

Toward an assessment of Targeted Osmotic Lysis as a potential novel therapy against Leishmaniasis

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URGENT DEMAND FOR NOVEL LEISHMANIA TREATMENTS

Leishmaniasis is an often-deadly infection caused by the protozoan parasite, *Leishmania*. It is found mainly in tropical and subtropical areas including South America and Africa. Parasite transmission occurs via the bite of a female sandfly, and around 1.2 million people are infected yearly. As eukaryotes, *Leishmania* share many key biological features with human cells. Thus, the development of effective, non-toxic, parasite-selective chemotherapies against *Leishmania* continues to be a challenging medical goal, somewhat analogous to difficulties associated with developing safe, effective anti-cancer treatments. In cancer patients, cancer cells retain many biological features of normal human cells, typically making it extremely difficult to selectively target them for therapy. Recently, studies from the Paul and Gould laboratories at LSUHSC have developed Targeted Osmotic Lysis (TOL). TOL has shown promise as an approach to selectively lyse cancerous cells through the concurrent electrical activation of VGSCs and pharmacological blockage of sodium (Na⁺) ATPase pumps, both of which have been found to be overexpressed in cancer cells. *Leishmania* also has sodium transporters and we recently identified LmjF.34.0480 as a candidate Voltage-Gated Sodium Channel (VGSC) gene in *Leishmania*. We hypothesize that *Leishmania* may possess functional VGSCs and sodium ATPase pumps that are quantitatively and/or qualitatively distinct from human sodium transporters such that we may use TOL therapeutically against leishmaniasis.

MECHANISTIC BASIS FOR TARGETED OSMOTIC LYSIS

The mammalian transmembrane sodium gradient is maintained by both VGSCs and ATPase-dependent sodium pumps. VGSCs open and close to allow an influx of Na⁺ across the cell membrane in response to changes in the membrane potential. A device was developed to generate a pulsed electric field (EF) that excites mammalian cells resulting in the generation of action potentials that open the sodium channels. This allows Na⁺ followed by water to enter the cell, due to osmosis. Cipargamin is a fast-acting and potent pharmacologic inhibitor of a P-type transporter Na⁺ ATPase in *Plasmodium* parasites and mammalian cells. Thus, in cancer cells that overexpress VGSCs and sodium pumps, simultaneously activating the VGSCs and pharmacologically blocking Na⁺ ATPases results in cellular swelling and lysis. We are conducting preliminary experiments to explore whether *Leishmania* have functional VGSCs and whether TOL may be potentially used to treat leishmaniasis.

METHODOLOGY AND GOALS

Leishmania amazonensis WT cells were cultured in M199 media supplemented with 10% FBS at 27°C.

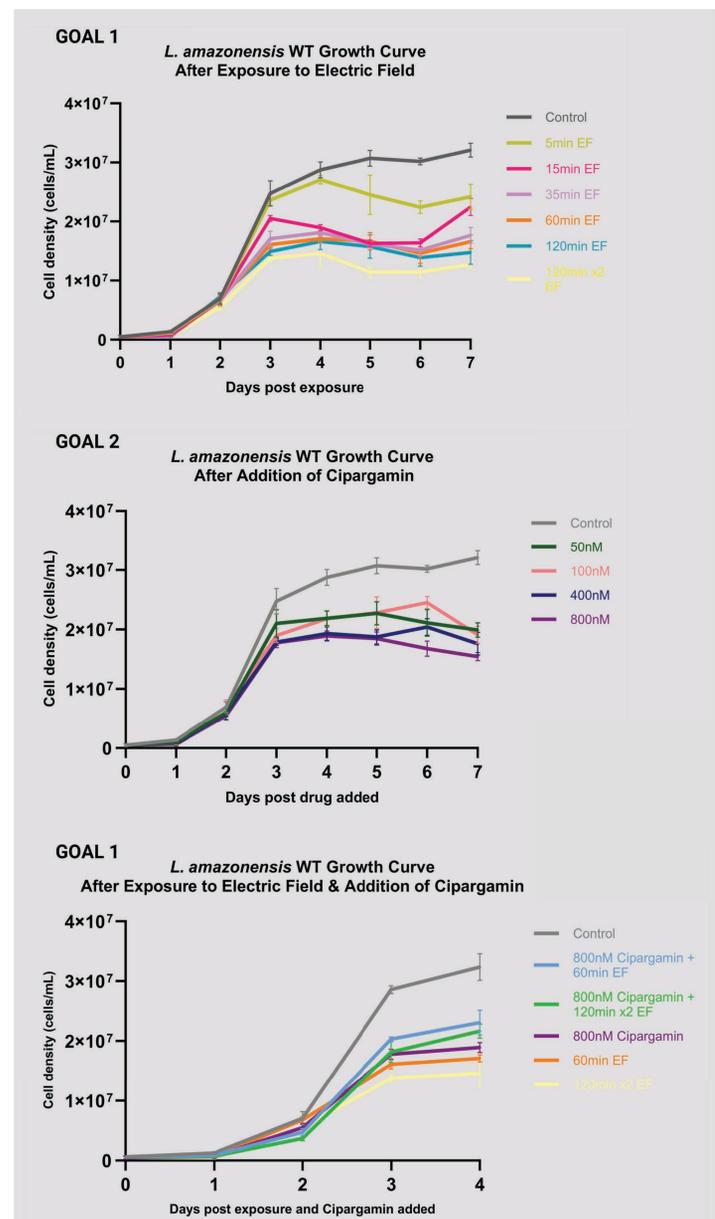
Day 4 parasites were diluted to 5x10⁵ parasites per mL and labeled as Day 0. The culture was then split into 10 experimental groups and 1 control. Daily cell counts over 7 days were obtained using a hemocytometer.

Goals:

- 1) Examine the effect of different doses of electrical pulsed field exposure upon *Leishmania* viability, to establish dose toxicity thresholds.
- 2) Examine the effect of different concentrations of Cipargamin, an inhibitor of the sodium ATPase pump PfATP4 in the malaria parasite, upon *Leishmania* viability, to establish dose toxicity.
- 3) Examine the combined effects of both Cipargamin and electric field exposure.



RESULTS: EXPOSURE OF *L. AMAZONENSIS* TO A PULSED ELECTRIC FIELD AND CIPARGAMIN



Electric Field: 5min, 15min, 35min, 60min, 2 hrs, 2hrs twice (24h apart)*

*The dose of 2hrs twice (24h apart) was used because this is similar to what has been used clinically to treat a human patient (Gould^{3rd}, Miller et al., 2021).

Parasite growth was impacted by exposure to the electric field. Compared to the control, cell density was lower even in the shortest amount of time (5min). The highest exposure (120minx2) resulted in growth over 2 fold lower by day 7 compared to the control.

Cipargamin: 50nM, 100nM, 400nM, & 800nM

The control had the highest cell density. All the cultures with Cipargamin added had slower growth rates. Increased concentration of Cipargamin resulted in lower growth rates overall.

Combination: 60mins EF+800nM, 2hr twice EF (24h apart) + 800nM, 60min EF, 120 min x2 EF, 800nM

The cultures with Cipargamin and exposure to the EF alone were started at a different cell density resulting in the combination experiments to have slightly higher initial cell density. The cultures with the most exposure time to the EF either with or without Cipargamin grew slower than the ones with 60min exposure.

DISCUSSION

Exposure of *L. amazonensis* to both the pulsed EF and Cipargamin treatments resulted in moderately impaired growth, in a dose-dependent manner. Interestingly, the clinically validated pulsed EF treatment showed the greatest inhibition of parasite growth. Paradoxically, combined treatments (EF+Cipargamin) appeared to marginally enhance growth, relative to single treatments, up to Day 4. Additional experiments will be required to confirm our preliminary findings and determine whether EF-based treatments are a promising new mode of therapy against parasitic diseases.

Unfortunately, we had less time to do more studies with TOL because of contamination issues. Contamination can happen from improper techniques, reagents or water used. I've learned about time constraints contamination can cause and how it affects projects. Lastly, I've learned more proper ways to handle and carefully work with samples. I learned how to operate GraphPad Prism in order to graph my data sets, and Biorender to put my poster together.

REFERENCES

- Gould^{3rd}, H.J., Paul D. (2022). Biomedicine 10040838
Gould^{3rd}, Miller et al., (2021). Current Oncology 28(3) 2115-2122