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COMPARATIVE STUDY OF BONE MARROW INDUCED BY PURIFIED BMP AND RECOMBINANT HUMAN BMP-2

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SUMMARY: Into a calf muscle pouch in Wistar rats, 50μg purified bone morphogenetic protein (pBMP) or 50μg recombinant human bone morphogenetic protein-2 (rhBMP-2) was implanted using atelopeptide type I collagen solution (CL) as a carrier. Three weeks later bone and bone marrow were induced in both groups. These induced bone and bone marrow were studied histologically.

In the pBMP+CL group (n=5), rich bone matrix and little bone marrow were observed. There was no fatty marrow or angioid tissue observed. In the rhBMP-2+CL group (n=5), bone matrix and rich marrow including fatty marrow and angioid tissue were observed around and among the bony trabeculae. It was suggested that a " self-supporting bone organ " was induced.

Recently, bone morphogenetic protein (BMP), which induces bone tissue ectopically, has received considerable attention and basic studies have been performed. However, very few have verified pure osteoinduction(1-9) and

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there is no comparative histological study of induced bone and bone marrow.

In this study, purified BMP (pBMP) and recombinant human BMP-2 (rhBMP-2) were implanted into a calf muscle pouch in rats. Three weeks later bone and bone marrow were induced in both groups. Those bone and bone marrow specimens were observed histologically using a light microscope.

MATERIALS AND METHODS

Animals: Ten Wistar rats (male ; 10 weeks old ; weight 210-260g.) were established into two groups (each n=5).

Implant materials: pBMP was a final purified BMP, which was refined from bovine bone to a single band by sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis (SDS-PAGE) using liquid chromatography following Bessho's method(2). It was confirmed in the previous study that this pBMP is soluble in vivo and requires a gradually emitting carrier to induce bone tissue(2,3).

rhBMP-2 (Butch Number 0213J01; Genetics Institute,Inc. Massachusetts, U.S.A.) was donated by Yamanouchi Pharmaceutical Co., Ltd.(Tokyo, Japan). The rhBMP-2 was suspended in a buffer (pH 4.5) of 5mM glutamic acid, 2.5% glycine, 0.5% sucrose and 0.01% Tween 80 and preserved at a temperature of -80 degree, then thawed at room temperature.

As a carrier in both groups 3mg/ml atelopeptide type I collagen solution (pH 3.0) (Cellmatrix LA; Nitta Gelatin Inc. Osaka, Japan)(CL) was used.

50μg pBMP or 50μg rhBMP-2, mixed with 3mg CL, was lyophilized. The material was compressed in the injection syringe to disk form, measuring 4.0mm in diameter and 1.5mm thick.

Surgical procedures: The disk was implanted into the calf muscle pouch of a rat, which was anesthetized intraperitoneally with sodium pentobarbital. The fascia and skin were sutured closely in both groups.

Sampling procedures: Three weeks later rats were sacrificed by overdose of sodium pentobarbital. Then the calf muscles of the rat were dissected en block. The palpable nodule with a layer of surrounding muscle was then excised. After the sample was fixed in 10% formalin neutral buffer solution (pH 7.4), it was decalcified by EDTA and stained by hematoxylin and eosin for microscopic observation.

RESULTS

Findings under light microscopy in each group are summarized in Table 1.

Group I (pBMP+CL group)(Fig.1): CL, used as a carrier, was not detected in the lump. Considerable trabecular bone, many osteoblasts, which lined the trabecular bone, and a few osteoclasts were observed. In the samples excised three weeks later, no cartilage or chondrocytes were seen. Immature mesenchymal tissue surrounded the trabecular bone. Partially among the trabeculum, a small area of bone marrow was observed. The marrow did not include fatty marrow or angioid tissue. Overall, the lump was constructed of large amounts of trabecular bone and a small amount of bone marrow.

Group II (rhBMP-2+CL group)(Fig.2): CL was not detected. On various sides of the trabecular bone, one or two-layered osteoblasts were observed. A few osteoclasts were observed. There was no cartilage or chondrocytes. Among the trabecular bone and at the center of the lump, much bone marrow was observed. The marrow tissue included fatty marrow and

Table 1 : Comparison of histological findings three weeks after the implantation between the pBMP+CL group and the rhBMP-2+CL group

		Group I pBMP + CL	Group II rhBMP-2 + CL
bone matrix	**(NB)**	+++	+
osteoblast	**(OB)**	++	+++
osteoclast	**(OC)**	+	+
immature mesenchymal cell	**(IM)**	++	+
bone marrow	**(BM)**	+	+++
fatty marrow	**(FM)**	−	++
angioid tissue	**(AT)**	−	++

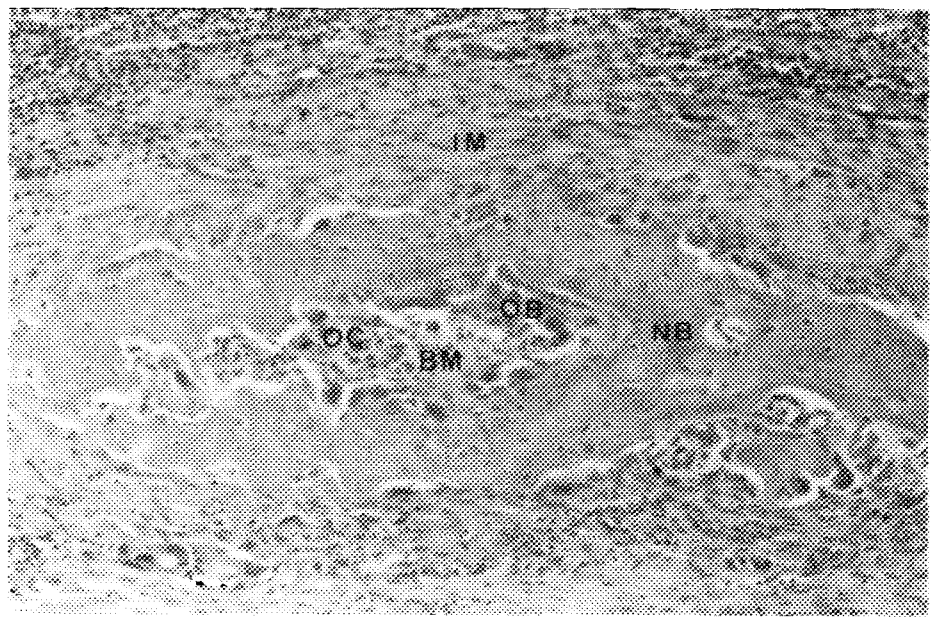

Figure 1 . Histological view three weeks after implantation of 50 μg purified BMP (pBMP) with atelopeptide type I collagen solution (CL) as a carrier. (decalcified by EDTA; HE stain; ×200.)
(BM: bone marrow; IM: immature mesenchymal cell; NB: bone matrix; OB: osteoblast; OC: osteoclast.)

angioid tissue pooling red blood cells. Overall, the lump was constructed of trabecular bone and rich bone marrow including fatty marrow and angioid tissue.

DISCUSSION

Some experiments of pure osteoinduction, using pBMP only or pBMP with a carrier other than bone matrix, were reported(1,4,7). Of these studies, only Urist's report(1) showed fatty marrow in the marrow tissue. There are very few experiments of pure osteoinduction using rhBMP-2, but in some of these reports, bone marrow with fatty marrow was shown(5,8,9). Induction of not only trabecular bone but also bone marrow indicates that osteogenesis was quite vigorous.

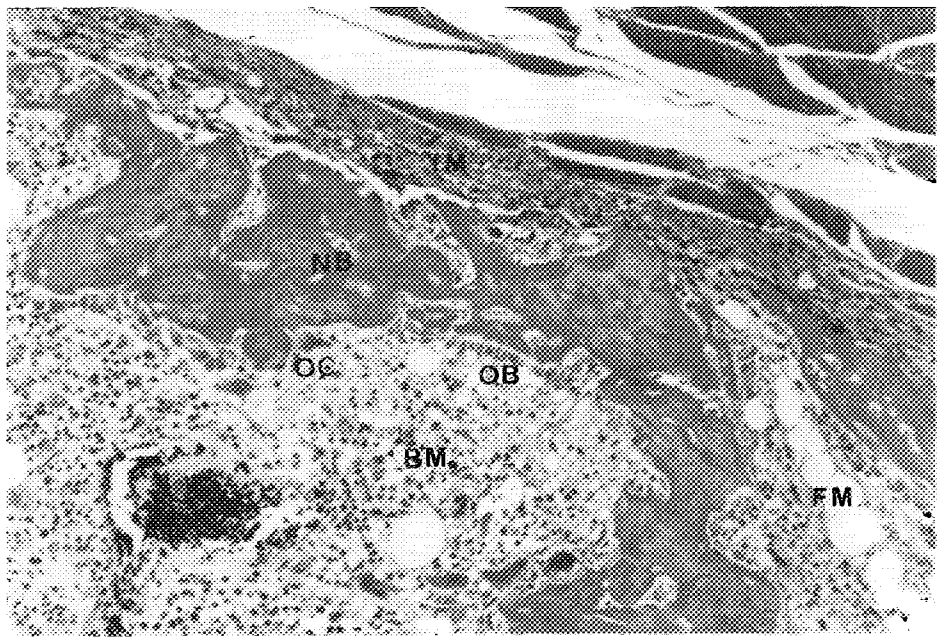

Figure 2 . Histological view three weeks after implantation of 50μg recombinant human BMP-2 (rhBMP-2) with atelopeptide type I collagen solution (CL) as a carrier. (decalcified by EDTA; HE stain; ×200.)
(AT: angioid tissue; BM: bone marrow; FM: fatty marrow;
IM: immature mesenchymal cell; NB: bone matrix;
OB: osteoblast; OC: osteoclast.)

To date, only Sampath et al. reported blood vessels invading induced bone marrow after implantation of collagenous demineralized bone matrix under the skin(10). There is no report of angioid tissue pooling red blood cells in the cavity as in our report. In our study, 50μg rhBMP-2, a relatively small amount, was implanted and angioid tissue was induced. It was initially proposed that BMPs act on immature mesenchymal cells, inducing osteoblasts and leading to osteogenesis, but immature mesenchymal cells are differentiated from committed progenitors to fibroblasts, reticulocytes, adipocytes or osteoblasts(11). Therefore, it is possible that rhBMP-2 participates in differentiation or induction of angioid

tissue in addition to osteoinduction. During osteoinduction using BMPs, various growth factors(12-17), interleukin 1B(18) and interleukin 6(17), were detected. Possibly they induce angiogenesis as a secondary effect. BMPs have been detected in bodily tissues other than bony tissue. Recently, the relation of BMPs with atherosclerosis was noted(19) and it is suspected that BMPs have unknown actions other than osteoinduction.

To maintain induced bone matrix, blood must be supplied to the bone tissue and the bone tissue must be remodeled continuously. Therefore, if much trabecular bone tissue is induced by BMPs and blood supply is insufficient, the bone tissue will be absorbed and the form of the induced bone will not be maintained. In this study, pBMP induced considerable trabecular bone, a small amount of marrow and no angioid tissue. To produce trabecular bone only, osteoinduction by pBMP is quite beneficial. However, rhBMP-2 induced trabecular bone and rich bone marrow including fatty marrow and angioid tissue. It is suspected that the whole bone tissue received a continuous blood supply and has the potential to become a " self-supporting bone organ ", in which bone tissue is remodeled continuously and the form is maintained over the long term.

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