Anti-Inflammatory Activity Through Fpr2 in a Mouse Model of Global Cerebral Ischaemia/Reperfusion.

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Ischaemic stroke is often followed by reperfusion of the ischaemic brain region with blood. While reperfusion is ostensibly beneficial, the blood carries with it a surge of leukocytes which interact with endothelial walls of the cerebral microvasculature before emigrating into surrounding tissue, where they propagate tissue damage. This archetypal inflammatory response is thought to limit recovery of the brain after initial excitotoxic cell death and provides an extensive opportunity for pharmacological intervention.

Members of the formyl peptide receptor family (FPR; Fpr in mouse) have been shown in several models to provide an endogenous mechanism for anti-inflammatory/pro-resolving activity. Our aim was to show that FPR ligands would reduce inflammation in global cerebral ischaemia and therefore be neuroprotective, providing evidence for a stroke therapy targeting the FPR system.

A model of global ischaemia/reperfusion (I/R) was developed in male C57BL/6 mice (21-25g) using bilateral common carotid artery occlusion (BCCAO) and reperfusion (5 min and 40 min, respectively), requiring no artificial ventilation. The resulting inflammatory response in the cerebral microvasculature could be viewed through a cranial window using intravital fluorescence video microscopy (IVM). Rhodamine 6G (100μL; 0.02%) was administered i.v. 5 min prior to IVM for visualisation of leukocytes under fluorescence.

Characterised FPR agonists—Ac2-26 (Annexin A1 pharmacophore; 100μg/mouse; n=6 mice/group) and lipoxin A4 (LXA4; 0.11μg or 1.06μg; n=3 mice/group) or its epimer, 15-epi-lipoxin A4 (15-epi-LXA4; 4μg; n=5)—were administered at the start of reperfusion. Inflammation was quantified from leukocyte-endothelial (L-E) interactions: rolling leukocyte flux (mm² sec⁻¹) and velocity (μm sec⁻¹), and leukocyte adhesion (mm²). Data are mean of each group ±SEM and analysed versus vehicle (100μL saline, except for 15-epi-LXA4 where vehicle = ethanol+saline) using an unpaired t test (P<0.05).

With respect to untreated animals, 15-epi-LXA4 caused a dramatic reduction the number of rolling (1.3±0.2 cells mm² sec⁻¹ vs. 0.5±0.2 cells mm² sec⁻¹) and adherent leukocytes (315.4±37.3 cells mm² vs. 53.7±14.7 cells mm²). These levels were comparable to sham-operated animals (0.4±0.1 cells mm² sec⁻¹ and 59.3±20.2 cells mm²). LXA4 produced no effect at the concentrations administered. Ac2-26 caused a marked decrease in cell adhesion (to 80.0±44.7 cells mm²) versus sham levels.

LXA4 and 15-epi-LXA4 are well established as ligands for FPR2/ALX (mouse orthologue Fpr2). Ac2-26 may activate all three human FPRs, therefore the mechanisms behind Ac2-26 anti-inflammatory activity in this model were investigated through use of combined treatment with Ac2-26 plus FPR antagonists Boc2 (pan-antagonist for FPRs; 2.5μg; n=4) or WRW4 (FPR2-selective; 553μg; n=4). Both antagonists eliminated the reduction in adhesion seen in animals treated with Ac2-26 alone (Ac2-26+Boc2, 529.2±48.7 cells mm²; Ac2-26+WRW4, 326.2±50.0 cells mm²). Interestingly, WRW4 administered independently of an agonist significantly increased cell rolling (to 6.7±0.4 cells mm² sec⁻¹), and was the only instance in which rolling velocity was slowed versus vehicle-treated mice (14.4±2.6μm sec⁻¹ vs. 34.7±5.1μm sec⁻¹). This suggests ‘agonistic’ pro-inflammatory properties of WRW4 via Fpr2.
We have shown that FPR agonists 15-epi-LXA₄ and Ac2-26 both reduce L-E interactions in an experimental model of global I/R, and that this activity is through FPR2/ALX (mouse orthologue Fpr2). These novel data suggest that FPR2/ALX may provide a therapeutic target in stroke.