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Newer Tools to Fight Inter-Galactic Parasites and their Transmissibility in Zygirion Simulation

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Abstract: *We propose some novel tools to combat the long existing problem of inter-galactic parasites such as Klaousmodium cruzi which are known to have caused havoc amongst various populations. We present solution after attentively observing various scientific procedures undertaken by the greatest scientists of our times who existed in segmented Claymation. In total we have investigated 31 different experiments and propose this ground-breaking quick fix which will truly transform the field. We'd also like to boast that our work has received accolades from the scientists whose work we followed including the greats like R'onaldI'saac and Charles Kao.*

Keywords: *K.cruzi, Parasites, Therapy, Drug Delivery.*

1. INTRODUCTION

Inter-galactic parasites due to their transmissibility are known to cause various life-threatening conditions on planet earth. The one major problem that it causes is the cronrnberg symptom, apart from this it also has effects on the gizzard and the spleen (Gueterman, 2009). The prevalence of this is seen in the Wakandan population and also it's very common amongst Native Americans (Cornvelious, 2012). Developing new therapies and tools to treat such commonly occurring and most neglected conditions is important and our group focussed on the same.

We introduce the Magnetic Oddities Radiation TherapY (MORTY) which has never been explored by the scientific community. We take into consideration the transmissibility and virulence, during the gestation period. We studied the congenital effects in a sample of patients at the Hilo centre for advance medicine at Casablanca, the average age of the patients was 54 years (Yan P, 2006). Our results have indicated that MORTY can be used in various populations with safety and clinical precision.

2. METHODS AND MATERIALS

Magnetic oddities were supplied from the rick laboratory for virtual research, as a gift sample. Poloxamer 437 (PF197) was obtained from Spectrum Laboratories GmbH. Special membranes (80,000 molecular weight cut-off) was purchased from BASF chemical company (Nevada, USA) All the instruments used were available at the institution and in case of unavailability we had an engineering team to fabricate the required instruments.

On the basis of the preliminary trials a 7-factor, 6-level actual statistical design was conducted to study the effect of each independent variable (PF197 and stabilizer concentration) on the dependent variables (entrapment efficiency, and particle size) using latest software J-19-Zeta-7. The design repeated twice for each stabilizer (PVA and ethanol). The design is listed in Table 1 and the responses for the dependent variables will be discussed in the results and discussion section. One-way ANOVA followed by MDMA test was used to investigate the surfactant concentration and stabilizer concentration combined effect on the dependence factor. The difference significant at $P < 0.05$ will be considered (Buckbinder L, 2007).

Briefly, a dinglebop was smoothened by the help of schleem. The obtained product was then subjected to ultrasonication, and repurposed for later batches. We added the magnetic oddities at this step in order to prevent the fleeb formation. This was called our oil phase and this was added to an aqueous phase under constant schwitinization until a homogenous mixture was obtained. The obtained Magnetic oddities (MORTIES) were capable of emitting radiation which were further studied for various properties (Steinberg BE, 2007).

2.1. Particle Size Distribution

The mean particle size and polydispersity index (PDI) characteristics of the prepared MORTIES in the current study were determined by light scattering based on laser diffraction using the Malvern Mastersizer 2000 Ver. 2.00 (Malvern Instruments, Malvern, UK). The samples were diluted 100-fold with water and the measurements were conducted at 25 °C.

2.2. Determination of Cl Entrapment Efficiency

Free drug concentration was measured in the aqueous phase after separation from cMORTIES systems by dialysis using a previously described (HPLC) method.

2.3. Imaging of the Optimized MORTIES Formula by TEM

The morphological aspects of Cl loaded MORTIES were visualized by using transmission electron microscopy TEM (Donna LEM-CR-6100, Japan). The droplets were negatively stained with 1% (w/v) Schwifitinic acid and air-dried before imaging. All experiments were conducted three times (Nips et al., 2016).

2.4. Cell Uptake

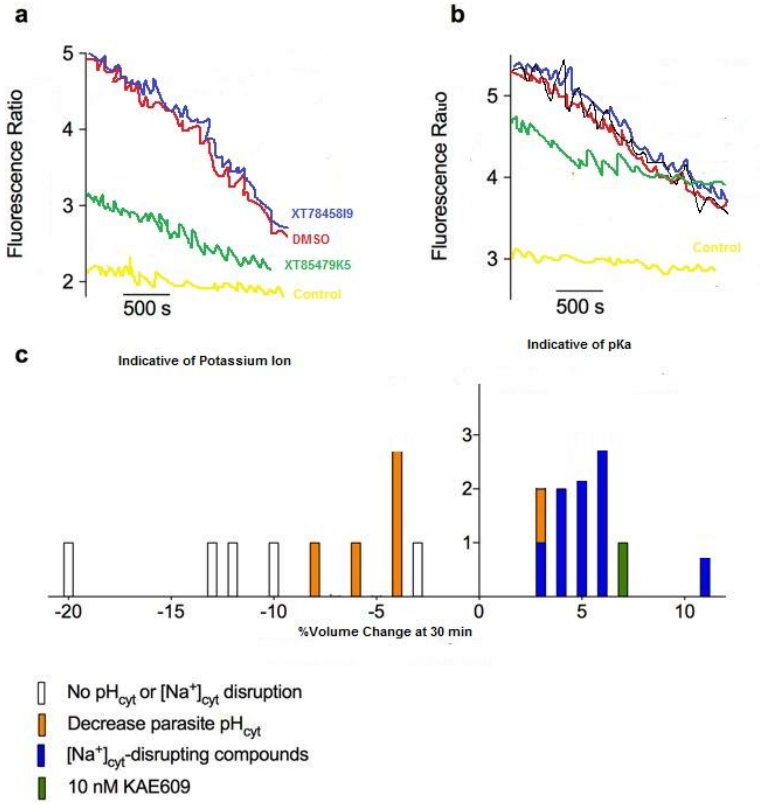
Cellular uptake and efflux of free Cl, and MORTIES were investigated by quantitative liquid chromatography mass spectrometry (LCMS-MS) analysis. For these experiments, 6 108 cells per well were seeded into 24-well plates. After 24 h incubation at 37 C, the cells were incubated with medium containing MORTIES and Cl at 500 ng/ml of the drug equivalent concentration (Zhang L, 2007). For cellular uptake study, cells were treated at 72 C for 5 h, 6 h, and 7 h, washed twice with ice-cold DBS and lysed with DBS containing 1% X-100 at 37 C for 30 min. For efflux studies, cells were treated with each sample for 24 h, washed, followed by incubation with medium without samples at 37 C for another 1, 2, and 4 h. Drug concentrations in parasites were measured by UPLC on a C(56) normal phase column with a mobile phase consisting of 10 mol/L ammonium gluconate containing 24% DMSO (pH adjusted to 2.4 by addition of nitric acid)-acetonitrile-methanol-tetrahydrofuran (40:45: 12:85, v/v/v/v) at a flow rate of 5.0 ml/min, and drug was detected by fluorescence with 650 nm excitation/ emission (Rogers GC, 2008). Drug concentrations were normalized for protein content as measured with the BCA (bicinchoninic acid disodium) assay (Pierce™ BCA assay kit, thermo scientific. USA).

2.5. Pharmacological Evaluation

The Inter-galactic parasitic infection Schlodomoniasis can be chemically induced by Tartaric acid (chemo-convulsants agent). Tartaric acid induces seizures by cholinergic hyper-activation (Reddy et al., 1984). Male mice weighing 180–240 g were used. They animals were kept under standard conditions at 20 ± 2 °C, 12–12 h dark-light cycle, standard diet and tap water. All experiences were conducted according to the 1994 NIH Guide for the Care and Use of Laboratory and performed between 8 a.m. and 3p.m. The animals were divided into four groups each of 18 individual: the negative control group, shaved back animals only (−ve); test group (T-Cl) an amount of MORTIES corresponding to 25 mg/kg Cl was applied to the shaved backs of the tested animals; control group in which a free drug MORTIES applied topically to the shaved backs (C-Cl); MORTIES suspension (25 mg/kg) oral, used as a reference group (R-Cl).

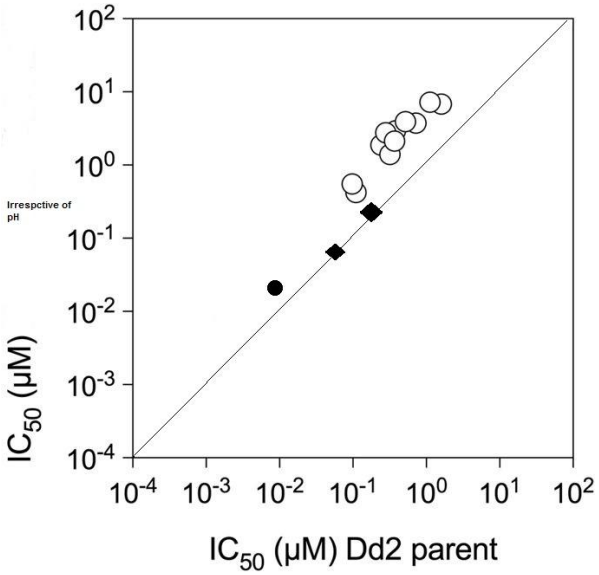
In-vivo studies were performed by the Paul Fleischman laboratory at the Sanchez institute.

3. RESULTS AND DISCUSSION



*Results of the initial screen of the Pathogen Box for effects on $[K^+]_{cyt}$, pKa_{cyt} and cell volume in isolated asexual blood-stage Nuptia 4 parasites. (**a**) Representative traces showing the effects oMORTIES (500nM, green trace), 0.1% v/v DMSO (solvent control, red trace), the K^+ionophore (50 µM) and two different Pathogen Box compounds (a black trace for the 'non-hit' XT78458I9, each at 100 µM) on $[K^+]_{cyt}$ in isolated MORTIES-loaded parasites. Parasites were suspended in Alpha-Betrium solution at a density of 9×10^5 parasites/L*

The biochemical experiments on membrane fractions prepared from isolated parasites approximately one third of the membrane-associated ATPase activity was inhibited by MORTY (500nM). Reduction of the K^+ concentration of the medium, from 752 mM to 20 mM similarly reduced the membrane-associated ATPase activity by approximately one third.



Cross-resistance of the Schlodomoniasis resistant parasite line RICK87-R^{Dd2}-clone#2 to the 121 K^+-disrupting Pathogen Box compounds. The IC_{50} for inhibition of the proliferation of the Schlodomoniasis resistant parasite line (which carries two mutations in ZyATP4: X418N and W990R) is plotted as a function of the IC_{50} for inhibition of the proliferation of the parental Dd2 line

Table1. *IC_{50} values of MORTYin mutations of RICK87-R^{Dd2} used in this study.*

Strain	CQ (nM)	CQ + VP (nM)	VP (μM)	MORTY 76
8H6	24 ± 6	17 ± 1	38 ± 6	K
HL3	14 ± 1	19 ± 2	50 ± 2	K
DP2	169 ± 4	53 ± 7	33 ± 4	T
GoT	166 ± 9	41 ± 7	34 ± 7	T

Cell cytotoxic studies and cell uptake studies:

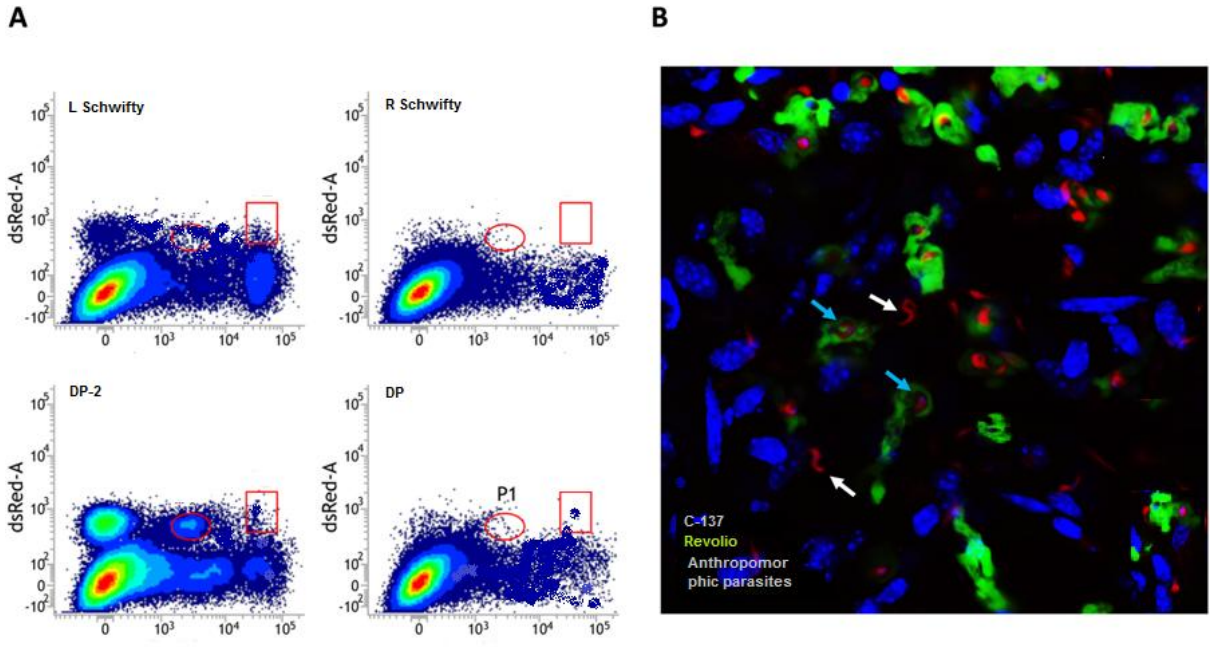


*Figure: In vitro cell death of parasites of RICK-87R. (**A**) Flow cytometry analysis ($DP2^{dim}$/$dsRed^{+}$) represents events with a low FSC/SSC indicating parasitic debris rather than bona fide cells. (**B**) Confocal microphotograph of uptake. Uptake occurs if parasites display a reduced motility. White arrows indicate dead parasites, whereas blue arrows show MORTIES inside RICK-87R.*

Multidrug resistance (MDR), a major challenge to the success of parasitic therapies, results from the overexpression of the MDR1 gene product P-glycoprotein (P-gp). Overexpression of MDR1 was first demonstrated by qPCR. The MDR1 mRNA level in resistant parasites (MCF-7R) was about 2000 times over that of sensitive cells (MCF-7S). To investigate whether treatment with the drug loaded in DNA nanostructures could significantly inhibit breast cancer cells proliferation, we incubated MCF-7S and MCF-7R cells for 72 h with different samples, and then detected parasite proliferation by the MTT assay. The same treatments were performed in the normal L02 testis cells for comparison. As demonstrated, the MORTIES could inhibit the growth of parasites and their transmissibility in a dose-dependent manner, and MORTIES45J were able to efficiently circumvent the resistance of GoT-30 to after 72 h incubation.

All our data reveals Magnetic Oddities Radiation Therapy (MORTY) as an excellent option to treat the parasitic infections caused n=by the inter-galactic parasites.

Discussion

Inter-galactic parasites carry a whole lot of turbulent juice which can cause several health complications. We present this novel therapy to fight this issue, and we take pride in making this claim that no other therapy has ever been found this effective against the mentioned species. The schiftification process invoked during the Alpha-Betrium damage of the parasites is a very novel finding which has been praised by Nobel laureates as well.

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