Can outgrowth endothelial cells restore neurovascular integrity and function after ischaemic stroke?

Aims: To assess whether outgrowth endothelial cells (OECs), a type of stem cells, can be used as efficacious therapeutic components to restore radically impaired neurovascular integrity and function after ischaemic stroke.

Background: Ischaemic strokes occur due to an interference with blood supply to the brain and is characterised by appearance of brain oedema and neurological deficits. Thrombolysis (blood clot dissolution) with recombinant tissue plasminogen activator (rtPA) remains the only approved pharmacotherapy for this disease. However, it can only be given to ~5% of patients due to a narrow time window for administration (the first 4.5 h of symptom onset) and elevated risk of bleeding beyond this. As each year ~150,000 people in the UK have a first stroke, it is important to discover new therapeutic components that can effectively prevent vascular leak and stimulate the generation of new neurons (neurogenesis) and vessels (vasculogenesis) to aid recovery of motor and cognitive functions.

Hypothesis: Considering the significant tubulogenic, migratory and proliferative capacities of OECs, we hypothesise that OECs will radically diminish the volumes of brain oedema and infarct by effectively repairing the damaged blood-brain barrier and improve neurological function by inducing vasculo-neurogenesis.

Experimental methods: Mononuclear cells will be extracted from the total bone marrow aspirates of the donor rats via density gradient centrifugation and cultured in endothelial cell growth medium-2 (EBM-2) until they acquir cobblestone morphology i.e. differentiate into OECs. Flow cytometry will be used to immunologically characterise OECs while modified Boyden chamber (migration), BrdU incorporation (proliferation) and matrigel assay (tubulogenesis) will test their functional aspects.

Transient focal ischemia will be induced in rats by transient middle cerebral artery occlusion (MCAO) before randomly allocating them into 3 different groups. Group 1: sham-operation + vehicle (500 µl EBM-2); Group 2: MCAO + vehicle and Group 3: MCAO + OECs (4×10^6 cells in 500 µl EBM-2; 24 h post-MCAO).

The newly generated cells of vascular and neuronal origin after MCAO will be determined in brain samples by double immunofluorescence staining of BrdU with CD31 and neuronal nuclei, respectively. Lesion volume and brain water content reflecting damage and oedema will be measured after MCAO. Lateralised adjusting steps and response to vibrissae stimulation will be used to assess sensory motor deficits while the novel object recognition task will be used to assess cognitive deficits. Vascular engraftment of OECs will be studied by detection of labelled Qdot via confocal microscopy.

The effect of OECs on cerebral oxidative stress, inflammatory responses and apoptosis will also be tested using a variety of sophisticated molecular biological methods like ELISA and fluorometry.

Expected outcomes and impact: Data generated will reveal whether late administration of OECs will restore normal neurovascular function in a clinically relevant model of ischaemic stroke.

Supervisor: Drs Ulvi Bayraktutan and Rebecca Trueman

Themes: Stroke, Vascular Health and Disease, Molecular Biology

Keywords: Ischaemic injury, blood-brain barrier, brain oedema, endothelial progenitor cells

Fee band: High cost laboratory-based research

Project availability: International students only