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Macromolecular prodrug of dexamethasone prevents particle-induced peri-implant osteolysis with reduced systemic side effects

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ABSTRACT

Aseptic implant loosening related to implant wear particle-induced inflammation is the most common cause of failure after joint replacement. Modulation of the inflammatory reaction to the wear products represents a rational approach for preventing aseptic implant failure. Long-term treatment using anti-inflammatory agents, however, can be associated with significant systemic side effects due to the drugs' lack of tissue specificity. To address this issue, *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymer–dexamethasone conjugate (P–Dex) was developed and evaluated for prevention of wear particle-induced osteolysis and the loss of fixation in a murine prosthesis failure model. Daily administration of free dexamethasone (Dex) was able to prevent wear particle-induced osteolysis, as assessed by micro-CT and histological analysis. Remarkably, monthly P–Dex administration (dose equivalent to free Dex treatment) was equally effective as free dexamethasone, but was not associated with systemic bone loss (a major adverse side effect of glucocorticoids). The reduced systemic toxicity of P–Dex is related to preferential targeting of the sites of wear particle-induced inflammation and its subcellular sequestration and retention by local inflammatory cell populations, resulting in sustained therapeutic action. These results demonstrate the feasibility of utilizing a macromolecular prodrug with reduced systemic toxicity to prevent wear particle-induced osteolysis.

1. Introduction

Total joint replacement has been performed in North America since the early 1970's and is considered to be the final treatment option to relieve pain and restore joint function in patients with advanced osteoarthritis or rheumatoid arthritis. There are over 600,000 total joint replacement surgeries performed annually in the United States, and the number is expected to increase to 4 million annually by 2030 [1,2]. Despite the significant progress in the implant design/materials and surgery techniques, the overall 10-year success rate for total joint replacement is ~90%, with close to 10% of patients ultimately requiring revision surgery [3]. Revision procedures are costly, surgically more challenging, and associated with a shorter duration of implant survival. Therefore, prevention of total joint replacement failure would be of great benefit to patients and the healthcare system.

Aseptic loosening of prosthetic implants due to peri-implant osteolysis is a common cause of failure after joint replacement [4]. Inflammation induced by wear particles generated from the prosthetic components is considered to be the major cause of osteolysis and aseptic implant loosening [5,6]. Wear debris are phagocytosed primarily by macrophages, leading to activation of pro-inflammatory cascades, resulting in osteoclast-mediated osteolysis at the bone-implant interface and eventual loss of fixation [7,8]. Thus, wear particle-induced inflammation plays a vital role in the development of wear particle-induced total joint replacement failure and consistent with this, modulation of inflammation has been proven to be an effective strategy to prevent wear particle-induced bone resorption [9–11].

Long-term modulation of inflammation is needed to prevent aseptic implant loosening. However, the lack of tissue specificity of anti-inflammatory drugs combined with their ubiquitous distribution may cause significant systemic adverse side effects after long-term use. For example, glucocorticoids (GC), a class of highly potent anti-inflammatory drugs, have been widely used in treating many inflammatory diseases, including rheumatoid arthritis, asthma, bronchospasm and inflammatory bowel diseases. The ubiquitous distribution of GC and their adverse systemic toxicities such as immunosuppression and secondary osteoporosis, however, have limited their therapeutic potential for many chronic inflammatory conditions.

In order to address the problem related to high off-target GC concentrations and associated systemic toxicity, we developed a *N*-(2-Hydroxypropyl) methacrylamide (HPMA) copolymer–dexamethasone conjugate (P–Dex) [12,13]. In previous studies, we have found that

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HPMA copolymer conjugated with an imaging probe could preferentially target to the sites of particle-induced inflammation prior to detectable osteolysis in a murine calvaria osteolysis model [14]. Based on these preliminary results, we hypothesized that P–Dex would preferentially target the site of peri-implant inflammation to effectively prevent osteolysis and avert the typical side effects associated with GC systemic exposure. This study was designed to directly validate this hypothesis.

2. Material and methods

2.1. Synthesis of macromolecular prodrug

P–Dex was synthesized by reversible addition–fragmentation chain transfer (RAFT) copolymerization as described previously [12]. Briefly, *N*-(2-hydroxypropyl) methacrylamide (HPMA), *N*-methacryloylglycylglycylhydrazyl dexamethasone and trace amount of *N*-methacryloylaminopropyl fluorescein thiourea (for visualization in column purification) [15] were copolymerized at 40 °C under argon for 48 h with 2,2′-azobisisobutyronitrile as the initiator and *S*,*S*′-bis(α, α′-dimethyl-α″-acetic acid)-trithiocarbonate as the RAFT agent. Resulting polymers were purified by LH-20 column (GE HealthCare, Waukesha, WI) and lyophilized. *N*-(3-Aminopropyl)methacrylamide hydrochloride (APMA, Polysciences, Inc. Warrington, PA) was added to the aforementioned co-monomers to obtain the APMA-containing P–Dex. Alexa Fluor 488 labeled P–Dex (P–Dex-Alexa) and IRDye 800 CW labeled P–Dex (P–Dex-IRDye) were obtained via polymer analogous reaction between APMA-containing P–Dex and NHS esters of the dyes [14].

2.2. Establishment of a murine prosthesis failure model

Poly(methyl methacrylate) (PMMA) particles (1–10 μm, Bangs Laboratories, Fishers, IN) were soaked in 70% ethanol overnight, then washed and suspended in sterile phosphate buffered saline (PBS). A limulus assay was performed using a Pyrosate® kit (Associates of Cape Cod, Inc., East Falmouth, MA) to confirm that the treated particles were endotoxin-free. All animal experiments were performed according to a protocol approved by University of Nebraska Medical Center Institutional Animal Care and Use Committee. Male Swiss Webster mice (10 weeks, Charles River Laboratories Inc., Wilmington, MA) were anesthetized with a mixture of xylazine and ketamine. The surgical site and wide margins (1 cm or more if possible) were clipped free of hair, scrubbed twice with betadine, and then wiped with 70% isopropyl alcohol. A distal to proximal incision along the medial aspect of the patella was made and the patella was reflected laterally to expose the femoral condyles. A 25-gauge needle was used to manually drill through the intercondylar notch to access the medullary cavity and the marrow cavity of the femur was reamed out to a depth of 5 mm from the condyles. The medullary canal was flushed with PBS. For the induction of osteolysis, PMMA particles (1 mg in 10 μL sterile PBS) were injected into the medullary canal before insertion of the pin. Then a 0.45 mm × 5 mm stainless steel pin was inserted into the medullary chamber of the distal femur. The patella was reduced to its anatomical position. The wound was cleaned, rinsed with saline containing antibiotics and closed with sutures in layers. Antibiotics (cefazolin sodium, 25 mg/kg, oral) and analgesics (buprenorphine, 0.5 mg/kg, s.c.) were given twice daily for three days after surgery. The position of the implant was validated with plain X-ray. PMMA particles (1 mg in 10 μL PBS) were injected into the knee twice a month postoperatively to induce osteolysis. For the control group, PBS was injected at the time of surgery and twice a month postoperatively. A total of 75 male Swiss Webster mice were used in this experiment. For prevention study, 30 mice were implanted with stainless pin and injected with PMMA in both sides. As the control group, 6 mice were implanted with the pin and injected with PBS on both sides instead of PMMA particles. The rest of the animals (39 mice) were used for optical imaging (6 mice), flow cytometry (9 mice × 3 repetition) and immunohistochemical analysis (6 mice). These mice were implanted with the stainless steel pin in both femurs and injected with PMMA in one side and PBS in the other.

2.3. In vivo treatment study

As shown in Table 1, 30 mice with pin implantation and PMMA particle infusion on both sides were randomly assigned into 5 groups for prevention study. One-month post surgery, the animals were given the following treatments: group 1, HPMA homopolymer without Dex (PHPMA, the amount of polymer used was equivalent to that in group 4, monthly i.v. injection, two injections in total); group 2, PBS (monthly i.v. injection, two injections in total); group 3, free Dex (total dose = 10 mg/kg, daily i.p. injections for two months); group 4, P–Dex (equivalent Dex dose = 10 mg/kg, monthly i.v. injection, two injections in total); group 5, P–Dex (equivalent Dex dose =5 mg/kg, monthly i.v. injection, two injections in total). The sixth group was designated as the control with pin implantation and PBS injection on both sides instead of PMMA particle infusion. At three months postsurgery, these mice were euthanized. The femurs and L4 lumbar vertebrae bodies of the animals were isolated and fixed with 4% buffered paraformaldehyde and subjected to micro-CT analysis.

2.4. Micro-CT evaluation

A high-resolution Skyscan 1172 micro-CT (Skyscan, Aartselaar, Belgium) was used for qualitative and quantitative analyses. For femurs, micro-CT focused on the distal part of the femur. The X-ray source was set at a voltage of 70 kV and a current of 141 μA with a fixed exposure time of 480 ms. Eight frames were averaged with a rotation step of 0.7° following an angle of 180°. The resolution was 5.5 μm using a medium camera pixel size (2000 × 1336) with Al 0.5 mm filter. The region of interest (ROI) was selected from 300 slices below the growth plate and extending distally for 100 slices, defined as a ring from the implant axis and with a radius of 0.6 mm. The pin was excluded from the ROI by setting the specific threshold. Three-dimensional reconstructions of the scanned region by the system reconstruction software (NRecon, Skyscan) and bone background segmentation were then performed. For quantitative analysis of the particle-induced osteolysis, the resident software (CTAn, Skyscan) was used to obtain specific morphometric parameters within the ROI, including bone volume (BV), tissue volume (TV), bone volume fraction (BV/TV), bone surface density (BS/TV), trabecular number (Tb. N) and structure model index (SMI) in 3D. In the two-dimensional (2-D) slice-by-slice analysis, average bone fragment area, average bone fragment numbers and mean polar moment of inertia (MMI) were calculated. To produce a visual representation of the results, 3D images were developed using the resident CT-Vol software. Methods to calculate the above mentioned bone parameters are described in detail on the Skyscan website.

Table 1
Animal treatment group arrangement.

Group	Challenged with	Treated with	Dosage	Administration frequency
1	PMMA	PHPMA	Equivalent to polymer amount in group 4	Monthly i.v. injection, two injections in total
2	PMMA	PBS	0.1 mL	Monthly i.v. injection, two injections in total
3	PMMA	Free Dex	Total Dex Dose = 10 mg/kg	Daily i.p. injections for two months
4	PMMA	P–Dex	Equivalent Dex Dose = 10 mg/kg	Monthly i.v. injection, two injections in total
5	PMMA	P–Dex	Equivalent Dex Dose = 5 mg/kg	Monthly i.v. injection, two injections in total
6	PBS	–	–	–