Supplemental Materials

Short Article title: Repurposing Quinacrine Against Ebola Virus Infection *In vivo*

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Short running title: Efficacy of Quinacrine against Ebola virus

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Table S1. NIAID *in vitro* virus testing of quinacrine.

	Cell line	**EC_{50} (µM)**	**EC_{90} (µM)**	**CC_{50} (µM)**	**SI_{50}**	**SI_{90}**
Herpes Simplex Virus 1	HFF	>1.2	>1.2	5.30	<4	<4
Vaccinia Virus	HFF	>1.20	>1.20	3.05	<3	<3
Chikungunya virus	Vero 76	3.2		10	3.1	
Dengue Virus 2	Vero 76	3.2		3.2	1	
Ebola virus	Vero 76	>12.3		>12.3	0	
Influenza A virus H1N1	MDCK	23		28	1.2	
MERS coronavirus	Vero 76	>4.2		4.2	0	
Poliovirus 3	Vero 76	32		32	1	
Respiratory synactial virus	MA-104	32		32	1	
Rift Valley fever virus	Vero 76	32		32	1	
Tacaribe virus	Vero	>28		28	0	
Venezuelian equine encephalitis virus	Vero 76	4		15	3.8	

West Nile virus	Vero 76	>7.5		7.5	0	
Yellow Fever virus	Vero 76	>2.8		2.8	0	
Zika virus	Vero 76	3.2		3.2	1	
Norovirus	HG23	>100	>100	>100	1	1
Human cytomegalovirus	HFF	>1.20	>1.20	5.26	<4	<4
Hepatitis C virus	Huh7	2.22	13.05	3.04	1	<1
Hepatitis B virus	HepG2 2.2.15	3.46	9.43	3.42	<1	<1

Text S1

Quinacrine (branded as mepacrine, atebrin, chinacrin, erion, acriquine, acrichine, palacrin, metoquin and halchin) was developed by IG Farbenindustrie in Germany during the 1920s and it has been used since the 1930's to combat malaria and was declared the official US treatment in 1943 by Thomas Parran Jr., the Surgeon General at the time (1). During WWII three million soldiers were administered the drug (100 mg orally) for up to four years as a prophylactic for malaria (2, 3). Quinacrine was then used worldwide as a treatment for lupus as early as the 1940's (1). It has additionally been shown to be an effective antiprotozoal against Giardia (4). Adverse reactions to quinacrine were found mostly to be minor, reversible and dose-dependent and include transient symptoms of mild headache, dizziness, or gastrointestinal symptoms (1). While rare, some serious adverse reactions have been associated with quinacrine such as: aplastic anemia, severe dermatitis, exacerbation of psoriasis, and psychosis (5). Quinacrine is however a known DNA intercalator and a mutagen in a mouse lymphoma cell line. *In vivo* this has not shown to be conclusive, where a mouse micronucleus assay was negative (6) and tumor formation results were mixed in mouse studies (7). In addition, human studies with intrauterine-administered quinacrine showed no statistically increased risk of cancer development (8-10). There have been multiple recent publications suggesting that quinacrine has the potential to be a potent antineoplastic drug (11-13) as well as *in vitro* inhibition of the propagation of prions (14-20), the causative agent of Creutzfeldt-Jakob disease. Since 2007, four clinical trials using quinacrine have been completed and there is currently one ongoing trial. Two of these trials were focused on the treatment of prion disease (NCT00104663, NCT00183092) and the others have concentrated on treating colorectal (NCT01844076, NCT00417274) and non-small cell lung cancer (NCT01839955).

Text S2

The Caco-2 data suggest excellent absorption, which agrees with the early published data showing an increase in plasma levels 2-4 hours after oral administration (2). Previously, quinacrine demonstrated low toxicity in mammals, with an LD_{50} of 900 mg/kg in rats (21, 22) after oral administration. While a maximum-tolerated dose study has not been performed in a mouse model, earlier studies had shown that daily oral doses of quinacrine at 75 mg/kg when administered to mice over a 4-week period showed no clinical aberrations (23). In a recent clinical trial to treat prion disease, quinacrine was given orally with a loading dose of 1 g over 24 h (200 mg every 6 h), followed by 100 mg three times daily for up to 2 years and showed no reported hematological toxicity, confirming the low general toxicity seen in humans (24). While absorption of quinacrine via i.p. administration has not previously been examined, intrapleural, intralesion/paralesion, and intrauterine routes have all been shown to have rapid absorption and distribution (25, 26). The pharmacokinetics of quinacrine have previously been described in rabbits via an intrapleural and intravenous route (25). Both routes showed similar kinetics, with a $t_{1/2}$ of approximately 26 hrs and a t_{max} of between 0-20 mins. Historical studies in a dog model have shown high spleen and liver distribution with minimal excretion (27). Mouse studies have also illustrated a similarly high distribution in the liver as well as the spleen (23, 28).

Text S3

NMR studies suggest the antimalarial mechanism involves inhibiting hemozoin formation (29), but this would not explain the mode of action required to inhibit *Giardia* as well (30, 31). A mechanism capable of mitigating the pathogenesis of both protozoa may be based on quinacrine acting as a lysosomotropic agent. *Plasmodium falciparum* has the highly acidic "digestive vacuole" compartment and the antimalarial chloroquine is known to accumulate in these, while chloroquine-resistant *P. falciparum* has reduced accumulation in these vacuoles (32). *Giardia* do have analogous "peripheral vacuoles" (33), suggesting that disruption of acidic compartment may be a possible overlapping mechanism. Quinacrine also has a potent anti-inflammatory effect that may be attributed to suppression of IFN-alpha and TNF-alpha expression, with reduced *in vitro* expression of these proteins in peripheral blood mononuclear cells (PBMC) from dermatomyositis (DM), cutaneous lupus erythematosus (CLE) and control patients (34).

Text S4

As the lysosomotropic amine concentration in the organelle increases the concentration of [H^+] decreases. Ultimately, there are many types of acidic vesicles shown to sequester basic molecules, including endocytic compartments, secretory vesicles, multi-vesicular bodies, etc. (35). Quinacrine has been shown to have substantial lysosomal sequestration with an approximate 50-fold enrichment in the lysosome as compared to the cytosol in MDR HL-60 cells (36). While the effects on endosomal pH have not been investigated with quinacrine, the related antimalarial chloroquine has been extensively studied. Poole and Ohkuma showed a strong linear relationship between pH and log [M] of chloroquine within the lysosomes of mouse peritoneal macrophages, with a 0.5 pH increase at 1µM, 1 pH increase at 10µM, and 1.5 pH increase at 100µM (37). A similar, concentration-dependent pH change was also shown in freshly-isolated mouse hepatocyte lysosomes with chloroquine (38). This is slightly contradicted by findings by MacFarlane *et al.*, which suggested that perinuclear vesicles incubated with the antimalarials chloroquine and hydroxychloroquine at 5 µM for 5-10 mins had no effect on pH, but at higher concentrations (25µM and higher) this agreed with the findings of Poole and Ohkuma (39). It has also been demonstrated that the uptake of weak bases into lysosomes is a progressive process, which may take 20- 60 min or longer to go to completion (37), suggesting that 5-10 mins may not have been sufficient time to reach equilibrium. In contrast, Kuznik *et al*. determined that endosomal pH did not change with 4 µM chloroquine in multiple types of primary cells, but the method used for quantification was very different (quantified by a change in the ratio of a pH-sensitive:pH-insensitive fluorophores; incubation time was not described) (40). These findings suggest the lysosomotropic amine endosomal pH effect is a real phenomenon, which is both concentration- and time-dependent.

Much is known regarding the EBOV life-cycle, including the infiltration of endosomal system of the host organism. The current evidence suggests that once EBOV is trafficked via the endosomal system, the cysteine proteases cathepsin B and cathepsin L prime the viral glycoprotein (GP) by removing ~60% of the amino acids of GP1, including the glycan cap, with cathepsin B being responsible for the majority of the cleavage (41, 42). This cleavage is necessary for the late endosomal membrane fusion of EBOV, which also requires the primed GP1 to interact with the endosomal transmembrane Niemann-Pick C1 (NPC1) protein (43-45). This cleavage is necessary for EBOV entry (46, 47) and cathepsin B works ideally at low pH (pH 5.0-5.5), with a retention of only ~20% activity at pH 7.5 (48). This indicates that a pH increase in late endosomes would interfere with EBOV entry by inhibiting GP priming. While still speculative, the accumulation of this evidence may point to a potential mechanism of action for quinacrine against EBOV and possibly other human pathogens described earlier.

Supplemental References

1. Wallace DJ. 1989. The use of quinacrine (Atabrine) in rheumatic diseases: a reexamination. Semin Arthritis Rheum 18:282-96.
2. Anonymous. 1946. PLASMA quinacrine concentration as a function of dosage and environment. Arch Intern Med (Chic) 78:64-107.
3. Jailer JW. 1945. Fluorescent Microscopic Study of the Physiological Distribution of Atabrine. Science 102:258-9.
4. Munoz Gutierrez J, Aldasoro E, Requena A, Comin AM, Pinazo MJ, Bardaji A, Oliveira I, Valls ME, Gascon J. 2013. Refractory giardiasis in Spanish travellers. Travel Med Infect Dis 11:126-9.
5. Ehsanian R, Van Waes C, Feller SM. 2011. Beyond DNA binding - a review of the potential mechanisms mediating quinacrine's therapeutic activities in parasitic infections, inflammation, and cancers. Cell Commun Signal 9:13.
6. Clarke JJ, Sokal DC, Cancel AM, Campen DB, Gudi R, Wagner VO, San RH, Jacobson-Kram D. 2001. Re-evaluation of the mutagenic potential of quinacrine dihydrochloride dihydrate. Mutat Res 494:41-53.
7. Cancel AM, Dillberger JE, Kelly CM, Bolte HF, Creasy DM, Sokal DC. 2010. A lifetime cancer bioassay of quinacrine administered into the uterine horns of female rats. Regul Toxicol Pharmacol 56:156-65.
8. Kessel E. 1996. 100,000 quinacrine sterilizations. Adv Contracept 12:69-76.
9. Dabancens A, Sokal DC, Pruyas M, Rivera M, Zipper J. 1995. Prevalence and standardized incidence rates of preclinical cervical pathology among 1,061 women sterilized with transcervical quinacrine hydrochloride pellets. Fertil Steril 64:444-6.
10. Sokal DC, Zipper J, Guzman-Serani R, Aldrich TE. 1995. Cancer risk among women sterilized with transcervical quinacrine hydrochloride pellets, 1977 to 1991. Fertil Steril 64:325-34.
11. Jani TS, DeVecchio J, Mazumdar T, Agyeman A, Houghton JA. 2010. Inhibition of NF-kappaB signaling by quinacrine is cytotoxic to human colon carcinoma cell lines and is synergistic in combination with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) or oxaliplatin. J Biol Chem 285:19162-72.
12. Mohapatra P, Preet R, Das D, Satapathy SR, Choudhuri T, Wyatt MD, Kundu CN. 2012. Quinacrine-mediated autophagy and apoptosis in colon cancer cells is through a p53- and p21-dependent mechanism. Oncol Res 20:81-91.
13. Preet R, Mohapatra P, Das D, Satapathy SR, Choudhuri T, Wyatt MD, Kundu CN. 2013. Lycopene synergistically enhances quinacrine action to inhibit Wnt-TCF signaling in breast cancer cells through APC. Carcinogenesis 34:277-86.
14. Doh-Ura K, Iwaki T, Caughey B. 2000. Lysosomotropic agents and cysteine protease inhibitors inhibit scrapie-associated prion protein accumulation. J Virol 74:4894-7.
15. Korth C, May BC, Cohen FE, Prusiner SB. 2001. Acridine and phenothiazine derivatives as pharmacotherapeutics for prion disease. Proc Natl Acad Sci U S A 98:9836-41.
16. Mays CE, Joy S, Li L, Yu L, Genovesi S, West FG, Westaway D. 2012. Prion inhibition with multivalent PrPSc binding compounds. Biomaterials 33:6808-22.

17. Barret A, Tagliavini F, Forloni G, Bate C, Salmona M, Colombo L, De Luigi A, Limido L, Suardi S, Rossi G, Auvre F, Adjou KT, Sales N, Williams A, Lasmezas C, Deslys JP. 2003. Evaluation of quinacrine treatment for prion diseases. J Virol 77:8462-9.
18. Fasano C, Campana V, Griffiths B, Kelly G, Schiavo G, Zurzolo C. 2008. Gene expression profile of quinacrine-cured prion-infected mouse neuronal cells. J Neurochem 105:239-50.
19. Klingenstein R, Lober S, Kujala P, Godsave S, Leliveld SR, Gmeiner P, Peters PJ, Korth C. 2006. Tricyclic antidepressants, quinacrine and a novel, synthetic chimera thereof clear prions by destabilizing detergent-resistant membrane compartments. J Neurochem 98:748-59.
20. Ryou C, Legname G, Peretz D, Craig JC, Baldwin MA, Prusiner SB. 2003. Differential inhibition of prion propagation by enantiomers of quinacrine. Lab Invest 83:837-43.
21. Al Asmari AK, Al Sadoon KT, Obaid AA, Yesunayagam D, Tariq M. 2017. Protective effect of quinacrine against glycerol-induced acute kidney injury in rats. BMC Nephrol 18:41.
22. Mushett CW, Siegel H. 1946. Hematological changes following the administration of large doses of quinacrine hydrochloride. Blood 1:537-47.
23. Yung L, Huang Y, Lessard P, Legname G, Lin ET, Baldwin M, Prusiner SB, Ryou C, Guglielmo BJ. 2004. Pharmacokinetics of quinacrine in the treatment of prion disease. BMC Infect Dis 4:53.
24. Collinge J, Gorham M, Hudson F, Kennedy A, Keogh G, Pal S, Rossor M, Rudge P, Siddique D, Spyer M, Thomas D, Walker S, Webb T, Wroe S, Darbyshire J. 2009. Safety and efficacy of quinacrine in human prion disease (PRION-1 study): a patient-preference trial. Lancet Neurol 8:334-44.
25. Bjorkman S, Elisson LO, Gabrielsson J. 1989. Pharmacokinetics of quinacrine after intrapleural instillation in rabbits and man. J Pharm Pharmacol 41:160-3.
26. Laufe LE, Sokal DC, Cole LP, Shoupe D, Schenken RS. 1996. Phase I prehysterectomy studies of the transcervical administration of quinacrine pellets. Contraception 54:181-6.
27. Shannon JA, Earle DP, Brodie BB, Taggart JV, Berliner RW. 1944. The Pharmacological basis for the rational use of atabrine in the treatment of malaria. J Pharmacol Exp Ther 81:307-330.
28. Ahn M, Ghaemmaghami S, Huang Y, Phuan PW, May BC, Giles K, DeArmond SJ, Prusiner SB. 2012. Pharmacokinetics of quinacrine efflux from mouse brain via the P-glycoprotein efflux transporter. PLoS One 7:e39112.
29. Bohle DS, Dodd EL, Pinter TB, Stillman MJ. 2012. Soluble diamagnetic model for malaria pigment: coordination chemistry of gallium(III)protoporphyrin-IX. Inorg Chem 51:10747-61.
30. Requena-Mendez A, Goni P, Rubio E, Pou D, Fumado V, Lobez S, Aldasoro E, Cabezos J, Valls ME, Trevino B, Martinez Montseny AF, Clavel A, Gascon J, Munoz J. 2017. The Use of Quinacrine in Nitroimidazole-resistant Giardia Duodenalis: An Old Drug for an Emerging Problem. J Infect Dis 215:946-953.
31. Pant KC, Ray HN. 1942. Quinacrine in the Eradication of Giardia Lamblia Infection. Ind Med Gaz 77:469-470.
32. Saliba KJ, Folb PI, Smith PJ. 1998. Role for the plasmodium falciparum digestive vacuole in chloroquine resistance. Biochem Pharmacol 56:313-20.
33. Adam RD. 2001. Biology of Giardia lamblia. Clin Microbiol Rev 14:447-75.

34. Alves P, Bashir MM, Wysocka M, Zeidi M, Feng R, Werth VP. 2017. Quinacrine Suppresses Tumor Necrosis Factor-alpha and IFN-alpha in Dermatomyositis and Cutaneous Lupus Erythematosus. J Investig Dermatol Symp Proc 18:S57-S63.
35. Kaufmann AM, Krise JP. 2007. Lysosomal sequestration of amine-containing drugs: analysis and therapeutic implications. J Pharm Sci 96:729-46.
36. Duvvuri M, Gong Y, Chatterji D, Krise JP. 2004. Weak base permeability characteristics influence the intracellular sequestration site in the multidrug-resistant human leukemic cell line HL-60. J Biol Chem 279:32367-72.
37. Poole B, Ohkuma S. 1981. Effect of weak bases on the intralysosomal pH in mouse peritoneal macrophages. J Cell Biol 90:665-9.
38. Myers BM, Tietz PS, Tarara JE, LaRusso NF. 1995. Dynamic measurements of the acute and chronic effects of lysosomotropic agents on hepatocyte lysosomal pH using flow cytometry. Hepatology 22:1519-26.
39. Manzel L, Strekowski L, Ismail FM, Smith JC, Macfarlane DE. 1999. Antagonism of immunostimulatory CpG-oligodeoxynucleotides by 4-aminoquinolines and other weak bases: mechanistic studies. J Pharmacol Exp Ther 291:1337-47.
40. Kuznik A, Bencina M, Svajger U, Jeras M, Rozman B, Jerala R. 2011. Mechanism of endosomal TLR inhibition by antimalarial drugs and imidazoquinolines. J Immunol 186:4794-804.
41. Dube D, Schornberg KL, Shoemaker CJ, Delos SE, Stantchev TS, Clouse KA, Broder CC, White JM. 2010. Cell adhesion-dependent membrane trafficking of a binding partner for the ebolavirus glycoprotein is a determinant of viral entry. Proc Natl Acad Sci U S A 107:16637-42.
42. Lee JE, Fusco ML, Hessell AJ, Oswald WB, Burton DR, Saphire EO. 2008. Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor. Nature 454:177-82.
43. Miller EH, Obernosterer G, Raaben M, Herbert AS, Deffieu MS, Krishnan A, Ndungo E, Sandesara RG, Carette JE, Kuehne AI, Ruthel G, Pfeffer SR, Dye JM, Whelan SP, Brummelkamp TR, Chandran K. 2012. Ebola virus entry requires the host-programmed recognition of an intracellular receptor. EMBO J 31:1947-60.
44. Cote M, Misasi J, Ren T, Bruchez A, Lee K, Filone CM, Hensley L, Li Q, Ory D, Chandran K, Cunningham J. 2011. Small molecule inhibitors reveal Niemann-Pick C1 is essential for Ebola virus infection. Nature 477:344-8.
45. Carette JE, Raaben M, Wong AC, Herbert AS, Obernosterer G, Mulherkar N, Kuehne AI, Kranzusch PJ, Griffin AM, Ruthel G, Dal Cin P, Dye JM, Whelan SP, Chandran K, Brummelkamp TR. 2011. Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. Nature 477:340-3.
46. Chandran K, Sullivan NJ, Felbor U, Whelan SP, Cunningham JM. 2005. Endosomal proteolysis of the Ebola virus glycoprotein is necessary for infection. Science 308:1643-5.
47. Schornberg K, Matsuyama S, Kabsch K, Delos S, Bouton A, White J. 2006. Role of endosomal cathepsins in entry mediated by the Ebola virus glycoprotein. J Virol 80:4174-8.
48. Werle B, Julke B, Lah T, Spiess E, Ebert W. 1997. Cathepsin B fraction active at physiological pH of 7.5 is of prognostic significance in squamous cell carcinoma of human lung. Br J Cancer 75:1137-43.