Initial Calcification of Bone Formation in Ectopic and Osteogenic Tissue Induced by BMP-Collagen Composites

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Abstract: In this study, the initial osteoinductive capacity and bone forming patterns induced by BMP were studied using routine histology and electron microscopy .The pattern of initial calcification is different in induced bone formation at heterotopic sites. Three experiments were conducted; BMP and atelocollagen complexes were implanted into subcutaneous and subperiosteal tissues of rats. Initial calcification multi potentially started in matrix vesicles of osteoid tissue as well as in carrier collagen fibers. Bone formation occurs either via preexisting cartilage or directly according to the geometry of carriers used and the micro environmental conditions. In this study we conducted an ultra structural analysis of subcutaneously implanted both bovine extracted BMP and rhBMP with collagen carriers and subperiosteally implanted rhBMP-collagen composites in rats to understand the effects of carrier and environmental conditions on bone formation.

Keywords: bone formation, BMP, collagen, initial calcification, ultra structural analysis, TEM

Introduction

BMPs have a unique ability to alter the differentiation pathway of mesenchymal cells toward chondrogenic and osteogenic lineages with the ultimate induction of enchondral bone at ectopic sites. It has been reported that the implementation of partially purified BMP-carrier complex in heterotopic sites induces cartilage formation first and then bone formation through a normal endochondral sequence. Mesenchymal cells migrate and condense into the region of the implant, chondroblasts are derived from the early appearing mesenchymal cells forming cartilage, the cartilage gradually become hypertrophic and gradually replaced by bone. However direct bone formation without cartilage was also reported recently. Ectopic bone formation is induced by the implantation of extracted bovine BMP or recombinant rhBMP 2 protein with a carrier. Cell differentiation pattern induced by BMP was dependent on the cell environment and the geometry of carrier. In this study, we examined cell differentiation and initial calcification in BMP or rhBMP 2-collagen carrier implanted into rats by using transmission electron microscopy (TEM).

Materials and Methods

Bovine BMP was prepared as reported before (Nagai et al., 1995). RhBMP 2 was obtained from Yamanouchi Co ltd, Tokyo. Combination of BMP with collagen was done as reported previously (Nagai et al., 1995). Thirty male Wistar rats (about 100g in weight) were subjected to intra peritoneal anesthesia with pentobarbital sodium. The back was shaved, disinfected with iodine and alcohol and cut by blind dissection to form subcutaneous pockets. BMP and the collagen composites were implanted in the subcutaneous tissues. At 1, 2 and 3 weeks after implantation, the composites were removed and prepared for histological and ultra structural analyses.

In the first experiment, a mixture of bovine extracted BMP and type collagen were implanted in the subcutaneous tissue of 4 weeks old rats. In the second experiment, a composite composed of rhBMP 2 and collagen I was implanted in the subcutaneous tissues of 8 months old rats, while in the third experiment a composite of rhBMP 2 and collagen I was implanted in the subperiosteal membrane of the heads of 8 months old rats to observe chondro-osseous tissue formation. The composites were removed after 1, 2 and 3 weeks. Histological analysis of the induced bone formation was done using routine microscope as well as TEM.

Results and Discussion

In normal bone formation initial calcification occurs in matrix vesicles in newly formed chondro-osseous tissue. However, initial calcification multi potentially started in matrix vesicles of osteoid tissue as well as in carrier collagen fibers. We used bovine purified BMP in experiment 1. This BMP is not pure and includes many other proteins and some other materials such as histones. Thus, BMP complex with other proteins are shown in electron microscope as dark oval shapes (Fig.1A). Calcification commenced both in collagen fiber-BMP complex areas (Fig.1A) and in osteoid tissue matrix vesicles near an osteoblast (Fig.1B). These calcifications progressively became bigger and finally congregated to result in bone tissue (Figs. 1C and 1D). The calcification process in experiment 2 using rhBMP 2 was similar to experiment 1. However, rhBMP 2 is pure and does not include some other molecules such as protein and histones. Therefore, we cannot demonstrate the structure of BMP-protein complex as shown in experiment 1 with TEM. Collagen fibers were used as a core for initial calcification in rhBMP 2-collagen implant. Calcification pattern was the same as in the experiment 1 as seen both in osteoid tissue matrix vesicles and in collagen fibers (Fig. 2 A and B respectively). These calcifications also merged to form bone tissue finally (Fig. 2C). In experiment 3, we found a different calcification pattern similar to endochondral ossification. Chondrocyte like cells, which were surrounded by chondro-osseous matrix containing abnormal collagen fibrils, were noticed 2 weeks after implantation into the subperiosteal membrane of

heads of rats (Fig. 3A). This chondro-osseous matrix was seen at the margins of the rhBMP-collagen composite.

It is generally believed that mechanical compression and oxygen concentration are related to the chondro-osteogenic cell differentiation. When the cells are firmly packed in the very narrow spaces, so that the concentration of oxygen was likely to be very low, cartilage formation occurs first. Vascularization and oxygen supply to implant area was probably poor in sides of rhBMP 2-collagen composites which resulted in differentiation of mesenchymal cells to chondrocyte-like cells. Chondrocyte-like cells were also confirmed by TEM (Fig. 3B). Abnormal collagen fibrils were noticed by TEM (Fig. 3C). Initial calcification pattern was similar as in experiments 1 and 2. Calcification started both in matrix vesicles of chondro-osseous tissue and carrier collagen fibers.

In conclusion, initial calcification started multi potentially both in new osteoid tissue and in collagen carrier induced by BMP-collagen complex.

It is well known that BMP induces chondro-osseous tissue formation through mesenchymal cell differentiation similar to endochondral ossification in normal bone. However, recent studies reported that BMP induces bone formation directly without going through cartilage formation. The cell differentiation pattern induced by BMP depends upon the nature of tissue environment that the carrier provides. In this study, we developed an experimental system, which supports this hypothesis

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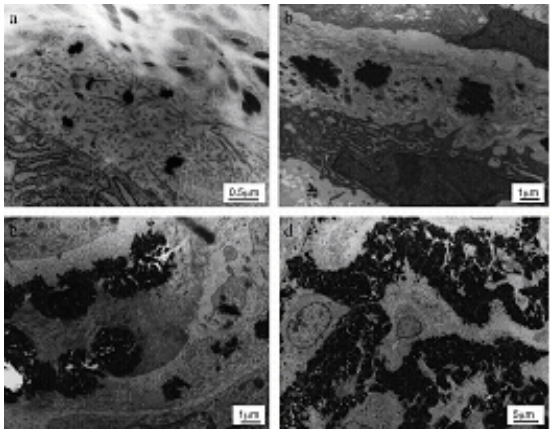

Fig.1. BMP-protein complex as dark oval shape and the border between osteoid and carrier are noticed. Enlarging calcifications started both in osteoid tissue and collagen carriers are merging to result in bone tissue. 2 weeks after implantation with bovine extracted BMP–collagen carrier. TEM. Fig.1a: x18000, 1b: x6000, 1c: x6000, 1d: x1800

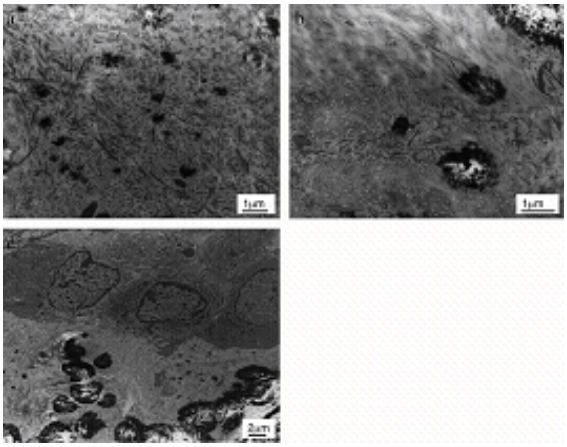

Fig.2. Calcification starts both in osteoid tissue and carrier collagen fibrils and finally congregates. 2 weeks after implantation with rhBMP 2-collagen carrier. TEM. Fig.2a: x18000, 2b: x12000, 2c: x2400

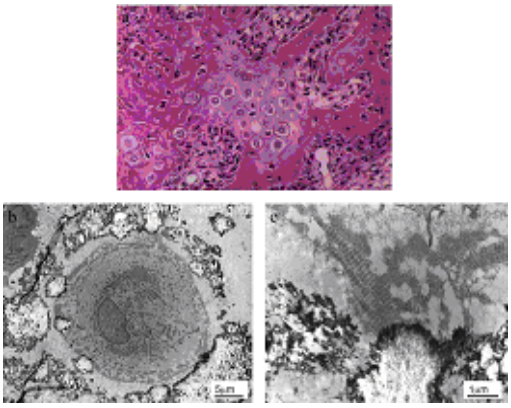

Fig.3. Chondrocyte like cells are seen (3a). Chondrocyte is confirmed by TEM (3b). Abnormal collagen fibrils are noticed (3c). 2 weeks after implantation with rhBMP 2-collagen carrier. Fig 3a (H&E): x460, 3b(TEM): x2100, 3c(TEM): x9000