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Release of recombinant human bone morphogenetic protein 2 from a newly developed carrier

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Abstract

After implantation of a polymer-coated gelatin sponge (PGS) containing either 0.4 or 1.0 mg of ^{125}I-rhBMP-2 for each 1 cm^3 of PGS into the right ulnar of rabbits, changes in the level of radioactivity at the implant site and in the blood were measured for 21 days after implantation, and the cumulative excretion ratio of radioactivity in the urine and feces was calculated. For both doses, radioactivity at the implant site was eliminated biphasically. The concentration of trichloroacetic acid (TCA)-precipitable radioactivity in the blood reached a maximum 6 h after implantation, at which time it was equivalent to 1.41% of the administered dose (0.4 mg/cm^3). The remaining radioactivity was eliminated rapidly thereafter, falling below the detection limit within 48 h. The $t_{1/2\alpha}$ was about 0.1 days, the $t_{1/2\beta}$ was about 3 days, and the mean resident time (MRT) value was about 4 days. By 17 days after implantation, 88.1% of the administered radioactivity had been excreted in the urine, and 1.7% had been excreted in the feces. TCA precipitation test results indicated that most of the radioactivity excreted in urine was a low-molecular weight decomposition product. At 21 days after implantation, the radioactivity of the PGS implant site had declined to 0.5% of the administered amount. Autoradiographs of the implant site taken 28 days after implantation revealed that, at both doses, the residual radioactivity was confined to the area of the implanted PGS. These results indicate that PGS retains an appropriate amount of recombinant human bone morphogenetic protein 2 (rhBMP-2) at the orthotopically implanted site for at least 21 days enough to induce bone regeneration. Thus, PGS shows great clinical potential as a carrier for rhBMP-2.

Keywords: Gelatin; Bone morphogenetic protein; Pharmacokinetics; Controlled release

1. Introduction

From the time that recombinant human bone morphogenetic protein 2 (rhBMP-2) was cloned by

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Wozney et al. (1988), many investigations have been conducted to find systems suitable to deliver rhBMP-2 so that it can stimulate bone regeneration in patients who have bone defects in orthopedic and maxillofacial surgery. To achieve this goal, it should be noted that an optimal rhBMP-2 carrier meets the following requirements: (1) to retain BMP at a bone reconstruction site for a period of time sufficient to induce bone formation; (2) to possess a porous structure to allow cellular infiltration and proliferation as well as vascular invasion; (3) to maintain its shape against pressures exerted by soft tissue; (4) to have low or no toxicity; (5) to be inert, not to interfere with BMP activity; (6) to be replaced easily with new bone; and (7) to possess appropriate mechanical strength so that it can be cut or molded into various shapes to fit bone reconstruction sites.

A rat ectopic bone formation assay has been commonly used to evaluate the suitability of subcutaneously implanted carriers (Reddi and Huggins, 1972). This assay can directly demonstrate the ability of bone morphogenetic protein to generate bone in combination with carriers. Results from a series of rat ectopic bone formation assays (Yokota et al., 2001) indicated that a biodegradable carrier which was made of gelatin reinforced with poly D,L-lactic-co-glycolic acid (PLGA) was a potential carrier for rhBMP-2. The results of those studies showed that PLGA-coated gelatin sponges (PGSs) containing rhBMP-2 maintained their three-dimensional structure after subcutaneous implantation; soft X-ray roentgenograms revealed that this carrier resisted the pressure-induced collapse caused by soft tissue that occurred during the rapid bone formation period. However, the retention and release of rhBMP-2 in vitro and in vivo were not fully evaluated during that study. In order to use rhBMP-2 therapeutically, the behavior of rhBMP-2 contained within a PGS at an orthotopically implanted site must be evaluated. In addition, the effect of body fluid accumulation at surgical sites must be considered, because infiltration of blood is often observed at the kind of surgical site where the rhBMP-2/PGS is to be applied. Therefore, this study was conducted to characterize the release of ^{125}I-rhBMP-2 from PGS, both in vivo after implantation into rabbit ulnae and in vitro after incubation in rat serum.

2. Materials and methods

2.1. Materials

rhBMP-2 was manufactured by Genetics Institute, Inc. (Cambridge, MA) using a Chinese hamster ovary (CHO) cell expression system. Purified (98%) protein was dissolved in a glycine–glutamic acid buffer. The Iodo-Gen reagent was purchased from Pierce (IL). PGS with a bulk gelatin sponge density of 18 mg/cm^3 was prepared according to a previously described process (Yokota et al., 2001). All other reagents were analytical grade chemicals.

2.2. Animals and serum source

All experiments involving animals conformed to the guidelines for animal care established by Yamanouchi Pharmaceutical Co., Ltd.

Male Japanese white rabbits (16–19 weeks old) purchased from Japan Experimental Animals Co., Ltd. (Tokyo, Japan) were used. Rabbits were housed individually in cylindrical urine collection cages (Natsume, Tokyo, Japan). These cages have a diameter of 36 cm and a height of 30 cm, and are equipped with stainless steel lattice sides and stainless steel mesh floors. During acclimation, rabbits were given solid rabbit food (RC-4; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water ad libitum, but beginning 3 days before PGS implantation, a 20 mM NaI solution was provided as the drinking water.

Male Fisher rats, 4 weeks old, were used to obtain serum for the in vitro release study.

2.3. In vitro release of rhBMP-2 from PGS

2.3.1. Radioiodination and preparation of rhBMP-2 solutions

^{125}I-labeling of rhBMP-2 was performed with the Iodo-Gen reagent (1,3,4,6-tetrachloro-3α,6α-diphenyl glycouril) as described previously (Yokota et al., 2001). Part of the resulting stock ^{125}I-

labeled rhBMP-2 solution was diluted with sufficient non-radiolabeled rhBMP-2 solution to make two rhBMP-2 solutions with concentrations of 0.12 and 0.50 mg/ml.

2.3.2. Measurement of rhBMP-2 retained in PGS after incubation of serum or buffers

Two hundred and sixty microliters of the ^{125}I-rhBMP-2 solution was added to an 8 mm × 8 mm × 5 mm (0.32 cm^3) piece of PGS. The solution and PGS were incubated together for 30 min at room temperature, and then centrifuged at 1500 rpm ($800 \times g$) for 15 min to remove unincorporated ^{125}I-rhBMP-2. A gamma counter (Aloka, Tokyo, Japan) was used to measure the amount of radioactivity remaining in PGS. Next, 320 μl of rat serum or one of the buffers was added to the ^{125}I-rhBMP-2-containing PGS and was incubated at room temperature for up to 120 min. After incubation, the sponges were centrifuged at 1500 rpm ($800 \times g$) again to remove the fluid. The amount of radioactivity remaining in PGS was measured with the gamma counter, and the final amount of ^{125}I-rhBMP-2 remaining in PGS was calculated from the total radioactivity in the original ^{125}I-rhBMP-2 solution of 0.12 mg/ml.

2.4. In vivo elimination of rhBMP-2 from PGS implanted at ulnar defect sites in rabbits

2.4.1. Preparation of PGS implants soaked in ^{125}I-labeled rhBMP-2 solution

Part of the stock ^{125}I-labeled rhBMP-2 solution was diluted with sufficient non-radiolabeled rhBMP-2 solution to make two solutions with rhBMP-2 concentrations of 0.4704 and 1.176 mg/ml. Eight hundred and fifty microliters of one of these solutions was added drop-wise to separate 2 cm × 1 cm × 0.5 cm (1 cm^3) pieces of PGS to wet them uniformly; these final concentrations corresponded to 0.4 and 1.0 mg/cm^3 of PGS, respectively. These PGSs, designated ^{125}I-rhBMP-2/PGS, were implanted into rabbits as described in the following section.

2.4.2. Implantation of ^{125}I-rhBMP-2/PGS

A 1.5 cm segmental gap was created in the right ulna of each rabbit, and ^{125}I-rhBMP-2/PGS, prepared as described in Section 2.4.1 and shaped to fit the defect, was implanted. The schematic illustration is shown in Fig. 1. The weights of the implanted ^{125}I-rhBMP-2/PGS blocks ranged between 0.451 and 0.643 g. Rabbits were anesthetized by intravenous injection of pentobarbital (30 mg/kg body weight) preoperatively.

2.4.3. Measurement of radioactivity at the implant site

A scintillation probe (G1LE; Bicron, Solon, OH) connected to a ratemeter (TRM-B12, Bicron) was used to measure radioactivity at the implant site immediately after the implantation procedure; 30 min after implantation; 1, 2, 4, 6, and 8 h after implantation; and 1, 2, 4, 7, 10, 14, 17, and 21 days after implantation. To prevent inaccurate measurement caused by radioactivity located at sites other than the implant site, the thoracic and abdominal regions of the rabbits were covered with lead sheets and the probe was placed 5–15 cm away from the implant site (right forelimb). On each measurement day, the radioactivity of a standard source containing 0.5 ml of the corresponding ^{125}I-rhBMP-2 solution was measured from the same distance, and the ratio of the radioactivity measured at the implant site to that of standard was calculated.

The ratio of the radioactivity value at each measurement point to the radioactivity value immediately following administration was calculated. These values were plotted against time. WinNonlin software (Pharsight, Mountain View, CA) was used to determine the pharmacokinetics of rhBMP-2 from this plot. The mean resident time (MRT) was calculated from the following equation using the area under the radioactivity–time curve (AUC) and the area under the moment curve (AUMC); the trapezoidal method was used to determine these values using data collected from the time of administration until the last measurement point (21 days after implantation).

$$\mathrm{MRT} = \frac{\mathrm{AUMC}}{\mathrm{AUC}}.$$

A two-exponential model was used to calculate the elimination half-lives $t_{1/2\alpha}$ and $t_{1/2\beta}$.

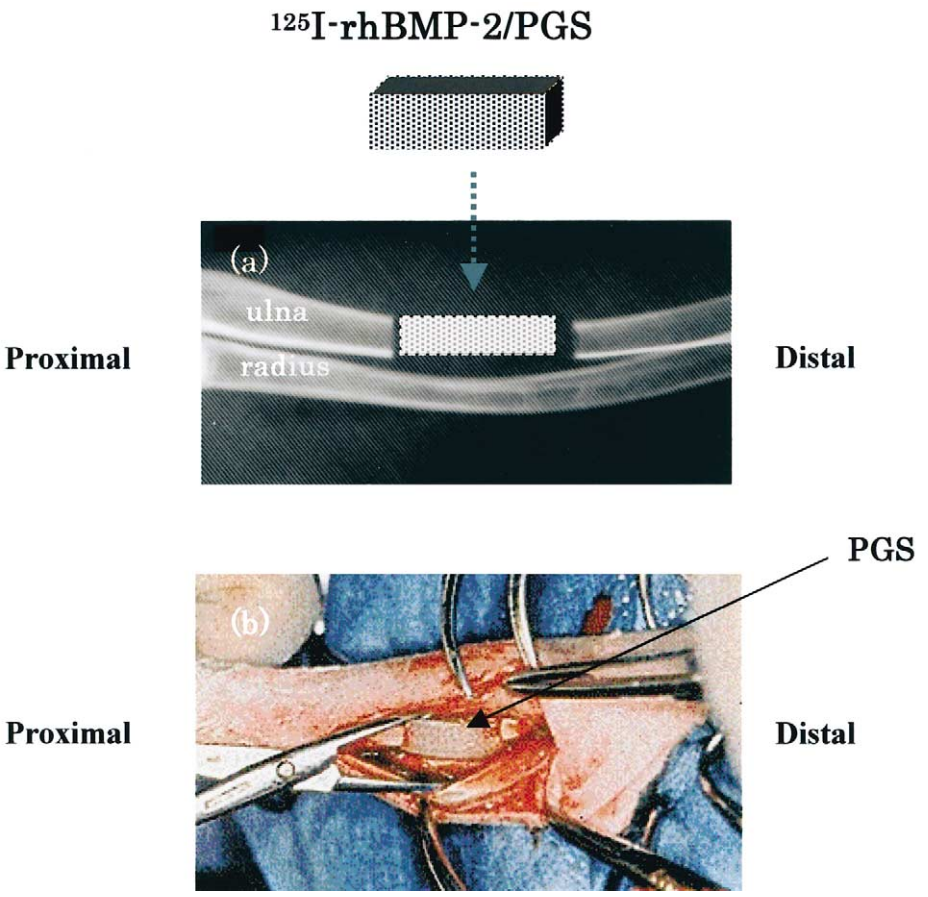

Fig. 1. Implantation of ^{125}I-rhBMP-2/PGS into the right ulna of rabbits: (a) scheme of implantation; (b) PGS after soaking with ^{125}I-rhBMP-2 was cut into suitable size for the defect and implanted into the defect site.

2.4.4. Measurement of radioactivity in urine and feces

Urine and feces were collected during the following time intervals after surgery from each rabbit implanted with 0.4 mg/cm^3 ^{125}I-rhBMP-2/PGS: 0–1, 1–2, 2–3, 3–4, 4–6, 6–8, 8–10, 10–13, 13–15, 15–17, 17–20, and 20–22 days. The total weight of urine collected during each sampling period was measured, and then 1 ml of each sample was used to measure the amount of radioactivity as described in Section 2.4.6. The total amount of feces collected each day was used to measure the amount of radioactivity. Radioactivity was measured using the gamma counter. The cumulative percentages of radioactivity excreted to urine and to feces were then calculated.

2.4.5. Measurement of radioactivity in blood

A 0.3 ml blood sample was collected at 30 min and 1, 2, 4, 6, 8, and 24 h after surgery from an auricular vein of rabbits implanted with 0.4 mg/cm^3 ^{125}I-rhBMP-2/PGS. A 0.1 ml of the blood obtained was used for measurements of radioactivity as described in Section 2.4.6.

2.4.6. Measurement of TCA-precipitable radioactivity in blood and urine

To 0.1 ml of the blood sample obtained as described in Section 2.4.5, 0.9 ml of phosphate-buffered saline (PBS) and 1 ml of 20% (v/v) trichloroacetic acid (TCA) were added, and the solutions were mixed in a vortex mixer. These mixtures were then centrifuged at 800 × g, and the

radioactivity in each supernatant and pellet was measured by the gamma counter.

2.4.7. Autoradiography of the implant site

On day 28 after implantation, the animals were euthanized, and right forearms were harvested. A 30-μm thick, longitudinal tissue section of bone was prepared from the implant site of a rabbit implanted with 0.4 mg/cm^3 and from a rabbit implanted with 1.0 mg/cm^3 ^{125}I-rhBMP-2/PGS. After drying, the sections were exposed to a BAS imaging plate, and image analysis of the residual radioactivity was performed with BAS2000 image analysis equipment (Fuji Photo Film, Tokyo, Japan).

2.5. Data analysis

The results of in vitro release of rhBMP-2 are shown as mean ± S.D. of the samples examined.

3. Results

3.1. In vitro release of rhBMP-2 from PGS

Fig. 2 shows the ability of rat serum and the two incubation media to leach rhBMP-2 from PGS. The amount of rhBMP-2 retained in PGS after the addition of rat serum or the incubation media are expressed as the percentage of the amount of rhBMP-2 initially retained in PGS before the addition of rat serum or the incubation media. The amount of rhBMP-2 retained in PGS exposed to serum gradually decreases with the incubation time. After 120 min of incubation with serum, the amount of rhBMP-2 retained in PGS decreased to approximately 40–50% of the amount of rhBMP-2 initially retained in PGS. In contrast, approximately 90% of the initially retained rhBMP-2 remained in PGS during incubation in 10 mM PBS (pH 7.4) for up to 120 min. Only after 30 min of incubation in 20 mM histidine–0.5 M arginine buffer, pH 6.8, the amount of rhBMP-2 retained in PGS decreased to approximately 24% of the amount initially retained. All these effects are independent of the amount (concentration) of rhBMP-2 initially retained in PGS.

3.2. Elimination of radioactivity from the implant site

Fig. 3 shows changes over time in the amount of radioactivity at the implant site after implantation of ^{125}I-rhBMP-2/PGS containing 0.4 or 1.0 mg of ^{125}I-rhBMP-2 per cm^3 of PGS into a bone gap induced in the right ulna of rabbits. Table 1 shows some of the relevant pharmacokinetics of this process. Radioactivity at the implant site is eliminated in an apparent biphasic manner, independent of rhBMP-2 concentration, with a $t_{1/2\alpha}$ of 0.1 days and a $t_{1/2\beta}$ of 3 days. The MRT value is approximately 4 days, and does not change depending on rhBMP-2 concentration. By 21

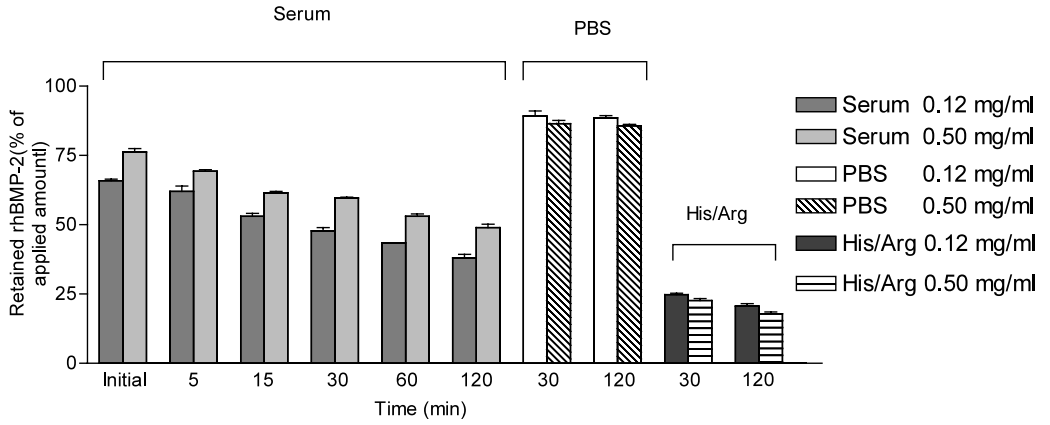

Fig. 2. Effect of incubation in rat serum or incubation media on rhBMP-2 retention in PGS ($n = 3$).

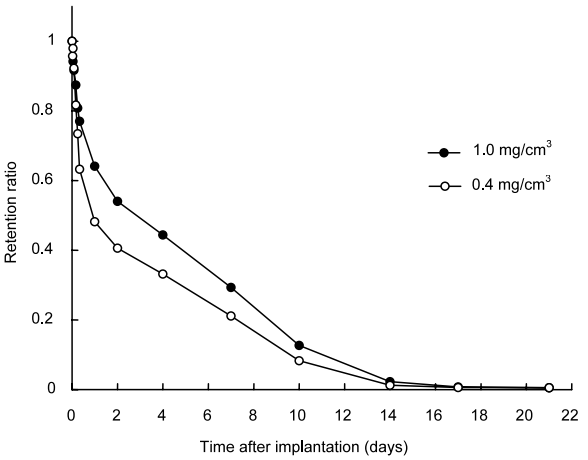

Fig. 3. Elimination profile of radioactivity from the implant site after implantation of ^{125}I-rhBMP-2/PGS containing a dose of 0.4 or 1.0 mg/cm^3 into a right ulna bone defect site in rabbits. Note that the cumulative amount of radioactivity excreted in the urine is inverse to the radioactivity remaining at the implant site. The values represent the mean of data from two rabbits for each rhBMP-2 dose.

Table 1
Pharmacokinetics of radioactivity remaining at the implant site after implantation of ^{125}I-rhBMP-2/PGS into a right ulna bone defect site in rabbits

Dose[a] (mg/cm^3)	$t_{1/2}$[b] (days)		$MRT_{0-21\ days}$[c] (days)
	α	β	
0.4	0.12	2.99	4.10
1.0	0.06	3.04	4.28

[a] Dose represents concentration of rhBMP-2 per volume of PGS.
[b] Calculated using two-exponential analysis.
[c] Estimated using non-compartmental analysis.

days after implantation, the radioactivity had declined to 0.5% of the administered amount.

3.3. Excretion of radioactivity to urine and feces

Fig. 4 shows the cumulative excretion of radioactivity after implantation of ^{125}I-rhBMP-2/PGS containing 0.4 mg of ^{125}I-rhBMP-2 per cm^3 of PGS into a bone gap induced in the right ulna of rabbits. By 17 days after implantation, 88.1% of the administered radioactivity had been excreted in urine, and 1.7% had been excreted in feces. The

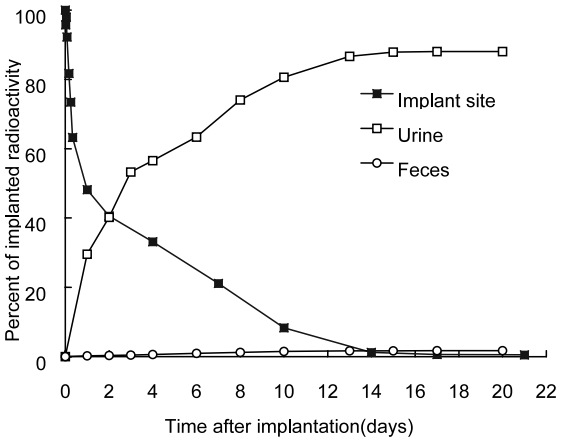

Fig. 4. Amount of radioactivity excreted into urine and feces after implantation of ^{125}I-rhBMP-2/PGS containing a dose of 0.4 mg/cm^3 into an ulna bone defect site in rabbits. The values represent the mean of data from two rabbits.

increases in the cumulative excretion of radioactivity are inversely related to the elimination of radioactivity from the implant site (Fig. 3).

3.4. Radioactivity in the blood

Fig. 5 shows the amount of radioactivity in the blood after implantation of ^{125}I-rhBMP-2/PGS containing 0.4 mg of ^{125}I-rhBMP-2 per cm^3 of PGS into a bone gap induced in the right ulna of rabbits. Radioactivity in the blood reached a

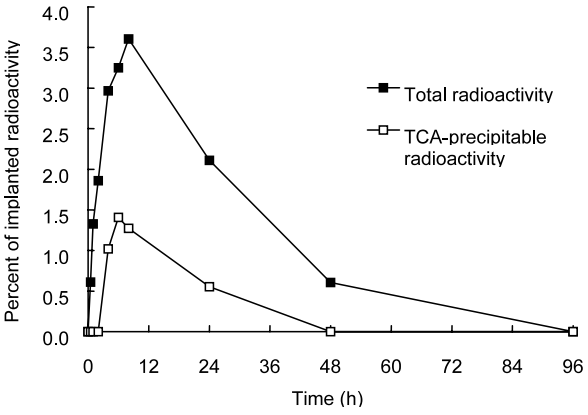

Fig. 5. Radioactivity in the blood after implantation of ^{125}I-rhBMP-2/PGS containing a dose of 0.4 mg/cm^3 into a right ulna bone defect site in rabbits. The values represent the mean of data from two rabbits.

maximum 8 h after implantation; the value at that time was 3.6% of the administered radioactivity. TCA-precipitable radioactivity reached a maximum 6 h after administration; the value at that time was 1.41% of the administered radioactivity. Subsequently, remaining radioactivity was eliminated rapidly, declining below the detection limit by 48 h after implantation for TCA-precipitable radioactivity, and by 96 h after implantation for total radioactivity.

3.5. Autoradiography of the implant site

Only a trace amount of radioactivity remained 28 days after implantation of ^{125}I-rhBMP-2/PGS. Autoradiograms of tissue sections prepared from the implant sites are shown in Fig. 6. At both doses, 0.4 and 1.0 mg/cm^3, the radioactivity remaining on the 28th day was confined to an area that matched the shape of the implanted PGS, and bone formation was observed on the periphery of PGS.

4. Discussion

In vivo bone formation caused by rhBMP-2 requires a carrier that will hold rhBMP-2 in the needed place until bone induction can be elicited (Doi, 1993). In order to investigate the dose-dependent action of rhBMP-2, it is essential to confirm that the administered rhBMP-2 is retained at the implant site at biologically active concentrations. In developing a new carrier, estimates of the amount of drug at the implant site (not an ectopic site) are considered essential if the relationship between efficacy and the ability to retain rhBMP-2 is to be ascertained. Given these considerations, the elimination behavior of rhBMP-2 from the implant carrier and the dose-dependent action of rhBMP-2 in inducing osteogenesis were assessed using PGS, a newly developed carrier.

It should also be noted that the possibility of influence of bleeding cannot be ruled out when any carrier is surgically inserted into a bone defect site. Accordingly, a thorough assessment of any potential carrier must include the effect of blood,

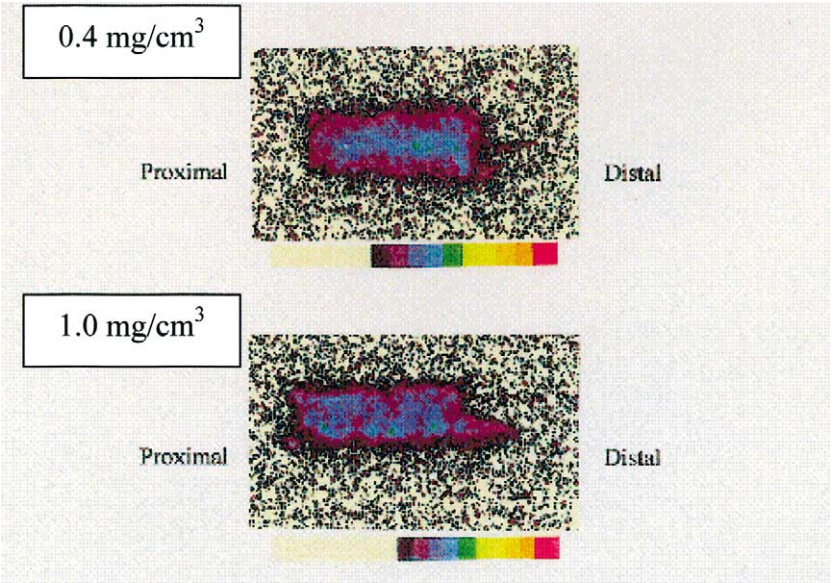

Fig. 6. Autoradiogram of ^{125}I-rhBMP-2/PGS implantation site taken 28 days after implantation into a right ulna bone defect site in rabbits. Note that the radioactive signal is confined to the area of the implant.

especially serum, present at the implant site on the retention of rhBMP-2 in the carrier.

To investigate the possible effect of serum for PGS at a surgical site, in vitro studies were first conducted before studies in rabbits were initiated. A common technique used for in vitro release testing is to immerse impregnated carrier samples in a solution whose composition is clearly defined, such as PBS, and then measure the amount of the target molecule in the supernatant without centrifugation. However, in order to investigate the direct effect of serum, a centrifugation method was used. This method seemed to provide a more accurate measure of retained rhBMP-2 as indicated by results from a previous report that showed three types of incorporation of rhBMP-2 in PGS: retention in the void spaces of PGS, absorption into the gelatin sponge hydrogel, and adsorption onto gelatin molecules (Yokota et al., 2001).

Fig. 2 shows rhBMP-2 retained in PGS after centrifugation is time dependently released into serum. In contrast, PBS appears to have a rather weak effect on the release of rhBMP-2, indicating that PBS is not a suitable solution to evaluate the release of rhBMP-2 from PGS. These results suggest that certain serum components facilitate the release of rhBMP-2 from PGS. Interestingly, the results for the histidine–arginine buffer, which has a pH similar to that of PBS, exhibited a significant ability to release rhBMP-2. Since guanidine hydrochloride has been successfully used to purify BMPs from bone (Sampath and Reddi, 1981; Wang et al., 1988), this provides a possible explanation why the arginine-containing buffer is an efficient agent for the extraction and solubilization of rhBMP-2 from PGS because arginine has a guanidino group.

Another possible interpretation of these results is that hydrolysis of gelatin by proteases in serum results in the fragmentation of the gelatin, which causes the subsequent release, into serum, of a gelatin fragment to which rhBMP-2 is bound. However, it should be noted that proteases in serum also might digest rhBMP-2 itself, leading to release of the radioactive rhBMP-2 fragments. Taken together, the results indicate factors, either proteases or compounds having arginine-like function, exist in serum that contribute to the solubilization of BMPs from bone and other matrix.

Next, the in vivo retention of biologically active amounts of rhBMP-2 by the PGS carrier was evaluated. Because the radioactivity elimination profile is similar at both the 0.4 and 1.0 mg/cm^3 concentrations, as shown in Fig. 3 and Table 1, the results confirmed that rhBMP-2 is retained in amounts proportional to the administered concentration, at least for concentrations up to 1.0 mg/cm^3. The results also show that at least two processes for elimination are involved since the elimination profile of radioactivity is biphasic (Fig. 3).

This biphasic profile might result from the following processes in consideration that PGS is a sponge-like carrier which is reinforced by evenly coating the surface of each gelatin sponge pore with PLGA, and has the ability to retain rhBMP-2 in its pores and gelatin sponges (Yokota et al., 2001). As shown in Fig. 2, the retained rhBMP-2 after centrifugation to remove the rhBMP-2 residing in the pores of PGS is released after the addition of serum in vitro. Consequently, the rapid elimination observed in the early post-implantation phase might be caused by both the leakage of rhBMP-2 residing in the sponge pores and the certain level of dissociation of rhBMP-2 from the gelatin sponge in PGS because of the influx of blood and other body fluids. Secondly, the subsequent gradual elimination phase probably involves the liberation of rhBMP-2 that occurs during PGS degradation, especially hydrolysis of the gelatin sponge. But this hydrolysis of gelatin may be rather delayed due to the coating of the gelatin sponge surface with PLGA in comparison to "uncoated" gelatin sponge itself as discussed previously (Yokota et al., 2001), which means that longer retention of rhBMP-2 will be expected. These explanation also suggests that the residual retention of rhBMP-2 might be achieved by the inability of blood and other body fluids to permeate the newly generated calcified bone in and/or surface of PGS, resulting in the inability to release rhBMP-2 from the remaining PGS. The initial burst-like release of rhBMP-2 may contribute to the chemotaxis of osteoprogenitor cells

and then the following gradual release may enhance the bone induction.

It has been reported that bone formation occurs 28 days after implantation at sites that have implants impregnated with either 0.4 or 1.0 mg rhBMP-2 per cm^3 sponge; additionally, fresh bone cortex had formed on the surface of the implant region and residual PGS is observed at the implant site (Kokubo et al., 1996). This observation was also confirmed in this study. Radioactivity measurement (Fig. 3) and autoradiography (Fig. 6) confirmed that a trace amount of ^{125}I-rhBMP-2 remained at the PGS implant site; the amount remaining was less than 0.5% of the initial dose (0.5% at 21 days after implantation). These results indicate that a biologically active amount of rhBMP-2 had been retained by PGS during bone growth, and consequently, the site at which bone induction is elicited can be controlled by the shape of PGS as demonstrated during a previous study (Yokota et al., 2001).

How bone induction is elicited is also partially explained by the results that rhBMP-2 signal transmission was mediated by receptors present on surface of the certain kinds of cells (ten Dijke et al., 1994; Penton et al., 1994; Rosenzweig et al., 1995; Letsou et al., 1995; Itoh et al., 2001). However, rhBMP-2 concentration greater than a certain level would be required for its bone-inducing action. It has been reported that the dissociation constant (K_d) for rhBMP-2 from its receptors is 35–230 pM, or about 1–7 ng/ml (Iwasaki et al., 1995). The results of this study thus demonstrate that the rhBMP-2 concentration is maintained at or above the K_d level for at least 3 weeks after implantation. Although radioactivity was used as a marker in this study, and rhBMP-2 was not directly measured or traced, the fact that a calcified bone matrix developed was confined at the implantation site (Kokubo et al., 1996) provides strong evidence for the specific biologic action of the implanted rhBMP-2 on local osteoblast precursor cells.

Finally, the cumulative amount of radioactivity excreted in the urine is inverse to the radioactivity remaining at the implant site (Fig. 4). This result suggests that rhBMP-2, once eliminated from the implant site, is rapidly excreted. Since most of the radioactivity excreted in urine is TCA-soluble (data not shown), rhBMP-2 is probably rapidly degraded, either at the implant site or in the blood, to a low-molecular weight decomposition product. The results also show the biphasic nature of rhBMP-2 elimination since TCA-precipitable radioactivity in blood reached a maximum (1.41% of the initial dose) 6 h after implantation, and declined below the limit of detection within 48 h (Fig. 5). These results suggest that after implantation, the released ^{125}I-rhBMP-2 is rapidly decomposed and excreted.

5. Conclusions

After implantation of ^{125}I-rhBMP-2/PGS, radioactivity at the implant site is eliminated biphasically. The value of $t_{1/2\alpha}$ is about 0.1 days, the value of $t_{1/2\beta}$ is 3 days, and the MRT value is about 4 days. The concentration of rhBMP-2 at the implant site was maintained at or above the reported K_d level for at least 3 weeks after implantation. Autoradiography of the implant site, 28 days after implantation, revealed that a trace amount of residual radioactivity in the shape of the implanted PGS remained at the implant site. These results indicate that PGS retains a sufficient amount of rhBMP-2 for at least 21 days to induce bone regeneration at the orthotopically implanted site. Consequently, PGS has great potential as a carrier of rhBMP-2 for clinical use.

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