Early Detection and Therapeutic Intervention of Peri-prosthetic Inflammation and Osteolysis Using HPMA Copolymer Conjugates

Ren, K; Purdue, P E; Burton, L; Chen, F; Fehringer, E V; Goldring, S R; Wang, D

1University of Nebraska Medical Center, Omaha, NE; 2Hospital for Special Surgery, New York, NY
dwang@unmc.edu

Introduction:
Roughly 1.5 million total joint arthroplasty procedures are performed annually worldwide to treat end-stage joint disease. The overall 10-year success rate for total joint replacement is ~90%, indicating that close to 10% of patients will require revision surgery, which is associated with a poorer outcome and a shorter duration of implant survival. Wear particles generated from the articulating surfaces of prosthetic components and wear particle-induced inflammation are the major cause of aseptic implant loosening and clinical failure after total joint replacement. The wear particles have been shown to activate macrophages, and to induce a granulomatous inflammatory reaction that results in osteoclast-mediated peri-implant osteolysis at the bone-implant interface leading to loss of fixation. There is a need for early detection techniques and treatment of this pathological condition prior to the loss of implant fixation.

This study was undertaken to establish a diagnostic tool for early detection of wear particle-induced inflammation and associated peri-implant osteolysis to determine if N-(2-Hydroxypropyl)methacrylamide (HPMA) copolymer-Dex conjugate (P-Dex), a macromolecular prodrug designed for arthrotropic treatment of inflammatory arthritis would ameliorate peri-implant osteolysis.

Methods:
1. Synthesis of HPMA copolymer conjugates

The near infrared dye labeled HPMA copolymer (poly(HPMA-co-APMA-co-IRDye 800CW)) and P-Dex were synthesized according to established methods reported in literature.

2. In vivo mouse calvaria resorption model

After removal of the periosteum from the calvaria of Swiss Webster mice with a 25G needle, polymethylmethacrylate (PMMA) particles (1-10 μm) or saline were injected directly onto the removal site. This procedure has been proved to be reliable in induction of a granulomatous inflammation associated with osteoclast-mediated osteolysis.

3. In vivo imaging and P-Dex treatment analysis

All animal procedures have been approved by Institutional Animal Care and Use Committee of Nebraska Medical Center. One day post induction, poly(HPMA-co-APMA-co-IRDye 800CW) or P-Dex were administered (i.v.) to the mice. The animals injected with dye were then imaged using the IVIS® 200 Imaging System to evaluate the biodistribution of the HPMA copolymer for the next 6 days.

4. Micro-CT

The calvaria were removed and scanned in 70% ethanol solution in a Scanco μT35 (Scanco Medical, Brütisellen, Switzerland) μCT system with a resolution of 15 microns at regular contrast conditions (25KVP, 145μA, 0.36 degrees angular rotation step).

5. Histological evaluation

After decalcification in 10% EDTA, the calvaria were paraffin embedded for sectioning. The sections (5μm) were stained with hematoxylin & eosin (H & E) for histological evaluation. Osteoclast-like cells in whole calvaria were identified by tartrate-resistant acid phosphatase (TRAP) staining using a commercial kit (387A, Sigma).

6. Macrophage culture

Macrophages were isolated from normal human donors and cultured for 24-48 hr in alpha-MEM medium containing 10% human M-CSF, pulsed for 4 hours with P-Dex (5μM) or Dex (0.6μM), washed and replenished with fresh medium. After 24 hr, cells were either analyzed by confocal microscopy or challenged with inflammatory mediators. For confocal microscopy, cells were treated with LysoTracker Red DND-99 and Hoechst 33342 to stain the lysosomal and nuclear compartments, respectively. For inflammatory challenge, cells were treated with 40pg/ml LPS +/- PMMA particles (30 particles/cell) for 6-24 hr. Conditioned media were then analyzed for inflammatory cytokines (TNF, IL1, IL6) by ELISA.

7. Statistical methods

Comparisons were made between two groups by two sample t-test. A value of P < 0.05 was considered statistically significant.

Results:

Compared to the ubiquitous and transient distribution of poly(HPMA-co-APMA-co-IRDye 800CW) in the sham induction (saline) group, IVIS images showed clear preferential and long-lasting (detectable 7 days post-administration) distribution of the polymer dye to the sites of calvarial implanted with PMMA particles. TRAP staining confirmed the presence of osteoclasts associated with focal osteolysis in the PMMA-implanted group. Micro-CT demonstrated the amelioration of PMMA particle induced bone resorption in the P-Dex treated group, compared to the group with no treatment. Macrophage culture studies revealed that the P-Dex was taken up by PMMA particle-activated macrophages, and could effectively down-regulate the level of inflammatory cytokines, including TNF and IL-1. This observation is consistent with a local macrophage-mediated prodrug retention and activation mechanism.

Discussion:

HPMA copolymers are biocompatible, nonimmunogenetic and nontoxic water-soluble polymers that have been used extensively as drug carriers. When tagged with imaging probes, the polymer carrier preferentially extravasates and accumulates at the site of peri-prosthetic inflammation prior to the development of severe osteolysis. When conjugated with gamma or positron emitters, this system can be employed in human subjects with implanted orthopedic devices to screen for early signs of peri-implant inflammation and evolving osteolysis. Further studies are necessary to optimize the system for both detection and treatment purposes. In these studies, Dex was selected for the conjugate drug because of its demonstrated potent anti-inflammatory properties. In the future, this system can be adapted for additional biologically active molecules that exhibit similar anti-inflammatory and/or immunosuppressive properties.

Reference:

Paper No. 191 • 56th Annual Meeting of the Orthopaedic Research Society