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Feasibility of Using a Bone-Targeted, Macromolecular Delivery System Coupled with Prostaglandin E₁ to Promote Bone Formation in Aged, Estrogen-Deficient Rats

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Abstract

Purpose—Macromolecular delivery systems have therapeutic uses because of their ability to deliver and release drugs to specific tissues. The uptake and localization of HPMA copolymers using Asp₈ as the bone-targeting moiety was determined in aged, ovariectomized (ovx) rats. PGE₁ was attached via a cathepsin K-sensitive linkage to HPMA copolymer–Asp₈ conjugate and was tested to determine if it could promote bone formation.

Materials and Methods—The uptake of FITC-labeled HPMA copolymer–Asp₈ conjugate (P-Asp₈-FITC) on bone surfaces was compared with the mineralization marker, tetracycline. Then a targeted PGE₁-HPMA copolymer conjugate (P-Asp₈-FITC-PGE₁) was given as a single injection and its effects on bone formation were measured 4 weeks later.

Results—P-Asp₈-FITC preferentially deposited on resorption surfaces, unlike tetracycline. A single injection of P-Asp₈-FITC-PGE₁ resulted in greater indices of bone formation in aged, ovx rats.

Conclusions—HPMA copolymers can be targeted to bone surfaces using Asp₈, with preferential uptake on resorption surfaces. Additionally, PGE₁ attached to the Asp₈-targeted HPMA copolymers and given by a single injection resulted in greater bone formation measured 4 weeks later. This initial *in vivo* study suggests that macromolecular delivery systems targeted to bone may offer some therapeutic opportunities and advantages for the treatment of skeletal diseases.

Keywords

bone formation; HPMA; macromolecular therapeutics; prostaglandin; rats

Introduction

Improvements in drug targeting and delivery systems have resulted in improved therapeutic applications for many diseases. The use of water-soluble polymer–drug conjugates (macromolecular therapeutics) offers some advantages including improved water solubility of

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hydrophobic low molecular weight drugs with concomitant improvement of bioavailability, protection of unstable drugs from degradation, longer-lasting circulation in the bloodstream, decreased non-specific toxicity of the conjugated drug, and increased accumulation of the drug at the target location (1,2). Macromolecular delivery systems have been particularly successful to target anti-cancer drugs to solid tumors (3–5).

We have developed some bone-targeted, water-soluble polymer drug delivery systems that may have uses for a variety of skeletal diseases. These macromolecular therapeutic systems are based on *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers. The HPMA copolymer system can be selectively targeted to bone by attaching a bone-seeking compound, such as a bisphosphonate or D-aspartic acid peptides (6,7), although other molecules may also have activity (2). We have also recently demonstrated that HPMA copolymer conjugated with D-aspartic acid octapeptide (Asp₈) have some preferential uptake on resorption surfaces compared with HPMA copolymer conjugated with a bisphosphonate (8). This may permit improved selectivity for delivery of drugs to bone surfaces with differing physiological activities.

Successful application of macromolecular drug delivery systems has depended on the development of mechanisms to release the drug at the intended tissue or organ. For cancer therapeutics, enzyme-cleavable linkers are used to attach the drug to the polymer backbone. These small peptide, enzyme-cleavable linkages have rendered the delivery system more effective due to the selective release of the free drug (1,9–11). Because osteoclasts highly express cathepsin K, we have incorporated a cathepsin K-sensitive peptide linkage to attach drugs to the HPMA copolymer backbone (12). The premise is that by targeting the delivery system to resorption sites, osteoclast-derived cathepsin K at these sites will selectively release the free drug.

There are a variety of potential therapeutic agents that may exhibit improved therapeutic profiles when delivered to the skeleton by a macromolecular delivery system (2). In these initial *in vivo* feasibility studies, we used prostaglandin E₁ (PGE₁) as the “prototype” drug. PGE₁ is a potent and well-established anabolic agent in bone (13), and has a chemical structure that is favorable for attachment via a peptide linkage to the HPMA copolymer. PGE₁ is, however, very labile and to be effective it must be given by repeated injections or continuous infusion (13).

There were several purposes to this study. The first was to establish the uptake and tissue localization of HPMA copolymers using Asp₈ as the bone-targeting moiety in the aged, ovariectomized (ovx) rat model. The aged ovx rat is an accepted model to test potential anabolic therapies on indices of bone formation in a model of estrogen-deficiency. To illustrate the localization of the conjugates in bone and their association with bone formation sites, the conjugate was labeled with fluorescein isothiocyanate (FITC). Bone forming surfaces were labeled with the fluorescent marker tetracycline, which can be distinguished from the FITC label on the HPMA copolymer conjugates. The second purpose was to determine if a single administration of the Asp₈-containing HPMA copolymer conjugated with PGE₁ via a cathepsin K-sensitive peptide linkage (P-Asp₈-FITC-PGE₁; P is the HPMA copolymer backbone) would stimulate bone formation in the aged, ovx rat model. These studies confirm the skeletal uptake of the bone-targeted HPMA copolymer conjugates and demonstrate that a single injection of the P-Asp₈-FITC-PGE₁ conjugate promoted bone formation in the aged, ovx rat model.

Materials and Methods

Design and Synthesis of the Bone-Targeted HPMA Copolymer PGE₁ Delivery System

The macromolecular drug delivery system used in this study consisted of HPMA copolymers conjugated with D-aspartic acid octapeptide (Asp₈) and PGE₁. Asp₈ is the bone-targeting moiety that exhibits some preferential uptake for resorption domains (8). PGE₁ was attached to the HPMA copolymer via a cathepsin K specific tetrapeptide linker (Gly-Gly-Pro-Nle) and a self-eliminating 4-amino-benzyl alcohol structure as previously described (12). The rationale for the cathepsin K specific peptide linker is that because this enzyme is highly expressed in osteoclasts (14) and with the HPMA copolymer–Asp₈ conjugates preferentially attaching at resorption domains, this may permit the release of a drug in regions of bone resorption (15). The HPMA copolymer–Asp₈–PGE₁ conjugates were also labeled with fluorescein isothiocyanate (FITC) to permit the detection of the polymer conjugate in bone by fluorescence microscopy and to compare its uptake with sites of bone formation and resorption. The synthesis procedures and chemical structures have been described previously in detail (12, 16). In the present study the weight average molecular weight (M_w) of the conjugate was 37.3 kDa (polydispersity, 1.3) and the contents of the PGE₁ and D-Asp₈ were 6.1 and 11.8 wt.%, respectively. The structures of the P-Asp₈-FITC-PGE₁ conjugate and of control conjugates (P-Asp₈-FITC and P-FITC) are illustrated in Fig. 1; their composition is characterized in Table I.

Initial Uptake and Localization of P-Asp₈-FITC Conjugates in Cancellous Bone

To confirm the ability of the Asp₈ to target the HPMA copolymers to bone, 7 month old Sprague–Dawley rats that had been ovariectomized 3 months prior to the start of the study were first given a subcutaneous injection of tetracycline (25 mg/kg) to label bone formation surfaces. Two days later, the rats ($n=4$) were given an intravenous injection via the jugular vein of the FITC labeled HPMA copolymer–Asp₈ conjugate (P-Asp₈-FITC) or FITC labeled HPMA copolymer (P-FITC) as a control. Ten mg of P-Asp₈-FITC and P-FITC conjugates were dissolved in PBS and given in a volume of 0.2 ml. Twenty-four hours later the femurs, tibias and lumbar vertebral bodies were harvested, fixed and dehydrated in acetone and embedded undecalcified in methyl methacrylate. Sections of the bone were cut with a bone saw, mounted on plastic slides, ground to about 30 μ m in thickness and viewed under the microscope for the presence of the tetracycline which marked bone formation surfaces and the FITC indicating the uptake of the P-Asp₈-FITC and P-FITC conjugates. The FITC label on the copolymers and the tetracycline label can be readily distinguished using an epifluorescence microscope (Olympus BX41) equipped with filters with different excitation-emission spectra. The animal protocols were approved by the University of Utah Institutional Animal Care and Use Committee and the study adhered to the “Principles of Laboratory Animal Care” (NIH publication #85–23, revised in 1985).

The process of bone resorption by osteoclasts occurs when packets of bone are removed from a bone surface, leaving a scalloped-appearing surface. The individual pits are called “resorption pits” or classically termed “Howship’s lacunae”. When osteoclasts can be observed on these surfaces, they are typically called “resorption surfaces” or “active resorption surfaces”, but if osteoclasts are not observed or are not present, the surface is typically called an “eroded” surface. If an eroded surface then undergoes bone formation, and starts to become buried by new bone, it is typically called a “reversal” surface, indicating a bone remodeling reversal from prior bone resorption to bone formation (17). This terminology was used in the present study to identify these surfaces.

Four-Week Study in Aged, Ovariectomized Rats

The purpose of this initial *in vivo* feasibility study was to determine the effects of a single administration of the P-Asp₈-FITC-PGE₁ conjugate on indices of bone formation measured 4

weeks later. Retired female breeder Sprague-Dawley rats (Harlan) were obtained at about 9 months of age and allowed to recover from any prior lactations for a period of 7 months. The rats were then ovariectomized and 5 months later the rats were used in the study. The rats ($n=8$) were given a single iv injection of 10 mg of P-Asp₈-FITC-PGE₁ conjugate. FITC was added to the conjugates to determine the localization of the uptake and retention of the copolymers in the bone. Two control groups were used in this study: one group of rats ($n=5$) was given the targeted conjugates without PGE₁ (P-Asp₈-FITC) and the second group ($n=5$) was given P-FITC (without Asp₈ and PGE₁). All rats were given injections of tetracycline (25 mg/kg) as a bone formation marker on study days 15 and 25 and the rats were killed on day 28. The lumbar vertebral bodies were fixed and dehydrated in acetone, embedded undecalcified in methyl methacrylate and sections prepared as described above. Unstained sections were used for the histomorphometric analyses of the tetracycline fluorochrome and later the sections were stained with a Giemsa stain to assess the histological detail at sites where the P-Asp₈-FITC-PGE₁ conjugate had deposited.

Histomorphometric indices of bone formation were measured using the tetracycline fluorochrome labeling in the cancellous bone of the lumbar vertebral bodies. The measured indices included the single tetracycline- and double-tetracycline-labeled surfaces (sLS and dLS, respectively), mineralizing surfaces (MS), corrected mineral appositional rates (cMAR), and the surface-referent bone formation rates (BFR). The MS was calculated as the perimeter of the dLS plus one-half of the sLS. The MAR was calculated as the distance between the two tetracycline fluorochrome markers divided by the time between the administration of the markers (10 days) and corrected for section obliquity. The BFRs were calculated using the MS as expressed as the amount of new bone area formed per bone surface perimeter per day ($\mu\text{m}^2 \mu\text{m}^{-1} \text{day}^{-1}$). The histomorphometric measurements were conducted and expressed per established convention (18).

Statistics

The data are expressed as the mean $\pm$ SE and the significance was tested by analysis of variance following by a Fisher's PLSD test to determine the significance among individual groups. The data were considered to be statistically significant when $p<0.05$.

Results

Preferential Localization of the P-Asp₈-FITC Conjugates to Eroded and Resorption Surfaces

The FITC label attached to the HPMa copolymer-Asp₈ conjugate permitted the detection and localization of the polymer on bone surfaces when viewed by epifluorescence microscopy. Likewise, the tetracycline label permitted the detection of bone formation surfaces and could easily be distinguished from the FITC label by epifluorescence microscopy. In the cancellous bone from the tibia, femur and lumbar vertebral bodies, the P-Asp₈-FITC conjugate was observed on the bone surfaces with particularly visible uptake on eroded surfaces (Fig. 2a). Tetracycline, on the other hand, marked the surfaces that were undergoing active bone mineralization (Fig. 2a). There were some eroded surfaces where both labels could be observed and these probably represented remodeling "reversal" surfaces where bone resorption regions were transitioning into bone formation regions. There was no evidence from epifluorescence microscopy of any uptake in bone of the non-targeted P-FITC conjugate.

The distinction between the uptake of tetracycline on mineralizing surfaces compared with the uptake of P-Asp₈-FITC conjugate on eroded surfaces was particularly evident in the growth plate and primary spongiosa region of the metaphyseal regions of the long bones. Due to the growth process at the epiphyseal growth plate, the calcification region of the epiphyseal cartilage is spatially removed from the resorption zone where some of the calcified cartilage

is removed prior to the initiation of new primary bone formation. Tetracycline labeling was evident in the calcification zone of the epiphyseal growth plate and on some bone forming surfaces of the primary spongiosa (Fig. 2b,c). On the other hand, the P-Asp₈-FITC conjugate was evident primarily on the eroded surfaces in the adjacent metaphyseal primary spongiosa. When companion sections were viewed first under fluorescent light (Fig. 2c) and then stained and viewed by normal histology (Fig. 2b), it was evident that many of the surfaces that had P-Asp₈-FITC uptake were also active osteoclast resorption surfaces, confirming the preferential uptake of the P-Asp₈-FITC conjugates on resorption surfaces.

Greater Indices of Bone Formation at 28 days After One Injection of P-Asp₈-FITC-PGE₁ Conjugate

The ability of a single administration of the P-Asp₈-FITC-PGE₁ conjugates to increase bone formation was determined in aged, ovx rats. At 4 weeks after a single administration of the P-Asp₈-FITC-PGE₁ conjugate, the dLS, MS, and surface-referent BFR were significantly greater when compared with the rats given the conjugates that lacked PGE₁ (P-Asp₈-FITC) or lacked the bone-targeting Asp₈ moiety and PGE₁ (P-FITC; Table II). When viewed by epifluorescence microscopy, the FITC-labeled conjugates that contained the bone targeting Asp₈ moiety were clearly evident in the bones. However, no FITC label was observed in the bones from the rats given the HPMA copolymer without the Asp₈ moiety (P-FITC). The P-Asp₈-FITC-PGE₁ conjugate was often buried, frequently on scalloped-appearing prior eroded or reversal surfaces, but covered by new bone that was often tetracycline-labeled, indicative of new bone formation at these sites (Fig. 3).

Discussion

The present study provides some initial observations that suggest that bone-targeted, macromolecular delivery systems may have potential therapeutic applications for skeletal disorders. These initial results show that HPMA copolymers with a prototype drug (e.g. PGE₁) could be delivered and incorporated into skeletal tissues and exhibit a drug effect in the tissues. The use of the Asp₈ as the targeting moiety on the HPMA copolymer facilitated the uptake on bone surfaces with preferential localization to eroded and resorption surfaces in an aged animal model. The histological presence of osteoclasts on eroded surfaces that had incorporated the HPMA copolymer targeted to bone with Asp₈ confirmed that the polymer had some preferential deposition at sites of active bone resorption. The linkage of the known bone anabolic agent, PGE₁, to the HPMA copolymer via a cathepsin K sensitive linkage may facilitate the release of PGE₁ at sites of active bone resorption, permitting the PGE₁ to exert its known effects on the stimulation of bone formation (13). Bone formation responses were significantly greater at 28 days after a single injection of the P-Asp₈-FITC-PGE₁ conjugate when compared with P-Asp₈-FITC and P-FITC.

The P-Asp₈-FITC conjugate, but not P-FITC conjugate, had excellent uptake in skeletal tissues, indicating the importance of a bone-targeting group on sequestering the delivery system into bone. We have previously demonstrated that Asp₈ or bisphosphonate conjugates of HPMA copolymers are readily incorporated into bone (6). Interestingly, however, the HPMA copolymer-Asp₈ conjugates were found in initial studies in mice to show preferential uptake on resorption surfaces (7) and a similar observation was made in the present study in aged, ovx rats. The osteotropy of the hexapeptide of aspartic acid (Asp₆) was first described by Kasugai *et al.* (19) and later this group conjugated Asp₆ to estradiol and found that the conjugate maintained a positive bone effect but minimized other side effects when compared with unconjugated estradiol (20).

Recent pharmacokinetic, biodistribution, and SPECT-imaging studies have confirmed the skeletal uptake of HPMA copolymer-Asp₈ conjugates as a function of molecular weight and

relative content of the Asp8 bound to the HPMA copolymer (16). The higher molecular weight (96 kDa) HPMA copolymer–Asp8 conjugates had a longer circulating half-life, enhanced deposition to bone, but also enhanced deposition in soft tissues. We have also recently reported differences in the stability of HPMA copolymer–PGE₁ conjugates in plasma of different species (21). The conjugates were found to be stable in human plasma, but the release of PGE₁ from the HPMA copolymer conjugates was substantial in mouse and rat plasma. For these reasons, in this initial study in rats, we selected a lower molecular weight conjugate (37 kDa) that would be incorporated into bone but also have a relatively fast clearance rate to help minimize its deposition in soft tissues and also the release of free PGE₁ while in the blood. Because of these species differences in enzymatic cleavage of drugs that may be attached to the HPMA copolymer via an ester linkage, polymer conjugates tested in rodent preclinical studies may differ from those ultimately used in humans.

The relative binding of α -Asp8 and a bisphosphonate to hydroxyapatite (HA) surfaces with differing crystallinity was recently compared (8). Using atomic force microscopy, the bisphosphonate was found to have a stronger HA affinity than α -Asp8. When tested against HA with different crystallinities, Asp8 showed a stronger sensitivity to change of HA crystallinity. Newly formed bone usually has a lower crystallinity than older bone and it is the older bone that is usually undergoing bone resorption (22). This may help explain why there was uptake of bisphosphonate-HPMA copolymer–FITC conjugates on both formation and resorption surfaces (7), whereas there was better relative uptake of Asp8-HPMA copolymer–FITC conjugates on resorption surfaces where more mature bone with a higher crystallinity would be exposed. This difference may provide a valuable and unique opportunity to target therapeutics to different functional bone surfaces.

Attaching therapeutic agents to HPMA copolymers via enzyme sensitive peptide linkages has proven to be particularly useful in cancer therapeutics (1,10,11,23,24). In the present study, we attached the prototype drug, PGE₁, via a tetrapeptide (Gly-Gly-Pro-Nle) that is sensitive to degradation by cathepsin K. Cathepsin K is highly expressed in osteoclasts (14), although recent studies have reported some cathepsin K production in osteoblasts (25). Pan et al. (12) recently demonstrated that cathepsin K could release PGE₁ that was linked to HPMA copolymers via the tetrapeptide linker used in this study (Gly-Gly-Pro-Nle).

HPMA copolymers have been found to be nonimmunogenic (26,27). The biocompatibility of HPMA copolymers has been demonstrated in clinical trials (28,29). An important advantage of targeted-macromolecular delivery systems is that they facilitate the delivery and release of drugs to target organs and tissues, thus reducing the side effects that may occur if the free drug were given systemically. For example, in a human trial, HPMA copolymer–doxorubicin conjugates showed a four- to fivefold reduction in systemic toxicity compared with the free drug (28,30). Macromolecular delivery systems can also help render hydrophobic drugs more hydrophilic, thus potentially improving their pharmacological properties (1,6,9). Thus the use of macromolecular delivery systems may also permit the use of efficacious drugs that were abandoned due to systemic toxicity.

In the present study we selected PGE₁ as the “test” drug because it is a powerful stimulator of bone formation when given systemically or locally (13). E-series prostaglandins are labile compounds that when given systemically are rapidly metabolized. PGE's have not been further developed for the systemic treatment of bone diseases because of non-skeletal side effects. In the present study, a single injection of P-Asp8-FITC-PGE₁ conjugate resulted in substantially greater indices of bone formation measured at 28 days after administration. The P-Asp8-FITC-PGE₁ conjugates were observed buried in bone, frequently on prior eroded surfaces that had reversed and were undergoing bone formation. This would indicate a remodeling reversal (from

bone resorption to bone formation), perhaps due to the PGE₁ treatment, but this could not be specifically determined from this study.

A drug delivery system that preferentially targets skeletal tissues offers several pharmacological advantages. By targeting the skeletal tissues, the therapeutic agents may have a smaller uptake in other tissues where potential toxicity may be manifest. Additionally, lower total doses of the therapeutic agent may be given compared with doses given systemically to achieve a similar therapeutic effect. In the present study, greater effects on bone formation were observed over a 4-week period when a total amount of PGE₁ of about 0.6 mg per rat was administered as a single injection in 10 mg of the P-Asp₈-FITC-PGE₁ conjugate. This dose contrasts, for example, to systemic doses ranging from 0.3–6.0 mg PGE₂/kg given daily for up to several months to achieve anabolic responses in bone (31–33). Similarly, doses of PGE₁ ranging from 0.5–2.0 mg/week for 3 weeks were given by local infusion to stimulate periodontal tissue and alveolar bone formation (34). Thus, the total dose of PGE₁ used in the present study (about 0.6 mg) is a small fraction of PGEs given in other studies to achieve increases in bone formation rates. However, the present study is the first *in vivo* study of this bone-targeted HPMA copolymer conjugate and clearly further dose-, time-response and pharmacokinetics studies are needed to define the therapeutic profiles of the bone-targeted HPMA copolymer-PGE₁ conjugates.

Although PGE₁ was used in this feasibility study with very encouraging results, there are a number of potential other agents that may have beneficial effects when delivered through a bone-targeted macromolecular delivery system. The finding that the macromolecular delivery system may be differentially targeted to forming or resorbing surfaces, provides additional versatility to the system. For example the targeting of drug-containing polymers to active resorption surfaces may have uses in osteolytic diseases where resorption dominates, such as osteoporosis, periodontal disease, cancers, and cancer metastases.

In summary, a bone-targeted macromolecular delivery system is described that has some preferential localization at sites of active bone resorption in aged, ovx (estrogen-deficient) rats. Because of its preferential localization to resorption surfaces, the bone active test drug, PGE₁, was attached to the HPMA copolymer via a cathepsin K sensitive peptide linkage. Following a single administration of the P-Asp₈-FITC-PGE₁ conjugate to aged, ovx rats, bone formation rates were substantially greater than controls when measured 28 days later. Furthermore, local bone formation frequently occurred over sites where the P-Asp₈-FITC-PGE₁ conjugates had deposited in bone, suggesting local bone formation at resorption or reversal surfaces. These initial studies, combined with proven successes in cancer therapeutics, suggest that macromolecular delivery systems may have some significant advantages for the treatment of a number of important skeletal diseases.

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Fig. 1. Structure of the HPMA Copolymer Conjugates. **A** P-Asp₈-FITC-PGE₁ and P-Asp₈-FITC (where $x = 0$); **B** P-FITC.

Fig. 2.

Initial uptake and localization of P-Asp₈-FITC conjugates in bone compared with the uptake of tetracycline. **A** The P-Asp₈-FITC conjugates (*green-appearing label*) preferentially incorporate on scalloped-appearing eroded surfaces (*white arrows*) in cancellous bone. The tetracycline (*yellow-appearing label*) is incorporated onto active bone mineralization surfaces (*orange arrowheads*). **B**, **C** Stained (**B**) and unstained (**C**) section of the same region of the proximal tibial growth plate and primary spongiosa. Tetracycline (*yellow-appearing label*) incorporated into the mineralizing zone of the growth plate (**C**, *orange arrowhead*), as expected, whereas the P-Asp₈-FITC conjugates (*green-appearing label*) are localized in the resorption areas of the primary spongiosa (**C**, *white arrows*). The histology of these resorption zones with numerous osteoclasts (*orange arrows*) from the same section is illustrated in **B**. Magnifications: **A**=150×; **B**=125×; **C**=125×.

Fig. 3.

At 4 weeks after the administration of a single injection of the P-Asp₈-FITC-PGE₁ conjugate, the conjugate (*green-appearing label*) can be seen (*white arrows*) buried in the bone (**B**) with new bone formation, as indicated by the tetracycline labels (*yellow-appearing labels*) occurring over the same region (*white arrowheads*). Much of the buried P-Asp₈-FITC-PGE₁ conjugates appear to have been deposited on previously eroded, scalloped-appearing surfaces (*white arrows*). Magnification=250 $\times$.

Table I

Composition of the HPMA Copolymer Conjugates

Conjugate	M_w (kD)	Polydispersity	FTTC (wt.%)	D-Asp ₈ (wt.%)	PGE ₁ (wt.%)
P-Asp ₈ -FTTC-PGE ₁	37.3	1.3	4.7	11.8	6.1
P-Asp ₈ -FTTC	27.8	1.3	11.9	23.9	—
P-FTTC	27.2	1.3	4.8	—	—

Table II

Histomorphometric Indices of Bone Formation Measured in Cancellous Bone from the Lumbar Vertebral Bodies in Ovariectomized Rats 4 Weeks after a Single Injection of P-Asp₈-FITC-PGE₁ Conjugate

	dLS (%±SE)	MS (%±SE)	cMAR (µm/day±SE)	BFR (µm² µm⁻¹ day⁻¹±SE)
P-FITC	3.4±0.6	7.3±0.9	0.49±0.02	0.0362±0.0062
P-Asp ₈ -FITC	4.0±1.3	9.6±2.3	0.59±0.06	0.0541±0.0108
P-Asp ₈ -FITC-PGE ₁	10.2±0.9 ^a	19.2±2.3 [*]	0.60±0.02	0.1142±0.0077 [*]

Measurements include the percent of double-labeled surface (dLS), percent mineralizing surface (MS), the corrected mineral appositional rate (cMAR), and the surface-referent bone formation rate (BFR)

^{*} $p < 0.05$, significantly different from HPMa-FITC and Asp₈-HPMa-FITC controls