolR MUTANT

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Background. Shigella flexneri is estimated to cause more than 80 million dysenterial episodes each year and around 700,000 deaths worldwide (1), but no vaccine is available yet. Since non-living vaccines seem to be the safest option, our group has focused on the potential capacity of Outer Membrane Vesicles (OMVs) to provide protection against an infection of S. flexneri (2). However, the low yield obtained from natural production of OMV is still a challenge (3).

Objectives. The aim of this study was the construction of a S. flexneri 2a mutant with a non-polar deletion in tolR, one of the genes of the Tol–Pal system of Gram negative bacteria membranes, to increase the OMVs release rate.

Methods. We present a new OMV product obtained from a S. flexneri 2a ΔtolR mutant. Physical characterization, as well as a sensitivity study against different antibiotics and chemicals was performed in the new bacterial strain. A complete characterization of the new obtained OMV extract and a complete proteomic study were also carried out.

Conclusion. The tolR deletion led to an increase in the OMV yield production in more than 6 times as compared to the wild type strain. S. flexneri 2a ΔtolR mutant appeared to be more sensitive to different antibiotics and chemical compounds due to an envelope alteration; although no significant differences in OMV protein or LPS profiles were found. Considering these promising results, although further studies are needed, ΔtolR-OMV antigenic extract appears as a new vaccine candidate to face shigellosis.

References
(1) WHO. Guidelines for the control of shigellosis, 2005.

Acknowledgements
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THE INTRODUCTION OF AN ACTIVE CARBONIC ANHYDRASE ALLOWS CO₂-DEPENDENT BRUCELLA STRAINS TO GROW UNDER ATMOSPHERIC CO₂ CONCENTRATION

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Background

Brucellosis is an important zoonosis caused by bacteria of the genus Brucella. Animal vaccination is the main way to prevent this disease but there is no vaccine against B. ovis and protection is achieved using B. melitensis Rev1. Stopping vaccination with Rev1 when B. melitensis is eradicated leads to an increase in the number of infections caused by B. ovis and thus, research on specific vaccines is essential. Nevertheless, one of the main difficulties to develop a B. ovis-vaccine is the requirement of a high CO₂ atmosphere to grow.

Objectives

To analyze the mechanisms underlying CO₂ dependence in Brucella and to obtain a CO₂-independent B. ovis strain.

Methods

We first sequenced and analyzed the genes encoding carbonic anhydrases (CAs) I and II from B. ovis and two B. abortus strains (292 and 544). Then, we inserted the genes encoding CAI and/or CAII into the genome of the three strains using the mini-Tn7 system. We studied their growth under atmospheric conditions and their infection kinetics in mice.

Conclusions

In the case of B. ovis, both genes encoding CAI and CAII are disrupted. In contrast, although CAI is conserved in the two B. abortus strains, the lack of a functional CAII seems to be responsible for the requirement of a high-CO₂ atmosphere. Consequently, the introduction of an active CAII allows Brucella growth under atmospheric conditions, while the role of CAI remains to be unveiled. Interestingly, the introduction of a functional CAII into B. ovis does not affect the virulence of this strain, and therefore, it is an excellent background for the development of specific vaccines.

References


Acknowledgements

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References


Acknowledgements

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EXTRACTION AND COMPARISON OF CHEMICAL CONSTITUENTS OF ARTEMISIA ANNUA PLANTS

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1. INTRODUCTION AND OBJECTIVES

Artemisia annua is a Chinese tea plant used for its antimalarial activity due to the presence of artemisinin. It is reported that flavonoids and other chemical constituents present in the plant display antioxidant property contributing to the antimalarial effect. We conducted a qualitative and quantitative chemical comparison of components of plant samples found in 3 cities of Cameroon (Bangangté, Bandjoun, Dschang) and Luxembourg.

2. MATERIAL AND METHODS

On 4 g of dry leaves, we carried out successive extractions with methanol or ethanol, petroleum ether, diethyl ether, ethyl acetate, n-butanol and water were performed on samples from the 4 cities. Rates of polyphenols and totals flavonoids of different extracts were evaluated by ferric chloride and aluminum trichloride respectively, after validation of these assay methods. The flavonoids were then separated and identified by Thin Layer Chromatography (TLC).

3. RESULTS

Ferric chloride method was better than Folin method for quantifying polyphenols. Ethanol extract was higher in polyphenols (1.15%) than methanol (1.025%). As for flavonoids, diethyl ether extract contained $2.192 \pm 0.04g\ EQ/\ kg$ of dry leaves vs $0.912 \pm 0.04g\ EQ/\ kg$ for the n-butanol extract. Nevertheless, no significant difference was noted between levels of polyphenols and total flavonoids crude extracts in accordance to the place of harvest, except for the sample from Dschang which contains $2.38g\ EQ/kg\pm0.03$ of flavonoids. In the crude extract of Bangangté, TLC indicated nine spots which eight correspond to flavonoids, including kaempferol, flavonol aglycone which was clearly identified.

4. CONCLUSION

It is likely that the rate of polyphenols and total flavonoids of leaves of Artemisia annua in our study does not depend on the harvest region, except the original sample from Dschang. Whether the Dschang Artemisia annua antimalarial effect is modified need to be revealed.

Acknowledgements

I dedicate this work to my dear parents Jeanne and Jacques Lamero as well as to all those who supported me.
CATTLE AND OVINE BRUCELLOSIS IN ALGERIA: SEROLOGICAL STUDY AND BRUCELLA ISOLATION

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Brucellosis is a zoonosis afflicting many countries of the Mediterranean basin. In Algeria, human brucellosis is rampant with thousands of cases reported in 2016 but information on the animal disease is scarce. The aim of this study was (i) to assess bovine and ovine brucellosis presence in Algiers (where brucellosis vaccination is not implemented), (ii) to compare four serological tests under the conditions of resource-limited laboratories and (iii) to isolate Brucella from cattle in Medea. A total of 402 cattle and 203 ovine sera from two slaughterhouses in Algiers were examined by the Rose Bengal Test (RBT), complement fixation test, immunoprecipitation with native hapten and iELISA. In Medea, sera, retropharyngeal and mammary lymph nodes were obtained from 225 cattle from two slaughterhouses; sera were analysed by RBT and immunoprecipitation with native hapten, while lymph nodes were used to isolate Brucella. Twenty-four bovine sera were found seropositive and only 2 ovine sera were seropositive in Algiers, while in Medea, 24 sera were positive and 22 Brucella strains were isolated, including B. melitensis from cattle. This study shows that cattle brucellosis is a major problem in Algeria. Moreover, since the presence of B. melitensis in cattle is of particular concern for Public Health, more bacteriological studies are necessary. Similarly, studies in areas with dominance of ovine breeding are necessary. For serological studies, the results show that, in the absence of vaccination, simple tests like RBT and native hapten immunoprecipitation are not outperformed by complement fixation or iELISA.

Acknowledgements

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SOCIAL PROGRAM

Founded by the Romans and located on the Pilgrim’s Way to Santiago, Pamplona is now a modern and welcoming city with a wide range of activities that include walking around century-old walls and cobbled streets; resting in parks and terraces; trying its delicious tapas (or pinchos as they're known locally); visiting historical monuments; attending great shows or watching traditional sports.

WALKING TOUR IN PAMPLONA

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
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<tr>
<td>17.30</td>
<td><strong>Bus ride</strong> from the University of Navarra to the city centre.</td>
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<tr>
<td></td>
<td>On the bus we will get to know a few facts about Pamplona and its history and traditions.</td>
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<tr>
<td>18.00</td>
<td><strong>Cathedral.</strong> We will visit the gothic Cathedral of Pamplona (built in the 14th-16th century).</td>
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<td>We will have the opportunity to see the cathedral as well as the dining hall (<em>refectory</em>), the kitchen, the cloister and the bedrooms where the people working for the bishop used to sleep.</td>
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Meson del Caballo Blanco (The White Horse Inn). One of the most visited spots in Pamplona, with an incredibly beautiful view over the city walls (built in the 16th-18th century) and the mountains surrounding the valley of Pamplona.

This very old house (built in 1492) is now a bar/restaurant where we will have a drink and one of its really tasty pintxos (tapas or fingerfood).

19.00

Walking tour in the old quarter (city centre). We will have a look at the actual streets where bulls run during the festival of San Fermín. We will also have the opportunity to see the city hall, seat for the municipal government and the place where every 6th July the festival of San Fermín begins.

19.30
**Café Iruña.** The oldest café in the city (serving coffee and other drinks since 1888) and also known as Hemmingway’s Café, because this famous American writer used to hang out quite often in that place. We will have a look at the same decor (Liberty Style / Art Nouveau / Jugendstil) Hemmingway saw in the 1920s when he first visited the city of Pamplona.

20.15

![Image of Café Iruña interior]

**Dinner at Café Iruña.** We will have the opportunity to try some of the most typical dishes in the local cuisine and have a go at some of the wines produced in the region (red, white and rosé).

20.30

![Image of Café Iruña entrance]

![Image of Café Iruña dining area]