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Growth factors in orthopaedic surgery: demineralized bone matrix versus recombinant bone morphogenetic proteins

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Abstract

During recent decades the utilisation of growth factors, especially BMPs, has received an increasing interest in orthopaedic surgery. For clinical implantation the two main options are demineralised bone matrix (DBM) and recombinant bone morphogenetic proteins (rhBMP). Many clinical studies agree on an equivalent osteoinductive effect between DBM, BMPs and autologous bone graft; however, the different origins and processing of DBM and rhBMP may introduce some fluctuations. Their respective characteristics are reviewed and possible interactions with their effectiveness are analysed. The main difference concerns the concentration of BMPs, which varies to an order of magnitude of 106 between DBM and rhBMPs. This may explain the variability in efficiency of some products and the adverse effects. Currently, considering osteoinductive properties, safety and availability, the DBM seems to offer several advantages. However, if DBM and rhBMPs are useful in some indications, their effectiveness and safety can be improved and more evidence-based studies are needed to better define the indications.

Introduction

Bone growth, healing and remodelling require a complex cascade of events to produce or regenerate bone ad integrum. Many factors interact, involving cells and the physicochemical environment. Among them growth factors have received special attention over the last three decades [1, 2]. Growth factors have different origins in platelets, progenitor cells, osteoblasts and bone matrix [3–7].

Bone matrix represents a natural reservoir able to deliver various proteins and growth factors at the fracture site. In addition to collagen-I, bone sialoprotein (BSP) and osteopontin (OPN), insulin-like growth factor (IGF-I), transforming growth factor-β (TGF-β), acidic fibroblast growth factor (FGF-α), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) are found in significant amounts [8–10]. Their concentrations may vary in relation with donor, extraction techniques and processing.

The super family of TGF-β include the main bone morphogenetic proteins (BMP). Historically in 1945, Lacroix was probably the first to evoke the role of an “organizer” of the osteogenesis extracted from the epiphysis [11], and in 1947, he suggested calling it “osteogenin” [12]. Later on, in 1965, Urist introduced the term “autoinduction” [13] and in 1971, bone morphogenetic proteins [14]. In 1981, Sampath and Reddi [15] dissociated in demineralized bone matrix (DBM), the insoluble collagenous substratum and the soluble extract. Later on, BMP-3 was identified and called osteogenin [16] as proposed by Lacroix. All of these works have led to the identification of at least 20 different bone morphogenetic proteins that are in existence today (Table 1) [6, 17].

Bone Morphogenetic Proteins – Famille TGF-β (n>20)		
BMP-2	BMP-2a	Bone and cartilage morphogenesis , osteoinduction, osteoblast differentiation, apoptosis
BMP-3	Osteogenin	Negative regulator of bone morphogenesis
BMP-3b	GDF-10	Negative regulator of bone morphogenesis
BMP-4	BMP-2b	Teeth, cartilage and bone morphogenesis
BMP-5	-	Limb development, cartilage and bone morphogenesis
BMP-6	Vgr-1, DVR-6	Osteoblast differentiation, chondrogenesis
BMP-7	OP-1	Cartilage and bone morphogenesis
BMP-8	OP-2	Bone and cartilage morphogenesis
BMP-9	GDF-2	Bone morphogenesis
BMP-11	GDF-11	Axial skeleton patterning
BMP-12	CDMP-3, GDF-7	Ligament and tendon development
BMP-13	CDMP-2, GDF-6	Cartilage development and hypertrophy
BMP-14	CDMP-1, GDF-5	Chondrogenesis, angiogenesis

Table 1: BMPs table with function related to musculoskeletal tissues [6, 17]

Originally identified for their role in osteogenesis, they have other functions in the development of various organs and tissues. For their specific activities on bone, two of them are currently available and proposed for therapeutic treatment: BMP-2 and BMP-7. Some others, including BMP-4, BMP-6 and BMP-9, are under study [4, 6, 18].

In the cascade of sequences, they act at different stages of bone cell differentiation and during the different phases of fracture healing. Today, there is no consensus regarding their precise sequences of activity and possible synergies [6, 19–22].

Biological assessment

DBM and BMPs show various effects on different in vitro and in vivo models. However, the specific conditions in vitro cannot simulate the complex in vivo environment of bone, and the in vivo trials on animals show different sensitivities that do not represent a reliable evaluation of the effects on human subjects. While an in vitro or in vivo biological assay may be theoretically an ideal evaluation of the osteoinductive properties of DBM or BMP, no one is presently available to predict the clinical outcome [4, 5, 23–25].

Even in clinical studies, the comparisons are difficult between different protocols, indications and therapeutic associations. However, within the same study, using the same protocol to compare different groups, more reliable and coherent relations can be drawn. The autografts remain the most used reference to compare and evaluate the effect of growth factors. In the present review, we select a few clinical studies for their level of evidence and which are illustrative for their use of DBM or BMP in different clinical indications, i.e. closed or open fresh fractures, non-unions and lumbar and cervical fusions.

Clinical use of DBM

Hierholzer et al. [26] compared the effect of DBM to iliac crest autograft (ICA) in a series of atrophic non-unions of humerus treated by internal plate fixation (Table 2). While the mean age of non-union is older in the DBM group, there is no difference in the percentage of healing nor in the time to healing. They observe a significant morbidity at the donor site for the ICA.

	ICA	**DBM (Grafton®)**
N	45	33
Age of non-union	14 months	22.6 months
Healing	100%	97%
Healing time	4.5 months	4.2 months
Donor site morbidity	44%	

Table 2: Atrophic non-union of the humerus treated by internal fixation: comparison of the result between association with Iliac Crest Autograft (ICA) or Demineralized Bone Matrix (DBM) [26].

The same comparison, DBM versus ICA, was studied by Pieske et al. [27] on atrophic non-union of long bone treated by internal fixation. The groups were smaller but the observations were the same, i.e. no statistical difference between DBM and ICA in terms of healing and time to healing (Table 3).

	ICA	DBM (Grafton®)
N	10	10
Age of non-union	8.7 months	14.6 months
Healing	8	10
Healing time Xray month	10.9 (2-40)	11.9 (2-21)
Donor site morbidity	2	

Table 3:Atrophic non-union of long bones treated by internal fixation: comparison of the result between association with Iliac Crest Autograft (ICA) or Demineralized Bone Matrix (DBM) [27]

Several authors [28–31] used DBM as an ICA extender in posterolateral lumbar fusion. They compared the fusion between the two sides using the different procedures or between groups (Table 4). Using DBM as an extender makes those protocols less reliable as the association with more or less local autologous graft or ICA is not accurate. No difference between DBM and autograft was observed in those studies and they confirm the utility of DBM as graft extender [32, 33].

Graft	N	Study	Evaluation	Difference	Author
DBM (Grafton®) + local autog.	56	Retrospective	Xray	NS	Sassard 2000 [28]
vs ICA	52				
DBM (Grafton®) + ICA	120	Prospective, Multicentric	Xray	NS	Cammisa 2004 [29]
vs ICA					
DBM (Accell Connexus®) + ICA	33	Retrospective	Xray	NS	Schizas 2008 [30]
vs ICA	26				
DBM (Grafton®) + local autog.	30	Prospective, Multicentric,	Xray	NS	Kang 2012 [31]

vs	ICA	16	Randomised

Table 4: Comparison of the result between Iliac Crest Autograft (ICA) and Demineralized Bone Matrix (DBM) used as extender in Posterolateral Lumbar Fusion

Clinical use of DBM and BMP

In 1999, Geesink et al. designed an interesting prospective, randomized protocol [34]. They used patients undergoing a proximal tibial osteotomy. For the associated osteotomy of the fibula, they resected a segment of 15 mm and studied the evolution of this osteotomy comparing four groups: control (no associated treatment), DBM + glycerol (Grafton®), bovine collagen type-I sponge alone and BMP-7 on collagen sponge. The only weak point of this study is the small number (N = 6) of subjects in each of these groups (Table 5). Significantly more bone formation and bone bridges were observed for DBM and BMP-7. In addition, after one year, the mineral bone density was 100 % restored with DBM and 82 % with BMP-7 in comparison with an absence of evolution in the control and in the collagen sponge groups. The X-rays show a more homogenous callus formation with DBM while, with BMP-7, the periosteal activity seems more stimulated. Two patients in the group implanted with bovine collagen alone had anti-collagen reaction without clinical manifestation.

	N = 24	**No Pain**	**Xray**		**DEXA**
			New Bone	**Bridge**	
Control	6	6/6	3	0	0.44
DBM[1]	6	6/6	6	4	1.01
Collagen[2]	6	6/6	2	0	0.44
BMP-7[3] + collagen	6	3/6	5	5	0.82

1. DBM: 2.0ml Grafton® (+ glycerol)
2. Collagen: 1.0gr bovine type-I sponge
3. BMP-7: 2.5mg eptotermine α + bovine collagen sponge

Table 5: Model of fibular resection: after 12 months, comparison between control group, Demineralized Bone Matrix (DBM), collagen sponge alone and BMP-7 + collagen sponge [34]

Clinical use of BMP

In a large prospective, randomized and multicentric study, Govender et al. [35] compared the healing of open tibial fracture treated by intramedullary nail fixation (IMN) alone versus intramedullary nail (IMN) and BMP-2 on bovine collagen sponge (Table 6). They used two different concentrations of BMP-2 (0.75 mg/ml and 1.50 mg/ml) and observe for the highest a shorter time to healing for 50 % of the group, with less reinterventions and less infections. However, this study presents a bias as there are more reaming procedures in the group showing the best results (Table 7). Reaming debris is "a source of multipotent stem cells" [36] and "...a rich source of growth factors" [37, 38]. This may explain the differences observed.

	IMN alone	**+ 0,75mg/ml**	**+ 1,50mg/ml**
N	138	142	141
50% healed at	184 days	187 days	145 days
2nd intervention	46%	37%	26%
Wound healing at 6 weeks	65%	72%	83%
Infections in Gustilo IIIA/IIIB	44%	29%	24%
Hardware failure	22%	17%	11%

Table 6: Open tibial fracture treated by intramedullary nail (IMN): comparison of the result between IMN alone and IMN + 0.75 or 1.50 mg/ml BMP-2 [35]

	IMN alone	**+ 0,75mg/ml**	**+ 1,50mg/ml**
N	147	145	145
Reamed	27%	33%	41%

Table 7: Percentage of the reaming procedure in the different groups [35]

	IMNR alone	**+ rhBMP-2 1.50mg/ml**
N	138	139

Healing	67%	68%
2nd intervention	12%	12%
Infection	11%	19%

Table 8: Repeated study on open tibial fractures all reamed [39]

Based on the same protocol a second study on BMP-2 was repeated but with reaming procedure in all cases (Table 8). No significant difference was observed in this last study [39].

In a prospective, randomized study, Friedlander et al. [40] compared ICA to BMP-7 (3.5 mg/gr) in tibial non-unions treated by reamed intramedullary nail fixation. They observed no difference in the percentage of healing even if there are more atrophic non-unions in the BMP group. Also, they had less infection in this last group (Table 9).

	ICA	**rhBMP-7**	
N	61	63	
Healing (Xray-clinic)	85%	81%	NS
Atrophic	25%	41%	
Smokers	57%	74%	
Infection	21%	3%	
Donor site morbidity	20%		

Table 9: Tibial non-union treated by intramedullary nail (IMNR) reamed procedure: comparison between the results with Iliac Crest Autograft (ICA) and recombinant Bone Morphogenetic Protein 7 (rhBMP-7) [40]

In a small series, Maniscalco et al. [41] followed the time to healing of closed tibial fractures treated by external fixation in one group of seven without associated treatment and in a second group of seven with BMP-7. They observed no difference in the time to healing and concluded to the absence of indication in that case.

Like the DBM, BMP-2 is used in posterolateral lumbar fusion (PLF) as an extender of ICA compared to ICA alone. The use as an extender raises the same reservations we had previously with the DBM regarding the volume and percentage of autograft used. The conclusion was in favour of the use of BMP-2 (Table 10) [42, 43]. The same conclusion was made by Stambough et al. [44] in PLF using, in association with BMP-2, freeze-dried corticocancellous allograft and local autogenous bone graft (Table 10). They have no control group but they obtain a high fusion rate (97.2 %).

Graft	N	Study	Evolution	Union	Author
rhBMP-2 + ICA	39	Prospect	CT	97%	Singh, 2006 [42]
vs ICA	11		24 months	77%	
rhBMP-2 + local auto + freez-dried allograft	36	Prospect	CT 29 months	97.2%	Stambough, 2010 [44]

Table 10: Comparison of the result between Iliac Crest Autograft (ICA) and Bone Morphogenetic Protein 2 (BMP-2) used as extender in Postero Lumbar Fusion (PLF)

Several other studies used BMP-2 on collagen sponge with cage for anterior lumbar interbody fusion or in PLF in comparison with ICA and concluded with an equivalent effect [7].

However, in 2011, Carragee et al. [45, 46] reviewed the complications associated with the use of BMP-2 in spine surgery. They identified a significant number of industry-sponsored trials (N = 13) "remarkable for the complete absence of reported rhBMP-2 related clinical events". They strongly questioned the objectivity of those publications analysing the FDA data [46, 47]. They found neurological complications which may result from inflammatory reactions: adverse back and leg pain events, retrograde ejaculation [48] and post-op bladder retention. The delayed infections were increased. Bone resorption, osteolysis and implant displacement were observed [49, 50]. Other authors reported bone overgrowth into the spinal canal in PLIF and radiculitis, osteolysis and loss of alignment [46, 49, 51–53].

In cervical interbody fusion, wound problems, soft tissue swelling, airway compromise, graft subsidence and end plate erosion have been reported [49, 51, 54–57]. Finally, increased risk of malignancy is advocated with the highest doses of rhBMP-2 [46]. Caragee et al. also reported bias in PLF and PLIF study designs and ICA morbidity report at the donor site [46].

These observations were followed by more exhaustive reviews and meta-analysis in spine fusion. Some of them conclude: "in spinal fusion, rhBMP-2 has no proven clinical advantage over bone graft and may be associated with important harms, making it difficult to identify clear indication for rhBMP-2" [58] and at "24 months, rhBMP-2 increases fusion rates, reduce pain by a clinically insignificant amount, and increases early post-surgical pain compared with ICBG (iliac crest bone graft). Evidence of increased cancer incidence is inconclusive" [59]. More recently, Adams et al. [60] and Michielsen et al. [61] in PLIF using rhBMP-2 versus local bone autograft found an equivalent effect with an increased risk of complication.

Comment on the clinical evidence of the osteoconductive properties

In vitro and in vivo experimental models demonstrate osteoinductive effects of DBM and BMPs. However, those effects remain dependant on the model concerned and are difficult to be extrapolated systematically to a specific clinical situation. Qualitative and quantitative differences exist in between the observed effects.

In clinical applications, the clearest protocols are in favour of an equivalent osteoinductive effect of DBM, BMPs and autograft which per se could be considered as a positive and useful result. Even some of the adverse events related to rhBMPs (osteolysis, bone overgrowth, etc.), if they are detrimental in clinical situations, are proof of the action of BMPs but uncontrolled in the present state.

Despite the large generalisation of their use, we have not had much clear and robust evidence-based clinical studies.

Different parameters may interfere with the effectiveness of the products. They include the processing and packaging of the different labels, the concentration, the origin (variability of the donors, natural or synthetic, etc.), the carriers and the composite associations.

We review hereunder the main parameters which may influence the osteoinductive properties.

Availability and labels

DBM

DBM are produced by most of the current accredited public non-profit bone banks. Processed from human bone allograft, they are delivered as a simple (pure) particulate product without association and proposed in different granulometries. Industries also processed DBM but usually in association with carrier, some of them are supposed to have additional properties to promote osteogenesis. Presently, at least 15 companies propose no less than 35 derived products [5, 62]. In the following review on an arbitrary base, we mention as example only three of those products (Grafton® Putty, Osteotech; DBX® Putty, MTF/Synthes; Allomatrix® Injectable Putty, Wright Medical Technology).

Not all of them are available in every country and they may be withdrawn in some of them.

BMPs

Only BMP-2 and -7 were available in most countries [6, 62]. They are recombinant BMP. However, from the recent information we obtained, only rhBMP-2 (Infuse®) still remains distributed by Pfizer in the United States. The delivery of BMP-2 (InductOs®) was interrupted in 2011 in Europe by Pfizer and transfered to Medtronic BioPharma. The distribution of BMP-7 (Osigraft® in UK, OP-I® in US) was transferred in 2013 by Stryker to Olympus Biotech International who stopped delivery on August 1, 2014.

Composition

DBM

The public bone bank delivers a simple natural product. DBM are provided in different granulometries in vials of 2–3 g. As we already mentioned, they contain a natural cocktail of growth factors and of BMPs (Table 11). The powder can be implanted in association with simple freeze-dried bone chips (not demineralized) to improve volume, mechanical resistance and osteoconduction.

DBM simple (2-3gr)	
BMP-2	22 to 110 ng/g
BMP-4	5.45 ng/g
BMP-7	44 to 125 ng/g
Collagen-I human and NCPs	
Other BMPs, GDFs, TGF-β1	
DBX:	31% DBM (by wheigt) Na hyaluronate q.s. ad 0.5 to 10.0cc
rhBMP-2 (Infuse®)	12mg dibotermine α, 1.5mg/g
BMP-7 (Ossigraft®)	3.5mg eptotermine α, /g

Table 11: Bone Morphogenetic Proteins (BPMs) components: concentration of the different products.

n the products processed by industries, carriers are mixed to obtain different implantable preparations, one of the most used being the putty. Grafton® Putty mixes DBM fibers, containing less than 0.5 % $CaPO_4$ with glycerol, in presentation from 0.5 cc to 10 cc. DBX® Putty mixes 31 % DBM with a Na hyaluronate carrier presented in volumes of 0.5 cc to 10 cc. Allomatrix® mixes DBM with $CaSO_4$ powder and carboxymethyl cellulose presented in a volume of 1.5 cc to 15.5 cc.

BMP

rhBMP-2 (Infuse®, InductOs®) is presented in vials of 12 mg of dibotermine α with a concentration of 1.5 mg/gr on bovine collagen-I sponge. rhBMP-7 (Ossigraft®) is presented in vials of 1 g containing 3.5 mg of eptotermine α on bovine collagen-I and 2–3 cc of NaCl physiological saline (Table 11).

Influence of the origin on osteoinductive properties

DBM

Donor gender seems to have no significant influence [63, 64]. Donor age is more controversial, and some authors observe a decreased concentration in BMP-2 and BMP-4 with age [65, 66]. Significant osteoinduction is observed between 32.8 and 75.6 years [67]. A similar window effect with better osteoinduction is noted between 41 and 50 years for men and 51 and 60 years for women [64], while no difference was recorded between 133 men and 115 women, the youngest is equivalent to the 85 years old [68]. Gruskin et al. [5] conclude to a consensus on an arbitrary limit for the donors up to 70 years.

BMP

Extraction from natural material or synthetic processing will modify the dimeric association of BMPs. Purified BMPs (phBMPs) may be extracted from human bone by guanidine hydrochloride and purified by liquid chromatography [69]. Recombinant BMPs (rhBMPs) are obtained through recombinant DNA within mammalian cells. They are homodimeric. Comparison between the osteoinductive effects was in favour of the heterodimers. Using phBMPs versus rhBMP, ten times greater osteoinduction is observed on ectopic bone formation in rats [69], and heterodimers versus homodimers show five to ten times more osteoinduction on in vitro or in vivo models [70–73].

Influence of the processing

DBM

The usual physical and chemical processing aiming to clear the lipids and cellular debris to obtain freeze-dried bone matrix seems to have no influence. The demineralization may modify the osteoinductive properties depending of the length of exposure and the concentration of HCl [74–76].

The size of the grain has an effect as the optimal granulometry seems to be between 420 and 840 μm [5].

After lyophilisation, the deshydratation protects the osteoinductive properties regarding the condition and length of storage [77].

The final sterilisation may have a secondary toxic effect or alteration of the osteoinduction if ethylene oxide, glutaraldehyde or formaldehyde is used while merthiolate, gamma irradiation (≤25kGray) or electron beams preserve the osteoinductive properties [5, 24, 78–82].

BMP

To produce recombinant human BMP-2 or BMP-7 proteins (rhBMPs), a cDNA, constructed from the human BMP-2 or BMP-7 gene, is integrated to the genome of Chinese hamster ovary (CHO) cells. The correct location in the genome of the human DNA fragment allows transcription and translation to a recombinant protein during the CHO cells culture [83].

Carriers

Simple DBM depending on the granulometry do not need a carrier. The natural matrix allows a slow release of BMPs and other growth factors [8, 84, 85].

However, DBM powder alone depending on the indication has no biomechanical or osteoconductive properties and needs to be associated with freeze-dried (non demineralized) allograft-like bone chips. For the product processed by industries, depending on the indication, to facilitate the handling and the implantation the manufacturers propose different associations. For instance, to obtain a putty glycerol, Na hyaluronate or carboxymethyl cellulose are added.

rhBMPs are extremely soluble in vivo. Their fast elimination requires a carrier [86–89]. The elimination period of an adsorbed half dose may vary from a few days on blood clot to two weeks on calcium phosphate. The available rhBMP-2 and -7 are adsorbed on bovine collagen-I sponge.

Risk of viral transmission

DBM as an allograft-derived product is theoretically a possible vector; however, no one case of viral transmission was reported with DBM powder [90, 91]. This is due to the cumulative results of very strict regulations regarding allograft procurements and donor selection in the EU and the United States, the improvement of the immonoenzymatic tests duplicated in most of the bone banks by nucleic acid amplification test (NAT) test, the validation of the physical and chemical processing for the viral decontamination including HIV, hepatitis B and C and CMV, the quality assessment and the continuous survey of traceability and biovigilance procedure [91]. For these reasons, the safety of the DBM needs to be guaranteed by an accredited bone bank.

rhBMPs as a synthetic product does not present a risk of viral transmission.

Cost

The comparative costs are given as reference in relative units (Table 12). They are subjected to evolve during time and between countries. Today, the delivery of some of them is discontinued.

		U
Simple DBM	1 to 3 cc natural	1
Lyophilized bone chips	5 cc	1
Grafton® Putty	2.5cc	1
DBX® Putty	2.5cc	1
Allomatrix Injectable® Putty	7cc	2
rhBMP-2 Infuse® Inductos®	12mg	10
rhBMP-7 Ossigraft® OP-I®	3.5mg	13.5

Table 12: Cost of the different available products in relative units (1U ≅ 300€)

The simple DBM is the pure material without additive. The DBM proposed by the industry is mixed with carrier, in some of them DBM represents one third of the volume.

The total content of the rhBMP-2 and -7 are, respectively, 12 mg and 3.5 mg.

Discussion

A first question we have to consider is what should we expect biologically from the growth factors? Should we expect an acceleration of the time to healing? Probably not. If we have an homogenous group of simple closed fractures without significant devascularization and which are treated correctly from a biomechanical point of view, there seems to be no reason to interfere with the physiological healing process and "accelerate" the healing. This is illustrated for instance by the absence of effect observed by Maniscalco et al. with BMP-7 [41] reported previously in this paper and by the study of Ahn et al. [92] using DBM as an enhancer and not as an extender of local autologous graft in postero lumbar inter-body fusion (PLIF).

However, in more complex fractures with soft tissue damage, devascularisation, larger area of cell death or metabolic disease, the healing will be slowed or delayed, raising the risk of non-union. This could be aggravated by an inadequate biomechanical fixation. In that case, the use of the growth factors may have the effect of regulating or normalising the physiological process of healing and bone formation [18, 93]. It means that they could decrease the average time to healing and reduce the standard deviation around this mean. It is the reason why a statistical effect may be observed on open fractures and non-union where the physiological cascade is compromised. The same situation happens with spine fusion where the percentage of non-union is high with a larger standard deviation around the mean time to healing.

Also it is probably the reason why the best results observed are similar and not better than those obtained with autologous bone graft. It is already a significant advantage as the quantity of the bone substitute is theoretically unlimited and not associated with morbidity at the donor site.

When we compare the different ways to supply growth factors and especially the BMPs, we are facing a very paradoxical effect of dosimetry. For apparently the same osteoinductive effect (equal to autologous bone graft) the concentration of the BMPs in the DBM at the physiological level are in an order of magnitude of 10−6 (ng compared to mg) lower than those used for the rhBMP-2 and -7 alone (Table 11).

It seems that very high doses of the rhBMP-2 or -7 are needed to force the osteoinduction at the same level as the natural cocktail of BMPs and associated growth factors found in the DBM are able to obtain all together but at the physiological concentrations.

This observation seems to be supported by the observation of a better osteoinduction obtained by the heterodimers BMPs compared to homodimers like they are in the rhBMPs [68–71].

Between DBM and rhBMPs, it is difficult to demonstrate a difference in their osteoinductive properties. However regarding their adverse effects, the high dose of rhBMPs appears to be associated with more complications [46]. The implantation of rhBMPs close to the central nervous system was critical and reassessed by NASS [94] who tried to redefine the limitation

and more clear indications. Vukicevic et al. [18] analysed the molecular mechanism of some of these adverse effects. Application to the appendicular bone does not seem to raise the same problem. The risk of malignancy, evoked for the highest dose product (Amplify, 40 mg rhBMP-2 per level), was however difficult to establish considering the low incidence in the general population [46, 59, 94].

Presently, other BMPs like BMP-4, BMP-6 [18] and BMP-9 are studied and also the heterodimeric association of BMP-2/-7 [72, 95] and BMP-2/-6 [71]. Less antagonist reactions and noggin expression are associated with some of those BMPs [96, 97] and their heterodimers. Those associations are promising and will probably allow decreasing the present high concentration used to more physiological levels.

BMPs antagonists from different origins are regulating the BMPs activity during fracture healing [6, 21]. Extraction or control of noggin and antagonists from the DBM [98] could allow a better control of the BMPs activity at the fracture site [6, 18, 21, 99, 100].

A constant concentration of BMPs in DBM cannot be guaranteed [101]. Fluctuation may exist between different batches. Pooling of the different batches during processing could solve the problem but is not acceptable from our biovigilance recommendation and traceability rules [91]. However, the use of two to three vials from different donors in the same implantation should allow minimisation of the fluctuation. For the rhBMPs, their synthetic production guarantees a standard dose.

The putty, proposed under different labels, may have a better resistance to dispersion than simple DBM powder but the carriers introduce new variables in terms of DBM content, concentration and osteoinduction [18, 28, 102–104]. Nephrotoxicity of high doses of glycerol is observed in a rat model. Association with porcine gelatin or type-I bovine collagen may lead to some local reaction or chemical and physical alteration of the BMPs during shelf conservation.

In BMPs the bovine collagen-I sponge used as a carrier may generate an immunological response; however, no relation between immune response and treatment failure was established [6, 34].

There is a significant difference between the costs of the different products. The pure DBM powder is comparatively cheap but, for most of the indication, needs to be associated with lyophilized bone chips. In many countries, they are fully reimbursed by the social security system. In comparison, the mix proposed by some putty for an equivalent price is composed of one third of the pure DBM mixed with two thirds of a carrier with passive or active properties. The unitary dose of BMPs is considerably more expensive—at least ten times the price of the DBM dose.

Conclusion

DBM and BMPs are expected to have comparable effects following the available literature but need stronger evidence based on clinical studies and better definition of indications.

The main advantages of DBM on autograft are the absence of limitation in quantity, no associated surgical procedure and no morbidity at the donor site. On BMPs, the DBM offer a association of multiple growth factors at physiological doses, a natural on-site release with no

necessity for a carrier and a reasonable cost fully reimbursed by the social security system in some countries.

The disadvantage of the DBM is the possible fluctuation of osteoinductive properties between different vials and for the industrial composite side effects introduced by additional carriers.

rhBMPs are delivered in well-known doses but presently in a single homodimeric presentation at very high non physiological dose. This overdose may be responsible for adverse effects. In future, heterodimeric association and elimination of BMPs antagonist may allow reducing the high doses of the rhBMP. The control of the antagonist effect will also help to maximise the effect of the low dose of BMPs contained in the DBM.

Each rhBMP seems to act as a single key, the high dose allowing compensation for the absence of the other keys. The DBM have the advantage of combining the synergic effect of multiple keys but a standard dose cannot be guaranteed. This variability can be compensated using different vials. The action of DBM is close to the natural cascade in terms of dosimetry, complex association of growth factors and release in situ. They have less adverse effects and the cost is reasonable.

However, better standardization and non-bias clinical studies analysing the effectiveness of DBM and the new generations of BMPs are still required to evaluate more precisely their clinical indications and possible adverse effects.

Author's disclosure

Maurice Hinsenkamp is Honorary Medical Director of the nonprofit Tissue Bank (BTE) from the Hôpital Erasme at Université Libre de Bruxelles.

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