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Activation of cannabinoid receptor 2 attenuates synovitis and joint distruction in collagen-induced arthritis

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ABSTRACT

Objectives: Recent studies have suggested immunomodulatory and anti-inflammatory effects of cannabinoid receptor 2 (CB2R) activation, which is devoid of psychoactivity. We have demonstrated the expression of CB2R in synovial tissue from patients with rheumatoid arthritis (RA), and its specific activation shows inhibitory effects on fibroblast-like synoviocytes. However, it is still unclear whether selective activation of CB2R inhibits joint inflammation or protects joint damage in RA.
Methods: A murine model of collagen-induced arthritis (CIA) was used to evaluate the therapeutic efficacy of HU-308, a selective CB2R agonist. The disease severity was evaluated by semi-quantitative scoring of joint swelling, histological assessment of joint inflammation and structure, and radiographic assessment of joint destruction by using digital plain radiographs and micro-CT scans. The concentrations of various isotypes of anti-collagen II antibodies in sera and the levels of cytokines in culture supernatants were determined by ELISA.
Results: Compared with vehicle treatment, protective treatment with intraperitoneal injection of HU-308 (0.3–1.0 mg/kg) failed to decrease the incidence of the development of CIA, but it effectively suppressed the severity of the disease. In CIA mice, treatment with HU-308 significantly decreased joint swelling, synovial inflammation, and joint destruction, as well as serum levels of anti-collagen II antibodies. *In vitro*, HU-308 (1–10 μM) significantly suppressed the production of proinflammatory cytokines IL-6 and TNF-α from lipopolysaccharide-stimulated murine peritoneal macrophages with intact CB2R in dose-dependent manners. HU-308 failed to elicit any inhibitory effect of on lipopolysaccharide-stimulated macrophages from CB2R-knockout mice.
Conclusions: Activation of CB2R by HU-308 has therapeutic potential for RA to suppress synovitis and alleviate joint destruction by inhibiting the production of autoantibodies and proinflammatory cytokines.

Introduction

Rheumatoid arthritis (RA) is an immune-mediated inflammatory disease of unknown etiology, which is characterized by chronic inflammatory infiltration of the synovium, leading to eventual cartilage and bone destruction (Scott et al. 2010). In spite of significant improvements in the treatment of RA, there is still a need for the identification of new pathways involved in the modulation of inflammation in order to further increase efficacy, particularly in patients in whom the disease does not respond to current therapies.

Recently, the discovery of endocannabinoid system, especially two subtypes of cannabinoid receptors, has raised a great deal of interest in inflammatory diseases, including multiple sclerosis and RA (Klein 2005). Two accredited types of cannabinoid receptors, cannabinoid receptor 1 (CB1R) and cannabinoid receptor 2 (CB2R), were discovered in the early 1990s (Matsuda et al. 1990; Munro et al. 1993). CB1R exists primarily on central and peripheral neurons, and is associated with the psychoactive effects of cannabinoids. CB2R is predominantly expressed by cells of hematopoietic origin and is thought to mediate cannabinoid-induced immune modulation (Howlett et al. 2002). The level of their expression is

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dependent on the activation state of the cell and the type of stimulus (Lee et al. 2001). This type of distribution supports the prospect that anti-inflammatory and immunosuppressive CB2R-selective drugs without psychoactivity can be developed for the management of chronic inflammatory diseases. Indeed, selective CB2R agonists display beneficial anti-inflammatory and positive effects on experimental autoimmune encephalomyelitis (Kong et al. 2014; Ribeiro et al. 2013), atherosclerosis (Chiurchiu et al. 2014), endotoxin-induced sepsis (Gui et al. 2013), cystitis (Wang et al. 2014), liver disease (Guillot et al. 2014), pancreatitis (Michler et al. 2013), inflammatory bowel disease (Storr et al. 2009), and uveitis (Toguri et al. 2014).

Animal studies with cannabinoid-based drugs targeting joint disease were first reported in 1998, showing that ajulemic acid significantly reduced the severity of joint inflammation and synovitis in adjuvant-induced arthritis (Zurier et al. 1998). However, ajulemic acid has been demonstrated to have psychotropic effects (Dajani et al. 1999), because it binds to CB1R (Rhee et al. 1997). Studies on the role of selective CB2R activation in arthritis are, however, lacking.

HU-308 is a synthetic cannabinoid, and the affinity of HU-308 binding to CB2R is almost 300 folds of the affinity to CB1R (Hanus et al. 1999). So HU-308 is a novel specific agonist of CB2R. Functional studies show it inhibits forskolin-stimulated cyclic AMP production in CB2R-transfected cells, whereas it shows little effect in CB1R-transfected cells. Furthermore, HU-308 shows no psychoactivity (Hanus et al. 1999).

In 2008, the endocannabinoids, anandamide and 2-arachidonoylglycerol, were identified in the synovial fluids of RA patients, whereas neither of them was detected in the synovial fluids from normal volunteers (Richardson et al. 2008). This finding further makes the endocannabinoid system an attractive therapeutic target of RA. Moreover, we have demonstrated that both mRNA and protein of CB2R are expressed in synovial tissue and cultured fibroblast-like synoviocytes (FLS) isolated from RA patients, and the expression level of CB2R is upregulated by proinflammatory mediators. *In vitro*, HU-308 inhibited IL-1β-induced proliferation of RA-FLS as well as IL-1β-induced production of MMP-3, MMP-13 and IL-6 in RA-FLS in dose-dependent manners (Gui et al. 2014). These findings were highlighted as a clue to role of cannabinoid receptors in RA by a comment in *Nature Review Rheumatology* (Ray 2014). In addition, CB2R-deficient mice were characterized by increased osteoclast (the bone-resorbing cell) number. Activation of the CB2R by HU308 restrained trabecular osteoclastogenesis, by inhibiting proliferation of osteoclast precursors and expression of receptor activator of NF-κB ligand (RANKL) in bone marrow-derived osteoblasts/stromal cells (Ofek et al. 2006). HU-308 also attenuated ovariectomy-induced bone loss through the respective suppression of osteoclast number and stimulation of endocortical bone formation. No protective effects of HU-308 were observed in ovariectomized CB2R-knockout mice (Sophocleous et al. 2011), further supporting only CB2R mediates the effect of HU-308. These data imply that selective activation of CB2R may inhibit bone erosion caused by osteoclasts in RA. However, it is still unclear whether selective activation of CB2R inhibits joint inflammation or protects joint damage in RA.

In the present study, we demonstrated a dramatic amelioration of symptoms and a marked alleviation of joint destruction in mice with collagen-induced arthritis (CIA), an animal model of RA, by treatment with HU-308. To elucidate the underlying mechanisms, we extensively studied the effect of HU-308 on the production of autoantibodies, and proinflammatory cytokines. We also reconfirm the specificity of HU-308 by using CB2R-knockout macrophages. Such insights would enable the development of optimal strategies for RA treatment.

Materials and methods

Reagents

HU-308 was purchased from Tocris Bioscience (Bristol, UK), and lipopolysaccharide (LPS) was from Sigma (St. Louis, MO, USA). Bovine type II collagen (CII), complete Freund's adjuvant and incomplete Freund's adjuvant were purchased from Chondrex (Redmond, WA, USA). Trizol, Oligo $d(T)_{18}$ primers, dNTP mixture, ribonuclease inhibitor, reverse transcriptase M-MLV, premix Ex Taq, SYBR premix Ex Taq were all purchased from TaKaRa Biotechnology (Dalian, China).

Animals

Male DBA/1 mice (8 weeks old) were obtained from SLRC laboratory animal Co. Ltd. (Shanghai, China). CB2R gene knockout ($CB2R^{-/-}$) mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and expanded under specific pathogen-free conditions at laboratory animal centre of Second Military Medical University. All animals were fed standard mouse chow and water *ad libitum*, and maintained under constant conditions (temperature: 20–25 °C; humidity: 40–60%; light/dark cycle: 12 h). All procedures were conducted in accordance with the university guideline and approved by ethical committee for animal care and the use of laboratory animals of Second Military Medical University.

Induction and assessment of arthritis

Bovine CII (4 mg/ml in 0.05 M acetic acid) was mixed in an equal volume of complete Freund's adjuvant containing 4 mg/ml of heat-inactivated *Mycobacterium tuberculosis*. The male DBA/1 mice were immunized intradermally at the base of tail with 50 μl of emulsion (100 μg CII) on day 0. On day 21, a booster immunization was given intraperitoneally (*i.p.*) with emulsion of bovine CII and incomplete Freund's adjuvant. From day 20, joint inflammation was graded daily on a scale of 0–4 per paw using a semiquantitative scoring system, where 0 = no erythema or swelling, 1 = erythema and mild swelling confined to the tarsal or ankle joint, 2 = erythema and mild swelling extending from the ankle to the tarsal, 3 = erythema and moderate swelling extending from the ankle to the metatarsal joints, and 4 = erythema and severe swelling encompass the ankle, foot and digits, or ankylosis of the limb. All four limbs of a mouse were assessed, and the maximum score for each animal was 16.

To investigate the therapeutic effects of HU-308, 30 CIA mice were randomly divided into 3 groups ($n = 10$), and were injected *i.p.* daily with HU-308 (0.3 or 1.0 mg/kg) or vehicle from day 20 (one day before the booster immunization) for 7 days. On day 38, all the mice were killed with an overdose of anesthetic, and the paws were removed for histological analysis and radiographic assessments.

Radiographic assessments

On day 38, hind paws of normal mice and CIA mice were collected for radiographic evaluation. High-resolution digital plain radiographs (24 kV, 40 mAs) of hind paws were taken using MX-20 Cabinet X-ray System (Faxitron, USA). The severity of bone erosion was ranked with the radiological score system as follows: 0, normal with intact bony outlines and normal joint space; 1, slight abnormality with any one or two of the exterior metatarsal bones showing slight bone erosion; 2, definite early abnormality with any 3–5 of the exterior metatarsal bones showing bone erosion; 3, medium destructive abnormality with all the exterior metatarsal bones as well as any one or two of the interior metatarsal bones showing definite bone erosions; 4, severe destructive abnormality with all the metatarsal bones showing definite bone erosion and