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14. ABSTRACT

In vitro IC50's were determined for seventeen newly synthesized compounds. Most compounds showed high therapeutic indexes by displaying strong *in vitro* efficacy against *L. major* and *L. donovani* parasites but low *in vitro* toxicity when tested in a macrophage cell line. During the fourth (and last year) of this grant no compounds were tested *in the in vivo* models of cutaneous leishmaniasis (CL.

During the course of this award we have worked in parallel to validate and establish a new sking PK model in BALB/c mice.The PK skin validation ended this year. This is a major accomplishment that allows us to use the major pharmacokinetic parameters obtained from both plasma and skin PK studies to shed light on the mechanism of action of Arylimidamide and Azole compounds and help determine if accumulation of the compound in the skin is needed for compound efficacy. This new approach might help explain why several Arylimidamide compounds that display high in vitro efficacy against several *Leishmania spp*. and the *in vivo* Visceral leishmania VL models do not have the same efficacy in the Cutaneous Leishmaniasis (CL) models. Based on this data, the synthetic and medicinal chemistry can draw conclusions, better understand the SAR, and lead the way for developing and synthesizing more efficacious compounds that can reach and eliminate the *Leishmania spp*.parasites in the skin.

15. SUBJECT TERMS
None listed

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1. INTRODUCTION:

Existing oral treatments of visceral and cutaneous leishmaniasis (VL and CL) have significant drawbacks to include serious side effects, variable efficacy, and expense. Intravenous treatment with liposomal amphotericin B (AmBisome) is expensive, lengthy, and impractical for deployed soldiers (treatment requires 21 days of intermittent IV therapy in a hospital setting). An inexpensive oral treatment for both VL and CL that provides consistent efficacy against all species of *Leishmania* that infect man is a clear unmet need. This proposal is focused on a group of arylimidamide compounds which showed initial potency against visceral leishmaniasis *in vitro* and efficacy against visceral leishmaniasis *in vivo*. These compounds also showed interesting synergy with azoles which enhanced the efficacy of the arylimidamide compounds. The element of work performed at WRAIR encompasses the testing of these arylimidamide analogues against species of *Leishmania* that cause CL.

2. KEYWORDS:

leishmaniasis, cutaneous, visceral, arylimidamide

3. OVERALL PROJECT SUMMARY:

1) Evaluation of the antileishmanial efficacy and pharmacokinetics of Arilymidamide-Azole combinations
2) Characterization of the biochemical effects of Arilymidamide-Azole combinations on Leishmania.

4. KEY RESEARCH ACCOMPLISHMENTS:

Key Research Accomplishments
In vitro potency of 17 A1A compounds as sole agents was demonstrated in an amastigote macrophage assay showing very promising IC_{50}s *L. major and L. donovani*, as well as low CC50 values against a macrophage cell line. Validation of a skin PKmodel in BALB/c mice is also a major accomplishment that allows us to use the major pharmacokinetic parameters obtained from both plasma and skin PK studies to shed light on the mechanism of action of Arylimidamide and Azole compounds and help determine if accumulation of the compound in the skin is needed for compound efficacy.
Accomplishments in Support of the Statement of Work
1a. Assess of *in vitro* efficacy of newly synthetized A1A compounds against intracellular *Leishmania.*
In vitro potency testing[2,4] (see Appendix Table 1 for detailed data) of A1A compounds demonstrated strong potency of these compounds as sole agents against *L. major* and *L. donovani*.

2a. *In Vivo* Compound Evaluations; There was no *in vivo* testing of Arylimidamide and Azole compounds in the *in vivo* models of CL during the fourth and the last year of this grant.

Other: Preliminary experimental results deriving from the newly validated PK skin in BALB/c mice (mouse species that is used in the efficacy models), show that Posaconazole, the newest triazole antifungal agent and the main Azole compound that is used in the Arylimidamide-Azole combinations, accumulates in BALB/c mice's skin during a 21 day treating period. Literature suggests that when multiple, similar doses of Posaconazole are given to humans, this compound accumulates over time in skin and nails.These exiting results show that Posaconazole was rightly chosen as partnering compound for the Arylimidamide-Azole combinations against CL and that the models of murine malaria can be used to mimick to a certain extend of what happens in humans that get the human equivalent dose of the same compound.

5. IMPACT:

The assessment of A1A compounds showed *in vitro* but were not tested for *in vivo* efficacy against CL (scale up synthesis did not happen in time). Based on our *in vitro* efficacy dayta (as well as in vitro efficacy data obtained in his laboratory), Dr. Werbovetz (who is our main collaborator in this grant), is continuing his efforts to test promising hit compounds in the *in vivo* models against VL.

6. CHANGES/PROBLEMS:

Because of the failure to demonstrate *in vivo* efficacy with Arylimidamide compounds as sole agents against the Old World *L. major* parasite in the MLS screen we did not conduct Arylimidamide-Azole Combinations efficacy testing during the past year. While the WRAIR team has run out of funds, Dr. Werbovetz (who is our main collaborator in this grant), has applied for a one year extension on the grant and is continuing his efforts to test compounds with high *in vitro* toxicities and low *in vitro* cytotoxicity (described above) in the *in vivo* models against VL.

7. PRODUCTS:

No product developed yet.

8. PARTICIPANTS:

Collaborations under this grant include investigators at Ohio State University, WRAIR, Georgia State University, the University of South Florida, and the University of Kansas.

9. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

1- Caridha D, Parriot S, Hudson TH, Lang T, Ngundam F, Leed S, Sena J, Harris M, O'Neil M, Sciotti R, Read L, Lecoeur H, Hickman M, Grogl M. 2017. Use of Optical Imaging Technology in the Validation of a New, Rapid, Cost-Effective Drug Screen as Part of a Tiered *In Vivo* Screening Paradigm for Development of Drugs To Treat Cutaneous Leishmaniasis. Antimicrobial Agents and Chemotherapy 61.

2- Validation of a New BALB/c mouse/L. guyanensis Footpad Model as Part of a Tiered *In Vivo* Screening Paradigm for Development of Drugs to Treat Cutaneous Leishmaniasis. Oral Presentation in the World Leishmania Conference, 2017. D. Caridha, M. Khraiwesh, B. Vesely, F. Ngundam, K. Butler, S. Leed, E. Penn, N. Roncal, K. Lawrence, R. Sciotti, L. Read, M. Grogl, C. Bane, C. Black, M. Kreishman-Detrick, M. Hickman.

10. INVENTIONS, PATENTS AND LICENSES

Nothing to report.

11. REFERENCES:

1. Grogl, M., Hickman, M. Ellis, W. Hudson, T. Lazo, J., Sharlow, E., Johnson, J., Berman, J., and Sciotti, R. Review: Drug Discovery Algorithm for Cutaneous Leishmaniasis. Am J Trop Hyg 88(2), pp. 216-221, 2013.

2. Khraiwesh, Mozna, Leed, Susan, Roncal, Norma, Johnson, Jacob, Sciotti, Richard, Smith, Philip, Read, Lisa, Paris, Robert, Hickman, Mark and Grogl, Max. Antileishmanial Activity of Compounds Derived from the Medicines for Malaria Venture Open Access Box Against Intracellular Leishmania major Amastigotes. American Journal of Tropical Medicine and Hygiene, published online 26 October 2015.
3. Canfield, C.J., Pudney, M., and Gutteridge, W.E. Interactions of Atovaquone and other antimalarial drugs against P. falciparum in vitro. Experimental Parasitology 80, 373-381, 1995.

4. Sharlow E, Leimgruber S, Murray S, Lira A, Sciotti R, Hickman M, Hudson T, Leed S, Caridha D, Barrios A, Close D, Grogl, M Lazo J. Auranofin Is an Apoptosis-Simulating Agent with in Vitro and in Vivo Anti-leishmanial Activity. ACS Chemical Biology, Dec 2013.

5. Caridha D, Parriot S, Hudson TH, Lang T, Ngundam F, Leed S, Sena J, Harris M, O'Neil M, Sciotti R, Read L, Lecoeur H, Hickman M, Grogl M. 2017. Use of Optical Imaging Technology in the Validation of a New, Rapid, Cost-Effective Drug Screen as Part of a Tiered In Vivo Screening Paradigm for Development of Drugs To Treat Cutaneous Leishmaniasis. Antimicrobial agents and chemotherapy 61.

12. APPENDICES:

In vitro potency of AA (IC_{50} values) compounds against amastigote-macrophage forms of *L. donovoni* and *L. major*, as well as in vitro toxicity data (CC_{50}) values against a $HEPG_2$ mactophage cell line are shown in Table 1.

In vitro IC50's were determined for seventeen newly synthesized compounds. Several compounds displayed strong *in vitro* efficacy and a low toxicity profile. Results are shown in the table below. Waiting for the partnering PI to scale up the lead compound synthesis for *in vivo* screens.

Compound	IC_{50} against *L. major* (μM)[a]	IC_{50} against *L. donovani* (μM)[a]	CC_{50} against HepG2 (μM)[a]
AA3-43	6.9	2.4 ± 0.7	13 ± 0
AA3-64	0.27	0.68 ± 0.27	Recheck
AA4-20	0.019	0.82 ± 0.25	7.6 ± 0.4
AA4-16	0.010	0.29 ± 0.04	3.1 ± 0.2
AA3-59	>20	2.2 ± 0.6	9.9 ± 0.4
AA3-70	1.9	1.2 ± 0.6	7.0 ± 0.3
AA3-56	8.9	1.2 ± 0.4	4.7 ± 0.7
AA3-69	0.10	0.15 ± 0.04	3.4 ± 0.4
AA3-94	0.098	0.46 ± 0.06	12 ± 3
AA4-59	19	0.78 ± 0.16	12 ± 3
AA3-95	0.14	0.19 ± 0.03	Recheck, about 8
AA4-60	0.81	0.77 ± 0.13	3.4 ± 0.6
AA4-39	>20	3.0 ± 1.2	7.3 ± 1.1
AA4-40	5.9	1.2 ± 0.4	4.1 ± 0.7
AA4-71	5.0	0.69 ± 0.28	3.3 ± 0.2
AA4-77	1.0	0.93 ± 0.29	4.5 ± 1.1
AA4-78	14	2.1 ± 0.4	5.1 ± 0.7

Table 1: In vitro efficacies of A1A compounds against *L. major* and *L. donovani*