International Orthopaedics (SICOT) (2001) 25:203–208
DOI 10.1007/s002640100262

REVIEW ARTICLE

M. Pecina · L.R. Giltaij · S. Vukicevic

Orthopaedic applications of osteogenic protein-1 (BMP-7)

Accepted: 9 April 2001 / Published online: 13 June 2001

Introduction

In 1965 Marshall Urist [46] found that in rabbits demineralized bone matrix was capable of inducing formation of mature bone ossicles filled with marrow elements, when implanted in an ectopic, non-bony site. Although neither identified nor characterized, Urist named this activity bone morphogenetic protein (BMP) [30, 45, 46, 47]. Reddi [32] and Sampath and Reddi [34] standardized this bioassay in rats, which allowed broad and reproducible screening of bone inductive activity in various fractions of non-collagen bone proteins [35, 36, 37, 38, 39]. The proteins were shown to exist across all species [34], which suggested that BMPs are highly conserved in mammals, and provided a basis for the isolation and characterization of these proteins from bovine bone matrix [4, 27, 37]. So far, it has been shown that different BMP-like molecules, such as single morphogens, have a site-specific multiple tissue induction potency [29]. For example, osteogenic protein (OP)-1 induces bone formation when implanted subcutaneously with collagen carrier into muscle or between bone fragments [37]. Based on the bovine osteogenic protein peptide sequence human cDNA was cloned [31] and recombinant human OP-1 (rhOP-1) was produced by recombinant DNA technology [37]. Since 1992, extensive clinical and preclinical research has taken place to investigate rhOP-1 in bone and cartilage regeneration and to make it available for routine clinical use [9, 10, 35, 50, 51]. In this review we will focus primarily on OP-1 (BMP-7) in preclinical and clinical studies related to orthopedics.

Dedicated to the late Dr. Marshall R. Urist, a great scientist, clinician and lecturer, who passed away on 4 February, 2001.

M. Pecina (✉)
Department of Orthopaedic Surgery,
School of Medicine University of Zagreb,
Salata 7, 10000 Zagreb, Croatia

L.R. Giltaij
Stryker Biotech, Notengaard 13, 3941 LV Doorn,
The Netherlands

S. Vukicevic
Department of Anatomy,
School of Medicine University of Zagreb,
Salata 11, Box 916, 10000 Zagreb, Croatia

rhOP-1 heals critical-sized diaphyseal defects

Numerous preclinical studies have proven the potency of rhOP-1 to heal critical-sized long bone defects. By definition these defects cannot heal without the addition of exogenous osteogenic stimuli. Implantation of rhOP-1-containing collagen matrix preparations into surgically created critical-sized diaphyseal segmental defects leads to regeneration of new bone that is fully functional both biologically and biomechanically. These results have been shown in rabbits [11, 12, 13]. Both the rate and quality of the osseous union were better than that achieved by autogenous bone graft controls. In the primate ulna defect model rhOP-1 was shown to be capable of healing defects that could not be healed with autogenous bone [13]. In all animal models, nearly 100% of the intact limb strength was achieved in the rhOP-1 groups, which was significantly greater than that achieved in equivalent defects, treated with autogenous bone. Well-remodeled new cortices with a medullary canal were formed. The medullary canal was filled with fully functional marrow elements. In humans, a prospective randomized and double-blinded trial was reported by Geesink et al. [19] showing that osteogenic activity of rhOP-1 was sufficient to repair a critical-size human fibula defect model. In patients undergoing high tibial osteotomy a standardized fibula defect of critical dimensions was created. The patients then received demineralized bone matrix, the collagen-matrix alone, or the rhOP-1 bound to collagen matrix as a carrier. When treated with rhOP-1 five out of six critical-sized defects were bridged by 4 months, while none of the collagen-alone implants were effective in bridging such a gap [19].

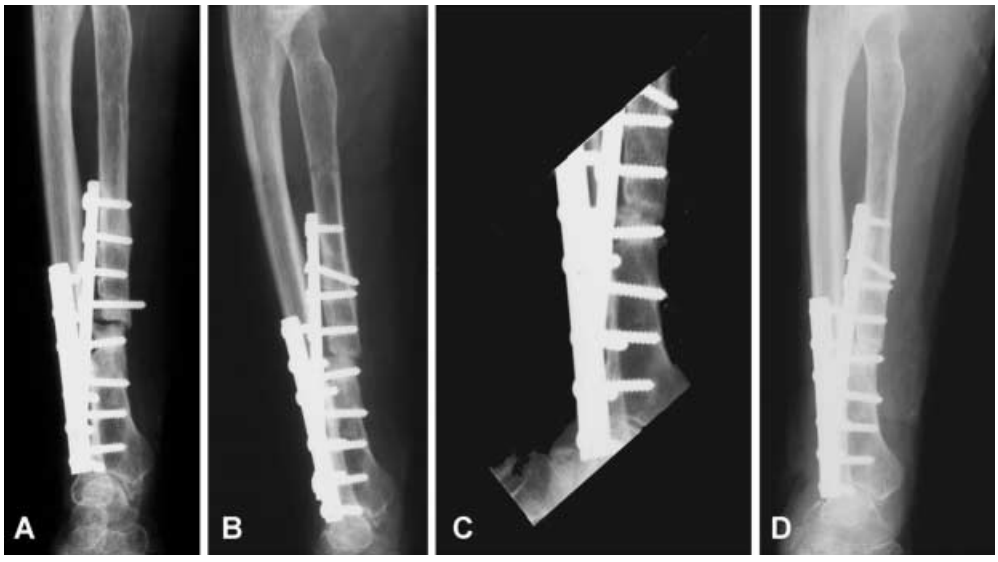

Fig. 1A–D Left radius of a 73-year-old female patient (K.V.). Internal fixation with plate and screws of fractured ulna and radius was performed in March 1999. Reoperation with plate and screws in October 1999 was unsuccessful. Seven months later no bone healing of radius was observed (**A**). Osteogenic protein-1 device was applied to radius during the third surgical procedure (**B**). After 3 months beginning of bone healing was observed (**C**) and complete bone bridging of the radius was present 6 months postoperatively (**D**)

rhOP-1 heals non-unions

The first prospective randomized clinical trial on a member of the BMP family compared rhOP-1 to autologous bone graft in resistant non-unions of the tibia [18]. In order to qualify for enrolment in this study, patients had to have an established tibial non-union for 9 months or more, and were in need of open bone grafting and intramedullary fixation. Patients with stiff hypertrophic non-unions in good alignment were excluded, since these patients would likely do well by intramedullary nailing alone. A total of 122 patients with 124 tibial non-unions were recruited in 18 centers in the United States. The rhOP-1 group was treated with an rhOP-1-containing implant, consisting of 3.5 mg rhOP-1 in 1 g bovine-derived non-soluble type I collagen matrix (known as 'OP-1 Device'). The study showed that the rhOP-1 implant and the autograft were comparable with respect to the clinical outcome parameters including resolution of pain and return to full weight bearing ambulation. The groups were comparable with respect to avoidance of the need for subsequent surgical treatment for persistent non-union. The rhOP-1 implant, however, eliminated the morbidity and pain associated with surgical harvest of autologous bone graft and was associated with a reduction of intraoperative blood loss and the rate of infection [18].

In another study Shimmin and Ruff [42] reported on 44 patients with resistant non-unions and a follow up longer than 5 months. Three patients had failed both radiological and clinical assessment; however, two of them were exhibiting bone formation before the hardware failed. Considering that all the patients had already previously failed conventional orthopaedic treatments, the bone forming response of rhOP-1 was encouraging. At the Department of Orthopaedic Surgery, Zagreb School of Medicine more than 20 patients were successfully treated for resistant non-unions with the OP-1 Device (Fig. 1 and Fig. 2).

rhOP-1 enhances bone graft incorporation and implant fixation

Bone implants coated with OP-1 in conjunction with a carrier material involving porous and smooth surfaced cobalt-chromium alloy implants were bilaterally placed transcortically through the femoral diaphysis of adult dogs. The dogs were killed after 3 and 6 weeks. Greater surface bone ingrowth and apposition was seen in the rhOP-1 treated implant, although there was little difference in mechanical fixation [10]. In another study, addition of OP-1 to morselized and impacted allograft in a dog model [43] resulted in significantly more bone formation and remodeling of the allograft in 3 weeks. Within the newly formed bone the trabecular structure was oriented towards the implant and optimal approximation on the implant surface was achieved. When combined with a (coralline) hydroxyapatite, the significant increase in bone ingrowth throughout the graft, the remodeling and the implant osseointegration led to a 900% increase in shear strength. Although the addition of rhOP-1 to the allograft did not increase the mechanical fixation at 3 weeks, it did so in the hydroxyapatite group with outcome comparable to that of the allograft group. More recently, it has been reported that the addition of OP-1 significantly increased energy absorption and new bone formation in apposition to the implant as compared to the allograft and/or hydroxyapatite alone [24]. These results suggest that OP-1 may be used to promote enhanced osseointegration of metal implants and can induce new bone formation in implant-bone interface gap space.

rhOP-1 increases remodeling and bone ingrowth in bone grafts and bone substitutes

Since the use of autograft results in extra morbidity, alternatives have become an accepted routine in bone sur-

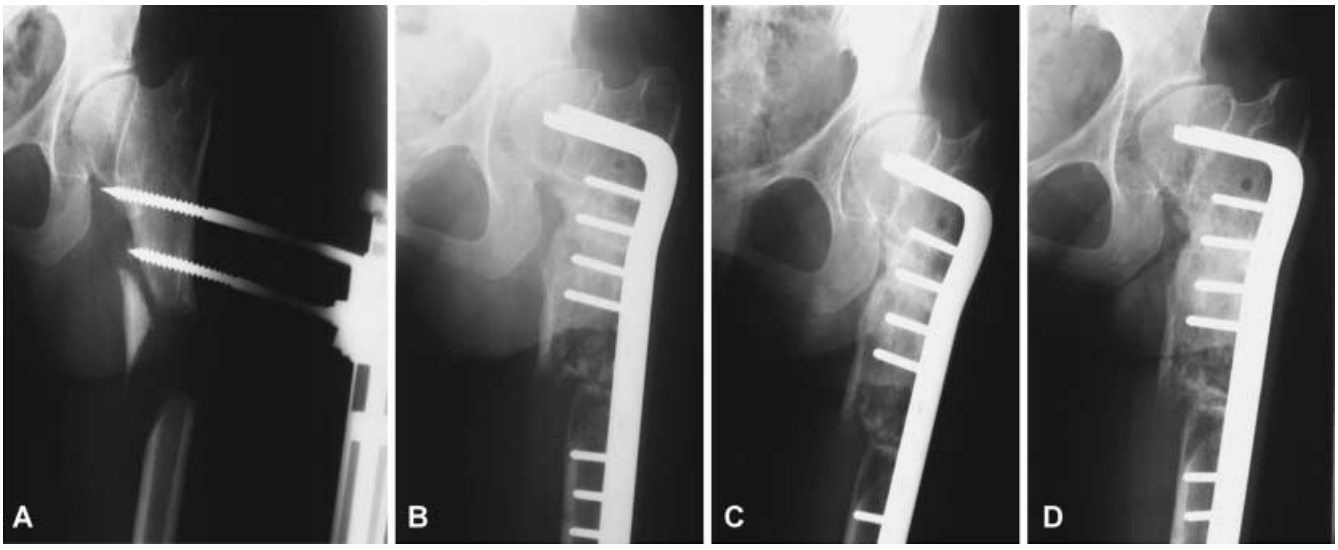

Fig. 2A–D A 32-year-old female patient (B.S.) with non-union 9 months after elongation of the left femoral bone by a Wagner procedure (**A**). After removal of the Wagner apparatus, osteosynthesis with a condylar plate was performed with addition of osteogenic protein-1 device and an autologous cancellous bone graft (**B**). 3 months after the procedure the beginning of bone healing was observed (**C**) and complete bridging of the defect was seen 6 months postoperatively (**D**)

gery. To date none of these have been established as a successful substitution for an autograft. Blokhuis and colleagues [3] created a 3 cm segmental defect in the tibia of 30 sheep. The defects were stabilized with an intramedullary-interlocking nail and were then left empty (*n*=6), treated with autograft (*n*=8), 10 ml HA (Endobone; Biomet/Merck GmbH, Germany) (*n*=8), or 10 ml HA plus 3.5 mg OP-1/1 g collagen matrix (*n*=8). After 12 weeks bone healing was evaluated radiographically and biomechanically. In all assessments both the autograft and the HA plus OP-1 groups outperformed the HA alone and the sham controls. The addition of OP-1 to HA granules considerably improved the bone healing. Since the results of HA plus OP-1 were comparable to autograft, HA plus OP-1 may be used as an alternative in the treatment of large bone defects [3]. Using OP-1 with different HA carrier materials in the healing of segmental defects in a rabbit ulna model, it has been demonstrated that the addition of rhOP-1 at low concentration to HA graft materials increased the amount of bone formation and incorporation [33]. Large 2.5 cm segmental ulna defects in dogs were filled with one of seven combinations: 100% OP-1 Device (3.5 mg rhOP-1/1 g type I collagen), 100%, 67% or 33% autograft, 100%, 67%, or 33% allograft with remaining percent made up of OP-1 Device [14]. The healing was studied radiographically until killing at 12 weeks. As early as 2–4 weeks significant bone formation was observed in all sites containing OP-1, where defects filled with either 100% autograft or 100% allograft showed no new bone formation on radiographs until 6 weeks. Allograft and autograft produced substantially less new bone formation. OP-1 Device treated sites showed new bone formation by 2 weeks, extensive new radiodense bone formation at 4 weeks, and bridging of the defects at 6 weeks. Mechanically, OP-1 Device alone tested at 100% of the contra-lateral intact ulna, whereas the autograft-alone bones tested at less than 50% of the contra-lateral intact ulna. This demonstrates that OP-1 Device, when used alone, was effective for the treatment of large segmental bone defects [33].

rhOP-1 provides a full alternative for autograft in spinal fusion

The search for suitable alternatives to conventional bone grafting techniques provides much of the basis for current spinal endeavors. Approximately 25% of patients with iliac crest donor sites reported significant pain lasting on average 5 years following surgery [44]. Unfavorable results with allogenic, xenogenic and artificial grafting materials in this indication limit the clinical application of these materials. Cook et al. [8] evaluated the efficacy of OP-1 in treating posterior spinal fusion segments in adult mongrel dogs at 6, 12 and 26 weeks post-implantation. Four sites on each animal received implants consisting of OP-1 on the standard collagen carrier, bone collagen carrier alone, autogenous iliac crest bone, or no implant material. OP-1 treated fusion segments attained a stable fusion by 6 weeks post-implantation and were completely fused by 12 weeks. The autograft sites demonstrated fusion at 26 weeks post-implantation. These results indicated that OP-1 is an effective bone graft substitute for achieving stable posterior spinal fusion in animal models. Magin and Delling [28] tested OP-1 in a sheep model for dorsal interbody fusion of the lumbar vertebral column by using a laterodorsal approach with transpedicular fixation. Three groups of ten sheep each were utilized. Implants consisted of autograft, deproteinized bovine hydroxyl apatite, or OP-1 in a collagen matrix. Biomechanically the greatest rigidity was seen in those treated with autograft and OP-1 [28].

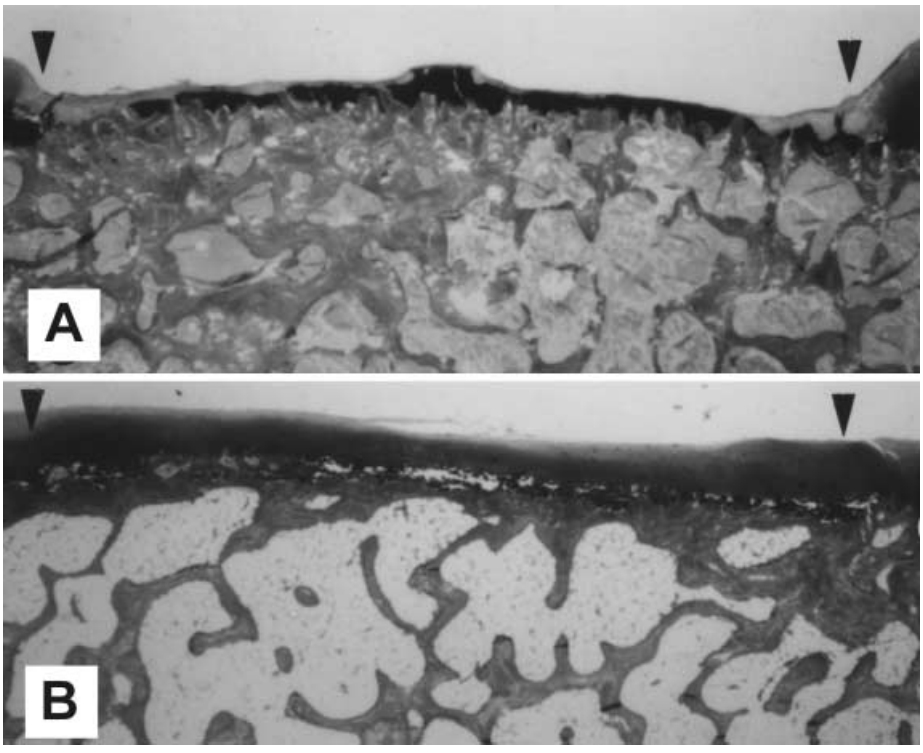

Fig. 3A, B Regeneration of articular cartilage at 6 months following surgery on the knee of a sheep and treatment with OP-1. **A** An empty defect treated with a acetate buffer vehicle. **B** A condylar defect (*arrows*) treated with OP-1 was filled with newly regenerated cartilage

The histological and histomorphometrical evaluations of the fusion with hydroxyl apatite demonstrate no continuous osseous structure, but only the formation of a tense pseudo-arthrosis. The bone density of the OP-1 induced fusions exceeded the bone density of fusions in the autograft group by an average of 40% indicating that the use of OP-1 can increase the reliability of dorsal vertebral interbody fusion, the degree of bone density in the area of the fusion, and can also lead to greater bone formation at earlier stages as compared with autograft in sheep [28]. Cunningham et al. [15] compared the efficacy of OP-1 with that of autograft for interbody arthrodesis by application of BAK-cages in the thoracic spine. For this he performed a multilevel thoracic decompression on three non-contiguous levels by thoracoscopic approach in 12 sheep. These sites were then randomly treated, with one of five modalities: destabilization alone, empty BAK-cage, autograft alone, BAK-cage plus autograft and BAK-cage plus OP-1/collagen carrier. Non-surgical levels were assessed for further control. Results of biomechanical analysis showed statistically higher segmental stiffness levels when comparing the control and experimental groups. Computed tomography (CT) and microradiography showed fusion for the destabilization alone group in one of six, BAK-cage alone in two of six, autograft alone in four of eight, BAK-cage plus autograft in five of eight and BAK-cage plus OP-1/collagen carrier in six of eight. This was confirmed histologically [15]. Taken together these studies suggested that OP-1 provided a viable alternative for iliac crest autograft in animal models; thereby obviating the need for a graft donor site and associated patient morbidity.

Cartilage and tendon repair by OP-1

Several investigators reported on chondrogenic effects of BMPs [1, 5, 16, 17, 27, 40, 41]. In cultured primitive anlagen of embryonic long bones, chondrogenesis was remarkably enhanced by OP-1, suggesting that OP-1 initiates differentiation of cartilage from perichondrium tissue in vitro [21, 26]. BMP-2 and OP-1 treated explant cultures of articular chondrocytes show enhanced synthesis of extracellular matrix and maintenance of cartilage phenotype [1, 6, 27]. In vivo studies demonstrated cartilage regeneration by OP-1 in animals both in subchondral and chondral knee joint defects at 3 and 6 months following implantation, respectively [20, 22, 23, 48, 49]. Jelic and collaborators [23] suggested that in the presence of full mechanical loading OP-1, delivered via a mini-osmotic pump, stimulates regeneration of chondral defects in sheep (Fig. 3). To evaluate the contribution of increased mechanical forces in cartilage regeneration, the efficacy of OP-1 in repair of thyroid cartilage defects has been studied [25]. Unlike control allograft implants, OP-1 enriched implants, dose-dependently induced bone, cartilage and ligament-like structures, suggesting that OP-1 can promote formation of multiple tissues in this specific microenvironment. Chubinskaya et al. [7] reported that the expression of OP-1 mRNA in human cartilage samples did not decrease with aging and was upregulated two-fold in osteoarthritic cartilage suggesting a role for BMPs in osteoarthritis. Aspenberg and Forslund [2] showed that tendon healing was stimulated with cartilage-derived morphogenetic proteins (CDMPs).

Conclusion

Bone induction by OPs/BMPs is one of the most important discoveries in the field of bone physiology and bone surgery. Osteogenic or bone morphogenetic proteins are growth and differentiation factors capable of initiating the recruitment, attachment, proliferation and differentiating of mesenchymal cells, leading to newly formed mature bone.

In the near future OP-1 containing implants could replace the conventionally employed autografts in repairing acute bone fractures and induce healing of nonunions. Such implants could promote osseointegration in joint arthroplasty and replace and/or improve performance of allografts and bone replacement biomaterials, while speeding up the process of bone formation and remodeling for defects and fusions.

With the availability of OP-1 (BMP-7) on a carrier, the orthopaedic and trauma surgeon will have a new and potent tool in regenerating bone when it does not spontaneously heal.

References

1. Asahina I, Sampath TK, Hauschka PV (1993) Human osteogenic protein-1 induces both chondroblastic and osteoblastic differentiation of osteoprogenitor cells derived from newborn rat calvaria. J Cell Biol 123:921–933
2. Aspenberg P, Forslund S (1999) Enhancement tendon healing with GDF 5 and 6. Acta Orthop Scand 70:51–54
3. Blokhuis TJ, den Boer FC, Bramer JA, Jenner JM, Bakker FC, Patka P, Haarman HJ (2001) Biomechanical and histological aspects of fracture healing, stimulated with osteogenic protein-1. Biomaterials 22:725–730
4. Celeste AJ, Ainnazzi JA, Taylor RC, Hewick RM, Rosen V, Wang EA, Wozney JM (1990) Identification of transforming growth factor beta family members present in bone-inductive protein purified from bovine bone. Proc Natl Acad Sci USA V87:9843–9847
5. Chang SC, Hoang B, Thomas TJ, Vukicevic S, Luyten FP, Ryba NJP, Kozak CA, Reddi AH, Moos M (1994) Cartilage derived morphogenetic proteins, new members of the TGF-beta superfamily predominantly expressed in long bones during human embryonic development. J Biol Chem 269:28227–28234
6. Chen P, Vukicevic S, Sampath TK, Luyten FP (1993) Bovine articular chondrocytes do not undergo hypertrophy when cultured in the presence of osteogenic protein-1. Biochem Biophys Res Commun 197:1253–1259
7. Chubinskaya S, Merrihew C, Cs-Szabo G, Mollenhauer J, McCartney J, Rueger DC, Kuettner KE (2000) Human articular chondrocytes express osteogenic protein-1. J Histochem Cytochem 48:239–250
8. Cook SD, Dalton JE, Tan EH, Whitecloud III TS, Rueger DC (1994) In vivo evaluation of recombinant human osteogenic protein (rhOP-1) implants as a bone graft substitute for spinal fusions. Spine 19–15:1655–1663
9. Cook SD (1999) Preclinical and clinical evaluation of osteogenic protein-1 (BMP-7) in bony sites. Orthopaedics 22:669–671
10. Cook SD, Rueger DC (1996) Osteogenic protein-1: biology and applications. Clin Orthop 324:29–38
11. Cook SD, Baffes GC, Wolfe MW, Sampath TK, Rueger DC (1994) Recombinant human bone morphogenetic protein-7 induces healing in a canine long-bone segmental defect model. Clin Orthop 301:302–312
12. Cook SD, Baffes GC, Wolfe MW, Sampath TK, Rueger DC, Whitecloud TS III (1994) The effect of recombinant human osteogenic protein-1 on healing of large segmental bone defects. J Bone Joint Surg [Am] 76:827–828
13. Cook SD, Wolfe MW, Salkeld SL, Rueger DC (1995) Effect of recombinant human osteogenic protein-1 on healing of segmental defects in non-human primates. J Bone Joint Surg [Am] 77:734–750
14. Cook SD, Salkeld SL, Brinker MR, Wolfe MW, Rueger DC (1998) Use of an osteoinductive biomaterial (rhOP-1) in healing large segmental defects. J Orthop Trauma 12:407–412
15. Cunningham BW, Kanayama M, Parker LM, Weis JC, Sefter JC Fedder IL, McAfee PC (1999) Osteogenic protein versus autologous interbody arthrodesis in the sheep thoracic spine. Spine 24:509–518
16. Delecrin J, Deschamp C (1999) Apports de l'experimentation animale dans l'evaluation preclinique des BMP. Rev Chir Orthop 86:153–154
17. Flechtenmacher J, Huch K, Thonar EJ, Mollenhauer JA, Davies SR, Schmid TM, Puhl W, Sampath TK, Aydelotte MB, Kuettner KE (1996) Recombinant human osteogenic protein-1 is a potent stimulator of the synthesis of cartilage proteoglycans and collagens by human articular chondrocytes. Arthritis Rheum 39:1896–1904
18. Friedlaender GE, Perry CR, Cole JD, Cook SD, Cernt G, Muschler GF, Zych GA, Calhoun JH, LaForte AJ, Yin S (2001) Osteogenic protein-1 (bone morphogenetic protein-7) in the treatment of tibial nonunions. J Bone Joint Surg [Am] 83 [Suppl 1]:S151–158
19. Geesink RGT, Hoefnagels NHM, Bulstra SK (1999) Osteogenic activity of OP-1 bone morphogenetic protein (BMP-7) in a human fibular defect. J Bone Joint Surg [Br] 81:710–718
20. Grgic M, Jelic M, Basic V, Basic N, Pecina M, Vukicevic S (1997) Regeneration of articular cartilage defects in rabbits by osteogenic protein-1 (bone morphogenetic protein-7). Acta Med Croatica 51:23–27
21. Haaijman A, DeSouza RN, Bronckers AL, Goei SW, Burger EH (1997) OP-1 (BMP-7) affects mRNA expression of type I, II, X collagen, and matrix Gla protein in ossifying long bones in vitro. J Bone Miner Res 12:1815–1823
22. Haspl M, Jelic M, Kos J, Vukicevic S, Pecina M (1999) Follow-up arthroscopy in sheep knee chondral defect regeneration. Bone 24:418A
23. Jelic M, Pecina M, Haspl M, Maticic D, Pirkic B, Vukicevic S (2001) The efficacy of OP-1 in sheep chondral defects regeneration. Growth Factors 18 (in press)
24. Jensen TB, Overgaard S, Lind M, Rahbek O, Bünger C, Søballe K (1999) Bone allograft, pro-osteon-200 and osteogenic protein-1 device around noncemented implants. Bone 24:428A
25. Katic V, Majstorovic L, Matacic D, Pirkic B, Yin S, Kos J, Martinovic S, McCartney JE, Vukicevic S (2000) Biological repair of thyroid cartilage defects by osteogenic protein-1 (bone morphogenetic protein-7) in dog. Growth Factors 17: 221–232
26. Klein-Nulend J, Semeins CM, Mulder JW, Winters HA, Goei SW, Ooms ME, Burger EH (1998) Stimulation of cartilage differentiation by osteogenic protein-1 in cultures of human perichondrium. Tissue Eng 4:305–313
27. Luyten FP, Yu YM, Yanagishita M, Vukicevic S, Hammonds RG, Reddi AH (1992) Natural bovine osteogenin and recombinant human bone morphogenetic protein-2B are equipotent in the maintenance of proteoglycans in bovine articular cartilage explant cultures. J Biol Chem 267:3691–3695
28. Magin MN, Delling AG (2001) Improved lumbar vertebral interbody fusion using rhOP-1: a comparison of autogenous bone graft, bovine hydroxylapatite (Bio Oss), and BMP-7 in sheep. Spine 26:469–478
29. Marie PJ (1997) Effects of bone morphogenetic proteins on cells of the osteoblastic lineage. J Cell Eng 2:92–99
30. Münzenberg KJ, Urist RM (1995) Eine Laudatio. Osteologie 4:199–200

31. Özkaynak E, Rueger DC, Drier EA, Corbett C, Ridge RJ, Sampath TK, Oppermann H (1990) OP-1 cDNA encodes an osteogenic protein in the TGF-beta family. EMBO J 9:2085–2093
32. Reddi AH (1998) Bone morphogenetic proteins: an unconventional approach to isolation of first mammalian morphogens. Nat Biotechnol 16:247–252
33. Rueger DC, Tucker MM, Salkald SL, Popich LS, Cook SD (1999) Studies evaluating the use of OP-1 (BMP-7) in the repair and regeneration of segmental bone defects. Bone 24: 412A
34. Sampath TK, Reddi AH (1983) Homology of bone-inductive proteins from human, monkey, bovine, and rat extracellular matrix. Proc Natl Acad Sci USA 80:6591–6595
35. Sampath TK, Rueger DC (1994) Structure, function and orthopaedic applications of osteogenic protein-1 (OP-1). Complications in Orthopaedics Winter:101–107
36. Sampath TK, Coughlin JE, Whetstone RM, Banach D, Corbett C, Ridge RJ, Ozkaynak E, Oppermann H, Rueger DC (1990) Bovine osteogenic protein is composed of dimers of OP-1 and BMP-2A, two members of the transforming growth factor-beta superfamily. J Biol Chem 265:13198–13205
37. Sampath TK, Maliakal JC, Hauschka PV, Jones WK, Sasak H, Tucker RF, White KH, Coughlin JE, Tucker MM, Pang RH, et al. (1992) Recombinant human osteogenic protein-1 (HOP-1) induces new bone formation in vivo with a specific activity comparable with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation in vitro. J Biol Chem 267:20352–20362
38. Sampath TK, Rashka KE, Doctor JS, Tucker RF, Hoffman FM (1993) Drosophilia transforming growth factor-β superfamily proteins induce endochondral bone formation in mammals. Proc Natl Acad Sci USA 90:6004–6008
39. Sampath TK, Reddi RH (1981) Disassociative extraction and reconstitution of extracellular matrix components involved in local bone differentiation. Proc Natl Acad Sci USA 78:7599–7603
40. Sellers RS, Peluso D, Morris EA (1997) The effect of recombinant human bone morphogenetic protein –2 (rhBMP-2) on the healing of full-thickness defects of articular cartilage. J Bone Joint Surg [Am] 79:1452–1463
41. Sellers RS, Zhang R, Glasson SS, Kim HD, Peluso D, D'Augusta DA, Beckwith K, Morris EA (2000) Repair of articular cartilage defects one year after treatment with recombinant human bone morphogenetic protein-2 (rhBMP-2). J Bone Joint Surg [Am] 82:151–160
42. Shimmin A, Ruff S (1999) Clinical use of recombinant human osteogenic protein-1 (rhOP-1). Bone. 24:424A
43. Søballe K, Hansen ES, B-Rasmussen H, Pedersen CM, Bünger C (1992) Bone graft incorporation around titanium alloy- and hydroxyapatite coated implants in dogs. Clin Orthop 274:282–293
44. Summers BN, Eisenstein SM (1989) Donor site pain from the ilium. A complication of lumbar spine fusion. J Bone Joint Surg [Br] 71:677–680
45. Urist MR, Strates BS (1971) Bone morphogenetic protein. J Dent Res 50 [Suppl 6]:1392–406
46. Urist MR (1965) Bone formation by autoinduction. Science 150:893–899
47. Urist MR (1995) The first three decades of bone morphogenetic protein research. Osteologie 4:207–223
48. Vukicevic S, Jelic M, Haspl M, Majstorovic L, Maticic Z, Katic V, Pecina M (1999) Thyroid and articular cartilage repair in dog and sheep by OP-1. Bone 24:423A
49. Vukicevic S, Martinovic S, Basic M, Jelic M (1999) Bone morphogenetic proteins: first European conference. Bone 24: 395–397
50. Vukicevic S, Paralkar V, Reddi AH (1993) Extracellular matrix and bone morphogenetic proteins in cartilage and bone development and repair. Adv Mol Cell Biol 7:207–224
51. Vukicevic S, Stavljenic A, Pecina M (1995) Discovery and clinical applications of bone morphogenetic proteins. Eur J Clin Chem Clin Biochem 33:661–671