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Oridonin inhibits IL-1β-induced inflammation in human osteoarthritis chondrocytes by activating PPAR-γ

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ABSTRACT

Osteoarthritis (OA), a progressive disease of the joints, affects millions of people worldwide. In the present study, we investigated the effects of oridonin, a diterpenoid isolated from *Rabdosia rubescens*, on IL-1β-induced inflammation using human osteoarthritis chondrocytes. The results showed that oridonin significantly suppressed IL-1β-induced MMP1, MMP3, and MMP13 production. IL-1β-induced NO and PGE_2 production, as well as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression were also attenuated by oridonin. Western blot analysis demonstrated IL-1β-induced NF-κB activation was reduced by oridonin. Furthermore, the expression of PPAR-γ was increased by oridonin in a concentration-dependent manner. PPAR-γ antagonist could reverse the anti-inflammatory activity of oridonin. The results suggested that oridonin could be a candidate agent for the treatment of OA.

1. Introduction

Osteoarthritis (OA) is a joint degenerative disease that seriously affects the quality of life of patients [1]. It is characterized by degeneration of articular cartilage, joint edge, and subchondral bone hyperplasia [2]. OA often causes by age, obesity, strain, trauma, joint congenital anomalies, joint deformities, and other factors [3]. OA often occurs in the middle-aged and elderly people, and the incidence rate is high [4]. Studies showed that > 50% of the people over 65 years old were OA patients [5]. OA brings huge economic burden to patients and their families. Therefore, to seek agents to treat OA is urgently needed. The pathogenesis of osteoarthritis is still unclear. Experiments have confirmed that the incidence of OA was closely related to inflammation-related mediators [6].

Oridonin, a diterpenoid isolated from *Rabdosia rubescens*, has been reported to have anti-inflammatory effects [7]. Oridonin has been reported to inhibit vascular inflammation *via* inhibiting NF-κB and MAPKs signaling pathways [8]. Oridonin was found to protect against diabetic nephropathy through inhibiting inflammatory response [9]. Oridonin has been reported to inhibit LPS-induced inflammatory cytokine production in RAW264.7 cells [10]. Furthermore, oridonin was found to inhibit LPS-induced acute lung injury in mice [10]. A previous study showed that oridonin could attenuate experimental colitis in mice [11]. However, whether oridonin has anti-inflammatory effects against OA remains unclear. In the present study, we used an IL-1β-stimulated human osteoarthritis chondrocytes model to investigate the anti-inflammatory effects of oridonin on OA.

2. Materials and methods

2.1. Reagents and materials

Oridonin (purity > 98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). MMP1, MMP3, and MMP13 ELISA kits were purchased from Biolegend (CA, USA). PGE_2 ELISA kit and recombinant human IL-1β were purchased from R&D systems (Minneapolis, MN, USA). GW9662 was purchased from Cayman chemical (AnnArbor, MI). NO assay kit was obtained from Nanjing Jiancheng Biotechnology Co., Ltd. (Nanjing, China). PPAR-γ, COX-2, and iNOS antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). NF-κB signaling pathway antibodies were obtained from Beyotime Institute of Biotechnology (Jiangsu, China).

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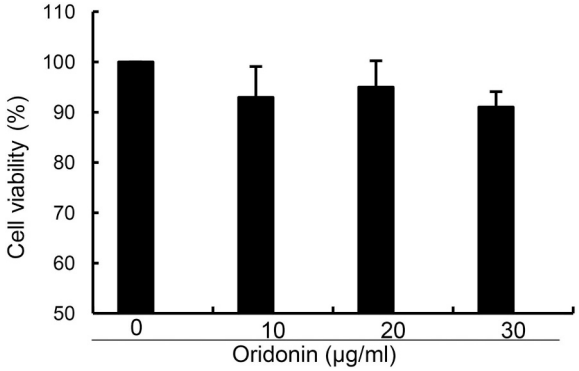

Fig. 1. Effects of oridonin on the cell viability of chondrocytes. The values presented are the means ± S.E.M. of three independent experiments. $^{**}P < 0.01$ *vs.* control group.

2.2. Cell culture and treatment

All the experiment of this study was in accordance with the Declaration of Helsinki and Tokyo and approved by the Ethics Committee of Harbin Medical University. Human articular cartilage samples were collected from 6 osteoarthritis patients (age: 52 ± 6). This experiment was informed and signed consent by the patients. The human chondrocytes were isolated and cultured as described previously [12] and cultured in DMEM with 10% FBS at 37 °C with 5% CO_2. Passages 2 to 4 were used in the present study [13]. The cells were treated with oridonin (10, 20, 30 μg/ml) 1 h before IL-1β treatment.

2.3. MTT assay

Chondrocytes were incubated with oridonin, at the concentration ranging from 0 to 30 μg/ml for 24 h. Subsequently, MTT was added to each well for 3 h. Then, the supernatants were discarded and DMSO (150 μl) was added to each well. The absorbance was detected at 570 nm on a microplate reader.

2.4. ELISA assay

Chondrocytes were pretreated with oridonin 1 h and stimulated with IL-1β (10 ng/ml) for 24 h. Then, the supernatants were collected and the contents of MMP1, MMP3, and MMP13 were detected using ELISA kits purchased from Biolegend (CA, USA). The level of PGE_2 was measured using ELISA kit purchased from R&D systems (Minneapolis, MN, USA).

2.5. NO assay

Chondrocytes were pretreated with oridonin 1 h and stimulated with IL-1β (10 ng/ml) for 24 h. Then, the supernatants were collected and the level of NO was measured by Griess reagent purchased from

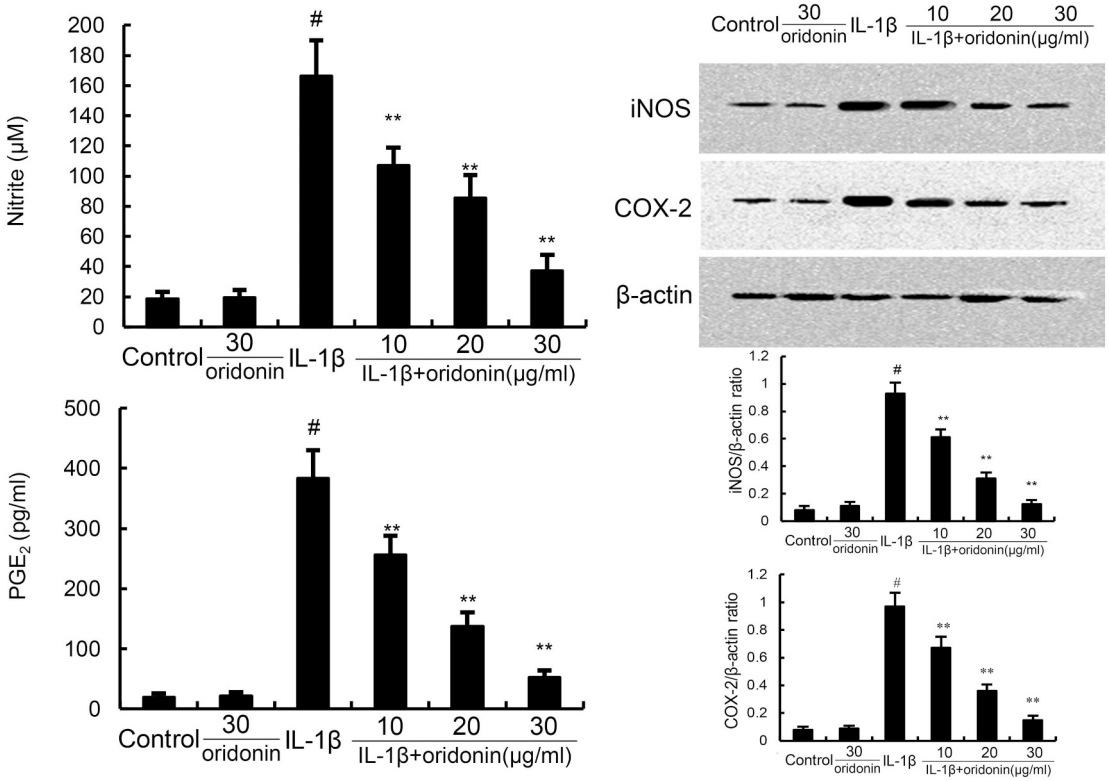

Fig. 2. Oridonin inhibits IL-1β-induced NO, PGE2 (c) production, and COX-2 and iNOS expression. The data presented are the means ± S.E.M. of three independent experiments. $^{\#}P < 0.05$ *vs.* control group; $^{**}P < 0.01$ *vs.* IL-1β group.

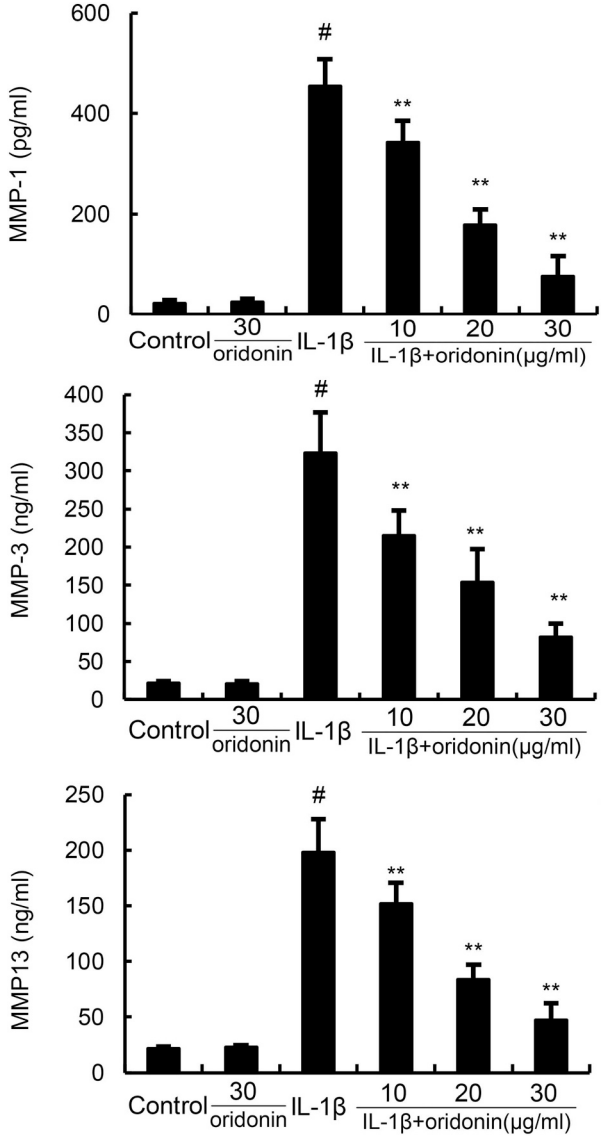

Fig. 3. Oridonin inhibits IL-1β-induced MMP1, MMP3, and MMP13 production. The data presented are the means ± S.E.M. of three independent experiments. $^{\#}P < 0.05$ *vs.* control group; $^{**}P < 0.01$ *vs.* IL-1β group.

Nanjing Jiancheng Biotechnology Co., Ltd. (Nanjing, China) according to the manufacturer's instructions.

2.6. Western blot analysis

Total proteins from chondrocytes were isolated using the Protein Extraction Reagent (Beyotime, China). The protein concentration was measured by BCA method and equivalent amounts of protein were separated by 12% SDS-PAGE. The proteins were transferred onto PVDF membranes and blocked with 3% BSA for 2 h. The membranes were washed three times and incubated with NF-κB, PPAR-γ, COX-2, and iNOS antibodies for 12 h. After washing three times, the membranes were incubated with secondary antibodies for 2 h. Finally, the membranes were detected by the enhanced chemiluminescence detection kit (Beyotime, China).

2.7. Statistical analysis

The results are shown as the means ± S.E.M of three independent experiments. The results were analyzed by one-way analysis using GraphPad Prism 7.0 software. $P < 0.05$ were considered to indicate statistical significance.

3. Results

3.1. Effects of oridonin on chondrocyte viability

The MTT assay showed that oridonin did not affect the viability of chondrocytes at the concentration ranging from 0 to 30 μg/ml. Therefore, we chose the concentration of 10, 20, 30 μg/ml in the subsequent experiments (Fig. 1).

3.2. Oridonin inhibits IL-1β-induced inflammatory mediator production

The effects of oridonin on inflammatory mediator production were measured in this study. As presented in Fig. 2, the production of NO and PGE_2 increased significantly after IL-1β treatment. However, the increases were dose-dependently inhibited by the treatment of oridonin. Furthermore, the expression of iNOS and COX-2 were increased significantly after IL-1β treatment. However, the increases were dose-dependently inhibited by the treatment of oridonin (Fig. 2). Treatment of oridonin alone did not affect the production of NO and PGE_2, as well as iNOS and COX-2 expression.

3.3. Effects of oridonin on MMP1, MMP3, and MMP13 production

The effects of oridonin on MMP1, MMP3, and MMP13 production were measured by ELISA. As presented in Fig. 3, the production of MMP1, MMP3, and MMP13 in the IL-1β group were higher than the control group. However, the levels of MMP1, MMP3, and MMP13 in oridonin-treated groups were much lower than the IL-1β group (Fig. 3). Treatment of oridonin alone did not affect the production of MMP1, MMP3, and MMP13.

3.4. Oridonin inhibits IL-1β-induced NF-κB activation

The effects of oridonin on NF-κB activation were measured by western blot analysis. As presented in Fig. 4, the expression of phosphorylation NF-κB p65 and IκBα in the IL-1β group were higher than the control group. However, the expression of phosphorylation NF-κB p65 and IκBα in oridonin-treated groups were much lower than the IL-1β group (Fig. 4).

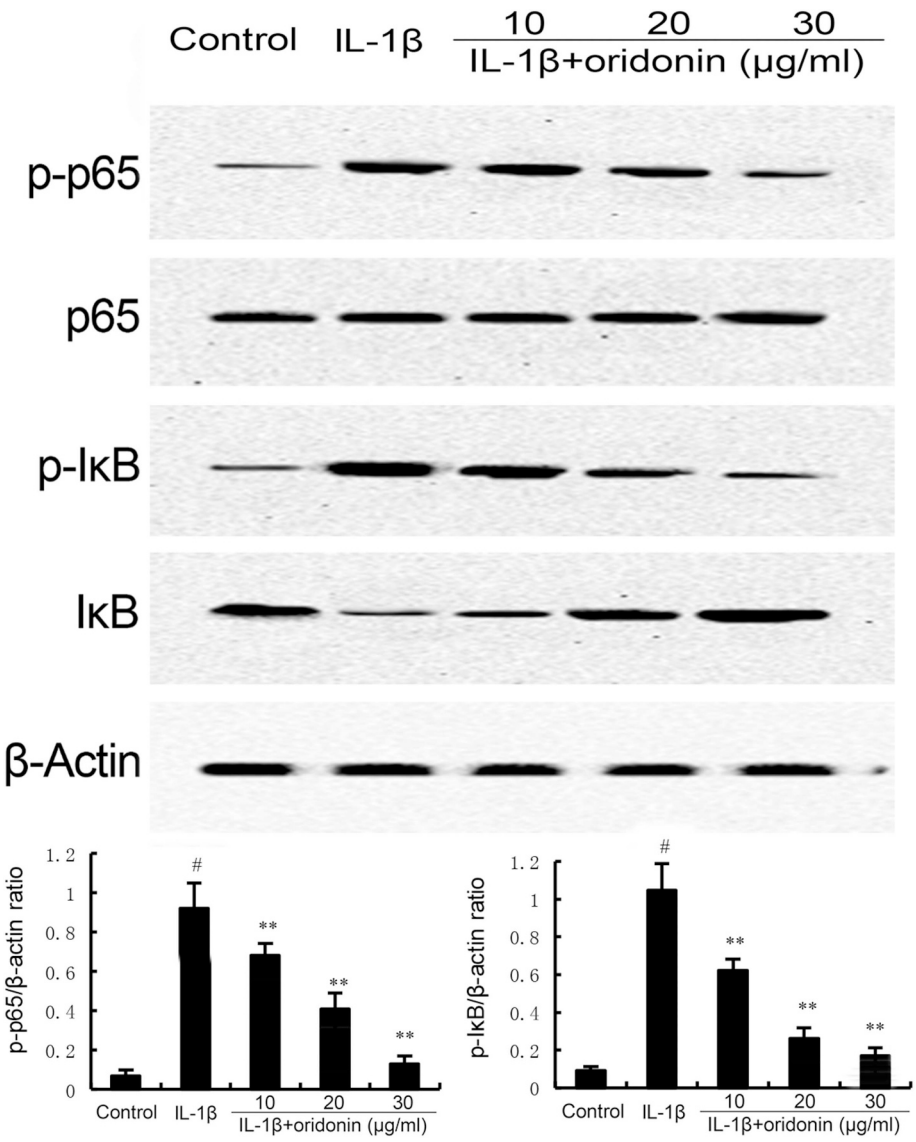

Fig. 4. Oridonin inhibits IL-1β-induced NF-κB activation and IκBα degradation. The values presented are the means ± S.E.M. of three independent experiments. $^{\#}P < 0.05$ *vs.* control group; $^{**}P < 0.01$ *vs.* IL-1β group.

3.5. Anti-inflammatory effects of oridonin was PPAR-γ dependent

The effects of oridonin on PPAR-γ expression were measured by western blot analysis. As presented in Fig. 5, the expression of PPAR-γ in the IL-1β group was lower than the control group. However, the expression of PPAR-γ in oridonin-treated groups was much higher than the IL-1β group (Fig. 5). In addition, the inhibition of oridonin on MMP1, MMP3, MMP13, PGE_2, and NO production were blocked by PPAR-γ inhibitor GW9662 (Fig. 6).

4. Discussion

Oridonin has been reported to have anti-inflammatory effects [14]. However, the effects of oridonin on OA have not been reported. To our knowledge, this is the first report showing that oridonin inhibited IL-1β-induced inflammatory response in chondrocytes. Our results showed that oridonin significantly inhibited IL-1β-induced inflammatory mediator production through activating PPAR-γ.

OA is a joint degenerative disease that characterized by degradation of articular cartilage. Matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes that have the ability to degrade the extracellular matrix of OA cartilage [15]. Studies showed that increased

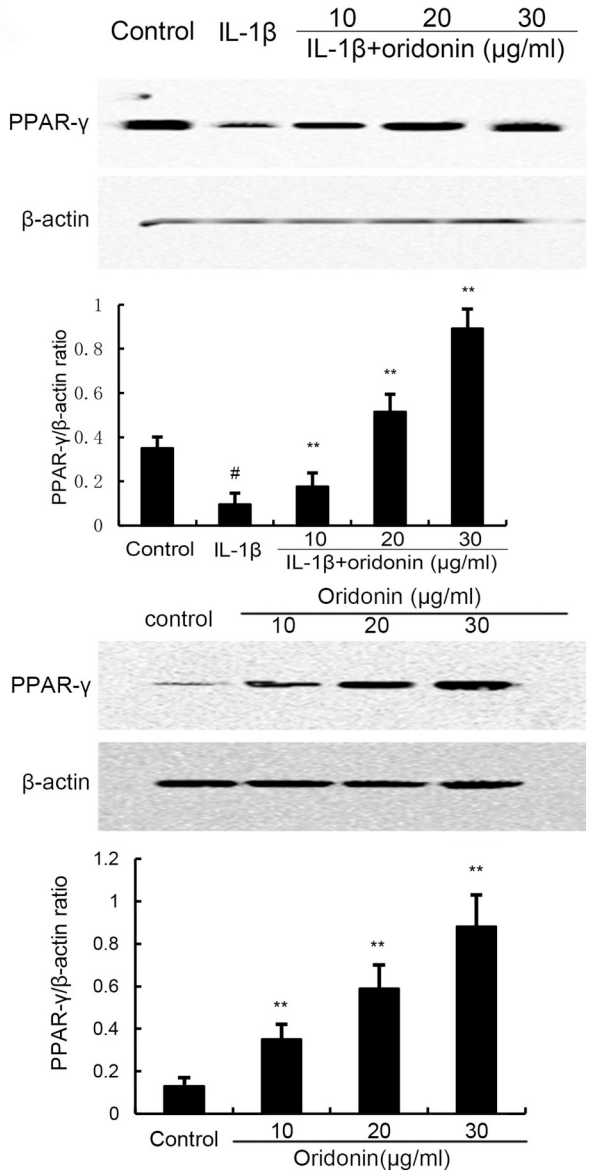

Fig. 5. Effects of oridonin on PPARγ expression. The values presented are the means ± S.E.M. of three independent experiments. $^{\#}P < 0.05$ *vs.* control group; $^{**}P < 0.01$ *vs.* IL-1β group.

expression of MMPs were associated with cartilage degradation [16]. Furthermore, increased inflammatory cytokines, such as IL-1β was observed in patients of OA [17]. IL-1β is abundantly synthesized by OA synovium and cartilage. Studies showed that IL-1β could induce the production of MMPs, such as MMP1, MMP3, and MMP13 [18]. In addition, IL-1β has been reported to be involved in OA pathophysiology and inhibition of IL-1β-induced inflammation could attenuate the pathophysiology of OA [19]. Therefore, we chose IL-1β as a stimulus in this study. In this study, our results showed that oridonin significantly inhibited IL-1β-induced MMP1, MMP3, and MMP13 production. Furthermore, IL-1β-induced PGE_2 and NO production were also inhibited by oridonin. NF-κB is involved in the regulation of inflammatory cytokine production. NF-κB contributes to the inflammation during the development of OA. Numerous literatures reported that inhibition of NF-κB activation could attenuate the development of OA [20]. Many herbal medicines protected OA through inhibiting NF-κB activation [21,22]. In this study, the results demonstrated that oridonin significantly inhibited IL-1β-induced NF-κB activation.

PPAR-γ is a membrane of nuclear receptor superfamily and activation of PPAR-γ could attenuate the development of cartilage lesions in OA [23,24]. Studies demonstrated that PPAR-γ agonist could inhibit the synthesis of cartilage degradation products and the production of inflammatory mediators [25]. Furthermore, PPAR-γ agonist has been known to inhibit the activation of NF-κB [26]. In the present study, we found oridonin had the ability to activate PPAR-γ and could be used as a PPAR-γ agonist. Inhibition of PPAR-γ by its inhibitor GW9662, the anti-inflammatory effects of oridonin were blocked. Therefore, our results suggested that oridonin exhibited its anti-inflammatory effects through activating PPAR-γ. In addition, studies showed PPAR-γ agonist could inhibit NLRP3 inflammasome [27]. A previous study demonstrated oridonin could inhibit NLRP3 inflammasome activation and could be used as a covalent inhibitor of NLRP3 [28]. This was consistent with our results which showed oridonin could activate PPAR-γ and used as a PPAR-γ agonist.

In conclusion, the data of the present study demonstrated that oridonin exhibited anti-inflammatory activity in IL-1β-stimulated chondrocytes. And activating of PPAR-γ may mediate the anti-inflammatory effects of oridonin.

Conflict of interest statement

All authors declare that they have no conflict of interest.

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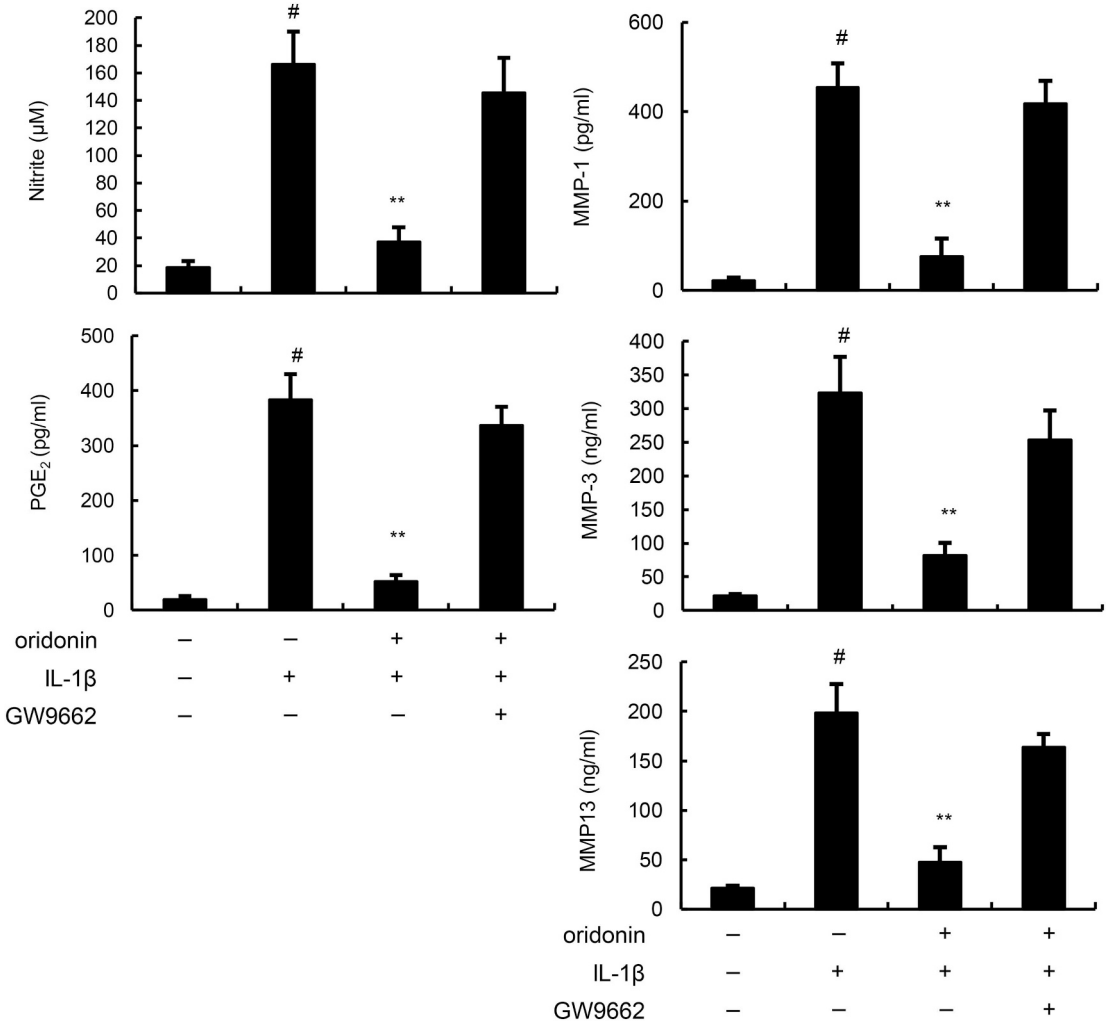

Fig. 6. The anti-inflammatory effects of oridonin were PPAR-γ dependent. For PPAR-γ inhibition assay, the cells were pretreated with oridonin (30 μg/ml) for 1 h, or 10 μM GW9662 for 30 min before oridonin incubation, and stimulated with IL-1β. The values presented are the means ± S.E.M. of three independent experiments. $^{\#}P < 0.05$ *vs.* control group; $^{**}P < 0.01$ *vs.* IL-1β group.

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