Targeting Apicoplast Structures in P. falciparum during the Pre-erythrocytic Stage

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Introduction
Although almost any American can acutely recall the fear and panic surrounding the recent Ebola outbreak of 2014, few are aware of the ongoing public health crisis caused by malaria. In fact, more people will die of malaria in a single day than have died in the past ten years of Ebola. Malaria infects anywhere from 350 to 500 million people a year, mostly young children in sub-Saharan Africa. The parasite Plasmodium is responsible for this vicious disease, with five species in particular able to infect humans. The most deadly species is Plasmodium falciparum, responsible for over 80% of infections. Because of this dynamic pattern of infection, vaccine research has a wide range of possibilities, from pre-erythrocytic targets to hemotocrit modifications (Zheng 2014).

Of particular interest to this research is the apicoplast, a plastid that likely arose from the engulfment of an organism of red algal lineage (Jéus 2014). Apicomplexan parasites cause many diseases, from malaria, toxoplasmosis, and coccidiosis. The apicoplast itself is a double membrane organelle that is crucial in the manufacturing and storage of lipids and chemicals. Although its exact role in the parasite is unknown, it must remain intact for parasitic survival (Foth 2003). The apicoplast possesses its own genes it can express, but generally the apicoplast proteome is encoded in the nuclear genome. Although they are non-photosynthetic, apicoplasts retain other typical plastid functionalities such as fatty acid synthesis, heme synthesis, and isoprenoid synthesis. The products of these pathways are essential to the parasite, which requires these structures in order to survive.

Hypothesis
By deepening our knowledge of this unique vestige of P. falciparum, we may identify its differential characteristics that ultimately render it vulnerable. Specific investigation into the molecular makeup of the organelle’s membrane may reveal structures able to be targeted by novel perforins. We hypothesize that the characterization of the membrane’s glycoproteins and other signaling structures would aid in the design of a drug that would lyse the structure, impairing the numerous functions that make it a necessary component of P. falciparum. Previous research conducted by Orier et al. resulted in the successful design of novel primers that targeted a highly conserved area in the apicoplast genome PFC10 for parasite detection (Orier 2015), yet our method will investigate a far simpler technique that seeks to lyse the apicoplast as a whole.

Specific Aims
The aim of research will be twofold: characterize the membrane structures of a P. falciparum apicoplast and design a drug that acts as a perforin to lyse the target apicoplast. Characterization will be accomplished through a variety of different isolation and identification techniques that will ultimately reveal detailed membrane structures unique to the apicoplast. A humanized mouse model transfected with human hepatocytes will act as the final source to culture the P. falciparum subjects. Although the apicoplast’s extracellular matrix likely contains numerous unique structures, we predict that specifically hijacking a transmembrane transport protein will offer the most direct way of interfering with membrane structure. Success of our novel drug will be tested in the aforementioned humanized mouse models. We predict that the lysing action of the proposed designer drug will render the parasite harmless, halting malaria in the pre-erythrocytic stage. By preventing the rupturing of the schizont, we expect that success and effectivity of our novel drug could be measured by the amount of merozoites found in the inoculated humanized mouse model (Ralph 2001). Lower counts of merozoites found in the red blood cells would suggest the viability of our simple lysing of the apicoplast model.

Experimental Procedure
I. In Vitro Characterization of the Apicoplast Membrane
In order to carry out extensive characterization of the apicoplast membrane, it would be necessary to first obtain a large library of viable P. falciparum. Because harvesting the parasite from infected humans would require a complex infrastructure of clinical sites as well as a questionable standard of informed consent, we propose to instead inoculate an experimental mouse with human liver cells that serve as done successfully by previous researchers (Vansbuskirk 2009). The successful transfecion of human hepatocytes into mice should pave the way for final infection with the P. falciparum parasite. In vitro characterization would be ideal, as membrane suspensions could be easily obtained after isolation of the apicoplast within the humanized mouse model. Membrane structure characterization would be carried out using a number of serological tests. Protein characterization in particular might be determined using affinity chromatography, which separates components from a bulk suspension. A nickel affinity column would provide the most specific way of isolating the protein, as the column would attract the fused nickel ions and proteins while allowing the bulk suspension to pass through.

These components might be isolated by in vivo extraction followed by the centrifugation of a cell and organelle suspension (Wise 2000). The extraction methods, as first described by Wise et al. in 2000 would be carried out with a scanning electron microscope, which could easily help distinguish the nano-sized particles of interest. Alternatively, novel techniques in atomic force microscopy, which passes a probe over a structure to collect information about the structure’s surface, would yield immediate and visual descriptions of the membrane of the apicoplast. Gentle sonication of a membrane is suggested to successfully free the various glycolytic components of a membrane (Foth 2003). After crystallizing these sugars with in situ solvent pairing, taking the melting point of the crystallized sugars might reveal the stereospecificity of any glucose enantiomers present on the surface of the membrane. Using a successive series of basic chemical tests that divided components based on water solubility, polarity, and size, specific components of the apicoplast would slowly start to give us a better understanding of the membrane we seek to compromise.

II. Introduction of Novel Drug
Site specific binding at almost all biological proteins, protozoan or other, provides the basis for our confidence in specifically targeting transmembrane proteins. Investigation into the stereospecificity of glycolytic markers on such proteins would increase the theoretical precision of our drug. Once the thorough characterization of a protein is complete, we expect that a molecule comparable to a perforin could be designed so as to inhibit protein function or degrade the protein altogether. By mimicking the signaling pathways of the proteins we characterize in the early stages of research, we are following a proven way of invading a membranous organelle (Botte 2013). Considerable caution must be taken to ensure that the perform-like drug does not target healthy hepatic cells. Because the apicoplast is an organelle already within the parasite, initial phases of drug testing may examine direct effects on the apicoplast rather than the entire P. falciparum organism itself. Success in the early phases might allow us to determine whether the drug possess the ability to penetrate not only the apicoplast membrane, but the P. falciparum membrane as well without degradation or phagocytosis.

Conclusion
Apicoplasts are a unique feature of Plasmodium falciparum that render it vulnerable to attack by perform-related drugs. Although scientists have linked its existence to a few basic, yet necessary functions, precise membrane characterization has yet to be executed. Current research regarding apicoplasts has focuses strongly on genomic sequencing and preand post-transcriptional interference as a way to target P. falciparum. If our detailed characterization can provide descriptive qualities unique to the apicoplast structure, the creation of a perforin molecule with similar signaling mechanisms can rupture the apicoplast membrane and ultimately render the parasite immobile before infection spreads to the blood.

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