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# Anti-inflammatory And Analgesic Effects Of Endocannabinoids In A Mouse Model Of Acute Arthritis

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Knee joints are known to possess an elaborate endocannabinoid system that helps regulate synovial blood flow and joint pain (McDougall JJ et al, Br J Pharmacol 153: 358, 2008; Schuelert N & McDougall JJ, Arthritis Rheum 58: 145, 2008). Local inhibition of endocannabinoid hydrolysis by intra-articular injection of the fatty acid amide hydrolase (FAAH) inhibitor URB597 has been shown to reduce nociceptor activity and pain behaviour in a rodent model of osteoarthritis. The current study aimed to see whether FAAH inhibition could alter pain perception and leukocyte trafficking in acutely inflamed mouse knee joints.

Male c57/bl6 mice were housed 1-5 per cage in a temperature-controlled environment on a 12hr light-dark cycle. Hindlimb weight bearing was assessed as previously described (McDougall JJ et al, Pain 123: 98, 2006) where the weight borne by the hindlimbs was quantified by an incapacitance tester (measurement duration = 1s). Following a baseline measurement, acute synovitis was induced by intra-articular injection of 2%kaolin/2%carrageenan into the right knee joint with a 24hr recovery period. On the day of testing, mice were treated with either vehicle (10% DMSO; 10% cremophor in 0.9% saline) or URB597 (3mg $kg^{-1}$ s.c.) ± either the $CB_1$ receptor antagonist AM251 (0.2mg $kg^{-1}$ s.c.) or the $CB_2$ receptor antagonist AM630 (0.2mg $kg^{-1}$ s.c.). Weight bearing was then measured at 0, 30, 60, 120 and 180min.

Leukocyte trafficking in synovial blood vessels supplying acutely inflamed knees was measured by intravital microscopy as previously described (Andruski B et al, Am J Physiol 195: R814, 2008). Briefly, deeply anaesthetised mice received an intravenous injection of 0.05% rhodamine 6G to label circulating leukocytes. The knee joint was exposed and a postcapillary venule identified for analysis under a fluorescent microscope. Rolling leukocytes were identified as moving slower than circulating blood, while adherent leukocytes were stationary in the blood vessel for at least 30s. Leukocyte kinetics were measured at 0, 1, 5 10, 20, 30 and 60min after topical administration of either vehicle or URB597. Separate cohorts of animals were also examined with URB597 in the presence of either AM251 or AM630.

Local administration of URB597 to acutely inflamed knees reduced hindlimb incapacitance by 26.8 ±8% 30min after injection ($P<0.05$, $n = 12$, one-way ANOVA). This anti-nociceptive effect of the FAAH inhibitor was blocked by AM251 ($P<0.05$), but not with AM630. With intravital microscopy, URB597 caused a significant inhibition of leukocyte rolling and adhesion ($P<0.0001$) with the maximal effect occurring 20min after administration. Leukocyte rolling was blocked by AM251 and AM630 while adhesion was unaffected by cannabinoid receptor antagonism.

This study shows that local administration of URB597 causes a reduction in pain and leukocyte trafficking in acutely inflamed mouse knee joints. These effects are mediated by articular cannabinoid receptors showing that endocannabinoids could be harnessed to help treat inflammatory joint disease.