Effect of Interleukin-6 on PMMA Mediated Induction of COX-2 Gene Transcription

**Introduction:** Osteoclastic bone resorption in wear debris mediated periprosthetic osteolysis is regulated at several levels by a number of pro-inflammatory cytokines including Interleukin-1, Interleukin-17, Tumor Necrosis Factor-α, and receptor activator of NF-κB ligand (RANKL). 

In addition to these factors, a number of studies have demonstrated a role for COX-2 (Cyclo-Oxygenase2) as a potential mediator in osteolysis and implant loosening. Expression of COX-2 enzyme can be induced by pro-inflammatory cytokines TNF-α and IL-1. COX-2 expression results in increased PG2 production which stimulates RANKL expression and osteoclast activation. PMMA has been shown recently to inhibit IL-6 cytokine function by inhibiting activation of JAK/STAT signaling. Interleukin-6 is a pleiotropic cytokine that has recently been shown to play a major role as an anti-inflammatory cytokine capable of down regulating bone loss associated with inflammation.

The hypothesis for this study was that PMMA is capable of inducing COX-2 gene expression in human PBMC’s and that exogenous addition of IL-6 would inhibit PMMA induced COX-2 expression. To test our hypothesis, the present study was designed to examine the effect of PMMA on COX-2 gene transcription and the role of IL-6, if any, on PMMA mediated COX-2 expression in human peripheral blood mononuclear cells in vitro using semi-quantitative RT-PCR.

**Materials and Methods:**
- **Preparation of Particles:** Commercially pure polymethylmethacrylate particles (Polysciences Inc.; average diameter: 5.86 μm) were sterilized by 70% Ethanol treatment for 48 hours and resuspended in complete RPMI-1640 medium (Gibco-BRL) supplemented with 10% heat-inactivated FBS (Hyclone). Only endotoxin-free particles were used for this study as determined by limulus assay.

- **Co-culture of Human PBMCs/PMMA and Semi-quantitative RT-PCR:** Human PBMCs were isolated from buffy coat from healthy donors using Lymphoprep fractionation (Gibco-BRL). The isolated PBMC’s were resuspended in complete RPMI-1640 medium and incubated with PMMA particles for 3 hours at a 1:30 cell to particle ratio. To examine the effect of IL-6, human PBMCs were pre-treated with human IL-6 (R&D Systems; 25 ng/ml) for 1 hour prior to coincubation with PMMA particles. Cells without IL-6 and particles served as negative control.

**RT-PCR:** Total cellular RNA was isolated in each condition by cell lysis using RNA-zol (Gibco-BRL). RT-PCR was carried out using cDNA (reverse transcription of RNA) and primer specific for human COX-2 gene. The results were expressed in comparison with control gene human Actin.

**Results:** Human PBMCs following incubation with PMMA particles showed significant increase in COX-2 gene transcription at 3 hour time point with respect to control (cells only). Cells treated with IL-6 alone failed to show any induction in COX-2 expression. Interestingly, treatment of human PBMCs with IL-6 for 1 hour before PMMA challenge significantly inhibited PMMA induced COX-2 expression. The validity of these findings are supported by uniform expression of control gene human Actin. (Figure 1)

**Discussion:** Bone remodelling is a dynamic process regulated by several factors. Cyclo-oxygenase(COX-2) has been shown to play a role in wear debris mediated periprosthetic osteolysis. The results of this study clearly demonstrate that human PBMC’s exposed to PMMA particles for 3 hours increase their expression of COX-2 gene. The fact that pre-treatment with IL-6 for one hour resulted in reversal of PMMA induced COX-2 gene expression is interesting. It is perhaps further indirect evidence that PMMA induced periprosthetic osteolysis may be the result of inhibition of the anti-inflammatory function of IL-6 in addition to the activation of pro-inflammatory cytokines.

**Conclusion:** PMMA particle exposure in human PBMC’s results in increased COX-2 gene expression. Pretreatment of PBMC’s with IL-6 prior to PMMA particle challenge inhibits the increased expression of COX-2.

**References:**

**Figure 1**

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