TARGETING THE COMPROMISED BRAIN ENDOTHELIAL BARRIER FUNCTION DURING CEREBRAL MALARIA WITH AT2 RECEPTOR AGONISTS

Strengthening of inter-cellular junctions of endothelial cells would facilitate important translational applications for a variety of diseases where endothelial integrity is compromised. As a first model, we have chosen cerebral malaria (CM), which remains the deadliest manifestation of malaria. It is caused by Plasmodium falciparum infected erythrocytes (iRBC) adhering to host brain endothelial cells and compromising the blood brain barrier. While anti-malarial drugs clear parasites from the blood, they do not have specific effects against CM. We have found that P. falciparum-iRBC-induced disruption of human brain microvascular endothelial cell junctions and development of CM in mice was prevented by the activation of the angiotensin (Ang) II receptor type 2 (AT2). Ang II is only a biased agonist of the G-protein coupled receptor AT2, since it does not activate G- proteins via the receptor. We have discovered the real endogenous agonist for AT2 (EA), which activates Gαs and protects against disruption of endothelial integrity. Based on in silico modelling and in vitro experiments, we have also identified a first AT2-specific non-peptidic receptor agonist (SNPA) that also activates Gαs. We hypothesize that activation of specific intracellular signaling pathways of AT2 protects human brain and retinal endothelial cells from P. falciparum-induced disruption of inter-endothelial junctions, thus maintaining endothelial function, reducing/preventing edema and hemorrhages and thus CM and related retinopathy. We will first identify which AT2-mediated intracellular signaling pathways are key in the protection of endothelial integrity, by testing 4 differently acting compounds: Ang II, EA, SNPA and the non-specific agonist C21, in AT2- transfected cells and in human and murine brain endothelial cells and quantifying intracellular signaling molecules important in barrier integrity. A pharmacological approach and targeted inhibition of AT2 by siRNA in primary human (brain, retina) and mouse (wild-type and AT2-deficient) endothelial cells will identify the most efficient agonist in brain endothelial protection. We will determine the specific effects of the agonists interacting with AT2 on endothelial activation, junction integrity, and on selected second messengers. Finally, we will test how treatment with the different agonists affects the outcome of CM and related retinopathy in wild-type and AT2-deficient mice, whereby analysis of the retina offers scope for investigation of brain microvascular function. The main goal of this project is to identify a lead compound, which can stimulate specific intracellular signaling at the AT2 receptor to mediate essential protection of endothelial integrity. Our experiments will lay the foundation for the development of a small molecule drug to be immediately tested in clinical trials for the treatment of the life-threatening pathology of CM, with possible future applications in other hemorrhagic diseases. This proposal is submitted under the US-Ireland R&D Partnership Programme, which is focused on the development of new therapeutic approaches. Funding is requested only for the US component, since the Irish funding agencies have already committed their financial support conditional on a positive NIH funding decision.

Public Health Relevance Statement:

The loss of endothelial integrity is a common pathology of a number of different life-threatening diseases resulting in edema and hemorrhages. Using cerebral malaria as a model, we have found that activation of a particular angiotensin receptor strengthens the junctions between endothelial cells in the brain and protects against disease pathology. We intend to identify compounds that can trigger this protective mechanism with the aim of developing treatments for diseases with compromised endothelial integrity and, in particular, for cerebral malaria.