INTRODUCTION

Previous reports of our laboratory indicated that dexamethasone treatment shifts the characteristics of osteogenesis into adipogenesis in a cloned pluripotent mesenchymal cell from bone marrow (D1-cell)(3). The conversion of the D1-cells into adipogenic cells upon steroid treatment in vivo was also demonstrated by transfected a traceable gene in D1-cell, and the lipid-lowering agent was noted to prevent these effects(4,5). Glucocorticoid-induced osteonecrosis of femoral head, along with fatty marrow changes, in chicken model was also observed to be similar to that occurs in humans(6). These findings suggested that the shift of osteogenesis to adipogenesis might be one of the important mechanisms involved in the pathogenesis of steroid-induced osteonecrosis and osteoporosis. Furthermore, steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs) have been indicated to have suppressive effects on bone remodeling. Our previous results showed that NSAIDs suppressed proliferation and induced cell death in cultured osteoblasts, suggesting these effects might be one the mechanisms contributing to their inhibitory effects on bone remodeling in vivo(6,7). In this study, we further investigated the steroid and NSAID effects on the cytotoxicity, proliferation, and differentiation in D1-cells.

METHODS

The D1-cell, a cell line cloned from bone marrow cells of Balb/c mice, is a pluripotent stem cell exhibits osteogenic properties in Dulbecco Modified Medium. We tested the lactate dehydrogenase (LDH) leakage, thymidine incorporation, cell cycle kinetics and the mRNA expression of osteocalcin, and adipin of D1-cells upon 24-hr treatment of dexamethasone, indomethacin, ketorolac, piroxicam or diclofenac in a broad concentration range (10^(-9)-10^(-3)M). The proliferation, cytotoxicity, and the osteogenic and adipogenic properties were compared among drug treated and non-treated control D1-cell cultures.

RESULTS

The results showed that dexamethasone inhibited thymidine incorporation and increased LDH leakage of D1-cells at concentrations of 10^(-7)-10^(-3)M. Indomethacin, ketorolac and diclofenac significantly inhibited the DNA synthesis of D1-cells at concentrations of 10^(-4)-10^(-7)M, while piroxicam suppressed the proliferation only at higher concentration (10^(-4)M) (Fig.1). These NSAIDs showed significantly arrest the cell cycle of D1-cells at G0/G1 phase (Fig. 2). Indomethacin, ketorolac and diclofenac significantly increased LDH leakage at concentration of 10^(-5)M, while piroxicam showed nonsignificant cytotoxicity for D1-cells (Fig.3). The mRNA expression of osteocalcin measured by RT-PCR showed that indomethacin had similar inhibitory effect as dexamethasone in D1-cells, while the other 3 NSAIDs revealed mild effects (Fig.4).

DISCUSSION

Our results showed that steroidal and non-steroidal anti-inflammatory drugs have cytotoxic and proliferation inhibitory effects on D1-cells, and revealed a concentration dependent manner. The effective concentrations of dexamethasone, indomethacin, ketorolac and diclofenac on the proliferation of D1-cells included the theoretic therapeutic concentrations (dexamethasone, 10^(-7)M; NSAIDs, 10^(-5)M). Higher concentration of these drugs revealed cytotoxic effects in D1-cells. Piroxicam showed less cytotoxicity and suppressive effect on D1-cells than the other 3 NSAIDs. These results suggest that the effects of proliferation suppression and cytotoxicity of both steroid and non-steroidal anti-inflammatory drugs on pluripotent stem cells may contribute to their suppressive effect on bone remodeling. The inhibitory effect of dexamethasone on proliferation of stem cells may be one of the mechanisms to elucidate the pathogenesis of steroid-induced osteoporosis and osteonecrosis. Our results demonstrated that the effects of indomethacin on the cytotoxicity, proliferation and osteogenesis in stem cells are similar to those of dexamethasone, suggesting indomethacin may affect the osteogenesis as the dexamethasone does.

REFERENCES