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## Activation of endothelial cells and its potential involvement in blood-brain barrier damage in cerebral malaria: an in vitro study

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### Abstract

Cerebral Malaria (CM), a severe complication in *Plasmodium falciparum* infection is classified as one of the main causes of mortality in malaria. The clinical outcome of CM, broadly varies from recovery to a lethal endpoint. Investigation of post-mortem brain tissue of CM patients demonstrates BBB breakdown (microhaemorrhages) with loss of tight junction proteins (TJPs), occludin, claudin-5 and ZO-1 in endothelial cells. Interestingly, this was observed in vessels in the presence and in the absence of sequestered PRBC. This suggested the mobilisation of direct and indirect mechanisms following PRBC sequestration. In previous studies, the endothelial cell monolayer integrity was reduced when co-cultured directly with PRBC. To investigate the indirect effect of sequestration to the BBB integrity, human brain endothelial cells (tHBEC) were first co-cultured with PRBC after which, the co-culture supernatant was harvested and analysed for the presence of candidate soluble factors and functional assays performed. Interestingly, analysis of the co-culture supernatant showed the induction of the ADAMTS family of protease, ADAMTS-4. In addition, differential regulation of ADAMTS-1 and the matrix metalloproteases (MMP) family proteases, MMP-2 and MMP-9 was observed. Treatment of freshly cultured tHBEC monolayer with the co-culture supernatant demonstrated up to 2-fold reduction in tHBEC monolayer integrity within 3 hours, measured using FITC-dextran permeability assay. This was accompanied by reduction in the TEER and the endothelial cell TJPs. We propose that endothelial cell-derived proteases in the co-culture supernatants that are released as a result of interaction with PRBC, during sequestration; contribute to BBB breakdown in CM.

### Keywords

Cerebral malaria, matrix metalloproteases, trans-endothelial electrical resistance, tight junction proteins.