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Original article

Activation of type 2 cannabinoid receptor (CB2R) by selective agonists regulates the deposition and remodelling of the extracellular matrix

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ABSTRACT

Remodelling of the extracellular matrix and accumulation of fibronectin and collagen type I play critical roles in scar formation following glaucoma filtration surgery. The transforming growth factor β1 (TGF-β1) signal transduction pathway is involved in this process in human Tenon's fibroblasts (HTFs). The type 2 cannabinoid receptor (CB2R) is an important member of the cannabinoid receptor family of G protein-coupled receptors. In this study, we investigated the effects of the CB2R agonists HU308 and JWH133 on the deposition of newly formed extracellular matrix (ECM) and the contractility of HTFs. CB2R was expressed in HTFs. Notably, the CB2R agonists HU308 and JWH133 ameliorated TGF-β1-induced generation of fibronectin, types I and III collagen, and the expression of matrix metalloproteinase 1 (MMP-1) and MMP-3. In addition, the CB2R agonists HU308 and JWH133 ameliorated TGF-β1-induced matrix contraction and remodelling in a dose- and time-dependent manner, respectively. HU308 and JWH133 also suppressed the TGF-β1-induced activation of mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinase 1/2 (ERK1/2), p38, and c-Jun N-terminal kinase (JNK). Based on our results, agonistic activation of CB2R exerts a protective effect on scarring during the healing of wounds from glaucoma filtration surgery.

1. Introduction

Excessive subconjunctival fibrosis and postoperative scarring cause failure of glaucoma filtration surgery [1]. Human Tenon's fibroblasts (HTFs) play an essential role in subconjunctival wound healing and have been considered the main effector cell involved in the scarring response. Complex molecular events at the operation site, such as excessive cell proliferation [2] and enhanced expression of extracellular matrix (ECM) proteins [3], are associated with the wound healing response. Excessive proliferation of HTFs stimulates the secretion of numerous ECM components, including collagenous and elastic fibres, leading to excessive subconjunctival fibrosis in the connective tissue. Excessive deposition of ECM components and aberrant contraction of subconjunctival tissue resulting from HTF-mediated activities at the wound site play critical roles in cases with significant surgical failure [4]. The cytokine transforming growth factor-β (TGF-β) has been reported to be involved in the pathological process of scar formation. Based on accumulating evidence, TGF-β1 markedly elevates the production of fibronectin and collagen type I by regulating the expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in HTFs [5]. Notably, MMPs and TIMPs may be the most important enzyme system involved in the pathogenesis of conjunctivochalasis. Significant upregulation of MMP-1 and MMP-3 expression has been observed in both conjunctival tissues and fibroblasts [6]. MMP-1, also known as interstitial collagenase, cleaves the triple helix of fibrillar collagens I, II, and III. MMP-3, also known as stromelysin-1, degrades collagens III, IV, IX, and X, laminin, proteoglycans, and fibronectin. Interestingly, a TGF-β1 treatment activates the mitogen-activated protein kinase (MAPK) signalling pathway in HTFs [7]. Activation of the MAPK pathway by TGF-β1 is associated with the TGF-β-stimulated expression of collagen types I and III in HTFs. Notably, the MAPK pathway has been reported to play a crucial role in scar formation [8]. In addition, overproduction of TGF-β1 is associated with tissue fibrosis and ECM remodelling in subconjunctival tissues [9]. Inhibition of fibrosis in HTFs mediated by the TGF-β signal transduction pathway has been considered as an important strategy for the prevention of postoperative scar formation [10].

The biological effects of cannabinoids are mainly mediated by two cannabinoid receptor subtypes, type 1 cannabinoid receptors (CB1R) and type 2 cannabinoid receptors (CB2R), both of which are important

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members of the cannabinoid receptor family of G protein-coupled receptors [11]. CB2R is predominantly expressed on immune cells, whereas CB1R is mainly expressed in the brain [12]. As a $G_{i/o}$ protein-coupled protein, CB2R modulates various intracellular signal transduction pathways. The expression level of CB2R has been reported to increase in tissues upon pathological stimulation [13]. Activation of CB2R by highly selective CB2R agonists regulates antigen presentation, cytokine production and apoptosis in response to extracellular stress [14]. CB2R is expressed on the surface of microglial cells in several pathological conditions. Selective CB2R stimulation inhibits microglial reactivity and promotes a neuroprotective phenotype. Palmitoylethanolamide (PEA), an endocannabinoid (eCB)-like compound, was recently shown to induce morphological changes associated with a reactive microglial phenotype, including increased phagocytosis and migratory activity, by upregulating CB2R expression in mononuclear phagocytic cells [15]. HU308 and JWH133 are the two most important CB2R-selective agonists used to study the physiological roles of CB2R in the pathological processes of various diseases [16]. For example, the selective CB2R agonist HU-308 ameliorates synovitis and joint destruction in collagen-induced arthritis by suppressing the production of the proinflammatory cytokines interleukin 6 (IL-6) and tumour necrosis factor (TNF)-α from lipopolysaccharide-stimulated murine peritoneal macrophages expressing intact CB2R in a dose-dependent manner [17]. The CB2R-selective agonist AM1241 ameliorates myocardial interstitial fibrosis by inhibiting the TGF-β1/Smad3 signal transduction pathway [18]. However, no previous reports have described the involvement of CB2R in the deposition and remodelling of the ECM and its preventative effects on subconjunctival fibrosis in cells and tissues during wound healing. In the current study, we investigated the expression patterns of CB2R in primary cultured human Tenon's fibroblasts (HTFs) and examined whether activation of CB2R by the agonists HU-308 and JWH133 affected TGF-β1-induced ECM synthesis and remodelling.

2. Materials and methods

2.1. Cell culture and exposure to TGF-β1

All experiments were conducted in compliance with the basic principles of the Declaration of Helsinki. The experimental protocol used in this study was approved by the Human Ethics Committee of Nanjing Medical University. Written informed consent was obtained from all subjects. Small Tenon's capsule specimens were excised from 10 individuals during strabismus surgeries. Tenon's capsule fibroblasts were isolated as previously described [19]. Isolated cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin) at 37 °C in a 5% CO_2 environment. Recombinant TGF-β1 was obtained from R & D Systems (Minneapolis, MN, USA). HU308 (#3088, R & D Systems, US); JWH133 (#1343/10, R & D Systems, US); AM630 (#1120/10, R & D Systems, US) were dissolved in dimethyl sulphoxide (DMSO) at the concentration of 5 mM and stored at −20 °C. HTFs were incubated with HU308 (2.5 μM in DMSO) or JWH133 (0.5 μM in DMSO) for 24 h prior to exposure to TGF-β1 (2 ng/ml) for 3 d to determine the effects of the CB2R-selective agonists HU308 and JWH133 on ECM deposition.

Cells were first incubated in a collagen gel for 24 h in the absence or presence of HU308 at concentrations of 0.5, 2.5, and 10 μM or JWH133 at concentrations of 0.1, 0.5, and 2 μM, and then incubated in the absence or presence of TGF-β1 (2 ng/ml) for 3 d to determine the concentration-dependent effects of HU308 and JWH133 on TGF-β1-induced collagen gel contraction mediated by HTFs. Cells were incubated in collagen gel with 2.5 μM HU308 or 0.5 μM JWH133 for the indicated times (1, 2, 3, or 4 d) in the absence or presence of transforming growth factor-β1 (TGF-β1; 2 ng/ml) to determine the time-dependent effects of HU308 and JWH133 on TGF-β1-induced collagen gel contraction mediated by HTFs.

2.2. Collagen gel contraction experiment

A mixture of relaxed HTFs and free-floating collagen gel was prepared as previously described [20]. HTFs at a density of 1.1×10^6 cells/ml, 3 mg/ml type I collagen, Modified Eagle's Medium (MEM) (10×), and reconstitution buffer were mixed together at a volume ratio of 2:7:1:1. The mixture was then placed in a 24-well cell culture plate (0.5 mL/well) coated with 1% bovine serum albumin (BSA). After gelation, the collagen gel was detached from the wall and the bottom of the culture plate. Then, 0.5 mL of serum-free MEM containing TGF-β1 and/or HU308 and JWH133 was placed on top of each gel. The degree of gel contraction was indexed based on daily measurements of the gel diameter.

2.3. Reverse-transcription polymerase chain reaction (RT-PCR)

Total intracellular RNA was isolated from cells using Qiazol reagent (Qiagen, Germany). The quality and concentration of isolated RNA were evaluated using a NanoDrop spectrophotometer (Thermo Scientific, USA). Equal amounts of total intracellular RNA (2 μg) from each sample were transcribed into cDNAs using a TaqMan reverse transcription kit (Thermo Fisher Scientific, USA). The expression patterns of CB1R and CB2R genes in HTFs were determined by PCR using synthesized cDNAs. The following primers were used in this study: human CB1R forward, (5′-CCTTTTGCTGCCTAAATCCAC-3′), human CB1R reverse (5′-CCACTGCTCAAACATCTGAC-3′), human CB2R forward, (5′-TCAACCCTGTCATCTATGCTC-3′), human CB2R reverse (5′-AGTCAGTCCCAACACTCATC-3′), human β-actin forward (5′-TGACCCAGATCATGTTTGAG-3′), human β-actin reverse (5′-TTAATGTCACGCACGATTTCC-3′).

2.4. Western blot

Cell lysates were extracted from HTFs using cell lysis buffer (Sigma-Aldrich, USA) and incubated on ice for 10 min. Protein lysates were quantified using a BCA protein assay kit (Bio-Rad Laboratories, USA). Protein samples were resolved by SDS-PAGE and blotted onto PVDF membranes. PVDF membranes were then blocked with 5% non-fat milk. Blots were sequentially incubated with primary antibodies and horseradish peroxidase (HRP)-conjugated secondary antibodies overnight (1:1000; Cell Signaling Technology, USA). Blots were developed using an enhanced chemiluminescent system (Pierce Biotechnology, USA). The following antibodies were used in this study: CB1R rabbit monoclonal antibody (1:1000, #93815, Cell Signaling Technology, USA), CB2R rabbit polyclonal antibody (1:500, #ab3561, Abcam, USA), collagen type I rabbit monoclonal antibody (1:500, #84336, Cell Signaling Technology, USA); collagen type III rabbit polyclonal antibody (1:500, #ab7778, Abcam, USA), β-actin mouse monoclonal antibody (1:10,000, #ab8226, Abcam, USA), MMP-1 rabbit polyclonal antibody (1:2000, #ab38929, Abcam, USA), MMP-3 rabbit polyclonal antibody (1:2000, #ab53015, Abcam, USA), p-ERK1/2 rabbit polyclonal antibody (1:2000, #9101, Cell Signaling Technology, USA), ERK1/2 rabbit monoclonal antibody (1:5000, #9102, Cell Signaling Technology, USA), p-p38 rabbit monoclonal antibody (1:1000, #4511, Cell Signaling Technology, USA), p38 rabbit monoclonal antibody (1:3000, #8690, Cell Signaling Technology, USA), p-JNK rabbit monoclonal antibody (1:1000, #4668, Cell Signaling Technology, USA), and JNK rabbit monoclonal antibody (1:5000, #9252, Cell Signaling Technology, USA).

2.5. Statistical analysis

Experimental results are expressed as means ± standard errors of the means (SEM) using unpaired *t*-tests or one-way or two-way ANOVA with multiple correction tests. $P < 0.05$ was considered a significant difference.

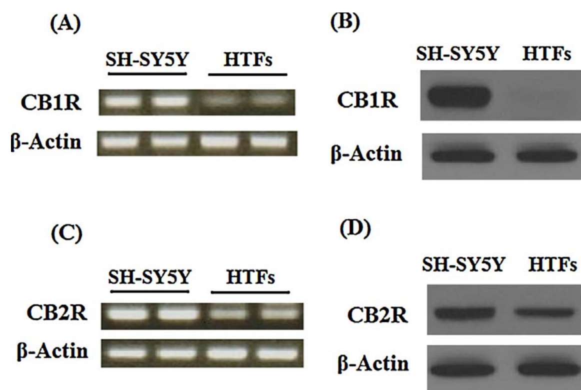

Fig. 1. Expression of CB1R and CB2R in human Tenon's fibroblasts (HTFs). (A) The expression of the CB1R mRNA was determined using RT-PCR. Human SH-SY5Y cells were used as a positive control. (B) Western blot analysis of the expression of the CB1R protein. (C) The expression of the CB2R mRNA was determined using RT-PCR. Human SH-SY5Y cells were used as a positive control. (D) Western blot analysis of the expression of the CB2R protein. Experiments were repeated 3 times.

3. Results

3.1. CB2R is expressed in HTFs

Firstly, the expression patterns of CB1R and CB2R were evaluated in HTFs. According to the results of the RT-PCR analysis, a minimal amount of the CB1R mRNA was detected in HTFs (Fig. 1A). However, the expression of the CB1R protein was undetectable in western blots of HTFs (Fig. 1B), whereas the CB1R protein was detected in human SH-SY5Y cells (a positive control). Molecules have been shown to have distinct expression patterns at the protein and mRNA levels. The presence of the CB1R mRNA does not necessarily indicate that the mRNA is being translated. If the transcript is being translated, HTFs may exert some regulatory effect on CB1R expression at the translational level or produce posttranslational modifications. Notably, the expression of the CB2R mRNA (Fig. 1C) and protein (Fig. 1D) was detected. Therefore, we hypothesized that CB2R, but not CB1R, might play a more essential role in regulating the physiological effects of HTFs.

Secondly, the effects of TGF-β1 on CB2R expression were investigated. Interestingly, according to the western blot results, the TGF-β1 treatment resulted in a sustainable concentration-dependent increase in CB2R expression at concentrations of 0.5 to 5 ng/ml (Fig. 2), suggesting that CB2R may be associated with the physiological function of TGF-β1 in HTFs. However, the expression of the CB1R protein was not detected in the presence or absence of TGF-β1 (data not shown). Therefore, we next investigated the effects of CB2R agonists on TGF-β1-induced collagen production and contraction in HTFs.

3.2. Activation of CB2R reduced the synthesis of fibronectin and collagen types I and III

The selective CB2R agonists HU308 and JWH133 were used to investigate the effects of CB2R on the production of ECM components, such as fibronectin and collagen types I and III. Western blots show that TGF-β1 (3 days, 2 ng/ml) significantly increased the levels of fibronectin and collagen types I and III. Administration of HU308 and JWH133 markedly ameliorated TGF-β1-induced ECM protein synthesis. The CB2R-selective antagonist AM630 was used to suppress CB2R activation and further verify the involvement of CB2R. Interestingly, treatment with AM630 (100 nM) significantly blocked the inhibitory effects of HU308 and JWH133 on the production of fibronectin and collagen types I and III (Fig. 3A and B), confirming the involvement of CB2R in this process.

MMPs are important regulators of cell-mediated collagen deposition. We thus determined the effects of HU308 and JWH133 on the release of MMPs and TIMPs by HTFs. As shown in the western blot analysis, the TGF-β1 treatment significantly elevated the expression of MMP-1 and MMP-3 (Fig. 4A and B), which was partially reversed by addition of the CB2R-selective agonists HU308 and JWH133 to the culture supernatants. Western blot analyses with TIMP-1 and TIMP-2 antibodies revealed that neither TGF-β1 nor CB2R agonists (HU308 or JWH133) affected the abundance of these proteins in the culture supernatant (Fig. 5).

3.3. Effects of CB2R agonists on TGF-β1-induced collagen gel contraction mediated by HTFs

We next evaluated whether CB2R agonists have any effect on HTF-mediated collagen gel contraction induced by TGF-β1. HTFs were incubated with various concentrations of HU308 (0.5, 2.5, 10 μM) and JWH133 (0.1, 0.5, 2 μM) for 24 h and then treated with TGF-β1 (2 ng/ml) for 3 days. HU308 (Fig. 6A) inhibited TGF-β1-induced collagen gel contraction by 30%, 48%, and 55% at the concentrations of 0.5, 2.5, and 10 μM, respectively. In addition, HU308 (2.5 μM) also inhibited TGF-β1-induced collagen gel contraction (Fig. 6B). JWH133 (Fig. 7A) reduced TGF-β1-induced collagen gel contraction by 22%, 43%, and 50% at concentrations of 0.1, 0.5, and 2 μM, respectively. Consistently, JWH133 (0.5 μM) also inhibited TGF-β1-induced collagen gel contraction induced by (Fig. 7B).

3.4. Effects of CB2R agonists on TGF-β1-induced activation of the MAPK pathway in HTFs

The MAPK pathway plays a critical role in TGF-β1-induced ECM deposition and remodelling in HTFs [21]. As shown in Fig. 8, TGF-β1 significantly increased the phosphorylation of ERK1/2, p38, and JNK, which was partially reversed by HU308 (2.5 μM) and JWH133 (0.5 μM). Notably, treatment with the CB2R-selective antagonist AM630 (100 nM) obviously suppressed the inhibitory effects of HU308 and JWH133 on the phosphorylation of these kinases, confirming the

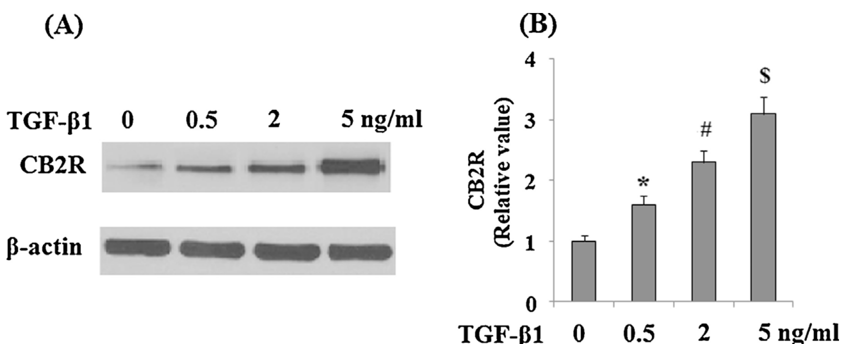

Fig. 2. TGF-β1 increased CB2R expression in human Tenon's fibroblasts (HTFs). HTFs were treated with various concentrations of TGF-β1 for 24 h. (A) Western blot analysis of CB2R. (B) Quantitative analysis of western blots (means ± SEM, *, #, $, $P < 0.01$ compared with the previous group, n = 6).

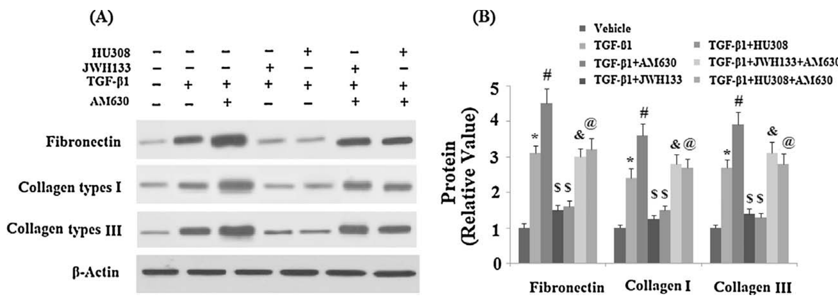

Fig. 3. Effects of the CB2R-selective agonists HU308 and JWH133 on ECM deposition by HTFs. (A) Cells were incubated with HU308 (2.5 μM) or JWH133 (0.5 μM) in the presence or absence of the CB2R-selective antagonist AM630 (100 nM) for 24 h prior to exposure to TGF-β1 (2 ng/ml) for 3 days. Western blot analysis of fibronectin and collagen types I and III in primary cultured human Tenon's fibroblasts. (B) Quantitative analysis of western blots (means ± SEM, *, $P < 0.01$ compared with the vehicle control; #, $P < 0.01$ compared with the TGF-β1 treatment; $, $P < 0.01$ compared with the TGF-β1 treatment; &, $P < 0.01$ compared with the TGF-β1 + HU308 treatment group; @, $P < 0.01$ compared with the TGF-β1 + JWH133 treatment group, n = 5-6).

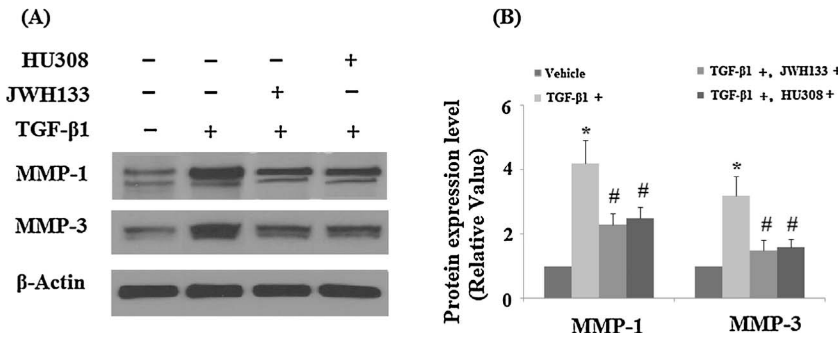

Fig. 4. Effects of the CB2R-selective agonists HU308 and JWH133 on MMP expression in HTFs. (A) Cells were incubated with HU308 (2.5 μM) or JWH133 (0.5 μM) for 24 h prior to exposure to TGF-β1 (2 ng/ml) for 3 days. Matrix metalloproteinase-1 (MMP-1) and MMP-3 levels were determined by western blotting. (B) Quantitative analysis of western blots (means ± SEM, *, $P < 0.01$ compared with the vehicle control; #, $P < 0.01$ compared with the TGF-β1 treatment, n = 6).

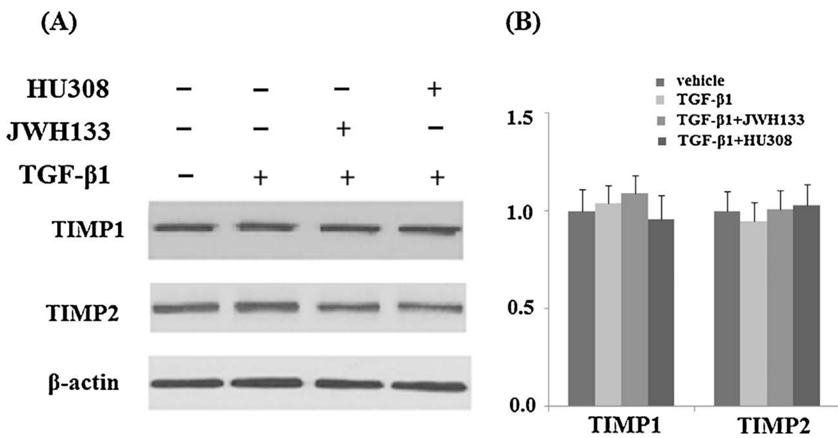

Fig. 5. Effects of the CB2R-selective agonists HU308 and JWH133 on TIMP expression in HTFs. (A) Cells were incubated with HU308 (2.5 μM) or JWH133 (0.5 μM) for 24 h prior to exposure to TGF-β1 (2 ng/ml) for 3 days. TIMP-1 and TIMP-2 levels were determined by western blotting. (B) Quantitative analysis of western blots (means ± SEM, n = 6).

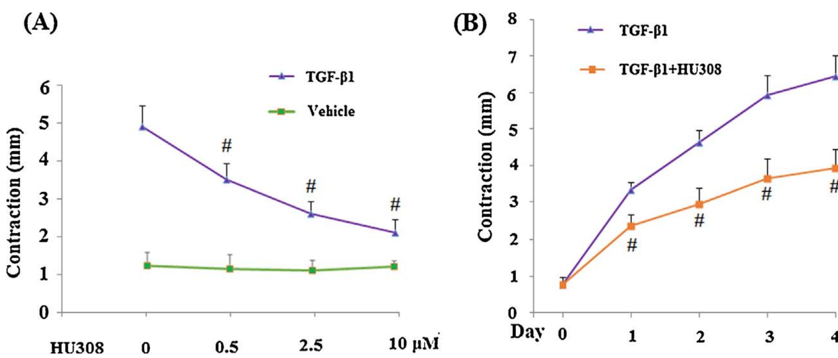

Fig. 6. Effects of the CB2R-selective agonists HU308 on TGF-β1-induced collagen gel contraction mediated by HTFs. (A) Cells were first incubated in collagen gels for 24 h in the absence or presence of 0.5, 2.5, or 10 μM HU308, and then additionally incubated for 3 days in the absence or presence of transforming growth factor-β1 (TGF-β1; 2 ng/ml), after which the change in gel diameter was measured. (B) Cells were first incubated in collagen gels with 2.5 μM HU308 for the indicated times (1, 2, 3, or 4 days) in the absence or presence of transforming growth factor-β1 (TGF-β1; 2 ng/ml), after which the change in gel diameter was measured (means ± SEM, #, $P < 0.01$ compared with the TGF-β1 treatment, n = 5-6).

involvement of CB2R. In addition, knockdown of CB2R using CB2R siRNA interference also abolished the inhibitory effects of HU308 and JWH133 on the phosphorylation of ERK1/2, p38, and JNK (data not shown). However, the total levels of ERK1/2, p38, and JNK were not affected.

4. Discussion

Pharmacological prevention of conjunctival scar formation after glaucoma filtration surgery remains a major clinical challenge in ophthalmology [22]. TGF-β1 has been considered a key target involved in regulating the wound healing response [23]. HTF-mediated inflammatory processes and fibroblast proliferation induced by TGF-β1 are involved in the formation of conjunctival scars [24]. Excessive deposition of collagen and fibronectin driven by TGF-β1 is a key step in scar formation following glaucoma revision surgery [25]. In addition, collagen and fibronectin are involved in the contractility of HTFs cultured in 3D collagen gels [26]. In the present study, we indicate that

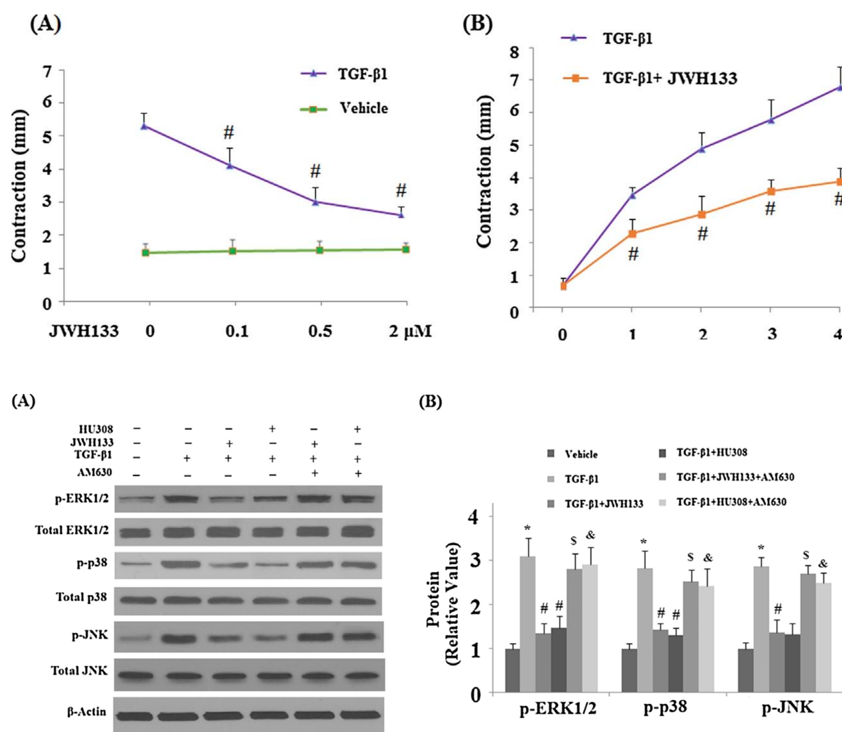

Fig. 7. Effects of the CB2R-selective agonists JWH133 on TGF-β1-induced collagen gel contraction mediated by HTFs. (A) Cells were first incubated in collagen gels for 24 h in the absence or presence of 0.1, 0.5, or 2 μM JWH133, and then additionally incubated for 3 days in the absence or presence of transforming growth factor-β1 (TGF-β1; 2 ng/ml), after which the change in gel diameter was measured. (B) Cells were incubated in collagen gels with 0.5 μM JWH133 for the indicated times (1, 2, 3, or 4 days) in the absence or presence of transforming growth factor-β1 (TGF-β1; 2 ng/ml), after which the change in gel diameter was measured (means ± SEM, #, $P < 0.01$ compared with the TGF-β1 treatment, n = 5–6).

Fig. 8. The CB2R-selective agonists HU308 and JWH133 attenuated TGF-β1-induced activation of the MAPK signalling pathway. (A) Cells were first incubated in collagen gels with TGF-β1 (2 ng/ml) in the presence or absence of HU308 (2.5 μM), JWH133 (0.5 μM), or the CB2R-selective antagonist AM630 (100 nM) for 24 h. The levels of phosphorylated ERK1/2, p38, and JNK were measured by western blotting. (B) Quantitative analysis of protein expression (means ± SEM, *, $P < 0.01$ compared with the vehicle control; #, $P < 0.01$ compared with the TGF-β1 treatment; $, $P < 0.01$ compared with the TGF-β1+ HU308 treatment group; &, $P < 0.01$ compared with the TGF-β1+ JWH133 treatment group, n = 5–6).

TGF-β1-induced synthesis of collagen types I and III and fibronectin was ameliorated by the CB2R-selective agonists HU308 and JWH133 and was associated with the inhibitory effects of these drugs on TGF-β1-induced collagen gel contraction mediated by HTFs.

Previous studies have reported the involvement of MMPs in fibrosis and scarring surrounding the surgical canal [27,28]. Moreover, certain anti-glaucoma medications alter the expression levels of matrix metalloproteinases (MMPs) [29]. Fibroblasts from patients with conjunctivochalasis show markedly increased expression of MMP-1 and MMP-3 transcripts compared with conjunctival fibroblasts from normal individuals [30]. Both HU308 and JWH133 blunted the levels of MMP-1 and MMP-3 expression in the present study. Concordantly, HU-308 was shown to inhibit IL-1β-induced expression of MMP-3, MMP-13, and IL-6 in fibroblasts from subjects with rheumatoid arthritis [31]. In addition, JWH-133 suppressed the activities of MMP-9 and MMP-12 in microglia [32]. Regulation of CB2R activity has been considered an important strategy for the treatment of ECM-associated diseases. JWH133 inhibits the production of IL-6, MMP-3, and CCL2 by tumour necrosis factor (TNF)-α-stimulated fibroblast-like synoviocytes (FLS) derived from rheumatoid joints, as well as osteoclastogenesis of peripheral blood monocytes. JWH133 administration reduced the arthritis score and infiltration of inflammatory cells in a rodent model of arthritis [33]. In addition to fibrillar collagen I, collagen III, and fibronectin, MMP-1 and MMP-3 also target other types of fibrillar collagens, laminin, and proteoglycans. Thus, the inhibitory effects of HU308 and JWH133 imply that these compounds have a potential role in regulating ECM formation.

The MAPK signalling pathway plays various roles in maintaining cell proliferation and differentiation. Various upstream regulators and downstream molecules of the MAPK pathway are widely distributed in the eyes. Notably, the MAPK signalling pathway is associated with TGF-β1-induced scar formation [34]. Phosphorylation of members of the MAPK pathway plays a critical role in TGF-β1-mediated collagen I production [35]. TGF-β1 induces the activation of the ERK, JNK and p38 signalling pathways in HTFs [36]. Blockade of MAPK p38 prevents TGF-β1-induced transdifferentiation of myofibroblasts, thereby causing an anti-fibrotic effect [37]. Hence, the MAPK and JNK pathways are recognized as potential therapeutic targets for regulating the uncontrolled fibrotic response at the fibroblast level. The MAPK signalling pathway also plays a key role in regulating collagen gel contraction. Importantly, collagen gel contraction induced by the anti-glaucoma drug latanoprost was reduced by inhibitors of ERK (PD98059 and ERK inhibitor II), p38 (SB203580), and JNK (JNK inhibitor II) in a previous study [38]. In the present study, TGF-β1 induced the activation of the ERK, p38 and JNK signalling pathways in HTFs, which was prevented by the administration of the two CB2R-selective agonists HU308 and JWH133, suggesting that these effects contribute to the inhibitory effects of CB2R activation on TGF-β1-induced ECM synthesis and collagen gel contraction mediated by HTFs. The effects of CB2R on the activation of the MAPK signalling pathway remain controversial. In contrast to the results of our current study, Lambert and Fowler reported that CB2R activation induces the phosphorylation of ERK1/2, p38, and JNK through the G protein pathway. Consistent with our current findings, treatment with the CB2R-selective agonist JWH133 decreases ERK and p38 phosphorylation in a concentration-dependent manner. However, the mechanisms underlying the inhibitory effects of CB2R agonists on TGF-β1-induced MAPK activation remain poorly characterized. Further studies will be helpful in clarifying the underlying mechanisms.

Endogenous cannabinoids bind to both CB1R and CB2R, both of which are coupled to a subclass of G proteins that inhibit guanine nucleotide-binding and adenylyl cyclase activity [39]. Although a 56% difference in their respective amino acid sequences has been noted and the receptors have different functions, both CB1R and CB2R are considered therapeutic targets for anti-fibrosis drugs. For example, the administration of curcumin reduced liver fibrosis and inhibited ECM expression by modulating the CBR system in hepatic stellate cells [40]. In addition, CB2R activation was recently shown to aid in wound healing by reducing inflammation, accelerating re-epithelialization, and attenuating scar formation [41]. The current study is the first to report that CB2R is expressed in human Tenon's fibroblasts (HTFs). In HTFs, MMPs regulate the balance of ECM metabolism [42]. As ECM-degrading enzymes, MMPs are divided into different subgroups, including collagenases and gelatinases. MMP-1, MMP-3, and MMP-8 are collagenases and are capable of cleaving intact fibrillar collagen, including collagen types I and III [43]. Gelatinases, including MMP-2 and

MMP-9, cleave denatured collagen and collagen type IV [44]. CB2R activation suppresses the expression and activity of gelatinases, such as MMP-2 and MMP-9 [45]. However, little information regarding the effects of CB2R on collagenases is available. In the current study, activation of CB2R by its agonists HU308 and JWH133 prevented the TGF-β1-induced increase in the expression of collagenases, such as MMP-1 and MMP-3. Notably, we show for the first time that CB2R activation reduced the levels of collagen types I and III, two fibril-forming collagens, in human Tenon's fibroblasts (HTFs). These results suggest a novel inhibitory effect of CB2R on scarring.

Based on our findings, CB2R is expressed in HTFs. Activation of CB2R by the selective agonists HU308 and JWH133 suppressed TGF-β1-induced ECM synthesis and reduced the contractility of HTFs *in vitro*. Thus, activation of CB2R by agonists might exert protective effects on scarring during tissue wound healing following glaucoma filtration surgery.

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