Title: Angiotensin II Type 2 Receptor Blockade is a novel target for murine osteoarthritic pain

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Intro: Pain in osteoarthritis (OA) is the main presenting symptom in OA; almost 75% of patients report constant pain (ARUK Report 2017). New targets for pain are an unmet clinical need, with nerve growth factor (NGF) currently at the forefront of novel analgesia development. Recent research suggests that the angiotensin II type 2 (AT2) receptor has a role in neuropathic and chronic inflammatory pain. Several preclinical and clinical studies show analgesic efficacy upon treatment with an AT2 antagonist (Lancet, 2014). The AT2 receptor is expressed by small diameter nociceptive fibres and activation by angiotensin II leads to neurite outgrowth, similar to that seen with NGF (Chakrabarty, J Pain 2013). Here we explore the relationship between NGF and the AT2 pathway and test the analgesic efficacy of AT2 receptor antagonism in a surgical model of murine OA.

Methods: Partial meniscectomy (PMX) or sham surgery was performed in 10-week old C57BL6 mice. Whole knee gene expression was measured at 2, 4, 6, 8, 10, and 12 weeks after surgery. NGF, angiotensinogen (AGT), angiotensin-converting enzyme (ACE), angiotensin II type 1 (AT1) and AT2 receptors were quantified by RT-PCR.

In an accompanying behavioural experiment (n=40 PMX, n=10 Sham), spontaneous behavioural pain responses were assessed using Linton incapacitance testing, which measures the percentage of hindlimb weight distribution. Measurements were initially biweekly until the onset of OA pain, then weekly. At the time of established pain (13 weeks after surgery), animals were treated with the AT2 receptor blocker EMA200 (also known as PD123319 (Tocris)) (n=20 PMX, n=5 Sham) or with vehicle (n=20 PMX, n=5 Sham), over a course of 3 weeks (3 injections in total per mouse). Animals in the treatment group were injected with single doses of 1mg/kg, 3mg/kg, or 10mg/kg EMA200 i.p., whilst the non-treatment group always received a vehicle injection. The injection schedule was randomized, with a week-long wash-out phase before the next injection and the experimenter remained blinded to treatment and operation. Over the course of the experiment each mouse received every dose of drug. Analgesic response was assessed 1hr, 3hrs, and 24hrs after injection.

Results: PMX surgery led to painful behaviour starting at 8 weeks after surgery. Compared to its sham control, NGF expression increased significantly from 8 weeks after PMX surgery compared, corresponding to the time animals first exhibited painful behaviour. AGT was significantly down-regulated at week 2 and week 4 after surgery but increased from 6 weeks post-surgery. There was no mRNA regulation of ACE, AT1 or AT2 receptors over the time course.

In the AT2 receptor blocker study, established pain was abrogated when using 1mg/kg and 3mg/kg doses at 1 and 3 hours after injection. Painful behaviour had returned to baseline by 24 hours. EMA200 at 10mg/kg was not analgesic.

Discussion: We have demonstrated a temporal relationship between painful behaviour and regulation of NGF in a surgical model of murine OA. AT2 receptor blockade was analgesic at low doses but not at 10mg/kg, which may be due to reduced bioavailability of the drug due to solubility issues. When examining the angiotensin pathway only, AGT was regulated in the joint over the post-surgical time course, increasing first at 6 weeks and then remaining significantly elevated during established pain. These results describe a novel target for pain in OA that may be driven by local regulation of AGT in the damaged joint. Whether and how AGT relates to NGF-dependent OA pain is currently unknown.

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