CFID Undergraduate Summer Research Proposal

Project title: *Putative neuroprotective agents for cerebral malaria*

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Introduction

Globally, malaria is a major public health concern. Although intervention strategies have allowed for a reduction in deaths due to malaria, the numbers remain high, especially in Sub- Saharan Africa.[1] Malaria is the fourth most common cause of death for children under five years, accounting for more than 400,000 deaths in 2015.[2] Spread by mosquito vectors, the infection is most commonly caused by *Plasmodium falciparum*, the most virulent of malaria species, with symptoms varying due to host factors.[3,4] In the most severe cases, *P. falciparum* can cause pathology in the brain, resulting in cerebral malaria (CM), for which mortality rates, with treatment, are 15%-20%.[2] CM is associated with sequestration of *P. falciparum* to the endothelial lining of blood vessels[2,4] and is characterized by impaired consciousness, seizures and even coma.[5] Those who survive are at risk of neurocognitive deficits.

One commonly observed outcome in post-mortem brain tissue of CM patients is disruption of the brain microvasculature, suggested to be a consequence of parasite sequestration.[2,5] An immunohistochemical analysis revealed upregulation of adhesion molecules ICAM-1 and E-selectin, in vascular endothelium containing sequestered infected erythrocytes.[6,7] Plasma samples indicate elevation of vasoactive compounds such as vascular endothelial growth factor (VEGF). Although this is not common across all patients, African children with CM in particular show evidence of cerebral oedema and excess movement of proteins like

albumin across the blood brain barrier, further suggesting damage to the brain microvasculature.[8,9] Moreover, there is evidence of reduced junctional proteins, particularly in blood vessels that have *P. falciparum* sequestration.[7] Junctional proteins normally function to limit passage of molecules between cells. Thus, it is hypothesized that elevation of VEGF, known to increase endothelium permeability, and subsequent downregulation junction proteins is likely responsible for increased permeability of the blood brain barrier in CM.[10,11]

This study focuses specifically on the VEGF receptor-2 (VEGFR-2) pathway for its effect on junctional proteins and vascular permeability. VEGFR-2, like other VEGFRs is a receptor tyrosine kinase. Its general, its sequence of signalling activity involves activation by ligand-binding to the extracellular domain, dimerization, and signal transduction via kinase activity.[12,13] Notably, the tyrosine kinase domain serves as a binding site for adaptor molecule TSAd, which through c-Src activation, leads to dissociation and internalization of vascular endothelial cadherin (VE-cadherin).[11,13] Because internalization of VE cadherin is, at least in part, responsible for endothelial membrane permeability, it is hypothesized that targeting adjunctive therapies to this VEGFR-2 pathway may improve outcomes in the neurocognitive sequelae of CM.[11] Specifically, this study looks at using tyrosine kinase inhibitors (TKI) to prevent signal transduction right from the receptor tyrosine kinase.

Three FDA approved pharmaceuticals of interest are Sunitinib, Pazopanib, and Imatinib. Sunitinib is a multitargeted TKI, that has been approved for treatment in renal cell carcinoma and gastrointestinal stromal tumours, both of which involve angiogenesis via VEGFR pathways.[14] Pazopanib, also a multitargeted receptor TKI has been used to as an antiangiogenic in pediatric sarcoma as well as renal cell carcinoma.[15] Imatinib is a TKI used in cancers such as chronic myeloid leukemia,

demonstrating higher efficacy compared to the previously used interferon alfa plus cytarabine therapy.

Objective

Using existing knowledge about the VEGFR-2 pathway and tyrosine kinase inhibitors that have proven to be effective, this study aims to fill a knowledge gap in adjunctive therapies

for cerebral malaria. The goal of this research project is to facilitate the development of adjunctive therapies for CM by repurposing currently approved pharmaceuticals, sunitinib, pazopanib, and imatinib, as putative neuroprotective agents in endothelial barrier dysfunction due to *P. falciparum* in experimental CM.

Hypothesis

We hypothesize that the pharmaceutical agents of interest, as clinically approved tyrosine kinase inhibitors, can reduce blood brain barrier permeability in an experimental *in vitro* model of cerebral malaria.

Proposed methods

This study will test putative neuroprotective agents using an *in vitro* model of cerebral malaria. Immortalized human cerebral microvascular endothelial cells (hCMEC/D3) are a logistically pragmatic and representative cell line as a model for the blood brain barrier in studies of pathology and drug transport.[16,17] The *in vitro* model used in this research will consist of hCMEC cultured in tissue culture flasks (Corning) at 37 °C and 5% CO_2 with endothelial cell growth medium, EndoGRO. $1x10^5$ hCMEC/D3 will be seeded into 24-transwell plates inserts with polycarbonate membranes and 0.4um pores and grown to a confluent monolayer

(~3 days). Once a monolayer is established, experimental and control conditions will be set up.

Experimental conditions will contain $1x10^6$ *P. falciparum*-infected red blood cells (iRBCs) added to the transwell insert with and without one of three pharmacological agents: sunitinb, pazopanib, or imatinib. Blood will be collected from volunteer, O+ donors who were not previously diagnosed with malaria. Red blood cells will be infected with *P. falciparum* clone 3D7 samples, synchronized by selective sorbitol lysis, and cultured in RPMI 1640 medium until the trophozoite stage. iRBCs will be magnet purified before addition to transwell inserts. Control conditions will include uninfected blood cells with and without the pharmacological agent of interest, as well conditions of media only or hCMEC/D3 only. Pharmacological agents will be added at concentrations reflective of physiological bioavailability.

Membrane permeability will be assessed by transendothelial electrical resistance (TEER) and macromolecule flux. TEER will be measured using an EVOM voltohmeter, while macromolecule flux will be measured by fluorescence intensity of fluorescein-labeled dextran (495/519 nm emission wavelength) in the transwell insert compared to the well in which it is placed. Higher TEER is associated with lower membrane permeability. Higher fluorescence intensity in the well is indicative of increased permeability. Each condition will have at least three replicates. TEER and fluorescence will be measured at various time points, including 1 hour and 24 hours following the addition of an inflammatory challenge and/or a TKI.

Knowledge translation, impact, and future directions

Results of this study will be shared with the academic community through conferences and publications, where possible. It is expected that at least one of the pharmaceuticals of interest will successfully rescue the *P. falciparum* induced permeability of our

in vitro model of the blood blain barrier. If so, the next stages of this study will focus on identifying specific proteins and mechanisms that may be involved in permeability or rescue of permeability. At a later stage, experiments will be pursued in an *in vivo* model and if successful, to a clinical trials. Because these pharmaceuticals of interest are FDA-approved, this translation will be more efficient. The outcomes are hoped to bring improved treatment and reduced mortality and morbidity for pediatric patients with cerebral malaria, especially in the most resource poor regions of the world.

References

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