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Research paper

Intraarticular slow-release triamcinolone acetate reduces allodynia in an experimental mouse knee osteoarthritis model

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ABSTRACT

Intraarticular steroid injection has been the mainstay of short-term treatment of knee osteoarthritis (OA) pain. However, the duration of therapeutic effect from a single injection is not as long as desired. In this study we use a viscous formulation of triamcinolone acetate (TCA) in hyaluronic acid to prolong the anti-allodynia effect of that steroid. OA was induced in mice by a partial medial meniscectomy. Over time the animals' developed a mechanical allodynia in the injected leg. Mice were then given a single intraarticular injection of TCA in a short-acting DMSO formulation, or a standard commercial suspension, or the drug formulated in 5% hyaluronic acid for slow-release. Control injections in OA mice were PBS or 5% hyaluronic acid vehicle. Mechanical allodynia was then monitored over the therapeutic period. Organotypic spinal cord slices and DRG culture were performed to assess whether TCA attenuates expressions of pain mediators induced by interleukin 1β. TCA 40 μg in a fast-releasing DMSO formulation produced relief from mechanical allodynia for a few days compared to PBS control injections ($P = 0.007$). Similarly, the commercial suspension of TCA 40 μg also produced relief from mechanical allodynia for a few days compared to PBS control injections ($P = 0.001$). However, TCA 100 μg in 5% hyaluronic acid produced relief from mechanical allodynia for at least 28 days compared to PBS control or 5% hyaluronic acid vehicle injections ($P = 0.0005$). Furthermore, TCA significantly suppressed expression of pain mediators induced by interleukin 1β in spinal cord and DRG organotypic culture. Intraarticular TCA in a sustained release formulation of viscous 5% hyaluronic acid will produce a long-term attenuation of mechanical allodynia in the OA knees of mice.

1. Introduction

Joint degeneration caused by osteoarthritis (OA) leads to chronic knee joint pain that affects >100 million individuals all over the world. Pain accounts for most primary care visits for OA, and is one of the key reasons why people with OA choose to have knee replacement surgery (TKR) (Altman et al., 1995). In the United States alone, there are almost one million TKR each year to treat OA and OA pain, with the projected number of TKR for 2030 expected to reach 3.5 million (Kurtz et al., 2007). Prior to TKR, knee pain patients will have used noninvasive non-surgical alternatives such as systemic NSIADs and analgesics for years. For more severe pain, intraarticular (IA) steroid injections have

Abbreviations: OA, osteoarthritis; TCA, triamcinolone acetate; TKR, knee replacement surgery; IA, intraarticular; DRG, dorsal root ganglion; NGF, nerve growth factor; CGRP, calcitonin gene-related peptide; TNFα, tumor necrosis factor-α; CCL2, chemokine (C-C motif) ligand 2; HA, hyaluronic acid; DMM, destabilization of the medial meniscus; HBSS, Hanks Balanced Salt Solution; IL-1β, interleukin 1β; qPCR, quantitative PCR.

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been the mainstay of short-term treatment of knee OA pain (Dieppe et al., 1980; Valtonen, 1981; Raynauld et al., 2003; Arroll and Goodyear-Smith, 2004). However, the duration of therapeutic effect from a single injection is not as long as needed for a chronic pain condition, (Dieppe et al., 1980; Arroll and Goodyear-Smith, 2004) and so patients may drop the therapy due to injection frequency. To extend the period of pain relief, there are ongoing clinical trials on the efficacy of new IA triamcinolone acetate (TCA) formulations (Petrella et al., 2015; Bodick et al., 2015).

Physiological mechanisms of pain operate at the local joint level, the dorsal root ganglion (DRG) level, spinal level and higher brain processing centers. Several nociceptive molecules are recruited into the OA joint, including nerve growth factor (NGF), calcitonin gene-related peptide (CGRP), tumor necrosis factor-α (TNFα) and chemokine (C-C motif) ligand 2 (CCL2) and can cause activation of peripheral nociceptors (Kc et al., 2016; Dong et al., 2015; Dawes et al., 2013). During chronic OA, the nociceptive system can become sensitized, leading to a heightened sensitivity. Steroids are proven, both scientifically and clinically, to be effective at reducing pain in knee OA. It has been suggested that TCA exert a potent anti-inflammatory effect on joints by inhibiting inflammatory cytokines and thus reduces pain (Evans et al., 2014). However, the mechanism by which TCA alleviates knee OA pain is still not clearly defined.

In this study we use a viscous formulation of triamcinolone acetate (TCA) in hyaluronic acid (HA) to prolong the anti-allodynic effect of that steroid. While the use of HA alone as an IA-injected viscosupplement to reduce knee pain is a controversial issue (Jevsevar et al., 2015), we are using HA to entrap the TCA molecule and thus slow its release. Furthermore, to delineate analgesic mechanism of TCA, we also performed organotypic culture of spinal cord slices and DRG.

2. Methods

2.1. OA model

The study was approved by the IACUC of Rush University Medical Center. OA was induced by partial medial meniscectomy in C57/BL6 mice (20 g) (Knights et al., 2012). Briefly, mice were anesthetized with 1.5% isoflurane (Abbott Laboratories) in oxygen and the left knee was shaved and prepared for aseptic surgery. A medial para-patellar arthrotomy exposed the anterior medial meniscotibial ligament, which was elevated with a microprobe and severed using curved dissecting forceps. The medial meniscus was freed from its attachments to the margin of the tibial plateau and approximately one third of the medial meniscus was removed (approximately 1 mm of tissue). The patella was repositioned, and the skin incision closed with 5-0 polypropylene sutures. This surgically-induced OA model is slightly modified from OA model of destabilization of the medial meniscus, referred to as DMM. In prior studies this medial meniscus transection model has been shown to produce cartilage degeneration and mechanical allodynia by 4 weeks post-surgery (Knights et al., 2012).

2.2. Evaluation of mechanical allodynia

Force withdrawal thresholds, in response to mechanical stimuli, were assessed using von Frey filaments applied to the plantar hindpaw, using an iterative up-down method (Chaplan et al., 1994). Animals were tested prior to and at 3 day intervals after OA induction surgery to determine when mechanical allodynia had developed (force withdrawal threshold < 2 g) (Kc et al., 2016).

2.3. Drug treatment

Once mechanical allodynia developed (at least 6 weeks after surgery), mice were briefly anesthetized with 1.5% isoflurane and given a single percutaneous intraarticular injection, through a 30-gauge needle, of drug or control solution. Withdrawal thresholds were assessed using von Frey filaments until the therapeutic effect had worn off in the drug group (threshold back below 2 g).

Experiment 1 (fast-release; 2 groups): Active drug was TCA powder (Ark Pharm Inc., Libertyville, IL), dissolved in DMSO at 8 mg/mL so a 5 μL injection was 40 μg total dose. Control injection was PBS.

Experiment 2 (slower-release; 2 groups): Active drug was a commercial TCA suspension (Kenalog-10; Bristol-Myers Squibb) at 10 mg/mL so a 4 μL injection was 40 μg total dose. Control injection was PBS.

Experiment 3 (sustained-release; 3 groups): Active drug was 50 mg TCA powder hand-mixed into 2.5 mL (2.5 g) of 5% HA solution producing a clear 20 mg/mL TCA mixture; so a 5 μL injection was 100 μg total dose. To produce the 5% HA vehicle, HA powder (sodium hyaluronate; 1000 kDa; Novozymes, Cincinnati, OH) was dissolved in PBS to produce a very viscous (200,000 cP), but easy to inject clear solution. The 5% HA solution was autoclaved for sterility. One control injection was PBS, and the other 5% HA alone.

The 40 μg TCA dose for Kenalog-10 was based on the manufacturer's recommendation of 2.5 mg to 5 mg (assume 4 mg average) for the initial dose for an IA injection. Assuming a normal mouse knee joint synovial volume of 4–5 μL (based on our preliminary dye-injection experiments) and a normal human knee joint synovial volume of 0.5 mL (Courtney and Doherty, 2005), 100× larger, the equivalent mouse dose was estimated as 4 mg/100 = 40 μg (Experiment 2). We used the same 40 μg dose for the TCA-DMSO injections (Experiment 1). For the TCA-HA injections (Experiment 3) we choose a larger dose, 100 μg, since with the expected release to be spread out over weeks we wanted our daily dose to be above a threshold, so we would have an effect within the first few days after injection (as with TCA-DMSO or TCA-Kenalog).

2.4. Organ culture experiments

Organotypic slice cultures of spinal cord and dorsal root ganglia (DRG) were prepared from adult mice (20 g) as previously described (Aoki et al., 2007; Marsh et al., 2000). After decapitation, the spinal cord and bilateral lumbar DRGs from L1 to L6 were aseptically removed and placed into ice-cold Hanks Balanced Salt Solution (HBSS) (Life Technologies, Carlsbad, CA). Lumbar spinal cords were transversely sliced into 1 mm thickness for spinal cord organ culture. The ganglia and spinal cord slices were transferred in serum free DMEM/F12 (Life Technologies, Carlsbad, CA) containing interleukin 1β (IL-1β) (10 ng/mL) (PeproTech, Rocky Hill, NJ), TCA in DMSO (1 mg/mL) or combination of IL-1β and TCA and incubated for 24 h at 37 °C in a humidified 95% air/5% CO_2 incubator. At the end of the culture period the ganglia and spinal cord slices were harvested, quickly frozen on dry ice, and stored at −80 °C until they were assayed.

2.5. Reverse transcription and quantitative polymerase chain reaction

Total RNA was isolated using the Trizol reagent (Life Technologies, Carlsbad, CA) following the instructions provided by the manufacturer. Reverse transcription (RT) was carried out with 1 μg total RNA using the qScript™ cDNA SuperMix (Quanta Biosciences Inc., Gaithersburg, MD) for first strand cDNA synthesis. For quantitative PCR (qPCR), cDNA was amplified using the MyiQ Real-Time PCR Detection System (Bio-Rad Hercules, CA). Relative mRNA expression was determined using the $^{\Delta\Delta}C_T$ method, as detailed by the manufacturer (Bio-Rad Hercules, CA). GAPDH was used as an internal control. The values represent the mean of three separate experiments. The primer sequences will be provided upon request.

2.6. Statistics

Withdrawal thresholds (g) over time were compared between groups with repeated measures general linear model (SPSS software).