Introduction

Aseptic loosening and peri-implant osteolysis are important obstacles for long-term survival of total hip arthroplasty (THA). Peri-implant tissues associated with osteolysis are composed of a foreign body granuloma with macrophages, giant cells and internalized wear debris. These tissues also secrete potent bone resorbing mediators such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) which also enhance osteoclast differentiation and activity. Currently, bisphosphonates are widely used in the treatment of metabolic bone diseases to inhibit osteoclasts and control bone resorption(1). Around joint replacements, bisphosphonates are effective in preventing wear debris-induced bone resorption(2). Thus, the purpose of this study was to investigate the role of various bisphosphonates (Alendronate, Zoledronate) in modulating the levels of the key bone resorbing cytokines IL-1β and TNF-α.

Materials and Methods

1. Tissue Collection

Capular tissues were obtained during primary THA’s for osteoarthritis (n=12, 3M, 9F, 46-75 years). Interfacial tissues surrounding the femoral stem were collected during revision surgeries for aseptic failure of uncemented (n=4), and cemented (n=6) THA’s (n=10, 5M, 5F, 41–96 years). Tissue samples were transported from the operating room immediately to the laboratory in PBS and minced under sterile conditions.

2. Tissue Culture

Approximately 400 mg of minced tissue was cultured in six wells of a 12-well plate in 2 ml DMEM supplemented with 100U/ml penicillin, 100g/ml streptomycin, 50 µg/ml gentamycin, and 10% FCS. Tissues were divided into 3 groups; Group A: no treatment; Group B: alendronate treatment (10⁻⁸ M); and Group C: zolendronate treatment (10⁻¹ M). Cultures were continued for 24 hr in a humidified atmosphere of 5% CO2 in air at 37°C.

3. Reverse transcription-polymerase chain reaction (RT-PCR)

Total cellular RNA was isolated in each condition using TRIzol® reagent (Gibco-BRL). cDNA was reverse-transcribed from the total RNA. And alterations in the gene expression of IL-1β and TNF-α were assessed via PCR amplification by using specific primer pairs. GAPDH transcription and amplification was used as a housekeeping gene. PCR products were electropheretically separated using 1.5% agarose gel with ethidium bromide.

4. ELISA

 Supernatant was collected from all tissue specimens after 24-hrs of culture and stored at -80°C till ELISA analysis was conducted. The supernatants were assayed for IL-1β and TNF-α levels (R&D Systems). The ELISA data was analyzed by ANOVA and two tail t-test for comparisons among the treatment groups. All p-values were compared to an alpha of 0.05 for statistical significance. RT-PCR results were assessed qualitatively.

Results

IL-1β levels in peri-implant tissues (33.2 pg/ml±40.0) were similar to that in osteoarthritis (23.9 pg/ml±4.53). TNF-α levels after 24-hrs of tissue culture were significantly lower in osteolysis cases (22.8 pg/ml±15.9) relative to osteoarthritis capsular tissues (38.6 pg/ml±11.4)(p<0.05). With both these cytokines, alendronate treatment resulted in slightly higher levels of cytokine release than non-treated controls (Fig 1). Similarly, zolendronate treatment tended to be lower than non-treated and alendronate groups. IL-1β and TNF-α mRNA expression was evaluated via RT-PCR and the results corroborated with an increase in gene expression of IL-1β and TNF-α in alendronate treated groups, and a decrease with zolendronate treatment (Fig 2).

Discussion

Periprosthetic osteolysis is mediated by various pro-inflammatory cytokines (TNF-α, IL-1, and IL-6) that enhance osteoclast differentiation and activity. The findings here that TNF-α levels were lower in osteolysis compared with osteoarthritis cases were consistent with previous reports from this laboratory(3).

Alendronate is an aminobisphosphonate with a potent anti-resorptive action. Adam et al suggested that patients treated with aminobisphosphonates such as alendronate cause an elevated acute inflammatory response(4). This is similar to the finding in our study that alendronate treatment caused a trend of higher cytokine levels in the peri-implant tissues. Zoledronate did not cause such an increase and actually tended to result in lower levels of cytokines in tissues. Zoledronate is a new generation bisphosphonate that inhibits osteoclast bone resorption, and is currently in clinical trials for hypercalcemia following metastasis. This drug is very attractive because it is given intravenously and one IV treatment is effective for upto one year. Our studies demonstrate the differential effects of bisphosphonates on peri-implant tissues and the promising effect of zolendronate in treating osteolysis.

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References


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