Adaptability and flexibility, paradigms of survival in *Leishmania* parasites

Alicia Ponte-Sucre*, Maritza Padrón-Nieves, Emilia Díaz-López

Laboratory of Molecular Physiology, Institute of Experimental Medicine, Faculty of Medicine, Universidad Central de Venezuela'

***aiponte@gmail.com**

Summary

The Molecular Physiology Laboratory was created in 1994, a few months upon the return of Alicia Ponte-Sucre (APS) to the Chair of Physiology, after completing her Doctorate in Sciences at the Central University of Venezuela. Although the academic career of APS had begun in 1981, when she joined the Department of Physiological Sciences, Faculty of Medicine, UCV as an Instructor, it was at the end of her doctorate when she decided to become independent and organized her research group. The leitmotif of the Molecular Physiology Laboratory has been to understand fundamental processes involved in essential functions related to homeostasis and preservation of life. The main focus in their research has been understanding the physiological mechanisms responsible for drug- susceptibility/resistance, in the metabolically flexible parasites such as *Leishmania* and to a lesser extent *Trypanosoma*. The comprehensive approach that this laboratory has made in this field of health research is based on the training that APS, as well as her colleagues, have in multiple areas of knowledge including, biochemistry, biophysics, cellular biology, physiology and pharmacology. In 1988 when APS began her doctorate, she decided to direct her research towards this area of knowledge, closely related to health and development, that constitute important challenges in areas of the world that include her country of origin, Venezuela. Since then, her laboratory has focused on the understanding the physiology and exploring the pharmacology of neglected tropical diseases (NTDs), such as leishmaniasis and trypanosomiasis, produced by the unicellular parasites *Leishmania* and *Trypanosoma*. This short article summarizes the scientific production, teaching activities, and services provided by the Molecular Physiology Laboratory upon reaching 25 years of existence.

Key words: *Leishmania*, *Trypanosoma*, Molecular Physiology, Neglected Diseases

Introduction

Search for knowledge identifies human beings. Since ancient times, the how and why of the surrounding world are challenging questions. The seventeenth century represented a hallmark with the improvement of the scientific method. Since then, human beings began to systematically accumulate knowledge and build human capacity, behaviors that favor the optimization of our environment and destiny. Consequently, scientists assumed great responsibility before society. Doing science (being curious and searching for answers) is incorporated into our DNA. This condition exalts our human trait, and creating knowledge expands our horizon to improve the living conditions of the society in which we are immersed.

Since the second half of the twentieth century the complexity of doing science has increased significantly, in line with the scale of the challenges we dare to face. The consequence has been the conformation in the 21st century of large-scale collaboration models, or "Big Science." [(1)] Large-scale science complements the creative work of individual researchers, who explore the limits of nature; Big Science is based on concepts, techniques, instruments and reagents that, in a coordinated and systematic way, identify challenges whose solution exceeds the strengths of an individual researcher.

An example of this, from a global perspective, refers to one of the best examples of international solidarity of the 21st century, the decision taken by the 193 member states of the United Nations in 2000 to approve the Millennium Development Goals (MDGs)), subsequently extended for the 2015-2030 period to the Sustainable Development Goals (SDGs). In 2000, the international community committed itself to working together and coordinating efforts to achieve a better world by 2015 [(2, 3)] and later by 2030. [(4)] The MDGs catalyzed coordination, research and solidarity efforts without precedents, thus exposing the best of our human condition.

Three of the eight MDGs are directly related to the area of health. ODM-4, ODM-5 and ODM-6, and ODM-8 to research and development. However, despite the enormous effort invested so far, 5 years after leaving 2015, we are far from achieving the MDGs globally. [(5)] In this arduous mission researchers and academics carry out tasks of great commitment, linked to the possibility of improving the living conditions of the society, including health. It is in this subject where my professional life is inserted.

As a graduate of the Andrés Bello Catholic University, School of Education, Venezuela, my academic career began formally in 1981, upon entering the Department of Physiological

Sciences, Faculty of Medicine, UCV as an Instructor (equivalent to Assistant Professor). I joined this Chair after completing my studies at the Venezuelan Institute of Scientific Research (IVIC) and graduating from the *Magister Scientiarum* in Biology, mention Physiology and Biophysics. I initiated the Molecular Physiology Laboratory (LFM) in 1994, a few months upon returning to the Chair of Physiology, after completing my fourth level of studies and obtaining the degree of Doctor in Sciences (Pharmacology, 1993, UCV). Since 1988, when I started my doctorate, I oriented my research interest towards issues related to health and sustainable development. The laboratory studies leishmaniasis and trypanosomiasis, neglected tropical diseases (NTDs), produced by the single-celled parasites *Leishmania* and *Trypanosoma*.

Control and prevention of these diseases is closely related to experimental research, due to the urgency of establishing adequate diagnoses and offering optimal chemotherapies, achieving appropriate control and/or elimination of vectors and, optimizing guidelines to be applied in epidemiological, sociocultural and health contexts, common in areas of the world where these diseases are endemic. The organisms that cause these conditions are extremely flexible and have sophisticated survival mechanisms, implying that the challenge we face indicates that our work must produce useful knowledge for solving the ailments caused by these parasites.

The leitmotif of the LFM has been to understand fundamental processes involved in essential functions related to homeostasis and preservation of life, with a focus on the study of the mechanisms responsible for drug- susceptibility, or resistance, in these metabolically flexible parasites. The integral approach of the LFM has been based on the training of its members in areas including biochemistry, biophysics, cell biology, physiology and pharmacology.

But why physiology of parasites? Physiological sciences are an example of integration in which knowledge is organized according to broad and specialized interests. A physiologist, to study the mechanism of action of a drug, or the processes involved in parasite-host interaction, or the cellular mechanisms triggered in a parasite during the adaptation phenomena that support its survival at very high doses of chemotherapy, uses biochemical, histological, biophysical and physiological methods. The obtained results derive from concepts that could eventually be associated with the pathophysiology of the disease and the clinic outcome, according to the general principle of Claude Bernard (1859): *It is necessary to raise first of all the medical problem as it is given by the observation of the disease, and then experimentally analyze the pathological phenomena trying to give their physiological explanation.*

This implies a conceptual identity between biochemistry, physiology, pathology and pharmacology. At the same time this illustrates the perpetuation of the classical separation of these disciplines in the curriculum of medical studies, based on the intrinsic value of each, without affecting the object of inquiry of the physiological sciences as a whole [6], the function of an organism. As we believe in a transdisciplinary and interdisciplinary university, in which frontiers of knowledge surpass dogmatic canons, our research is consistent with these integration concepts.

This scheme includes teaching-learning methods based on the analysis of the philosophy of the medical science itself, as the "epistemology of interdisciplinarity", in tune not only with innovation, but with specific circumstances, such as break points of educational institutions, health centers [7], and in a Faculty of Medicine, as is the case. Therefore, among those who "do" physiological sciences, physiologists take the lead in how to link the other disciplines that make up the organization chart and integrate them. [7] This constitutes a natural process since physiological systems are only a context (organism, tissue, cell), where chemical processes (biochemistry), are regulated by molecules (pharmacology), and under strict control (functional feed-back loops), which if altered (pathology), break the physiological dynamics. This conceptual identity guides the research presented here around a concrete subject, the flexibility of *Leishmania*.

Lines of action

With the previous concepts in mind, the scientific activity of the LFM has focused on the following areas of interest:

- Parasite-host interaction in *Leishmania*,
- Adaptability of *Leishmania* and its involvement in drug resistance,
- Therapeutic tools against *Leishmania* and *Trypanosoma*,
- Edition of scientific books in the field of drug resistance,
- Human resources training.

I would briefly describe each of them.

In order to examine the **parasite-host interaction in *Leishmania***, the LFM partially based their research on previous data [8,9] in which it was shown that the enzyme pyruvate kinase (PYK) of *Leishmania amazonensis* has two isoforms with different kinetic and behavioral properties towards the activator, fructose, 2,6-bisphosphate (F2,6DP), which triggers the conversion of the enzyme from monomer to tetramer and inhibits the activity of the tetrameric form of PYK and activates the monomeric form of the enzyme. The LFM had also demonstrated the feasibility of studying the physiological properties (electrophysiological records of ion

channels) of plasma membranes, purified from *Leishmania mexicana* extracts, incorporated into artificial lipid membranes, and the susceptibility of *Leishmania* parasites to ionic channel inhibitors, at concentrations that do not affect the viability of host cells, macrophages. [10-13]

These studies were the base of the research that conformed part of the doctoral thesis of Prof. Maritza Padrón-Nieves [14], exploring the potential prognostic value of cellular markers and the therapeutic value of the combined use of a classic leishmanicide together with an ABC transporter blocker for leishmaniasis therapy.

On the other hand, and in order to examine the mechanisms involved in cell differentiation and in parasite-host cell interaction in *Leishmania*, the LFM, relied on previous research that demonstrated that the interaction of the parasite with host cells is a dynamic process, and that the exposure of host cells to *Leishmania* surface molecules, i.e., lipophosphoglycan, do not affect their antigen presentation properties, but inhibit their phagocytic activity -in the short term-, and their migratory properties, reinforcing the concept of the dynamic nature of the existing immune response Th1-Th2 in leishmaniasis. [14-18] The interest of the LFM in this area of research was initiated through many visits of Prof. Ponte-Sucre to the Zentrum für Infektionsforschung-University of Würzburg (1995-2001) as invited scientist and as a Humboldt (Georg Forster) fellow. Continuous visits up to date of Prof. Ponte-Sucre to the University of Würzburg, to attend invitations made by the Missionärztlisches Institut and the Department of Bioinformatic at the Biozentrum, have broadened even more the scope of the research made by the LFM.

In this sense it is essential to highlight the pioneering studies carried out by Prof. Emilia Díaz-López that include the adaptation of a methodology "a chemotaxis test performed by the capillaries-two chambers method ", as a simple and useful technique for the quantitative evaluation of the taxis in *Leishmania* [19] and its use to characterize the chemotactic effect of drugs based on modified polypeptides, fundamental amino acids in parasite metabolism, as well as the description of sensory and autonomic neuropeptide activities on parasite migration, and the expression of their membrane receptors on the surface of *Leishmania*. [20-22]

In summary, the work of the LFM has contributed to renew the way of studying experimental parasitology related to parasite-host interaction in *Leishmania* and *Trypanosoma*. [23] Th LFM uses simple innovation methods to update the way of doing science in this field of knowledge.

To better understand the disease, they began to ask questions directly to the parasite and unravel its biology. Their studies on mechanisms involved in cell differentiation and parasite-host interaction include the analysis of the parasite role in recognition, taxis, internalization, and infection, to describe the mechanisms involved, and identify parasite molecules that could affect the parasite-host interaction and could be used as tools to inhibit the internalization of the parasite by the host cell, and thus, the infection.

In order to examine the **adaptability and flexibility of *Leishmania*** and its implications for drug resistance, the LFM has characterized physiological consequences associated with drug resistance in these parasites.

Leishmania parasites are flexible, and able to implement multiple (host) evasion mechanisms. In fact, drug resistance is a daily challenge; it can be induced by improper use of medicines, and unfortunately it is spreading worldwide. The molecular mechanisms involved in the physiological selection of parasites with this phenotype are essential for their survival.

With their studies, the LFM has consolidated the concept that the expression of drug resistance in *Leishmania* includes, in addition to the increased expression of specific glycoprotein-P related genomic DNA, the alteration of functions that comprise, among others, the infectivity of the parasite (acid phosphatase and meta-1 protein), intracellular metabolism and oxidative phosphorylation (amino acids and carbohydrates essential for parasite survival), host-parasite interaction (lectin agglutination, membrane potential), and parasite morphology. [24-31]

Currently, the LFM research aims to validate the usefulness of selected cell markers in parasites isolated from patients with chemotherapeutic failure against classic drugs. [32-36]

In summary, the contributions of the LFM have been essential to incorporate in the field of parasitology the concept that drug resistance as a dynamic process that encompasses essential changes in the parasite physiology. [37] These pioneering studies are in line with work from other scientists that study whether such changes constitute epigenetic modifications that occur in parallel with the genetic changes associated with the expression of drug resistance. [38-40]

In addition, the LFM work adds to the enthusiasm of scientists worldwide to understand if these physiological events are modified in a coordinated manner, and if their systematic analysis and understanding can be useful in the design of chemotherapeutic approaches to multiple cell targets, thus identifying strategies to bypass drug resistance in *Leishmania*. [41]

Finally, their studies have incorporated the concept of "fitness" in the *Leishmania* lexicon [42,43], used primarily in viruses. [44] The importance of this concept is related to the molecular changes that occur in chemo-resistant parasites and how the understanding, identification and analysis of the elements that makes it up can be useful to guide patient therapy. This constitutes a crucial knowledge given that the main reliable method to assess the resistance of parasite isolates obtained from patients is the "amastigote-macrophage" in vitro model and that easy-to-use cellular and molecular markers of this condition have not been described for the clinical laboratory. [45] The research carried out by the LFM since 2000 emphasizes the latter point and highlights the need to describe cellular and molecular markers to be systematically used to identify drug-resistant phenotype in infectious parasites. Understanding the adaptation associated with this physiological state may be essential for the development of tools for disease control, especially in geographic areas where drug resistance is a common clinical problem. [46]

In order to find new **therapeutic tools** against *Leishmania* and *Trypanosoma*, the LFM, and colleagues have characterized the activity of natural products and compounds designed for specific targets against these parasites. [45-56] The interest of the LFM in this area of research was consolidated during the stay of Prof. Ponte-Sucre in Würzburg (2003-2007) as scientific staff and member of the central laboratory for the identification and characterization of natural products and compounds designed against specific targets (in bacteria, fungi and parasites), as anti-infectious agents. This work was carried out within the framework of the multidisciplinary and multicenter project (SFB630, financed by the German government): Recognition, Preparation and functional analysis of agents against infectious diseases. In these studies, the LFM and colleagues demonstrated the activity of compounds belonging to different chemical classes, and analyzed part of their mechanisms of action against *Leishmania* and *Trypanosoma*. [57-63]

One of the most relevant contributions of the LFM has been the leadership position it has played focusing on the *in vitro* activity of the compounds, and also in the need to evaluate the mechanism of action, pharmacodynamics, pharmacokinetics and, last but not least, the need to evaluate the compounds in *in vivo* trials, never forgetting that the ultimate goal of the project is to ensure that at least one compound reaches clinical trials.

Additionally, the LFM has been an advisor for projects related to the description of the antiviral, antitumor and immunomodulatory activity of plant extracts of the Euphorbiaceae family, [64] and the identification of the active compounds responsible for their biological activity. This

work has been carried out in collaboration with the University of Antioquia, Medellín, Colombia. The LFM has also been part of an Ibero-American network dedicated to the identification of disease markers, infection and susceptibility or resistance to visceral leishmaniasis in dogs, sponsored by the Ibero-American Science, Technology and Development Program (CyTED).

The members of the LFM are convinced that, in addition to doing research, it is essential to prepare and write comprehensive scientific books. Therefore, they have edited and published four books and contributed chapters corresponding to leishmaniasis, ABC transporters and drug resistance in two of them:

- Alicia Ponte-Sucre (Ed.). ABC transporters in microorganisms: Research, Innovation and Value as Targets against Drug Resistance, Horizon Scientific Press, Caister Academic Press, Norwich, UK (2009).
- Alicia Ponte-Sucre, Emilia Díaz, Maritza Padrón-Nieves (Eds.). Drug resistance in *Leishmania* parasites: Consequences, Molecular Mechanisms and Possible Treatments, SpringerVerlag (2013).
- Luis Germán Rodríguez LG, Alicia Ponte-Sucre (Eds.). ICT in the fight against neglected diseases: a Latin American vision, 2014. Fundación Telefónica, Venezuela, ISBN 978-980-271-460-5, http://www.fundaciontelefonica.com/arte_cultura/publicaciones-listado/pagina- item-publications /? itempubli = 313
- Alicia Ponte-Sucre, Maritza Padrón-Nieves (Eds.). Drug resistance in Leishmania parasites: Consequences, Molecular Mechanisms and Possible Treatments, SpringerVerlag (2018).

Finally, the LFM has done a tenacious work in **training human resources**, by providing space for research to undergraduate and graduate students of the different Schools of the Faculties of Medicine and Pharmacy. Students who have gone through the LFM have been trained in all the techniques and themes herein mentioned and have completed their studies with honors in most cases. It is noteworthy that, in recent years, the LFM has actively participated in the integration of students to the concept of research integrated into the medical career, through the Program of Stimulus to Research for Undergraduate Students, Faculty of Medicine (PEEI),

Moreover, the LFM has provided assistance to sections and academic personnel of the Institute of Experimental Medicine and any professor, research assistants, undergraduate and postgraduate students who have requested it. This support has covered areas including the loan of equipment or supplies, and academic advice. Additionally, the LFM has supported 27 Venezuelan students and teachers to go abroad to complete their third or fourth level studies.

In parallel to their docent and research activities, Professors Maritza Padrón-Nieves, Emilia Díaz-López and Alicia Ponte-Sucre have gone through all corresponding academic steps. They are

Full Professors. They have been teaching in various graduate courses at the School of Biology, and in the Faculties of Pharmacy and Medicine, UCV, and carry out administrative tasks of great responsibility in numerous UCV service areas and scientific organizations in and out the country.

In summary, the LFM has contributed to the understanding of the physiological mechanisms responsible for drug- resistance and susceptibility in *Leishmania* and *Trypanosoma*, by integrating the use of biochemical, biophysical, cell biology and pharmacological tools. Their studies and work contribute to promote the design of alternative therapies against these tropical diseases and have contributed to the education of many generations of medical doctors and postgraduate students in Venezuela.

Acknowledgements

Various national and international institutions have been fundamental in providing the resources to obtain the results summarized here: The Council for Scientific and Humanistic Development of the Central University of Venezuela (UCV), the Research Coordination of the Faculty of Medicine, UCV, the Ibero-American Program of Science, Technology and Development (CyTED), the Alexander von Humboldt Foundation (AvHF) -Germany, the International Academic Exchange Office (DAAD) -Germany, the German Research Council (DFG) -Germany, the University of Würzburg (Uni-Würzburg)-Germany, the Institute Missionärztlichen Institut -Würzburg-Germany, The Academy of Sciences "Siebold Colegium" (SCIAS) -Uni-Würzburg-Germany, Semmelweis University, Budapest, Hungary, University of Helsinki, Finland. To all of them our thanks for trusting in our work. We especially thank the students and fellow professors who during these years have relied in our work and have decided to develop their research challenges in the laboratory. Students and colleagues have been a constant source of inspiration. Their support, ideas and experimental work have been fundamental in the elaboration of this document that summarizes part of the work done in these last 25 years. Without them it would not have been possible.

References

References

1. Esparza J, Yamada T. The discovery value of “Big Science”. J Exp Med. 2007;204(4):70.
2. Naciones Unidas. Objetivos de Desarrollo del Milenio. Informe de 2012. Nueva York, 2012.

3. Gil González D, Palma Solís M, Ruiz Cantero MT, Ortiz Moncada MR, Franco Giraldo A, Stein A, Álvarez-Dardet Díaz C. El reto para la salud pública de los objetivos de desarrollo del milenio: un enfoque desde la epidemiología política. Gac Sanit. 2006;20(3): 61.

4. Sachs JD. From the millennium development goals to sustainable development goals. Lancet. 2012;379(9832): 2206-11. doi: 10.1016/S0140-6736(12)60685-0.

5. Lozano R, Gómez-Dantés H, Castro MV, Franco-Marina F, Santos-Preciado JI. Avances en los objetivos de desarrollo del milenio 4 y 5 en Mesoamérica. Salud Pública Mex. 2011;53(3): S295.

6. Ferreira JR. La enseñanza de las ciencias fisiológicas en la formación del médico. Educ Med Salud. 1975;9(1): 74.

7. Ferreira JR. Tecnología educacional en el proceso de formación de personal de salud. Educ Méd Salud. 1974- hist.library.paho.org. Id: 39518.

8. Ponte-Sucre AI, Alonso G, Martínez C, Hung A, Rivas L, Ramírez JL. Isolation of two pyruvate kinase activities in the parasitic protozoan *Leishmania mexicana amazonensis*. Archives of Biochemistry and Biophysics. 1993;300: 466.

9. Ponte-Sucre AI, Ramírez JL. Fructose 2,6 bisphosphate promotes the monomer-tetramer conversion of *Leishmania mexicana amazonensis* pyruvate kinase type two. Biological Research. 1993;26: 131.

10. DiFranco M, Villarroel A, Ponte-Sucre A, Quiñonez M, Drujan D, Dagger F. Incorporation of ion channels from the plasma membrane of *L. mexicana* into planar bilayers. Acta Científica Venezolana. 1995;45: 206.

11. Ponte-Sucre A, Campos Y, Vásquez J, Moll H, Mendoza-León A. Sensitivity of *Leishmania spp*. to glibenclamide and 4-aminopyridine: a tool for the study of drug resistance development. Memórias do Instituto Oswaldo Cruz. 1997;92: 601.

12. Ponte-Sucre A, Campos Y, Fernández M, Moll H, Mendoza-León A. *Leishmania sp*.: Growth and survival of *Leishmania sp*. are impaired by ion channel blockers. Experimental Parasitology. 1998;88: 11.

13. Ponte-Sucre A, Mendoza-León A, Moll H. Experimental leishmaniasis: synergistic effect of ion channel blockers and interferon-γ on the clearance of *Leishmania major* parasites. Parasitology Research. 2001;87: 27.

14. Padrón-Nieves M, Díaz E, Machuca C, Romero A, Ponte-Sucre A. Glibenclamide modulates glucantime activity and disposition in *Leishmania major*. Experimental Parasitology. 2001;121: 331.

15. Ponte-Sucre A, Heise D, Moll H. *Leishmania major* lipophosphoglycan modulates the phenotype and inhibits migration of murine Langerhans cells. Immunology. 2001;104: 462.

16. Ponte-Sucre A, Figarella K, Moll H. Experimental Leishmaniasis: The glibenclamide-triggered decrease in parasite growth correlates with changes in macrophage features. Inmunopharmacology and Immunotoxicology. 2001;23: 477.

17. Ponte-Sucre A, Scharner A, Moll H. El lipofosfoglicano de *Leishmania major* modula la expresión de receptores involucrados en la internalización del parásito en células de Langerhans de la piel. Acta Científica Venezolana. 2002;53: 218.

18. Ponte-Sucre, A. Modulación de la expresión del asa de regulación hierro libre-óxido nítrico sintetasa de células dendríticas por quelantes de hierro. Archivos Venezolanos de Farmacología y Terapéutica. 2006;25: 11.

19. Díaz E, Köhidai L, Ríos A, Vanegas O, Ponte-Sucre A. Ensayos de quimiotaxis in vitro en *Leishmania sp*. Evaluación de la técnica de los capilares-dos cámaras en promastigotes. Revista de la Facultad de Farmacia-UCV. 2011;74: 31.

20. Díaz E, Köhidai L, Ríos A, Vanegas O, Silva A, Szabó R, Mező G, Hudecz H, Ponte-Sucre A. *Leishmania braziliensis*: Cytotoxic and chemotactic effects of branched chain polypeptide conjugates with poly [L-Lysine] backbone. Experimental Parasitology. 2013;135(1):134.

21. Díaz E, Zacarías AK, Pérez S, Vanegas O, Köhidai L, Padrón-Nieves M, Ponte-Sucre A. Effect of aliphatic, monocarboxylic, dicarboxylic, heterocyclic and sulphur-containing amino acids on *Leishmania spp*. Chemotaxis. Parasitology. 2015;142(13):1621.

22. Febres A, Vanegas O, Gianmmaressi M, Gomes C, Díaz E, Ponte Sucre A. Is the activity of CGRP and Adrenomedullin regulated by RAMP (-2) and (-3) in Trypanosomatidae? An in-silico approach. Infect Genet Evol. 2018;61:197-206. doi: 10.1016/j.meegid.2018.04.003.

23. Ponte-Sucre A. An overview of *Trypanosoma brucei* infections: an intense host parasite interaction. Frontiers in Microbiology, Infectious Diseases, 7: article 2026. doi: 10.3389/fmicb.2016.02126.

24. García N, Figarella K, Mendoza-León A, Ponte-Sucre A. Changes in the infectivity, pyruvate kinase and acid phosphatase activity and p-glycoprotein expression in glibenclamide resistant *Leishmania mexicana*. Parasitology Research. 2000;86: 899.

25. Silva N, Ponte-Sucre A. ABC proteins in *Leishmania mexicana*: Modulation of parasite host cell interaction. Archivos Venezolanos de Farmacología y Terapéutica. 2001;20: 134.

26. Figarella K, Uzcátegui N, García N, Silva N, Camacho N, Ponte-Sucre A. Molecular pharmacology of chemo-resistant *Leishmania*. Archivos Venezolanos de Farmacología y Terapéutica. 2003;22:19.

27. Ponte-Sucre A. Physiological consequences of drug resistance in *Leishmania* and their relevance for chemotherapy. Kinetoplastid Biology and Disease. 2003;2:14 (http://www.kinetoplastids.com/home/).

28. Silva N, Camacho N, Figarella K, Ponte-Sucre A. Cell differentiation and infectivity of *Leishmania mexicana* are inhibited in an ABC-transporter blocker resistant strain. Parasitology. 2004;128: 629.

29. Uzcátegui NL, Figarella K, Camacho N, Ponte-Sucre A. Substrate preferences and glucose uptake in glibenclamide- resistant *Leishmania* parasites. Comparative Biochemistry and Physiology. 2005;140:395.

30. Machuca C, Rodríguez A, Herrera M, Silva S, Ponte-Sucre A. *Leishmania amazonensis*: metabolic adaptations induced by resistance to an ABC transporter blocker. Experimental Parasitology. 2006;114:1

31 Ramírez-Flamerich R, Padrón-Nieves M, Ponte-Sucre A. Detección de aislados quimio-resistentes en *Leishmania*: Enfoque a partir de un modelo macrófago humano-amastigotes. Rev. Dig. Post. Fac Med. 2018;7: 9.

32. Natera S, Machuca C, Padrón-Nieves M, Romero A, Diaz E, Ponte-Sucre A. *Leishmania sp.*: Proficiency of drug resistant parasites. International Journal of Antimicrobial Agents. 2007;29: 637.

33. Padrón-Nieves M, Díaz E, Romero A, Machuca C, Ponte-Sucre A. Valor pronóstico de los cambios fisiológicos asociados a la quimio-resistencia en *Leishmania*. Vitae, Academia Biomédica Digital. 2008;33: 7 (http://vitae.ucv.ve/)

34. Padrón-Nieves M, Ponte-Sucre A. Marcadores de resistencia en *Leishmania*: Susceptibilidad in vitro a drogas leishmanicidas vs. retención de calceina en aislados de pacientes venezolanos con Leishmaniasis Cutánea Difusa. Archivos Venezolanos de Farmacología y Terapéutica. 2013;32: 29.

35. Vanaerschot M, Dumet F, Roy S, Ponte-Sucre A, Arevalo J, Dujardin JC. Treatment failure in leishmaniasis: drug-resistance or another (epi-) phenotype? Expert Reviews Anti Infective Therapy. 2014;12:937.

36. Padrón-Nieves M, Díaz E, Machuca C, Rodríguez N, Cotrim P, Ponte-Sucre A. Correlation between glucose uptake and membrane potential in *Leishmania* parasites isolated from DCL-patients with therapeutic failure: A proof of concept. Parasitology Research. 2014;113:2121.

37. Padrón-Nieves, M., Alcázar, W., Ponte-Sucre, A. Adaptabilidad y Fracaso Terapéutico en *Leishmania*. Dermatología Venezolana. 2016;54: 9.

38. Dujardin JC. Structure, dynamics and function of *Leishmania* genome: resolving the puzzle of infection, genetics and evolution? Infect Genet Evol. 2009; 9:290.

39. Mukhopadhyay Ra, Mukherjee S, Mukherjee B, Naskar K, Mondal D, Decuypere S, Ostyn B, Prajapati VK, Sundar S, Dujardin JC, Roy S. Characterization of antimony-resistant *Leishmania donovani* isolates: Biochemical and biophysical studies and interaction with host cells. Int J Parasitol. 2011; 41:1311.

40. Scheltema RA, Decuypere S, T'kindt R, Dujardin JC, Coombs GH, Breitling R. The potential of metabolomics for *Leishmania* research in the post-genomics era. Parasitology. 2010; 137:1291.

41. Berg M, García-Hernández R, Cuypers B, Vanaerschot M, Manzano JI, Poveda JA, Ferragut JA, Castanys S, Dujardin JC, Gamarro F. Experimental resistance to drug combinations in *Leishmania donovani*: metabolic and phenotypic adaptations. Antimicrob Agents Chemother. 2015; 59:2242-55. doi: 10.1128/AAC.04231-14.

42. Ait-Oudhia K, Gazanion E, Oury B, Vergnes B, Sereno D. The fitness of antimony-resistant *Leishmania* parasites: lessons from the field. Trends Parasitol. 2011; 27:141.

43. Hendrickx S, Leemans A, Mondelaers A, Rijal S, Khanal B, Dujardin JC, Delputte P, Cos P, Maes L. Comparative Fitness of a Parent *Leishmania donovani* Clinical Isolate and Its Experimentally Derived Paromomycin-Resistant Strain. PLoS One. 2015; 10:e0140139. doi: 10.1371/journal.pone.0140139. eCollection.

44. Buckheit RW Jr. Understanding HIV resistance, fitness, replication capacity and compensation: targeting viral fitness as a therapeutic strategy. Expert Opin Investig Drugs. 2004; 13:933.

45. Croft SL, Olliaro P. Leishmaniasis chemotherapy- challenges and opportunities. Clin Microbiol Infect. 2011; 17:1478.

46. Ponte-Sucre A, Gamarro F, Dujardin JC, Barrett MP, López-Vélez R, García-Hernández R, Pountain AW, Mwenechanya R, Papadopoulou B. Drug resistance and treatment failure in leishmaniasis: A XXI century challenge. PLoS Negl Trop Dis. 2017;11(12):e0006052. doi: 10.1371/journal.pntd.0006052. eCollection.

47. Vicik R, Hoerr V, Glaser M, Schultheis M, Hansell E, McKerrow J, Holzgrabe U, Caffrey C, Ponte-Sucre A, Moll H, Stich A, Schirmeister T. Aziridine dicarboxylate inhibitor targeting the major cysteine protease of *Trypanosoma brucei* as lead trypanocidal agents. Bioorganic Medicinal Chemistry Letters. 2006;16:2753.

48. Ponte-Sucre A, Vicik R, Schultheis M, Schirmeister T Moll H. Aziridine-2,3-dicarboxylates: Peptidomimetic cysteine protease inhibitors with antileishmanial activity. Antimicrobial Agents and Chemotherapy. 2006; 50:2439.

49. Bringmann G, Kajahn I, Pedersen SEH, Reichert M, Faber JH, Gulder T, Brun R, Christensen SB, Ponte-Sucre A, Moll H, Heubl G, Mudogo V. Ancistrocladinium A and B, the first N, C-coupled naphthyl-dihydro-isoquinoline alkaloids, from Congolese Ancistrocladus species. Journal of Organic Chemistry. 2006;71:9348.

50. Ponte-Sucre A, Faber JH, Gulder T, Kajahn I, Pedersen SEH, Schultheis M, Bringmann G, Moll H. Activity of naphthylisoquinoline alkaloids and synthetic analogs against *Leishmania major*. Antimicrobial Agents and Chemotherapy. 2007; 51:188.

51. Muth M, Hoerr V, Glaser M, Ponte-Sucre A, Moll H, Stich A, Holzgrabe U. Antitrypanosomal Activity of Quaternary Naphthalimide Derivatives. Bioorganic Medicinal Chemistry Letters. 2007; 17:1590.

52. Caffrey CR, Weismann J, Swinerton RK, Kelly B, Fafarman AT, Rodgers M, Deady LW, Zorn JA, Walshe D, Debnath A, Land K, Beauchene J, Schreiber K, Cohen FE, McKerrow JH, Zhou YM, Doyle P, Moll H, Ponte-Sucre A, Schirmeister T, Saravanamuthu A, Fairlamb AH, Steverding D, May BCH. Bis-acridines as lead anti-parasitic agents: structure activity analysis of a discrete compound library in vitro. Antimicrobial Agents and Chemotherapy. 2007;51: 2164.

53. Goebel T, Ulmer D, Projahn H, Kloeckner J, Heller E, Glaser M, Ponte-Sucre A, Specht S, Sarite SR, Hoerauf A, Kaiser A, Hauber I, Hauber, J Holzgrabe U. In search of novel agents for therapy of tropical diseases and human immunodeficiency virus. Journal of Medicinal Chemistry. 2008;51: 238.

54. Ponte-Sucre A, Gulder T, Wegehaupt A, Albert C, Rikanović C, Schaeflein L, Frank A, Schultheis M, Unger M, Holzgrabe U, Bringmann G, Moll H. Structure-Activity Relationship and Studies on the Molecular Mechanism of Leishmanicidal N, C-Coupled Arylisoquinolinium Salts. Journal of Medicinal Chemistry. 2009;52: 626.

55. Ponte-Sucre A, Gulder T, Gulder T, Vollmers T, Bringmann G, Moll H. Alterations on the structure of *Leishmania major* induced by N arylisoquinolines correlate with compound accumulation and disposition. Journal of Medical Microbiology. 2009;59: 69.

56. Stich A, Ponte-Sucre A, Holzgrabe U. Do we need new drugs against Human African trypanosomiasis? Lancet Infectious Diseases. 2013;13: 733.

57.Berninger M, Schmidt I, Ponte-Sucre A, Holzgrabe U. Novel lead compounds in pre-clinical development against African Sleeping Sickness. Med Chem Comm. 2017;**8:**1872 – 1890. DOI: 10.1039/C7MD00280G.

58. Ponte-Sucre A, Bruhn H, Schirmeister T, Cecil A, Albert CA, Buechold C, Tischer M, Schlesinger S, Goebel T, Fuß A, Mathein D, Merget B, Sotriffer CA, Stich A, Krohne G, Engstler M, Bringmann G, Holzgrabe U. Anti- trypanosomal activities and structural chemical properties of selected compound classes. Parasitology Research. 2015;114:501.

59. Cecil A, Ohlsen K, Menzel T, Francois P, Schrenzel J, Fischer A, Dörries K, Selle M, Lalk M, Hantzschmann J, Dittirch M, Liang C, Bernhardt J, Oelschlaeger T, Bringmann G, Bruhn H, Unger M, Ponte-Sucre A, Lehmann L, Dandekar T. Modelling antibiotic and cytotoxic isoquinoline effects in Staphylococci and human cells. International Journal of Medical Microbiology. 2015;305:96.

60. Ponte-Sucre A. Availability and applications of ATP-binding cassette (ABC) transporter blockers. Applied Microbiology and Biotechnology. 2007;76:279.

61. Ponte-Sucre A, Díaz E, Padrón-Nieves M. Quantitative Structure-Activity Analysis of Leishmanicidal Compounds. In Chemoinformatics: Directions Toward Combating Neglected Diseases, 2012, pp. 37-57. Teodorico C. Ramalho, editor. E-book, Bentham Sciences, ISBN 978-1-60805-183-0. http://benthamscience.com/ebooks/9781608051830/index.htm.

62. Alcázar W, Silva López A, Alakurtti S, Tuononen ML, Yli-Kauhaluom J, Ponte-Sucre A. Betulin derivatives impair *Leishmania braziliensis* viability and host-parasite interaction by. Bioorganic and Medicinal Chemistry. 2014;22:6220.

63. Ponte-Sucre A. Propiedades químicas estructurales de compuestos que actúan contra el *Trypanosoma brucei*. Vitae. 2015;62: http://vitae.ucv.ve/?module=articulo&rv=118&n=512.

64. Llanes-Coronel DS, Gámez-Díaz LY, Suarez-Quintero LP, Páez LJ, Torres F, Echeverri F, Ponte-Sucre A, Patiño PJ, Trujillo-Vargas CM. New promising Euphorbiaceae extracts with activity in human lymphocytes from primary cell cultures. Immunopharmacology and Immunotoxicology. 2011;33:279.