The melanocortin receptor system as an anti-inflammatory therapeutic target for stroke

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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 including stroke. Targeting the inflammatory response in stroke may dramatically increase the 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 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 asses 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, male C57BL/6 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 venule 30-70μm in diameter and 100 μm in length was selected for video analysis. Leukocyte-endothelium interactions following 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 Significantly higher levels of rolling and adherent leukocytes were observed in mice subject to BCCAo (rolling = 42.08 ±0.35, adherence = 217.2 ±30.99) when compared with sham operated animals (rolling = 0.19 ±0.06, adherence = 42.08 ±14.35). Prophylactic treatment 30 minutes prior to ischemia with the pan receptor agonist α-MSH was shown to significantly reduce ischemia reperfusion induced leukocyte rolling (0.48 ±0.09) and adhesion (51.7 ±17.15). Treatment with the MC3 selective agonist, Dtrp8-γ-MSH, similarly showed a reduction in the adherence of leukocytes (121.19 ±23.70) however did not significantly reduce rolling. Antagonism of MC_3 and MC_4 using SHU9119 failed to increase adherence and rolling, in fact rolling was significantly decreased (0.7964±0.34). Combination treatment with α-MSH and SHU9119 revealed that SHU9119 did not abrogate the anti-inflammatory effects of α-MSH with levels being similar to α-MSH alone (rolling = 0.2318±0.65, adherence = 33.54 ±30.99).

Sham and BCCAo operated groups n=8, α-MSH treated n=3, Dtrp8-γ-MSH n=6, SHU9119 n=6, SHU9119+α-MSH n=6. Statistical evaluation was performed using ANOVA with Bonferroni test for post hoc analyses. $P<0.05$ was considered to be significant. All treatments were 10μg i.p.

Conclusions These preliminary results suggest that although selective activation of MC3 results in some attenuation of the local inflammatory response following stroke, MC_1 activation may be of particular importance in mediating these protective effects. To further characterise the contribution of each receptor subtype, additional treatments with selective agonists and antagonists must be preformed 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.