ADENO-ASSOCIATED VIRUS (AAV)-MEDIATED OPG GENE TRANSFER PROTECTS AGAINST PARTICULATE UHMWPE-INDUCED OSTEOLYSIS IN A MURINE MODEL

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Wear debris-associated osteolysis results in the loss of bony support of prosthetic joint components, leading to aseptic loosening and the failure of total joint arthroplasty. Osteoclast differentiation factor (ODF, also called RANKL or TRANCE) plays a critical role in osteoclastogenesis and contributes in the process of prosthetic joint loosening. Osteoprotegerin (OPG), a natural decoy receptor of ODF, has emerged as a potential therapeutic agent for treatment of osteolytic conditions including osteoporosis and rheumatoid arthritis. Utilizing an in vivo rAAV-mediated gene transfer technique, we report here that successful transduction of human OPG gene to a murine model of osteolysis resulted in protection against orthopaedic wear debris-induced bone loss.

**Methods:** Air pouches were established on dorsal region of BALB/c mice by repeated injection of sterile air. A section of femur or calvarium from a syngeneic donor was implanted into the pouch on day 6, and UHMWPE particles were introduced after 24 hours to provoke inflammation and osteolysis. The adeno-associated viruses coding human OPG gene (rAAV-hOPG) or LacZ control gene (rAAV-LacZ) at 1x10^8 i.u. were injected into the air pouches, and the tissue harvested 7 days after viral infection for histological and molecular analyses. The above animal experimental procedures were approved by the institutional Animal Investigation Committee. ELISA assay for hOPG was performed to detect the transgene production. Real time PCR was used to examine the expression changes of osteoclast markers (CTR, CPK) and proinflammatory cytokines (IL-1, IL-6 and TNFα). Fluorescent spectrophotometry was utilized to determine mobilized calcium concentration in pouch fluid as a measure of bone demineralization. Computerized image analysis system was employed to quantify the bone collagen content and inflammatory parameters of pouch membranes.

**Results:** Successful transgenes expression was confirmed by both detection of hOPG in pouch fluid and homogenate (70.1 pg/mg and 87.8 pg/mg protein, respectively) using ELISA, and positive X-gal staining of pouch tissue. Real-time PCR indicated that the expression of osteoclast markers calciitonin receptor (CTR) and cathepsin K (CPK) in the pouches with hOPG-transduction were significantly diminished in comparison with rAAV-LacZ transduced pouches (p<0.05). The mRNA level of the biological receptor for ODF (RANK) was also markedly decreased by the transduction and expression of hOPG (p<0.05). Interestingly, rAAV-hOPG transduction resulted in a significant reduction of IL-1 expression in the model, compared to either LacZ-transduced or non-viral control pouches; however, the reduction of either TNFα or IL-6 did not reach significance. Figure 1 summarizes the calcium concentration released into air pouch fluid. The expression of hOPG in the bone implanted pouch reduced bone calcium release by a mean of 31% (± 1.8%) compared with the calcium release in LacZ-transduced or non-viral control bone implants (p<0.04). Computerized image analysis of histology sections stained with modified Trichrome Blue revealed that expression of hOPG significantly protected against collagen loss from bone areas in close contact with inflammatory membranes (9.8% collagen loss), in comparison with similar regions in LacZ-transduced sections (22.1% loss, p<0.04) and in non-viral controls (27.7% collagen loss, p<0.01), respectively. There was no significant difference in bone collagen loss between LacZ-transduced and non-viral medium groups.

**Discussion:** We recently reported the development of a modified murine air pouch with bone implantation to study debris-associated bone resorption (1). With this model, we have now successfully transduced hOPG using rAAV in vivo transfer technique and investigated the protection effects of OPG against UHMWPE-induced bone resorption. Transgene-expressed OPG significantly inhibited osteoclastogenesis, as indicated by the reduction of CPK and CTR expression in the pouches. The protection effects of the gene therapy were also confirmed by the reduction of bone collagen loss and bone calcium release into the pouch fluid. Although OPG transduction did not have a significant effect on most parameters of particle-induced inflammation, IL-1 expression was significantly reduced 7 days after gene therapy. The data suggest that the murine air pouch model of bone resorption is a useful tool to screen therapeutic approaches for debris-associated osteolysis; and the gene therapy using rAAV-hOPG might be a feasible and effective therapeutic candidate to treat or prevent wear debris-associated osteolysis and aseptic loosening.

![Mobilized Calcium in pouch lavage](image)

Figure 1


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