Introduction: Osteoarthritis (OA), one of the most common forms of arthritis diseases, is a progressive degenerative joint disease with signs and symptoms of inflammation, leading to significant functional impairment and disability in older adults. Growing evidence indicates that metabolic factors play a key role in the progression of arthritis diseases. OA has recently been suggested to have a positive correlation with glucose imbalance, metabolic dysfunction, and diabetes mellitus (DM). Previous studies have found that high glucose decreases proteoglycan synthesis induced by IGF-1 in rabbit chondrocytes and induces initiation of the catalytic program such as induction of MMP-1 and MMP-13 in human chondrocytes. Several studies observed that peroxisome proliferator-activated receptors γ (PPARγ) agonists reduced the cartilage degradation in vitro and in vivo. However, the role of PPARγ in high glucose/hyperglycemia-induced chondrocyte/cartilage damage is still unclear. In this study, we hypothesized that high glucose/hyperglycemia down-regulated PPARγ expression and triggered inflammatory response and damaged chondrocyte/cartilage in which administration of PPARγ activator might reverse the alterations. We investigated the role of PPARγ in high glucose/hyperglycemia-triggered chondrocyte/cartilage damages by using a human chondrocyte culture model and a diabetic mouse model. The therapeutic potential of PPARγ agonist on high glucose/hyperglycemia-triggered chondrocyte/cartilage damages was also evaluated.

Methods: (1) Isolation and culture of chondrocytes from human articular cartilage: Cartilage specimens were collected from 10 people aged 32-65 years undergoing orthopedic surgery with written approvals from the institutional Ethics Committee at National Taiwan University Hospital, Taipei, Taiwan, and also from the patients. Chondrocytes were isolated by sequential enzymatic digestion at 37°C with 0.2% collagenase (type II) for four hours in DMEM. Isolated chondrocytes were filtered through 100 μm nylon filters. The cells were grown in the plastic cell culture dishes in 95% air-5% CO2 with Ham’s F-12 containing 10% FBS and 1% penicillin-streptomycin. Cells were subsequently cultured in Ham’s F-12 (Regular Glucose Medium, RGM, 10 mM glucose) or in the same medium supplemented with D-glucose to yield a final glucose concentration of 30 mM (High Glucose Medium, HGM). (2) Western blot analysis: The protein expressions of cyclooxygenase (COX)-2, collagen II, and PPARγ were determined. (3) Measurement of IL-6, MMP-13, and PGE2: The levels of PGE2, IL-6, and MMP-13 in culture media were quantified by using the commercially specific ELISA kits. (4) Experimental diabetic mice: Male ICR mice (4 weeks old) were obtained from the Animal Center of the College of Medicine, National Taiwan University, Taipei, Taiwan. This study was approved and conducted by the institutional animal care and use committee, College of Medicine, National Taiwan University. Mice were intraperitoneally injected with 100 mg/kg streptozotocin (STZ). Blood glucose levels reached more than 400 mg/dL were defined as diabetic hyperglycemia. STZ-induced diabetic mice were orally treated with pioglitazone (10 mg/kg) or vehicle for 4 weeks. The quantity of AGE adduct in blood samples was determined by AGE ELISA kit.
Blood glucose levels were determined using glucose colorimetric assay kit. (5) Histological analysis and immunohistochemistry: Femurs were fixed, decalcified, and dehydrated. Serial sections with a thickness of 5 μm were taken. Sections were stained with Safranin O. The expressions of COX-2, advanced glycation end-products (AGEs), PPARγ, and collagen II in cartilages were determined by immunohistochemical analysis. Articular cartilages were histologically examined and graded referring to the Mankin scale.

**Results:** The protein expression of COX-2 was time-dependently increased in human chondrocytes cultured in high glucose medium (HGM) for 1-24 h. The productions of PGE2, MMP-13, and IL-6 were also time-dependently increased in human chondrocytes by HGM for 1-24 h. HGM significantly down-regulated the protein expressions of PPARγ and collagen II in human chondrocytes. Pioglitazone could dose-dependently inhibit HGM-induced protein expression of COX-2 and productions of PGE2, MMP-13, and IL-6. Furthermore, pioglitazone could dose-dependently reverse HGM-inhibited collagen II expression. The HGM-induced down-regulation of PPARγ was also dose-dependently reversed by pioglitazone in human chondrocytes (Figure 1). The serum levels of MMP-13 and PGE2 were markedly increased in STZ-induced diabetes mice, which were significantly suppressed by the treatment of pioglitazone (10 mg/kg). Furthermore, blood glucose and AGEs levels were relative high and could be reversed by pioglitazone in STZ-induced diabetic mice. The score of histologic grading showed that cartilage destruction was significantly severe in diabetic mice and it could be significantly reversed by pioglitazone. The immunostainings of AGEs and COX-2 were markedly enhanced and the immunostainings of collagen II and PPARγ were markedly down-regulated in diabetic mouse cartilages, which could be effectively reversed by pioglitazone treatment (Figure 2).

**Discussion:** The in vitro and in vivo studies have correlated hyperglycemia with local and systemic toxicities to joint degradation in OA, resulted from high glucose concentration. Diabetes might be an independent risk factor for OA according to several epidemiological and experimental studies. Hyperglycemia has been observed to trigger inflammatory responses. In the present study, we also found that hyperglycemia could induce inflammatory responses in human chondrocytes and diabetic mouse cartilages. These findings imply that an independent hyperglycemia-related inflammation may play an important role in the progression of OA. PPARγ agonist pioglitazone is known as an anti-diabetic drug. Pioglitazone has been found to reduce cartilage lesions in a dog model of inflammation by reducing the synthesis of MMP-1 and iNOS via the inhibition of MAPKs and NF-kB signaling pathway. Our results suggest that down-regulation of PPARγ is involved in the high glucose/hyperglycemia-induced chondrocyte/cartilage damage and PPARγ activator pioglitazone could inhibit hyperglycemia-induced inflammatory responses in human chondrocytes and show chondroprotection on mouse cartilage damage in diabetic mice.

**Significance:** The findings of this study demonstrated that hyperglycemia down-regulated PPARγ expression in cultured human chondrocytes and diabetic mouse cartilage. PPARγ activation exerts anti-inflammatory effects on human chondrocytes under hyperglycemia and shows chondroprotection in diabetic mouse cartilage damage. Pioglitazone may consider as an adjunctive therapy for chronic joint diseases which traditionally treated only with symptom-alleviating NSAID.
Figure 1. High glucose down-regulated the expressions of PPARγ and collagen II and induced inflammatory responses in human chondrocytes that could be reversed by PPARγ activator pioglitazone. A. PPARγ expression; B. Collagen II expression; C. COX-2 expression; D. PGE2 production; E. MMP-13 production; F. IL-6 production. The results are presented as mean±SEM (n=5). * P<0.05 vs control; # P<0.05 vs HGM alone.

Figure 2. Hyperglycemia induced cartilage damage and up-regulated AGEs and COX-2 expressions and down-regulated collagen II and PPARγ expressions in STZ-induced diabetic mice that could be reversed by pioglitazone. (A) Histological changes in articular cartilages were detected by using Safranin O staining. (B) The immunohistochemical stainings in cartilage sections for AGE, COX-2, collagen II and PPARγ were shown. The results are presented as mean±SEM (n=4). * P<0.05 vs control; # P<0.05 vs STZ alone.

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