The participation and quantification of Lymphocyte Function Associated Antigen-3 (LFA-3) in vaso-occlusion in pregnant women with sickle cell disease and the efficiency of folic acid as a vaso-occlusive prophylactic.

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Aims:
- Identify the relationship between LFA-3 erythrocytic expression, pregnancy and sickle cell disease (SCD).
- Observe the suitability of folic acid as a potential anti-vaso-occlusive prophylactic.

Background

- Erythrocytes express adhesion molecules that are mediators of sickle cell adhesion. In sickle cell disease this adhesion leads to congestion in vasculature and consequently vaso-occlusive crises (Shiu, Udden & McIntire 2000).
- Lymphocyte function antigen-3 (LFA-3/CD58) is overexpressed on erythrocytes in neonates (Brousse et al 2014) but research into the adult sickle cell population with regards to LFA-3 is limited. LFA-3 is known to function as a cell contact/T-cell activation pathway (Arulanadam et al 1993).
- Therapeutic folic acid supplements are administered to sickle cell patients to reduce oxidative damage and downregulate ICAM-1, lessening vaso-occlusion (Jana et al 2018).
- Increased placental growth factor in SCD and pregnancy increases the risk of cerebrovascular events and spontaneous miscarriage (Brittain et al 2010) and is predicted to cause an increase in LFA-3 expression on the erythrocyte surface (Baptista et al 2016) highlighting the demand for a prophylactic.

Methodology

EDTA whole blood samples were obtained from Heartlands Hospital and University Hospital Coventry and Warwickshire Sample population groups:
- Pregnant and non-pregnant women with SCD
- Pregnant and non-pregnant non-sickle cell patients

LFA-3 Erythrocytic Expression

LFA-3 expression was identified on erythrocyte surface membranes using BD Accuri Plus flow cytometer and anti-CD58 monoclonal mouse FITC antibody. Each sample group was incubated with both 1mg/l folic acid suspension and anti-CD58 to determine the effect of folic acid on cell staining.

Adhesion Assay

A static adhesion assay was performed using HEPG2 cell monolayers. Isolated erythrocytes from each sample group were incubated with either anti-CD58 or folic acid before inoculating the confluent monolayers of HEPG2 cells. Images were captured via microscopy. Percentage epithelial damage was determined via ImageJ.

Results

Erythrocytic expression of LFA-3 (Figure 1)
- Antenatal sickle cell samples displayed a substantial increase in mean fluorescence in comparison to all sample groups (P<0.05).
- Significant reduction in CD58 fluorescence after folic acid incubation within both antenatal and non-antenatal sickle cell samples when compared to the antenatal control samples (P<0.05).

Static adhesion assay exploring reduction in epithelial damage (Figure 2)
- Erythrocytes incubated with anti-CD58 showed a 54.9% reduction in epithelial damage when comparing antenatal sickle cell patients to non-antenatal sickle cell patients.
- After incubating with folic acid, epithelial damage due to erythrocyte adhesion was increased compared to control samples in all sample groups except antenatal sickle cell samples. Providing evidence for the interception of vaso-occlusion.

Discussion and conclusion
- LFA-3 is essential in T cell activation (Bierer et al 1988). Lack of T cells in immunocompromised patients with SCD may promote upregulation of LFA-3 to activate present Th1 cells to protect mother and baby.
- Provides evidence of a correlational relationship between placental growth factor and LFA-3. Pregnancy and SCD combined contribute to increased LFA-3.
- LFA-3 could be an isoform of Folate Receptor-α and is upregulated during inflammatory crises to increase folate metabolism and reduce vaso-occlusion.
- LFA-3 overexpression without sufficient folic acid supply for uptake may explain the decrease in CD58 fluorescence.
- This research highlights both the importance of LFA-3 in the vaso-occlusive process and the demand for a vaso-occlusive prophylactic in patients with SCD during pregnancy. Further research would involve a pulsatile flow adhesion assay whilst inhibiting other known adhesion molecules to isolate and determine the exact function of LFA-3.

References


