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Immunopharmacology and inflammation

The monoacylglycerol lipase inhibitor JZL184 decreases inflammatory response in skeletal muscle contusion in rats

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ABSTRACT

Muscle wound healing process is a typical inflammation-evoked event. The monoacylglycerol lipase (MAGL) inhibitor (4-nitrophenyl)4-[bis(1,3-benzodioxol -5-yl)-hydroxymethyl]piperidine-1-carboxylate (JZL184) has been previously reported to reduce inflammation in colitis and acute lung injury in mice, which provide a new strategy for primary care of skeletal muscle injury. We investigated the effect of JZL184 on inflammation in rat muscle contusion model, and found decreased neutrophil and macrophage infiltration and pro-inflammatory cytokine expression. With extension of post-traumatic interval, myofiber regeneration was significantly hindered with increased collagen types I and III mRNAfibroblast infiltration as well as promoted fibrosis. Furthermore, 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-morpholin-4-ylpyrazole-3-carboxamide (AM281, a selective cannabinoid CB_1 receptor antagonist) and [6-iodo-2-methyl-1-(2-morpholin-4-ylethyl)indol-3-yl]-(4-methoxyphenyl)methanone (AM630, a selective cannabinoid CB_2 receptor antagonist) treatment alleviated the anti-inflammatory effect of JZL184. Our findings demonstrate that JZL184 is able to inhibit the inflammatory response and interfere with contused muscle healing, in which the anti-inflammatory action may be mediated through cannabinoid CB_1 and CB_2 receptors.

1. Introduction

Muscle injuries are one of the most common injuries, which are usually caused by contusion, strain or laceration (Jarvinen et al., 2007). After injury, muscle repair process can be temporally divided into three overlapping phases, the acute inflammatory and degenerative phase, the repair phase, and the remodeling phase. Inflammatory cells play an important role in the event. They phagocytize necrotic tissue and activate myogenic cells to differentiate and fuse into new myofibers (Baoge et al., 2012). However, the early inflammatory response after injury may be excessive and cause edema, resulting in anoxia and further cell death (Paoloni et al., 2009).

Monoacylglycerol lipase (MAGL) is a serine hydrolase that preferentially hydrolyzes monoacylglycerols to glycerol and fatty acid. In the endogenous cannabinoid system, MAGL is mainly responsible for hydrolyzing 2-arachidonoyl glycerol (2-AG), an endogenous ligand for cannabinoid CB_1 and CB_2 receptors (Labar et al., 2010; Mulvihill and Nomura, 2013). MAGL releases arachidonic acid for the synthesis of pro-inflammatory eicosanoids by hydrolyzing 2-AG in certain tissues such as the brain, liver, and lung (Nomura et al., 2011), which suggests that MAGL is closely involved in inflammatory response. Long et al. (2009a, 2009b) described a potent and selective inhibitor for MAGL termed (4-nitrophenyl) 4-[bis(1,3-benzodioxol-5-yl)-hydroxymethyl]piperidine-1-carboxylate (JZL184) in mice, which decreases 2-AG hydrolysis both in the central nervous system and peripheral tissues. Accumulated evidence demonstrates the protective and therapeutic effect of JZL184 on diseases having an inflammatory component. JZL184 treatment reduces inflammatory nociception through the activation of both cannabinoid CB_1 and CB_2 receptors in mice and rats (Ghosh et al., 2013; Guindon et al., 2011). In addition, JZL184 mitigates the inflammatory response in trinitrobenzene sulfonic acid-induced colitis and lipopolysaccharide-induced acute lung injury by a cannabinoid CB_1 and CB_2 receptor-dependent manner (Alhouayek et al., 2011; Costola-de-Souza et al., 2013). Since muscle wound healing is a typical inflammation-evoked event, we hypothesized that MAGL inhibition might alleviate inflammatory response of injured skeletal muscles, which may subsequently affect the skeletal muscle wound healing. In this study, we verified the hypothesis using JZL184 in a rat muscle contusion model.

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Using selective cannabinoid CB_1/CB_2 receptor antagonists, we also attempted to determine whether the effects observed after MAGL inhibition by JZL184 treatment were associated with activation of the cannabinoid receptors.

2. Materials and methods

2.1. Animal model of skeletal muscle contusion

All animal protocols were conformed to the "Principles of Laboratory Animal Care" (National Institutes of Health Publication no. 85-23, revised 1985) that sought to minimize both the number of animals used in a procedure and any suffering that they might experience, and were performed according to the Guidelines for the Care and Use of Laboratory Animals of China Medical University. A reproducible muscle contusion model in rats was described previously (Yu et al., 2010). Briefly, adult Sprague–Dawley male rats weighing 280–300 g were anesthetized by intraperitoneal injection with 2% sodium pentobarbital (30 mg/kg). The right hindlimb was positioned on a board in a prone position by extending the knee and dorsiflexing the ankle to 90°, and a single impact at velocity of 3 m/s was delivered to the gastrocnemius and soleus of the right posterior limb. The size of impact interface of the counterpoise (weighing 500 g) was 1.127 cm^2. After injury, each rat was housed individually and kept under a 12 h light–dark cycle. Rats were fed with commercial rat chow and water ad libitum. Rats were killed by intraperitoneal injection of an overdose of sodium pentobarbital. Gastrocnemius was taken and equally divided into two blocks. One block was used for morphological evaluation, and another was used for molecular biological assays. No bone fracture was detected at dissection.

2.2. Experimental protocol and grouping

Experiment 1: 60 rats were randomly divided into 2 groups (30 rats/group) according to treatments with JZL184 (Cayman Chemical, MI, USA) or vehicle alone. JZL184 (10 mg/kg, 2 ml/kg) was injected intraperitoneally immediately after contusion once a day for 5 days. Rats injected with equal volume of vehicle were used as control. JZL184 was dissolved in a vehicle containing saline:DMSO:Tween-80 in an 18:1:1 ratio. JZL184 dose and treatment were based on previous studies (Kerr et al., 2013; Long et al., 2009a, 2009b; Sciolino et al., 2011). Rats were killed at 1, 3, 5, 7, 9 and 14 days after contusion (5 rats at each time point in vehicle- or JZL184-treated group).

Experiment 2: 30 rats were divided into two groups that received JZL184 (10 mg/kg, 2 ml/kg) or vehicle. Within each group, the rats were further divided into 3 groups that received one of the following treatments 30 min before JZL184 or vehicle (5 rats in each group): 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N00 -morpholin-4-ylpyrazole-3-carboxamide (AM281, a selective cannabinoid CB_1 receptor antagonist, Tocris Bioscience, Ellisville, MO, USA, 3 mg/kg, 2 ml/kg), [6-iodo-2-methyl-1-(2-morpholin-4-ylethyl)indol-3-yl]-(4-methoxyphenyl)methanone (AM630, a selective cannabinoid CB_2 receptor antagonist, Tocris Bioscience, Ellisville, MO, USA, 3 mg/kg, 2 ml/kg) or vehicle. AM281 and AM630 were dissolved in the same vehicle as JZL184. AM281 and AM630 were chosen and administered based on previous studies (Arevalo-Martin et al., 2012; Costola-de-Souza et al., 2013; Sciolino et al., 2011; Yang et al., 2014). The rats were killed at 3 days after injury.

2.3. Immunohistochemical staining and morphometric analysis

The skeletal muscle specimens were immediately fixed in 4% paraformaldehyde in phosphate-buffered saline (pH 7.4). Fixed muscle specimens were embedded in paraffin. 5 μm-thick sections were prepared. The primary antibodies against rabbit anti-myeloperoxidase (MPO) polyclonal antibody (dilution 1:500; ab65871, Abcam, Cambridge, UK), mouse anti-CD68 monoclonal antibody (dilution 1:100; ab31630, Abcam, Cambridge, UK) or mouse anti-alpha smooth muscle actin (α-SMA) monoclonal antibody (dilution 1:200; ab7817, Abcam, Cambridge, UK) were used. Histostain-Plus kit (Zymed Laboratories, South San Francisco, CA, USA) was used according to the manufacturer's instructions. As immunohistochemical controls, some sections were incubated with normal rabbit/mouse IgG or phosphate-buffered saline (pH 7.4) in place of the primary antibodies. Nuclei were counterstained with hematoxylin. In addition, hematoxylin-eosin (H&E) staining and Masson's trichrome staining were conventionally conducted. Sections containing the largest contusion area were evaluated. MPO, CD68 or α-SMA positive cells were counted under the 400-fold magnification in the contusion zones. Five fields in the contused zones were randomly chosen for the calculations or evaluations in each section. The nucleus number of regenerating myofibers and collagen deposition areas were analyzed under the 200-fold magnification in the contusion zones. All measurements and data analysis were performed independently by two pathologists in a blind manner. Morphometrical analysis was performed using Image-Pro Plus 6.0 (Media Cybernetics, Rockville, USA).

2.4. Protein extraction and immunoblotting assay

The skeletal muscle samples were ground into powder with liquid nitrogen using a grinder and homogenized with a sonicator in RIPA buffer (sc-24948, Santa Cruz Biotechnology, CA, USA) containing protease inhibitors at 4 °C. After being prepared via standard procedures, protein samples (30 μg) were separated on 12% sodium dodecyl sulfate polyacrylamide electrophoresis gel and were transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). Then they were used to perform immunoblotting. Rabbit anti-IL-1β polyclonal antibody (dilution 1:1000; ab1832p, Chemicon, ON, Canada), rabbit anti-IL-6 polyclonal antibody (dilution 1:500; ab6672, Abcam, Cambridge, UK) and rabbit anti-TNF-α polyclonal antibody (dilution 1:1000; ab1837p, Chemicon, ON, Canada) were applied as primary antibodies, and rabbit anti-GAPDH polyclonal antibody (dilution 1:1000; ab37168, Abcam, Cambridge, UK) was used for relative protein quantification. The horseradish peroxidase conjugated goat anti-rabbit IgG (sc-2004, Santa Cruz Biotechnology, CA, USA) was diluted to 1:2000 and applied. The blotting was visualized with western blotting luminol reagent (sc-2048, SantaCruz Biotechnology, CA, USA) and by the Electrophoresis Gel Imaging Analysis System (MF-ChemiBIS 3.2, DNR Bio-Imaging Systems, ISR). Subsequently, densitometric analyses of the bands were semi-quantitatively conducted using Scion Image software (Scion Corporation, MD, USA).

2.5. Cytokine and chemokine analysis

A Quantibody Rat Inflammation Array 1 (QAR-INF-1-4, RayBiotech, Inc, Norcross, GA) was used to simultaneously detect the expression of interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-13, monocyte chemoattractant protein 1 (MCP-1), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) in the contused muscles. The assays were performed according to the manufacturer's instructions. Each cytokine was arrayed in quadruplicate, together with positive and negative controls. Fluorescent signals were visualized with a laser scanner (Axon GenePix; Molecular Devices, Sunnyvale, CA) set. Data were extracted with RayBio Q Analyzer software (RayBiotech, Inc, Norcross, GA). After subtracting background signals and normalization to positive controls, comparison of signal intensities for antigen-specific antibody spots between groups was utilized to determine relative differences in expression levels of each sample.

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