Introduction

Inflammation is an essential physiological response to noxious stimuli, aimed toward the restoration of homeostasis. However a disproportionate inflammatory response has been shown to play a major deleterious role in the pathogenesis of a wide variety of disorders and is increasingly being acknowledged as a major pathological factor in stroke.

Targeting the inflammatory response in stroke may dramatically increase the time window for therapeutic intervention, and improve functional outcome in this debilitating disease.

The melanocortin receptors are a family of G-protein coupled rhodopsin-like receptors with key regulatory functions in a wide variety of cellular processes. These receptors are increasingly being recognised as exciting pharmacological targets for a number of different pathologies. Potent anti-inflammatory actions have been attributed to the melanocortin receptors MC1, MC3 and MC4.

This project aims to assess the therapeutic value of melanocortin based anti-inflammatory therapy for ischemic stroke.

Methods

The Bilateral common carotid artery occlusion (BCCAo) mouse model of global stroke has been used to assess the role of inflammation in the cerebral microcirculation following stroke. Briefly, mice were subject to 5 minutes global brain ischemia followed by 40 minutes of reperfusion.

Intravital microscopy has been utilised to quantify the inflammatory reaction following stroke by allowing real-time in vivo visualisation of the leukocyte adhesion cascade in the pial venules of the cerebral microcirculation. An un-branched section of a pial venule 30-70µm in diameter and 100 µm in length was selected for video analysis. Leukocyte-endothelium interactions after ischemia-reperfusion were quantified in terms of rolling cell flux and adherent leukocytes, and expressed as cells/mm², leukocyte rolling velocity was expressed as µm/sec.

Results

Sham operated and BCCAo groups n=8 mice, α-MSH treated n=3, Dtrp8-γ-MSH treated n=6. Statistical evaluation was performed using ANOVA with Bonferroni test for post hoc analyses. P<0.05 was considered to be significant.
Significantly higher levels of rolling and adherent leukocytes were observed in mice subject to BCCAo (rolling = 42.08 ±0.35, adherent cells = 217.2 ±30.99) when compared with sham operated animals (rolling = 0.19 ±0.06, adherent cells = 42.08 ±14.35).

Prophylactic treatment 30 minutes prior to ischemia with the pan receptor agonist α-MSH (10µg i.p) was shown to significantly reduce ischemia reperfusion induced leukocyte rolling (0.54 ±0.06) and adhesion (76.4 ±25.3). Treatment with the MC₃ selective agonist, Dtrp8-γ-MSH (10µg i.p), similarly showed a reduction in the adherence of leukocytes (121.19 ±23.70). However despite perceived reduction in rolling when compared to BCCAo control animals these trends were not found to be statistically significant.

**Conclusions**

These preliminary results suggest that although selective activation of the MC₃ results in some attenuation of the inflammatory response following stroke, multiple melanocortin receptor subtypes may be involved, acting in a synergistic manner.

In order to deduce the relative contribution of each receptor subtype, treatments with different selective agonists and antagonists will be performed in both wild type and receptor mutant mice. To ascertain the therapeutic value of these effects, changes in infarct size and functional outcome will additionally be assessed.