¹²⁵I-labeled OP-1 is Locally Retained in a Rabbit Lumbar Fusion Model

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Abstract Osteogenic protein-1 is evolving as a potential bone graft alternative. Surgical site retention is important to maximize local osteoinduction and to limit peripheral effects. An established rabbit lumbar posterolateral fusion model was used to evaluate the systemic distribution and pharmacokinetics of locally applied osteogenic protein-1 delivered on a collagen carrier. L5-L6 intertransverse process fusions were performed on 27 New Zealand White rabbits. Radiolabeled ¹²⁵I-osteogenic protein-1 collagen putty was implanted. At intervals, whole blood, plasma, and excreta were analyzed for radioactivity with liquid scintillation counting. Surgical site and tissue radioactivity also were assessed by quantitative whole-body autoradioluminography of animals euthanized at times ranging from 6 hours to 35 days. Animals remaining at the final time were assessed for fusion with manual palpation, radiography, and histology. Limited distribution of radioactivity

was observed in the blood, plasma, and tissues apart from at the surgical site and in the urinary bladder and thyroid. The mean residence time for osteogenic protein-1 collagen putty was 10.4 ± 2.7 days. These excretion profiles and kinetic properties are similar to those described for recombinant human bone morphogenetic protein-2 in the rabbit model (mean residence times of 7.6 days and 10.2 days with different carriers).

Introduction

Posterolateral lumbar fusion is a common surgical procedure. Traditionally, intertransverse process fusion has been achieved using autogenous iliac crest bone graft. Autograft, however, is associated with potential limitations of supply [7], donor site morbidity [1], and fusion site pseudarthrosis [13]. Additionally, a second operative site increases blood loss, infection risk, and operative time [3]. These shortcomings have prompted investigation for graft alternatives with recent efforts focusing on bone morphogenetic proteins (BMPs) [7].

Bone morphogenetic proteins are osteoinductive molecules in the transforming growth factor-beta (TGF- β) superfamily. Applied in vivo, recombinant human BMPs (rhBMPs) induce undifferentiated mesenchymal stem cells to enter an osteogenic pathway [19]. One such rhBMP, osteogenic protein-1 (OP-1), also known as BMP-7, has been cloned and commercially developed by Stryker Biotech (Hopkinton, MA). The effectiveness of OP-1 has been shown in a rabbit posterolateral lumbar fusion model with consistent fusion rates of 100% (versus 63% for autograft) [5, 14]. Osteogenic protein-1 putty currently is approved in the United States under a Humanitarian Device Exemption for revision posterolateral lumbar fusion in which use of

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autograft is not feasible or is not expected to result in successful fusion. Additional clinical trials are ongoing with preliminary reports suggesting fusion rates similar to autograft in humans [10, 20].

Successful fusion requires OP-1 to be retained at the surgical site long enough to achieve osteoinduction. Carrier biomaterials act as frameworks for osteogenic cell colonization and improve localization of implanted protein [12, 16]. It is unclear whether fusion enhancement depends on the action of carrier-bound BMP or of freely diffusible BMP slowly released from the carrier [18]. Nevertheless, elucidation of retention kinetics may aid evaluation and development of BMP delivery systems to optimize clinical outcomes [12, 18].

Evaluation of rhBMP-2 (Medtronic Sofamor Danek, Memphis, TN) in a rat ectopic assay model has led to characterization of a biphasic BMP release profile. The initial burst release correlates with protein solubility [16, 17], whereas the gradual secondary release is governed by carrier properties [16, 22]. Mean residence time (MRT), the average time that implanted or bolus-administered drug molecules are retained at the site of interest, is an important predictor of osteoinductive potential. Delivery of ^{125}I -labeled rhBMP-2 on an absorbable collagen sponge (ACS) in the rat ectopic model yielded MRTs of approximately 3 days [18]. In a rabbit posterolateral lumbar fusion model, implantation of radiolabeled rhBMP-2 on a biphasic calcium phosphate-absorbable collagen sponge (BCP/ACS) combination or on BCP alone produced MRTs of 7.6 and 10.2 days, respectively, although this difference was not statistically significant [12].

Implant site rhBMP retention is important not only to maximize local osteoinductive activity [16, 18], but also to limit nonspecific effects associated with broad distribution. Although local treatment with the BMPs is not associated with a specific peripheral effect in toxicology studies, these proteins have been shown to have transforming effects in such diverse organs as the kidneys [21], heart [4], and brain [9]. Using scintigraphy to measure blood sample radioactivity, systemic exposure to rhBMP-2 in a rabbit spine fusion model has been judged to be negligible [12]. The actual delivery of implanted rhBMPs to tissues throughout the body, however, has yet to be systematically analyzed in a lumbar fusion model.

The purpose of this study was to document how well OP-1 implanted in the lumbar spine to induce fusion would be locally retained. The primary research objective was to characterize the excretion profile and mean residence time of OP-1 with liquid scintillation counting. The secondary objective was to determine the surgical site and total body distribution with quantitative whole-body autoradioluminography.

Materials and Methods

We performed single-level, intertransverse process fusions at L5-L6 on 27 New Zealand White rabbits (these animals have seven lumbar vertebrae) [2, 6]. Radiolabeled OP-1 was implanted in a commercial formulation of collagen putty (further described subsequently).

Before surgery, we collected blood and excreta to determine background radioactivity levels with liquid scintillation chromatography (LSC). Excreta were collected in metabolism cages equipped for the separate collection of urine and feces. We obtained blood samples from peripheral arteries (approximately 1.2 mL).

Three animals per time were euthanized at 6 hours and at 1, 3, 7, 14, 21, 28, and 35 days postoperatively. After the animals were euthanized, blood was drawn for radioactivity analysis, and we evaluated animals by quantitative whole-body autoradioluminography (QWBA) to assess radioactive OP-1 concentration at the surgical and remote tissue sites. The three remaining animals were euthanized on Day 35 for evaluation of fusion.

We collected urine and feces at 6 hours and subsequently at 24-hour intervals for the six animals being followed until 35 days. These samples, and cage washings collected at 72-hour intervals, were analyzed for radioactive content with LSC. We evaluated fusion in these animals by in vivo radiography. Lumbar spines of the three animals not processed for QWBA analysis then were explanted for additional fusion evaluation by manual palpation [2] and histology.

No autograft control group was included, because the study was designed to characterize retention kinetics of radiolabeled OP-1 in the described carrier. Because fusion rates of 100% have been seen consistently with OP-1 in this model [8], powered fusion analysis was not a primary objective. Rather, the three animals analyzed were included to confirm that radioactive labeling of OP-1 would not interfere with its osteoinductive properties. All histologic sections therefore were viewed by one individual (LC) blinded to the results of manual palpation. We selected the 35-day study period because prior investigations have shown fusions to be distinguishable from nonunions by this time [2, 8].

The implant for this study was composed of commercially available OP-1 putty (3.5 mg OP-1 with 1 g Type I collagen and 200 mg carboxymethylcellulose-sodium) supplemented with ^{125}I -labeled OP-1 (Amersham Biosciences, Piscataway, NJ). Dosing levels were selected to permit comparison with previous investigation of BMP pharmacokinetics in the rabbit lumbar fusion model [12]. Each implant was reconstituted with 2.5 mL of water for a final volume of 3 cc. Before dosing, we analyzed implant

radioactivity by liquid scintillation counting. The test material was prepared to achieve a target radioactivity level of 50 μCi per implant. We used half of the implant on each side.

Rabbits weighing 3.3 to 4.1 kg (average, 3.7 kg) were housed for a minimum of 9 days before surgery to permit acclimation and to ascertain background levels of radiation in excreta. We administered enrofloxacin (10 mg/kg) intramuscularly before surgery. Surgical anesthesia was achieved with subcutaneous injection of 0.01 mg/kg glycopyrrolate followed by 50 mg/kg ketamine and 5 mg/kg xylazine. We then intubated the rabbits, and isoflurane inhalation was used to maintain anesthesia.

The rabbits were shaved, positioned, and prepared in a standard surgical fashion. We made a dorsal midline incision in the lumbar region. The L5 and L6 transverse processes were identified and exposed through two paramedian fascial incisions. After irrigation with saline, the transverse processes were decorticated with a burr and the graft material described was placed using a spatula. The incision was closed and the rabbit extubated.

Buprenorphine (0.02 mg/kg twice daily) was delivered subcutaneously and 10 mg/kg enrofloxacin was given daily intramuscularly for 2 days after surgery for postoperative analgesia and antibiosis. We analyzed the gauzes and spatulas used to administer the implants for retained radioactivity, and these quantities were subtracted to determine the true doses administered. A mean of 2.0 mg of OP-1 per animal was achieved.

Veterinary staff monitored animal status daily, and surgical staples were removed on postoperative Day 14. During the acclimation and postoperative periods, all rabbits were given free access to 180 g per day of pelleted commercial laboratory diet. Animals were weighed weekly, and supplemental hay and vegetables were provided to animals with reduced appetite at any point during the study. Municipal tap water, routinely analyzed for impurities, was available on demand through an automated water system.

We measured radioactivity concentrations in blood, plasma, urine, feces, and cage wash by LSC. Blood samples were taken from a peripheral artery at 6, 24, and 72 hours and 7, 14, 21, 28, and 35 days postdose. Fecal homogenates were solubilized in soluene, decolorized, and mixed with liquid scintillation fluid. We mixed aliquots of plasma, urine, and cage wash directly with liquid scintillation fluid. Each sample was counted for 5 minutes or to a two-sigma error of 0.1%, whichever occurred first. Samples that exhibited radioactivity concentrations less than or equal to double background values were considered zero.

We processed all animals but the three retained for fusion analysis for evaluation with QWBA. Ten ^{125}I standard solutions ranging from 0.5 to 2000 nCi/g were prepared as markers for use in imaging plate exposures.

Concentrations were confirmed by LSC. After the animals were euthanized, they (three per time) were frozen in hexane and dry ice. Specimens were embedded in a 2% carboxymethylcellulose (CMC) medium using a freezing frame, and holes were made in each CMC block for the standard solutions. We obtained 30- μm sections using a Leica CM 3600 cryomicrotome (Wetzlar, Germany).

Sections were exposed to an imaging plate and read by the Fuji BAS-2500 scanner using Fuji Image Reader software version 1.1 (Tokyo, Japan). Using the images obtained, we quantified the amount of radioactivity in representative tissues and areas of focal collection with reference to a calibration curve generated from the standard solutions using Fuji Image Gauge Analysis software version 3.122.

For histology, specimens were fixed for 24 hours in 10% formalin and then transferred to 70% alcohol for at least 1 week. We subsequently dehydrated these specimens in 95% and 100% ethanol and cleared them in xylene. The undecalcified specimens were embedded in methylmethacrylate and 5- μm sections were cut using a microtome. Sections were stained with hematoxylin and eosin and Goldner's trichrome for microscopic examination.

We calculated whole blood, plasma, urine, feces, and cage wash concentrations of radioactivity in mass equivalents per gram ($\mu\text{g eq/g}$) on the basis of the specific activity of the formulated OP-1 (dpm/mg). The radioactivity concentration in blood and plasma samples was converted to mass equivalents per milliliter ($\mu\text{g eq/mL}$) on the basis of the density of rabbit blood and plasma, respectively. We calculated percent of dose values for urine, feces, and cage washings based on the total radioactivity measured in collected samples and the calculated dose administered to the animals.

Disposition of radioactivity in whole blood, plasma, and tissues was estimated using the mean total radioactivity in the respective samples. We calculated mean residence times using log linear transformation of the percent dose remaining (the initial dose minus cumulative excretion at time). Negative values were excluded and trend lines added. We calculated area under the radioactivity concentration versus time profile ($\text{AUC}_{0-\text{tlast}}$) using the linear trapezoidal method [15]. Group mean values and standard deviations were calculated, and results are reported as a value $\pm$ the standard deviation unless fewer than three values were obtained.

Results

Using LSC, residence time and routes of excretion for labeled OP-1 were confirmed and limited blood and plasma

distribution was observed. The MRT for OP-1 collagen putty was 10.4 ± 2.7 days. Excretion of radioactivity was primarily through the urine ($95.9\% \pm 9.4\%$). Lesser quantities of radioactivity were recovered in the feces ($5.7\% \pm 1.4\%$) and cage washes (less than $2.5\% \pm 1.0\%$). Radioactivity was excreted gradually up to 35 days postoperatively with total dose recovery values of $104.1\% \pm 9.8\%$. Extrapolated percentages of dose for blood and plasma remained less than 3% at all times. The highest concentrations of labeled OP-1 (t_{max}) in implanted animals were $0.239 \pm 0.074 \mu\text{g eq/mL}$ for blood and $0.263 \pm 0.074 \mu\text{g eq/mL}$ for plasma observed 24 hours

Fig. 1A–D Quantitative whole-body autoradioluminography images for ^{125}I -osteogenic protein-1 TCP putty at (A) 6 hours, (B) 7 days, (C) 21 days, and (D) 35 days show the main concentration of radiation at the surgical site.

postoperatively. Areas under the radioactivity concentration versus time curves for blood and plasma, which quantify total OP-1 absorption, were $34.2 \mu\text{g eq}\cdot\text{h/mL}$ and $26.4 \mu\text{g eq}\cdot\text{h/mL}$, respectively.

With QWBA, we observed concentrations of radioactivity to be highest at the surgical site, bladder, and thyroid, whereas they remained minimal in other tissues. Surgical site concentrations were consistently greater than those of all other tissue foci, indicating substantial implant retention (Fig. 1). At the initial observation point (6 hours), the concentration at the implant site assessed by QWBA was $264 \pm 84 \mu\text{g eq/g}$ and subsequently diminished gradually throughout the study period (Fig. 2). The surgical site concentration versus time AUC for OP-1 collagen putty was $3297 \mu\text{g eq/g}$ per day. Concentrations were below the limit of quantification for all times in the adipose tissue, brain, eye, pituitary gland, dorsal skeletal muscle, and spinal cord. In the other tissues analyzed, all concentrations decreased below the limit of quantification by postoperative Day 7.

Exclusion rates and radiographic findings were consistent with previous studies using OP-1 in the rabbit lumbar fusion model. One animal was euthanized before the scheduled time because of hindlimb neurologic change. This rate of one of 27 or 3.7% is comparable to those reported in studies using the same model [2, 11]. Radiographically, we observed osseous masses in all six animals at the 35-day postoperative time. Fusion was confirmed by manual palpation in all three animals not processed for QWBA [2]. In these three animals, we observed complete bone bridging between the L5 and L6 transverse processes in five of the six sites assessed with microscopy (Fig. 3).

Fig. 2 Quantitative whole-body autoradioluminography analysis shows surgical site retention of ^{125}I -osteogenic protein-1 (OP-1; $\mu\text{g eq/g}$) throughout the study period.

Fig. 3 A representative parasagittal section spanning from the L5 to the L6 transverse process shows bridging bone in the intertransverse region (Stain, Goldner's trichrome; original magnification, x2).

Discussion

Animal models and preliminary clinical studies have shown OP-1 putty effectively induces fusion in the posterolateral lumbar spine. As noted earlier, surgical site retention is important to enhance osteoinduction by maintaining a higher local concentration of BMP [16] and to limit systemic distribution. Nonetheless, these parameters have not been described for OP-1 putty for posterolateral lumbar spine applications.

Radiolabeling techniques assume the marker, ^{125}I , remains bound to the protein, which then can be quantified. In a rat model, low explant trichloroacetic acid (TCA) solubility counts have shown that BMP, at least initially, remains labeled [18]. Radioactivity therefore can be considered a useful, although not exact, proxy for in vivo BMP distribution.

Additionally, this model assumes adding ^{125}I does not interfere with the function of the BMP molecule. This is evidenced in the current study by the successful fusion in all animals followed to the final time of the study, a finding confirmed by multiple testing techniques.

Using LSC and QWBA, limited whole-body distribution of OP-1 was found. This is consistent with previous work characterizing rhBMP-2 pharmacokinetics in rabbits, which deemed systemic exposure to be negligible [12]. We found consistently low levels of radioactivity in blood and plasma. Less than 3% of the administered dose was present in blood at any time, and values decreased below 0.3% by the second week. Therefore, at no point was there high systemic exposure to the implanted OP-1.

Nonetheless, measures of blood and plasma radioactivity are only rough proxies for systemic rhBMP exposure. This study is the first to analyze the actual tissue distribution of implanted BMP in a rabbit lumbar arthrodesis model. Using QWBA, radioactivity concentrations in representative tissues and areas of focal collection were quantified and compared. As anticipated, we found the highest concentrations of radioactivity at the surgical sites. Tissue to plasma ratios generally remained constant except at the surgical sites, where ratios increased during the study interval, suggesting slow test article elimination.

Thyroid radioactivity concentrations did increase throughout the study. Previous TCA solubility findings,

however, indicate eventual BMP catabolism and ^{125}I release, rather than actual accumulation of OP-1, are likely explanations [12]. Relatively high concentrations (less than 2% of those at the surgical site) also were found in the urinary bladder wall and contents, consistent with the identification of renal excretion as the primary means of eliminating implanted radioactivity. In the heart and kidneys, tissues potentially influenced by the transforming effects of BMPs, radioactivity concentrations were less than or equal to $1 \times 10^{-4}\%$ of the surgical site concentration at all times and decreased below the limit of quantification by 72 hours. Concentrations in central nervous system tissues were below the limit of quantification throughout the study. Overall, results indicate limited and transient peripheral distribution of lumbar-implanted OP-1.

Surgical sites retained some radiolabeled OP-1 collagen putty for the duration of the study. The MRTs achieved compared favorably with those found for rhBMP-2 in a rabbit model [12]. Additionally, the excretion profile for OP-1 collagen putty was similar to those for rhBMP-2 in BCP-ACS and BCP carriers [12] with the majority of radioactivity gradually excreted in urine. Lesser quantities were excreted in feces and cage wash. As reported, implanted BMP was almost completely eliminated by 35 days postoperatively [12].

The pharmacokinetics of OP-1 had not been characterized in a lumbar spine arthrodesis model. Implantation with OP-1 collagen putty was found to yield disposition kinetics and excretion profiles comparable to those for other BMPs tested in the New Zealand White rabbit posterolateral lumbar fusion model. Distribution of lumbar-implanted OP-1 was minimal in peripheral tissues, whole blood, and plasma.

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