EFFECTS OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ON PROLIFERATION, CYTOTOXICITY AND APOPTOSIS IN CULTURED FETAL RAT EPIPHYSEAL-ARTICULAR CHONDROCYTES

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INTRODUCTION:
Previous reports indicated that nonsteroidal anti-inflammatory drugs (NSAIDs) suppress bone repair in vivo. Our previous study further found that ketorolac suppressed bone repair at the early stage of endochondral ossification. Furthermore, we also found that NSAIDs suppressed osteoblast proliferation in vitro, which may be one of the important factors contributing to their inhibitory effects on bone repair in vivo. Chondrocytic functions play an important role on endochondral ossification during bone repair process. We hypothesized that another possible mechanism of these inhibitory effects of NSAIDs on bone repair may be affecting the functions of chondrocytes. In this study, we investigated the effects of 4 common used NSAIDs and 2 cyclooxygenase-2 (COX-2) selective inhibitors on proliferation, cell cycle kinetics, cytotoxicity and cell death induction in cultured epiphyseal- articular chondrocytes obtained from knee of fetal rats.

MATERIALS AND METHODS:
Epiphyseal-articular chondrocytes were isolated from knees of fetal Sprague-Dawley rats at the 21st day of gestation. Cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% of fetal calf serum. Cultures were treated with indomethacin, ketorolac, diclofenac, piroxicam, celecoxib or DFU [5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-2(5H)-furanone], an analogue of rofecoxib, (10^-3-10^-10 M) for 6, 17 or 24 hr. DNA synthesis and cell cycle kinetics were examined by thymidine incorporation and flow cytometry respectively. Lactate dehydrogenase (LDH) leakage was tested for the cytotoxicity of NSAIDs. Terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) was used for staining the apoptotic cells in cultures.

RESULTS:
Our results showed that after 6 hr treatment of ketorolac or indomethacin significantly inhibited thymidine incorporation at concentration of 10^-6 M, while after 24 hr treatment of ketorolac at 10^-4 M or indomethacin at 10^-5-10^-6 M revealed inhibitory effect on DNA synthesis (Fig.1). We also found that these NSAIDs arrested cell cycle at G0/G1 phase (Fig.2). Furthermore, indomethacin and ketorolac also significantly induced cytotoxicity of chondrocytes at 10^-3-10^-4 M after 17hr-treatment, and at 10^-4 M after 24hr-treatment (Fig.3). Both diclofenac and piroxicam induced cytotoxicity of chondrocytes at concentration range of 10^-3-10^-6 M upon either 17 or 24 hr of treatment (Fig.3). Celecoxib (10^-3 M) significantly induced cytotoxicity after 24 hr treatment, while DFU (10^-4 M) had no significant cytotoxic effect on chondrocytes. The result of TUNEL staining revealed that after 24 hr treatment of indomethacin, ketorolac or diclofenac (10^-3 and 10^-4 M) chondrocytes significantly underwent apoptosis, while piroxicam has no significant effect on chondrocyte apoptosis (Fig.4).

DISCUSSION:
In this study, the results demonstrated that all the 4 tested NSAIDs (10^-5-10^-10 M) and celecoxib (10^-3-10^-6 M) had significant cytotoxic effects on epiphyseal-articular chondrocytes at concentration range covered the therapeutic concentrations (10^-3 M). One of the mechanisms of this cytotoxicity of NSAIDs on chondrocytes would be apoptosis. However, DFU showed no cytotoxic effect on chondrocytes. These results showed that these 2 COX-2 selective inhibitors revealed less cytotoxicity on chondrocytes than the traditional NSAIDs. On the other hand, higher concentration of ketorolac (10^-3 M) and 10^-4 M of indomethacin inhibited proliferation and arrested cell cycle in epiphyseal-articular chondrocytes. These results suggest that the inhibitory effects on proliferation, cell cycle arrest and cell death induction of NSAIDs on chondrocytes may play an important role on their suppressive effect on endochondral ossification during bone repair. In addition, these findings also imply that the NSAID effects on chondrocyte death may affect the normal endochondral ossification process of epiphyseal growth plate on and the homeostatic functions of articular chondrocytes.

REFERENCES: