EFFECT OF ETIDRONATE ON COX-2 INDUCTION AND PROSTAGLANDIN E₂ PRODUCTION BY TITANIUM STIMULATED MACROPHAGE-LIKE CELLS IN VITRO

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Introduction
The most common cause of failure of total joint replacements is aseptic loosening in association with osteolysis. Many reports have showed that cytokines secreted from macrophages, such as PGE₂, IL-1, IL-6, TNFα, play an important role in osteolysis.

Recently, some clinical studies have shown that bisphosphonate therapy has reduced periprosthetic bone loss after total joint replacements [1-2] Bisphosphonates are synthetic compounds with the ability to decrease bone resorption. In addition, it is reported that they also have anti-inflammatory and analgetic effects by reducing the secretion of pro-inflammatory cytokines [3]. The mechanism of bisphosphonate action on reducing bone resorption is complicated, involving both direct effects on the osteoclasts and indirect effects through the osteoblasts.

The purpose of this study was to examine the effects of etidronate on suppressing cyclooxygenase-2 (COX-2) induction and Prostaglandin E₂ (PGE₂) secretion from a macrophage-like cell line stimulated by titanium particles, as a model of metallic debris, and to verify one of the bisphosphonate mechanism to reduce bone resorption around the prostheses.

Materials and methods
Cell culture. Murine macrophage-like cells RAW 246.7 cell line (American Type Culture Collection, VA, USA) were cultured in Dulbecco’s modified Eagle’s Medium (DMEM, Sigma Chemical, MO, USA) supplemented with 10% heat inactivated fetal bovine serum (Sigma), 100 U/ml penicillin and 100μg/ml streptomycin in a humidified environment of 5% CO₂ at 37°C. Cells at a logarithmic phase of growth were plated in 6-well plates at 2×10⁵ cells/well in 2ml of DMEM.

Cell-particle co-culture. Titanium particles (less than 8 µm in diameter, provided by Stryker) were added to the wells to attain final concentration of 0.1, 1, 10, 100 nM, 1 and 10 µg/ml oligo (dT) primer, Reverse transcription 10Xbuffer, 10mM dNTP Mix and AMV Reverse Transcriptase (Promega Corporation, WI, USA). PCR conditions were denaturation at 98°C for nine minutes, followed by 38-40 cycles of denaturation at 98°C for one minute, annealing at 60°C for 30 seconds and extension at 78°C for one minute. Thereafter, a seven-minute extension time at 72°C was allowed.

Treatment with disodium etidronate (EHDP). The cells were treated with EHDP at concentrations of 0.1, 1, 10, 100 nM, and 1 µg/ml, together with the titanium particles at concentration of 1mg/ml. After a 24 hour culture period, total RNA was isolated and RT-PCR was done. The supernatants were also collected and assayed for PGE₂ as described above.

Results
Titanium particles stimulated induction of COX-2 and release of PGE₂ in a time- and dose-dependent manner. RT-PCR analysis showed that COX-1 mRNA was constitutively expressed in both cells with and without challenging the particles. In contrast, titanium particles stimulated the induction of COX-2 mRNA by the cells in a dose- and time-dependent manner. Similarly, ELISA analysis showed that titanium particles also stimulated the production of PGE₂ by the cells in the same manner, consistent with the expression of COX-2.

Discussion
Bisphosphonates are pyrophosphate analogues characterized by a P-C-P bond. Many bisphosphonates, such as etidronate, alendronate, and risedronate, have been synthesized and used in the treatment of bone diseases, like Paget’s disease, hypercalcemia of malignancy, and osteoporosis. Although the detailed mechanism of action of bisphosphonates is still unknown, it may be that several mechanisms are operating simultaneously. At the cellular level, the following mechanisms are said to be involved: (1) inhibition of osteoclast recruitment; (2) inhibition of osteoclastic adhesion; (3) shortening of the life span of osteoclasts due to earlier apoptosis; (4) inhibition of osteoclast activity. Some of these mechanisms are indirect effects through the osteoblasts.

PGE₂ stimulates osteoblasts to induce osteoclastic differentiation factor RANKL, which activates osteoclast recruitment and differentiation. The existence of titanium wear debris phagocytosed by macrophages and high PGE₂ levels in the tissues surrounding the loosened components after joint replacement has been reported. Thus it can be hypothesized that bisphosphonates reduce bone resorption through the blockage of PGE₂ produced by macrophages. This in vitro study showed an evidence to support this hypothesis.

Conclusion
1. Etidronate inhibits COX-2 and PGE₂ production of macrophage-like cells stimulated by titanium particles.
2. This could be one way etidronate reduces periprosthetic bone loss.

References
[1] Yamaguchi K et al: Cyclic therapy with etidronate has a therapeutic effect against local osteoporosis after cementless total hip arthroplasty. Bone 2003;33:144-159.

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