Recombinant human bone morphogenetic protein–7 and bone marrow as a substitute for bone graft in reconstruction defect of rabbit mandible

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ABSTRACT

Objectives: To obtain information on the feasibility of employing recombinant human bone morphogenetic protein-7 (rhBMP-7), mixed with bone marrow as a substitute for autologous bone graft.

Methods: We carried out the study in the University Clinic Center, Sarajevo, Bosnia and Herzegovina, from February 2002 to January 2004. Six New Zealand rabbits underwent hemiresection of the mandible. We placed rhBMP-7 in a concentration of 100 micrograms and collagen (ACS) as carrier mixed with bone marrow in the defects in 3 animals. In another group of 3, we restored the defect by placement of autologous bone graft harvested from the iliac crest. We evaluated the results by the activity of the alkaline phosphatase enzyme (ALP), CT assessment with bone mineral density (BMD) analysis, and clinical and histologic examinations.

Results: The ALP activity was significantly higher after 14 days, in the rhBMP7/ACS sites than the bone graft sites (30th day). Mean BMD of tissue induced by rhBMP-7/ACS on the 30th day was 510 mg/cm^3 in caudal, and 553 mg/cm^3 in sagittal plane, and bone graft tissue was 510 mg/cm^3 in caudal and 530 mg/cm^3 in sagittal plane. Clinical inspection and histologic examination on the 60th day showed complete bridged defects with abundant woven bone in 2 of 3 rhBMP-7/ACS sites (67%), and no incorporation of the autologous bone graft into the other groups treated sites.

Conclusion: The rhBMP-7/ACS mixed with bone marrow was very effective in establishing complete bone regeneration into the defects, indicating that it could be an adequate alternative for autologous bone transplantation.

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Mandible reconstruction is a major challenge in oral and maxillofacial surgery. The complete osseous regeneration of traumatic, developmental, postresection or congenital origin defects has been a matter of considerable clinical and surgical concern for many years. Autologous bone grafts, especially vascularized bone flap grafts, are widely used in practice,[1] and are considered the gold standard because of their osteoinductive, osteoconductive and osteogenic elements. However, an autologous

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bone graft has disadvantages, such as limited availability of donor tissue, donor site morbidity, difficulty in providing the desired shape, and extensive resorption from the original amount. A number of other type of treatments as a substitute for autologous bone graft were developed using demineralized bone matrix (DMB), or materials such as plastic, metals or a combination allograft. The optimal maxillofacial bone grafting technique has not yet been developed. The discovery of DBM and bone morphogenetic protein's (BMPs) as the most responsible agents for their osteo inductivity, traced a new way in the treatment of bone defects, which could change conventional surgery approaches (fixation, immobilization, auto-transplantation). The purification of the BMPs, which were extracted from decalcified bone, has been attracting attention since placement of this protein in muscle was found to result in ectopic bone formation.[2] In 1988, Wozney et al[3] succeeded in cloning a cDNA of BMP.[3] The BMP's have become the most promising osteoinductive substances for bone formation. Considerable interest has been focused on the application of BMP's for therapeutic use.[4-6] The development of effective reconstruction procedures using osteoinductive factors without the need for conventional bone grafting would have a tremendous impact on reconstructive surgery of the head and neck area. The purpose of this paper is to report the results of the repair of a surgically produced hemimandibular defect utilizing recombinant human BMP-7 (rhBMP-7).

Methods. The study was carried out in the University Clinic Center Sarajevo, Bosnia and Herzegovina, from February 2002 to January 2004. The procedure was performed on 6 experimental New Zealand rabbits, 9-12 months old, 3-4 kg weight. Animals were elected in 2 groups; First group of 3 animals received in the defect, rhBMP-7 and collagen (ACS) as carrier, commercially available as "Helistat", in a concentration of 100 microgram mixed with bone marrow. The second group of 3 animals received autologous bone graft from the iliac crest. The animals were anesthetized with an injection of 1 mg ketamine hydrochloride (veterinary Ketalar) administered intramuscularly. The mandible was exposed extraorally through a submandibular approach. A 20-mm full thickness defect of the mandible, from the incisive tooth to the angle of the mandible behind the back molar teeth was created using a dental micromotor and drill, simulating a clinical postresection or trauma defect (**Figure 1**). If such kind of defect, known in literature as non-critical size defect, is left untreated, the healing will be incomplete and fibrous and scar tissue will form, in addition to pseudoarthrosis and dysfunction. The mandible defect was firmly fixed bicortically with a titanium mini plate. On 3 animals, the created defects were treated with 100 micrograms rhBMP-7 in collagen and mixed with bone marrow obtained from the resection particle of the mandible. On the other 3 animals, defects were treated with autologous bone graft from the iliac crest. The bone graft was placed in the mandible bone defect and fixed with mini plates that provide the animals immediate function for taking food and water. All animals were treated with 1 gm of Flucloxacillin daily for 3 postoperative days. The results were assessed during a 2-month postoperative period, and alkaline phosphatase (ALP) enzyme activity was measured together with CT assessment, clinical inspection and histologic analysis. The ALP was measured on 5, 14, 21, and 30 postoperative days as a significant marker of osteoblast activity. Three dimensional CT images with slices 1 mm thick, were taken after 30 days. The reconstruction pictures were carried out from the sagittal and caudal plane. The bone mineral density (BMD, mg/cm^3) of the new-formed tissue was analyzed with GEANIE 2.0 software (BonAlyse Ltd.). Tissue with density over 213 mg/cm^3 was defined as calcified bone. After 2 months, all animals were sacrificed for clinical inspection of defects. Clinical observation was performed, and the treated sites were categorized as: 1) Total bridging of defect with newly formed bone tissue or integrated bone graft; 2) Incomplete bone bridging with newly formed bone tissue or bone graft; 3) Instead of new bone, tissue fibrosis tissue formed or resorbed bone graft. Following this, a piece of newly formed tissue was removed for histological examinations from the middle of the non-critical site treated defects.

Results. Clinical observations during the postoperative period showed swelling with abscess in the treated region in both groups. Swelling in the surgical site rhBMP-7/ACS treated animals regressed in 8 weeks, whereas the control group showed swelling and abscess presented for the whole 8 weeks on the surgical site. Except for the swelling, no other complications were observed throughout the time of the experiment with no evidence of systemic problems resulting from the implant. The mean values of ALP activity observed preoperatively and 5th, 14th, 21st, and 30th postoperative day are presented in **Table 1**. The significant higher mean values of ALP activity were detected on the 14th postoperative day in animals treated with rhBMP-7/ACS than in control group of bone graft treated sites on the 30th day (**Table 2**).

Figure 2 shows the characteristic CT scan of the rhBMP-7/ACS mandible defect treated site. Inside the defect, there was visually detected newly formed bone-like tissue. The average BMD values using the GENAUINE 2.0 analyzing program of the

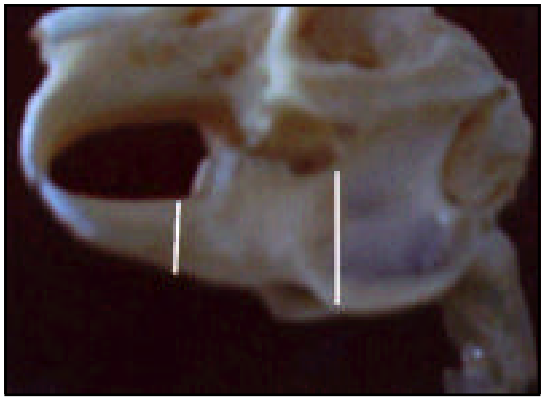

Figure 1 - Full thickness defect of the mandible from the incisive tooth to the angulus mandible behind the back molar teeth created for the study.

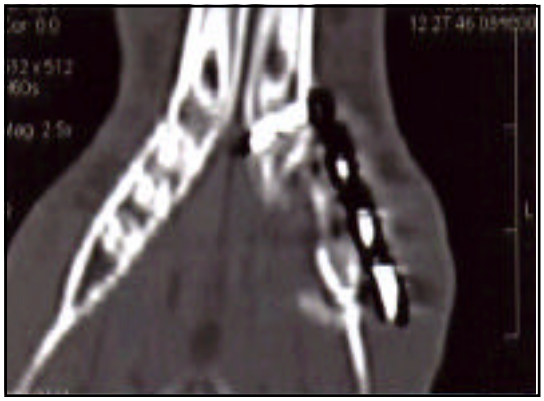

Figure 2 - A 3-dimensional CT scan evaluation of the bone defect implanted with recombinant human bone morphogenetic protein-7/ collagen.

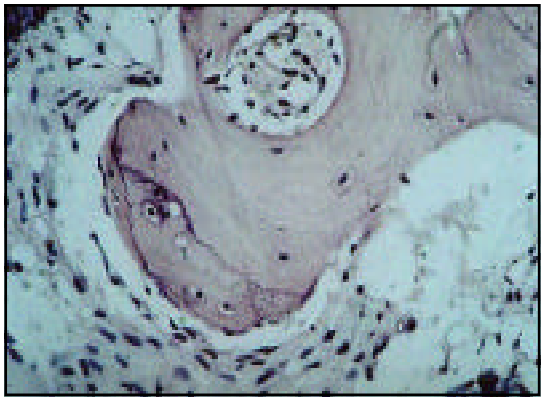

Figure 3 - Photomicrographs of histological section at high magnification (original magnification x 25) 60th postoperative day recombinant human bone morphogenetic protein-7/ collagen induced bone.

Table 1 - Mean values of ALP activity observed preoperatively and 5th, 14th, 21st, and 30th postoperative day.

Treated site	N	Mean	SD
rhBMP-7/ACS			
0 days	3	41.3	5.512
5 days	3	18.4833	1.8844
14 days	3	38.4967	16.2958
21 days	3	44.0333	14.7422
30 days	3	48.7267	6.58
Autologous bone graft			
0 days	3	29.0233	20.2757
5 days	3	27.4967	6.9253
14 days	3	22.6567	4.997
21 days	3	27.0033	2.4451
30 days	3	54.3833	9.3651

rhBMP-7 - recombinant human bone morphogenetic protein-7, ACS - collagen, ALP - alkaline phosphatase enzyme

Table 2 - Time course interval of the differences of mean values of ALP activity rhBMP-7/ACS and bone marrow treated species.

Treated site	t	df	Significant 2-tailed
rhBMP-7/ACS			
0 days	12.978	2	0. 006
5 days	16.989	2	0. 003
14 days	4.092	2	0. 05
21 days	5.173	2	0. 035
30 days	12.826	2	0. 006
Autologous bone graft			
0 days	2.479	2	0. 131
5 days	6.877	2	0. 02
14 days	7.853	2	0. 016
21 days	19.129	2	0. 003
30 days	10.058	2	0. 01

rhBMP-7 - recombinant human bone morphogenetic protein-7, ACS - collagen, ALP - alkaline phosphatase enzyme

Table 3 - Mean values of BMD on the 30th postoperative day rhBMP-7/ACS with bone marrow and bone graft treated sites.

Treated sites	Projection	N	Mean	SD	SEM
Autologous bone graft iliac crest	Caudal	3	510	44	25.4034
	Sagital	3	530.6667	53.2666	30.7535
RhBMP-7/ACS and bone marrow	Caudal	3	510.6667	87.8711	50.7324
	Sagital	3	553.6667	1153704	66.6091

rhBMP-7 - recombinant human bone morphogenetic protein-7, ACS - collagen, BMD - bone mineral density

rhBMP-7/ACS and bone marrow treated site, and autologous bone graft treated sites from both planes on the 30th postoperative day are shown in **Table 3**. Clinical inspection of defects showed that implantation of the combination of rhBMP-7/ACS and bone marrow induced bone formation with bridging of the mandible defects on the 60th day postoperatively in 2 animals, and in one fibrous tissue was observed. Use of the bone graft in the control group showed no incorporation of bone graft on the 60th day postoperatively in host bones of defects in any animal. Histological examination showed that rhBMP-7/ACS and bone marrow treated sites produced abundant woven bone mass with osteoblast and dense osteocytes arranged in large lacunae surrounded with newly formed capillary (**Figure 3**). The autologous bone graft treated site showed necrosis, inflammatory cell lymphocytes and traces of vascular elements.

Discussion. This study showed that the combination of the rhBMP-7/ACS and bone marrow had a synergistic osteoinductive effect, and produced bridging of the hemimandibular defect. It is already well known that bone marrow contains osteoprogenitor cells, and forms bone tissue in vivo and in vitro.[7,8] Excessive bone formations visually observed in our study on the 30th day on CT scan after rhBMP-7 implantation originated perhaps from an excess response of cells to rhBMP, and suggested that early exposure of bone marrow cells to the signal induction mechanism of BMP may produce and accelerate regeneration of mandible. The 3D CT evaluation used in this study made possible that precise visually observation inside defects and new formed tissue density analyzing with the GENAUINE 2,0 bone analyzing software. Lane et al,[9] showed that rhBMP-2 and bone marrow in combination are synergistic and superior for healing compared with an autogenous cancellous bone graft in a rat segmental bone defect model. Our study confirmed their result on a higher species. Seto et al,[10] obtained the best result in bridging 3 cm segmental mandible defects of the rhesus monkeys with 2:1 ratio of rhBMP-PGLA and bone marrow. Histological and gross examination as a definitive diagnostic technique confirmed induced dense bone formation and bridging bone defect with irregular trabecular pattern and little lamellar bone after 16 weeks. In our study, defects were bridged with more lamellar bone characteristics of the regenerating process. The more intensively metabolic response process in our study could be because of the different species used in research, and the age of animals with impact on slow metabolism and poor resource number of target osteoprogenitor cells. In our study, a concentration of 100-micrograma rhBMP-7 showed good osteoinductive potential. Mean values of BMD rhBMP-7/ACS and bone marrow induced newly formed tissue, showed no significant difference compared with bone graft treated sites. The optimal concentration of the rhBMP-7 and synergistic effect with bone marrow used in this study was confirmed by early increase of ALP activity observed on the 14th postoperative day, while bone graft side observed on the 30th day. The ALP enzyme is glycoprotein presented on the surface of the osteoblast cells membrane whose increase value is a clear indicator and marker of the osteoblastic function and beginning of the osteogenesis process. Yoshida et al,[11] on a partial defect of rabbit mandible, used a concentration of 10 micrograms of rhBMP-2 in porous hydroxyapatite and a telopeptide type I collagen as a carrier, and detected significant increases of ALP activity on the 21st day in the rhBMP-7 treated site. The concentration of 100 micrograms used in our study on hemiresection defects showed a more rapid result and beginning of the osteoinduction process. Other studies reported a dose dependent increase in bone induction by rhBMP-2.[12] Zezgula[13] examined the effect of different doses of rhBMP-2 in porous poly (DL-lactic acid) implant on bone formation of 20 mm defects of the radial diaphysis in rabbits, compared with a defect treated with autogenous corticocancellous bone graft. They showed dependent responses to rhBMP-2. The defect treated with 35 or 70 micrograms by histomorphometric data indicated that the amount of bone formation was equivalent to the amount in the defect treated with bone graft, and was significantly higher than in the defect treated with zero or 17 micrograms rhBMP-7, 4 weeks after implantation. Higher doses of rhBMP-7 used in this study either could be of membranous origin of mandible, or specifically conditions in maxillofacial regions for bone healing than on long bones. However, a high dose of BMP may have adverse pharmacological effects. For instance, increased dosage may lead to increased vascularity that causes excessive tissue edema. Marukawa et al,[14] in their research restored total hemiresection defects of 5-year old monkeys, 15 weeks after application with very heavy doses of growth factors, 9 mg per animal, which caused edema and swelling. It could produce severe pain in the maxillofacial region and other unexpected effects. To minimize the effective dose of BMP, a combination implant with bone marrow used in this study should be considered. Collagen as carrier for BMP's used in previous studies,[15-17] showed success in regenerating bone at the defect site. In this study, collagen was used as a carrier for rhBMP-7 as biocompatible and bioabsorbable natural material, which has a long record of safety in surgical applications such as absorbable hemostatic agents.

Our study showed that the combination graft of rhBMP-7/ACS and bone marrow could be a successful alternative in bone reconstruction.

However, judging from the results, it is not clear whether BMP's will definitely induce bone formation in humans. Maybe cells that migrate in to the BMP implants in humans have a longer life span and slower metabolic rates, and are thought to respond less to BMP than those cells in rabbits. The optimum concentration of implanted rhBMP-7 in accord with the environment of the treated site, and their contest of osteoprogenitor cells in combination with autologous transplant of bone marrow or mesenchymal stem cells as additional requirements, functional result and long-term observation, have to be considered before clinical trials.

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