INTRODUCTION:
Dexamethasone has been recommended for patients suffering from osteoarthritis (OA) with severe inflammation. Investigators have indicated that although dexamethasone inhibits the synthesis of inflammatory mediators in the inflamed joint, it may damage the cartilage. Our previous in vitro studies demonstrated that dexamethasone and several NSAIDs inhibited the synthesis of proteoglycan and collagen type II and facilitated the synthesis of collagen type X mRNA expression. SOX9, a transcriptional factor, is required for expressions of a series of chondrocyte-specific marker genes including Col2a1, Col9a2, Coll1a2 and aggrecan. On the other hand, it has been found that one of the main pathogenesis of OA is that articular chondrocytes undergo terminal differentiation, so that the chondrocytes become hypertrophy, synthesize collagen type X, form mineral formation and eventually undergo apoptosis. PTHrP, parathyroid hormone-related peptide, are reported to regulate endochondral ossification by inhibiting chondrocyte differentiation toward hypertrophy. Indian hedgehog (Ihh) is a master regulator of both chondrocyte and osteoblast differentiation during endochondral bone formation. Ihh stimulates chondrocyte proliferation and stimulates the production of PTHrP from perichondral cells and thereby delays chondrocyte hypertrophy. However, the influences of anti-inflammatory drugs on these gene expressions were rarely investigated. In this study, we tested the effects of dexamethasone, on the mRNA expressions of SOX9, Col2a1, Col10a1, aggrecan, PTHrP and Ihh in normal human articular chondrocytes.

METHODS:
Normal human articular chondrocytes were obtained from fresh cadaver-knees from a 23-year-old male that was supplied by the Hospital of Kaohsiung Medical University. The cartilage was minced and sequentially digested by hyaluronidase, pronase and collagenase. Chondrocytes released from tissue specimens were seeded into 15cm² culture dish in monolayer for cell expansion. Then chondrocytes were encapsulated in alginate beads and cultured for 7 days. Cells were treated with dexamethasone 10⁻⁹~10⁻⁷M after the 1, 5 and 7 day treatments (Fig. 3). The therapeutic concentration range of dexamethasone is 10⁻⁹~10⁻⁷M. Cells were harvested at the 1st, 5th, 7th day after drug treatments. The mRNA expressions of SOX9, COL2a1, aggrecan, COL10, PTHrP and Ihh were measured by RT-PCR. Sulfated glucosaminoglycan was measured by DMMB assay.

RESULTS:
The results showed that mRNA expressions of SOX9 (Fig.1), Col2a1, aggrecan (data not show) were significantly suppressed by dexamethasone (10⁻⁹~10⁻⁷M) upon either 1, 5 or 7 days of treatment in comparison to the control cultures. The results of sulfated glucosaminoglycan were consistent with the gene expression of aggrecan (Fig.2) The mRNA expression of Col10a1 was induced by dexamethasone 10⁻³~10⁻⁷M for 7 days. The therapeutic concentration range of dexamethasone is 10⁻⁹~10⁻⁷M. Cells were harvested at the 1st, 5th, 7th day after drug treatments. The mRNA expression of PTHrP was also suppressed by three different concentrations of dexamethasone (Fig. 3). Ihh was undetectable from cells with any treatment in this study (data not shown).

DISCUSSION:
Administrations of anti-inflammatory drugs are beneficial for OA patients by way of suppressing inflammation, and thus protecting the cartilage from the progress of OA. However, the direct effects of anti-inflammatory drugs on the functions of articular chondrocytes are also important issues to be concerned. In this study, we found that upon 1-7 day treatments of dexamethasone inhibited the mRNA expression of SOX9, Col2a1 (the most important collagen of normal articular cartilage) and aggrecan. From this result, we suggest that dexamethasone may inhibit SOX9 expression and subsequently down-regulate the expressions of Col2a1 and aggrecan. This effect of dexamethasone is similar to that of COX-2 inhibitors, but not non-selective NSAIDs, which were found in our Lab. Furthermore, dexamethasone effects on the induction of Col10a1 expression but suppression of PTHrP expression may promote cell undergoing terminal differentiation in normal human articular chondrocytes.

REFERENCES: