

Morphogenetic Protein rhBMP-2 and New Bone Formation

Proteína Morfogenética rhBMP-2 y Tejido Ósteoneoformado

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SUMMARY: This work aim to show by literature review the principal characteristics of morphogenetic proteins, in special of the rhBMP-2, with the major osteoinductive properties, presented in the prime works count from it discovery until actually, showing the most varieties and applications of this protein.

KEY WORDS: Bone; Morphogenetic protein; rhBMP-2.

INTRODUCTION

Over than thirty years, most researches have been realized with the objective to identify proteins capable to induce new bone formation and standard methods of biological application that can lead to decrease or elimination the bone graft (Nawata *et al.* 2005).

One decade ago, the bone morphogenetic proteins (BMPs) have been identified and purified from human bone matrix (Bessho *et al.* 1989). Since then, it has been identified 17 different proteins of the same family, that is, proteins with the same characteristics, some of synthesized by molecular biologic techniques, defined as recombinant human morphogenetic proteins or rhBMPs (Wozney, 1989; Celeste *et al.*, 1994; Urist, 1997; Bouxsein *et al.*, 2001). These BMPs belong to TGF growth factor superfamily, that involves polypeptides with common structural characteristics (Kingsley, 1994) and that have an important role in progenitor cells migration, mesenchymal cells proliferation, osteogenic and chondrogenic cells differentiation, vascular invasion and bone remodeling (Reddi, 1997; Rauch *et al.*, 2000; Spector *et al.*, 51, 2001).

Thus, the aim of this work is to present, by a literature review, the principal characteristics of bone morphogenetic proteins, in special, the rhBMP-2, since its discovery until the present time, and describe the most varies uses of this protein.

DISCUSSION

Bone morphogenetic protein characterization. Bone morphogenetic proteins have proliferative effects on different cellular types, showing chemotactic properties and being able to induce mesenchymal cells differentiation into osteoblastic and chondroblastic line cells (Reddi & Cunningham, 1993). Moreover, BMPs are powerful inductive of the osteogenic activity during the embryologic bone formation phase and in bone healing cases (Hogan, 1996; Rosen *et al.*, 1996). Among members of the extensive family of these proteins, the BMP-2, 4 and 7 have been demonstrated an important role on osteogenic process (Hogan; Rosen *et al.*).

The availability of recombinant human bone morphogenetic proteins allowed proving osteoinductive properties of these proteins, as well as the detailed characterization of this activity in vivo. The rhBMP-2 was the first molecule studied in detail (Wang *et al.*, 1990), being produced using Chinese hamster ovary cells. The purification and biochemical characterization of these molecules show that they are similar to BMP molecules found in bone (Israel *et al.*, 1992; Inoda *et al.*, 2004; Kamakura *et al.*, 2004). The rhBMP-2 implantation in an ectopic delivery system, shows that this molecule is osteoinductive, proven by histologic exams, where the implantation of different doses in different wait periods shows that new bone process with the use of rhBMP-2 resulted to a cartilaginous bone, in the

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first period and that only BMP molecule is capable to begin the new process *in vivo*. This study showed that BMP amount inserted is strongly related to new bone formation quantity, thus increasing the BMP quantity inserted, the new bone formation will occur faster. The bone healing can be observed in five days after the insertion, occurring this new bone formation concomitantly with the cartilaginous tissue formation, showing that the rhBMP-2 is capable to influence both, the cartilaginous and bone formation.

The BMP-2 use resulted in an increase on the BMP-4 expression. The recombinant human (rhBMP-4) seems to have similar activity to BMP-2 *in vivo*, inducing bone and cartilage formation in rat experiments. The BMP-6 and BMP-7 (Sampath *et al.*, 1992), as well as the BMP-2 and BMP4 are osteoinductive and chondroinductive, but it is difficult to compare the relative activities of all these molecules, because the researches have been used different methods (Table I) (D'Alessandro *et al.*, 1991; Cox *et al.*, 1991).

Action mechanism of bone morphogenetic proteins. An *in vivo* studies it was verified that BMPs can induce the new bone formation and cartilaginous tissue by a process that still was not been clarified and that involves the activity of a great number of local and systemic growth factors. Studies that involve individual characterization of these proteins is complicated by two reasons: first, because little comparisons about the different BMP types have been realized in the same cellular groups, and second, some studies have been realized with BMPs deriving from purified bone tissue, that generally contain different types of BMP molecules.

The treatment with BMPs result in mesenchymal cells differentiation into different cellular morphotypes (Yamaguchi *et al.*, 1991), an example, in C26 cells, the BMP-2 increase the alkaline phosphatase expression and the parathyroid hormone receptors (PTH) and also the osteocalcin expression, a marker for osteoblast differentiation.

Table I. Bone morphogenetic protein m RNA tissue and cell line sources.

BMP-2	Bone, spleen, liver, brain,	U-2 OS (osteosarcoma)
	lung, kidney, heart, placenta.	MG-63 (osteosarcoma)
		F9 (embryonal carcinoma)
BMP-3	Lung, brain	PC-3 (prostate)
		H128 (small cell lung carcinoma)
BMP-4	Bone, lung, kidney, brain, spleen, liver, heart, placenta	U-2 OS (osteosarcoma)
		MG-63 (osteosarcoma)
		PC-3 (prostate)
		DU-145 (prostate)
		F-9 (embryonal carcinoma)
BMP-5	Placenta	U-2 OS (osteosarcoma)
		MG-63 (osteosarcoma)
BMP-6	Calvaria, lung, brain, placenta, kidney, uterus, muscle, skin	U-2 OS (osteosarcoma)
		F-9 (+ RA + cyclic adenosine monophosphate)
		PC-3, DU-145, PAIII (prostate carcinomas)
BMP-7	Kidney, placenta, brain, calvaria, spleen, lung, heart, liver, adrenal, bladder	U-2 OS (osteosarcoma)

Generally, *in vitro* BMPs have significant effects on cells in multiple periods of the new bone formation, as also observed in *in vivo* studies.

Through chemotaxis, the BMPs can bring cells to implantation site and do with that undifferentiated mesenchymal cells become into specific cellular types that synthesize bone and cartilaginous tissue, as the osteoblast and chondroblast cells (Inoda *et al.*; Kamakura *et al.*).

In 1965, Marshall Urist discovered that decalcified bone matrix implanted at nonbone sites will induce the cartilaginous tissue and new bone formation (Urist, 1965). The author defined this process as autoinduction and later showed that protein extracts could be separate from decalcified bone and were responsible for this new bone formation (Urist *et al.*, 1973). In this model, new bone was formed following events well characterized in endochondral ossification, with cartilaginous tissue formation before the new bone formation (Urist *et al.*, 1979; Sampath & Reddi, 1981).

Development of the recombinant human morphogenetic protein. Bone can be described as having three components: a mineral component, a collagenous matrix and a growth protein component. Using experimental models in rats, some authors decalcified and separated the bone tissue in a component that contains a collagenous matrix and other component that contains growth proteins. The protein component was reconstituted with inactive matrix and implanted ectopically. New bone was observed after ten days. This growth factor component was called bone morphogenetic protein (Urist *et al.*, 1979; Sampath & Reddi).

Although the BMP has the capacity to induce new bone formation, it is difficult to obtain this protein by a purification process because small amounts are present in bone. Recent advances in biological molecular techniques provided the production of a great quantity of recombinant human proteins, like BMPs.

The development of the recombinant proteins (rhBMPs) began by individual members isolation of the morphogenetic proteins superfamily. Most of these proteins are members of the beta transforming growth factors (TGF-b) that constitute a superfamily proteins based in the homology sequence of the primary amino acid (Celeste *et al.*, 1990). The BMP-2 and BMP-4 recombinant are 92% identically and the BMP-5,6,7 e 8 are 82% identically (Wozney, 1995).

Isolating the BMPs in human corpses bone, only 0.1 µg BMP per kilogram of bone can be obtained. Already the rhBMP-2 can be produced in abundance. This protein has non-antigenic and non-immunogenic properties, not having risk of human disease transference because it is a protein produced by bio-engineered methods (Genetics Institute, Cambridge Mass, unpublished reports, 1995).

The osteoinductive activity of recombinant human bone morphogenetic proteins (rhBMPs) was analyzed in *in vivo* studies (Fujimura *et al.*, 1995; Kusumoto *et al.*, 1995). Due to osteoinduction power observed in these studies, is clearly the potential of these proteins in a great number of clinical applications, like reconstructive surgeries, bone defects provoked by pathological processes and physiological bone loss (Johnson *et al.*, 1988 a,b, 1990; Toriumi *et al.*, 1991; Marden *et al.*, 1994; Boyne, 1995).

Correlation between BMP and TGF-b. Despite of BMPs are members of the TGF-b superfamily, they have a few effects in common proven by *in vitro* studies in different cellular types. An example, TGF-b inhibit the phosphatase alkaline production in most cellular types, including C26, W-20-17 e MC3T3-E1 cells (Noda & Rodan, 1986; Katagiri *et al.*, 1990b), while BMPs have been increased in these cellular types. In contrast of BMPs, TGF-b suppresses the sulphate incorporation in any specific cellular types.

Proteins's carrier delivery. The morphogenetic protein of the classification 2, or rhBMP-2, has been despered interests for clinical applications, for this differentiated capacity to induce ossification and being commercially available.

However, *in vivo*, the rhBMP-2 spreads out quickly from the implanted place, when deposited in solution, what shortens your osteoinductor effects (Fujimura *et al.*). For this, recent studies have been demonstrated that the application of high dosages of these proteins directly on tissues is not so efficient in relation to osteogenic induction, because occur a fast protein metabolization (Wang *et al.*) however, small dosages, but supported, seem to produce satisfactory results (Yasko *et al.*, 1992; Gerhart *et al.*, 1993; Lee *et al.*, 1994; Bostrom *et al.*, 1995). Thus, the presence of a biomaterial carrier can influence in quantity of the new bone formation and decrease the quantity of protein that is necessary for new bone formation induction. Therefore, the role of carrier matrix is to immobilize the protein in site that was implanted for a period

sufficiently so that the cellular reply occurs.

Thus, substances capable to delivery BMPs to tissues for long periods, such as fibrin and collagen sponges, hydroxyapatite, calcium sulphate and synthetic materials like copolymers have been used as carriers in most studies (Kawamura *et al.*, 1987; Yamazaki *et al.*, 1988; Takaoka *et al.*, 1988, 1991; Desilets *et al.*, 1990; Ripamonti, 1991; Sato *et al.*, 1991; Matsuo *et al.*, 2003; Peterson *et al.*, 2005).

However, properties as controlled resorption, adequate density to support soft tissues, supported drug delivery, facility in manipulation and application, are desirable properties, however not always found in currently available materials (Bessho & Iizuka, 1995; Bessho *et al.*, 2002; Keskin *et al.*, 2005).

Treatments involving the use of the rhBMP-2. The effectiveness in use of the rhBMP-2, represented for the quantity and level of induction for the new bone formation is affected by the carrier that was used, animal specie and wait time until the animal sacrifice (Wozney, 1995; Zellin & Lindhe, 1997). Yasko *et al.* showed that a dose of 11 µg de rhBMP-2 for recovery a critical bone defect (5mm) in rat femurs- Sprague-Dawley, resulted in significantly new bone formation, with mechanic, radiographic and histologic evidences, but one dose of 1,4 µg produced new bone tissue but without these union evidences. Others studies also demonstrated that high rhBMP-2 levels are not effective, being of the carrier the role to provide a slow and gradual rhBMP-2 delivery (Zellin & Lindhe).

Some experiments were realized with the objective to test osteoinduction ability of bone morphogenetic proteins in healing process of the critical bone defects realized in most varied animal species, between them, in rats (Doll *et al.*, 1990; Mark *et al.*, 1990), rabbits (Moore *et al.*, 1990), dogs (Sato & Urist, 1985; Urist *et al.*, 1987b; Nilsson & Urist, 1991), sheeps (Lindholm *et al.*, 1988) and monkeys (Ferguson *et al.*, 1987). In all these studies, it has indications that BMP molecules are very useful in treatment of innumerable clinical conditions where aim is the new bone formation (Einhorn, 1992).

As new classes of treatments are evaluated for efficacy, their safety must be assessed. Of the recombinant proteins, rhBMP-2 has had extensive toxicological evaluations in both preclinical and clinical studies. These studies included rhBMP-2 in single and multiple doses, as well as teratology and fertility studies. Dosages of rhBMP-2 were administered intra-venously (IV) and were similar or the same accross the studies. In all studies, the rhBMP-2 dosages (per kilogram of body weight) were selected to constitute a range that was slightly lower to substantially higher than the amount of rhBMP-2 used in human clinical trials.

In acute intra-venously single-dose studies of up to 5.3mg/kg in the rat and dog, there no deaths related to treatment and no treatment related changes were observed for body weight, food consumption, blood pressure, electrocardiogram, clinical chemistries or urinalysis (Schaub, 1993). In the 28 day study of rats, no adverse events were noted. In the beagle dog study, dosages of 0.016 to 0.16mg/kg were administered intra-venously daily for 28 days. Again, there were no treatment related changes in clinical signs, clinical chemistries or urinalysis.

In teratology studies in the rat and rabbit, treatment of gravid rats and rabbits with rhBMP-2 did not produce systemic maternal toxicity or gross fetal abnormalities at dosages up to 1.6mg/kg/day. A rat fertility study showed no treatment related effects on maternal and paternal mating performance or reproductive parameter at dosages up to 0.16mg/kg/day (Genetics Institute, Cambridge Mass, unpublished reports, 1995).

CONCLUSIONS

Therefore, despite the rhBMP-2 osteoinduction properties already to be know and proven, new surveys must be realized with the objective to find a carrier substance that presents these characteristics and provide a slow and gradual rhBMP-2 delivery, what it is essential to get the clinical effect desirable.

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RESUMEN: Esta revisión de la literatura tiene como objetivo mostrar las principales características morfogenéticas, de la proteína rhBMP-2, de mayor propiedad osteoinductiva, estudiados desde su descubrimiento hasta la actualidad, señalando las diferentes utilidades y aplicaciones de esta proteína.

PALABRAS CLAVE: Hueso; Proteína morfogenética; rhBMP-2.

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