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Synergistic attenuation of chronic pain using mu opioid and cannabinoid receptor 2 agonists

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ABSTRACT

The misuse of prescription opiates is on the rise with combination therapies (e.g. acetaminophen or NSAIDs) resulting in severe liver and kidney damage. In recent years, cannabinoid receptors have been identified as potential modulators of pain and rewarding behaviors associated with cocaine, nicotine and ethanol in preclinical models. Yet, few studies have identified whether mu opioid agonists and CB2 agonists act synergistically to inhibit chronic pain while reducing unwanted side effects including reward liability. We determined if analgesic synergy exists between the mu-opioid agonist morphine and the selective CB2 agonist, JWH015, in rodent models of acute and chronic inflammatory, post-operative, and neuropathic pain using isobolographic analysis. We also investigated if the MOR-CB2 agonist combination decreased morphine-induced conditioned place preference (CPP) and slowing of gastrointestinal transit. Co-administration of morphine with JWH015 synergistically inhibited preclinical inflammatory, post-operative and neuropathic-pain in a dose- and time-dependent manner; no synergy was observed for nociceptive pain. Opioid-induced side effects of impaired gastrointestinal transit and CPP were significantly reduced in the presence of JWH015. Here we show that MOR + CB2 agonism results in a significant synergistic inhibition of preclinical pain while significantly reducing opioid-induced unwanted side effects. The opioid sparing effect of CB2 receptor agonism strongly supports the advancement of a MOR-CB2 agonist combinatorial pain therapy for clinical trials.

1. Introduction

Prescribed opioids, specifically those that bind mu-opioid receptors (MOR), have become some of the most highly abused drugs with deaths from drug overdose rising steadily over the past two decades (CDC, 2015). In 2007, the cost for prescription opioid abuse in the US was estimated at approximately $55.7 billion (Birnbaum et al., 2011); costs in Europe were nearly €4.2 billion (EMCDDA, 2010). Of the 1,244,872 emergency department visits in 2011, almost half involved opioid analgesics (Crane, 2015); 80% of new heroin drug users had previously abused prescription opioids (NPR, 2013). These statistics have led to increases in physician and patient concern over prescribing and using opioids for chronic pain, respectively (Balko, 2012).

Opioids such as morphine derivatives or oxycodone along with a mixture of NSAIDs are commonly prescribed for the treatment of acute and chronic pain (CDC, 2015). Monotherapy opioids are associated with undesirable side effects that include increased somnolence, constipation, cognitive impairment, hyperesthesia, respiratory depression with propensity towards addiction at doses that achieve analgesic efficacy (Chan et al., 1999; Heyman et al., 1988; Koch and Höllt, 2008; Ling et al., 1984). Opioid combination therapies such as Vicodin® (hydrocodone/paracetamol) and Percocet® (oxycodone/paracetamol), although synergistic as analgesics, have been limited due to the significant increase in liver and kidney damage (Mitka, 2014; Watkins et al., 2006). To overcome these opioid dosing obstacles of abuse and toxicity, alternatives to the current opioid or opioid-NSAID combination pain therapies are

Abbreviations: 2-AG, 2-arachidonoylglycerol; %MPE, percent maximal possible effect; A_{50}, potency; a-CSF, artificial spinal fluid; CB1, cannabinoid 1 receptor; CB2, cannabinoid 2 receptor; CPP, conditioned place preference; ED_{50}, effective dose at 50%; HPLC, high performance liquid chromatography; MOR, mu opioid receptor; SNI, spared nerve injury.

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urgently needed.

Cannabinoid 2 receptors (CB2) are G protein-coupled receptors primarily localized on cells within the immune system (Pertwee, 1997). Activation of CB2 by either endogenous or exogenous agonists attenuates both acute and chronic pain by inhibiting inflammation (Kinsey et al., 2011; Wilkerson and Milligan, 2011). Moreover, CB2 agonists have been shown to significantly inhibit thermal/mechanical hypersensitivity and spontaneous pain in preclinical models of neuropathic and bone cancer pain (Ibrahim et al., 2003, 2005; Lozano-Ondoua, 2013; Lozano-Ondoua et al., 2010). Treatment-resistant neuropathic pain disorders including HIV, multiple sclerosis, and chemotherapy-induced neuropathy have responded to cannabinoid intervention (Anand et al., 2009; Fine and Rosenfeld, 2014; Rahn and Hohmann, 2009). Moreover, CB2 agonists do not elicit many adverse effects including rewarding behavior alone and can reduce cocaine (Xi et al., 2011) nicotine (Navarrete et al., 2013) and ethanol-induced (Ortega-Álvaro et al., 2014) rewarding effects. To date, selective CB2 agonists (i.e., GW842166) have been well-tolerated in human clinical trials for the treatment of inflammatory pain (NIH, 2012). These collective data suggest that the co-administration of a cannabinoid and prescribed opioids would inhibit pain without increasing the misuse of opioids (Perron et al., 2015).

To address the increasing need to advance innovative strategies to treat chronic pain, we investigated whether dual-targeting of the mu opioid (MOR) and CB2 receptors would produce synergistic antinociception in preclinical models of acute, inflammatory, post-operative and neuropathic pain. We also tested whether the combination of a CB2 agonist and morphine would reduce morphine-induced constipation, conditioned place preference and dopamine release; critical limitations that must be addressed for successful clinical translation. Studies here strongly suggest that the combination MOR/CB2 agonists result in a synergistic analgesic effect in chronic models of pain while significantly reducing unwanted opioid side effects.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats weighing 250–300 g and male ICR mice weighing 15–25 g were obtained from Envigo (Indianapolis, IN). All procedures were approved by the University of Arizona Animal Care and Use Committee, and conform to the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health. Procedures were also in compliance to the guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and are in accordance to ARRIVE guidelines for reporting experiments involving animals or animal tissue (Curtis et al., 2015; McGrath and Lilley, 2015). Animals were maintained on a 12-hour light/dark cycle in a climate-controlled room and were provided with food and water *ad libitum*. To determine statistical significance, a power analysis was performed using GPower3.1 software to verify the number of animals needed for each experiment (Faul et al., 2009). A total of 273 animals were used herein.

2.2. Drugs

JWH015, a cannabinoid 2 receptor (CB2) agonist (CB_2 $K_i = 13.8$ nM, 30–80 fold selectivity versus CB_1) was obtained from Cayman Chemical (Ann Arbor, MI). JWH015 was dissolved in a vehicle solution of 10% dimethyl sulfoxide, 10% Tween-80, and 80% saline (Sigma, St. Louis, MO). The mu-opioid agonist (MOR) morphine sulfate was purchased from the NIDA Drug Supply program (Rockville, MD) and was dissolved in saline. Formalin solution was obtained by diluting 1.5% of formaldehyde in saline. Ketamine:xylazine (80 mg/kg: 12 mg/kg; Phoenix Pharmaceutical, St. Joseph, MO) was used to anesthetize animals in order to insert microdialysis probes into the nucleus accumbens. The antibiotic gentamicin (Phoenix Pharmaceutical, St. Joseph, MO) was provided as a single subcutaneous dose (8 mg/kg). Drugs were weighed out and dissolved in vehicle daily, prior to use. Cocaine hydrochloride was purchased from the NIDA Drug Supply program (Rockville, MD) and used as a positive control in microdialysis and HPLC studies. Finally, 5% and 2.5% isofluorane (Sigma, St. Louis, MO) mixed in 2.5 L/min of oxygen was delivered through a nose cone and used to induce and maintain anesthesia, respectively, for paw incision and SNI surgeries.

2.3. Antinociceptive responses

2.3.1. Measurement of thermal tail withdrawal latency (tail flick assay)

Male ICR mice were used in all tail flick studies. The distal two-thirds of the tail were immersed in a circulating water bath maintained at 52 °C, and latency to withdraw the tail was recorded (tail withdrawal latencies). Following baseline testing, mice were given a systemic injection of vehicle, morphine (1, 3, 10 mg/kg, i.p.), JWH015 (1, 10, 100 mg/kg, i.p.), or a fixed dose combination of morphine and JWH015. As a control, the vehicle for both morphine and JWH015 were injected and tail withdrawal latencies recorded. Mice were re-tested using the tail flick water bath every fifteen minutes over a 1-hour time course. The maximal effect (cut-off latency) was defined at 10 s, in order to prevent possible tissue damage. Any animal that reached the cut-off latency was returned to their cage and received the maximum latency score.

2.3.2. Measurement of inflammatory pain (formalin flinch test)

The formalin flinch test is a well-recognized, acute inflammatory pain assay characterized by a biphasic response (Tjølsen et al., 1992). Male ICR mice received an intraplantar injection of 1.5% formalin into their left hind paw 15 min before testing. Animals were placed in a Plexiglas chamber and the number of flinches observed was recorded for one hour in 5 min bins. Flinching behavior was characterized as a rapid upward movement of the injected hind paw. As a control, vehicle only (saline) was injected into paws, and flinches recorded. Mice were evaluated for antinociception after systemic (intraperitoneal, i.p.) application of drug treatment as compared to vehicle control. Systemic injections of vehicle, morphine (0.1, 0.3, 0.6, 1 mg/kg, i.p.), JWH015 (0.1, 1, 3, 10, 30, 100 mg/kg, i.p.) or a fixed dose combination of morphine and JWH015 were given to separate mice. The number of flinches in all groups were reassessed for 1 h. Flinches recorded from 0 to 10 min after formalin is noted as the first phase while the second phase of flinching was recorded from 11 to 60 min.

2.3.3. Measurement of post-operative pain

For paw incision, male Sprague-Dawley rats were induced at 5% and maintained on 2.5% isoflurane mixed in 2.5 L/min of air delivered through a nose cone. A 1-cm longitudinal incision was made through the skin and fascia on the plantar surface of the left hind paw. The plantaris muscle was elevated and incised longitudinally. Following incision, the muscle remained intact and the skin was affixed with two 3-0 silk sutures. Rats were allowed to recover in their home cages for a 24-hour period before they were behaviorally tested (Brennan et al., 1996). Animals were administered morphine (1, 3, 10 mg/kg, i.p.), JWH015 (1, 3, 10 mg/kg, i.p.) or a fixed dose combination of morphine and JWH015 for behavioral assays and compared to vehicle-treated animals. Behavioral assays included testing for thermal and mechanical hypersensitivity in