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Original Article

rhBMP-2 applied as support of distraction osteogenesis: a split-mouth histological study over nonhuman primates mandibles

Alan Scott Herford[1], Marco Cicciù[2], Livium Florian Eftimie[1], Megan Miller[1], Fabrizio Signorino[1], Fausto Famà[3], Gabriele Cervino[2], Giuseppe Lo Giudice[2], Ennio Bramanti[2], Floriana Lauritano[2], Giuseppe Troiano[4], Lorenzo Lo Muzio[4], Luigi Laino[4]

[1]Oral Maxillofacial Department, Loma Linda University, Loma Linda California, USA; [2]Department of Morphology Medical Sciences and Odontostomatology, University of Messina, Messina, Italy; [3]Department of Human Pathology, University of Messina, Italy; [4]Department of Clinical and Experimental Medicine, Foggia University, Foggia, Italy

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Abstract: Objective: The purpose of this experimental study was to analyze whether the addition of the recombinant human bone morphogenetic protein type 2 (rhBMP2) to a collagen matrix applied on a distraction osteogenesis device would help healing in the middle new formed bone. A histologic split mouth study on Macaca Fascicularis mandibles was performed. Material and methods: A surgical procedure was conducted on 6 Macaca Fascicularis to obtain 12 bilateral mandibular alveolar defects. All the defects were then reconstructed with a mixture of autogenous bovine bone using osteodistraction device. Two groups, with a total of 12 defects were created: The first group (CONTROL) to study just the effect of the distraction osteogenesis device, the second group (TEST) to compare the effect of rhBMP2 over the bone healing. Results: After 3 months histological evaluation of the mandibles revealed, new bone formation in both groups studied. The control group showed a better regeneration in the midollar bone despite test group recorded more cortical bone. Conclusion: The addition of rhBMP2 into the between of the bone fragment divided by the distraction osteogenesis device induced a rapid increase in hard and soft tissue healing. Moreover, a quick cortical bone formation has been obtained where BMP2 have been applied.

Keywords: Bone regeneration, growth factors, rhBMP2, distraction osteogenesis

Introduction

The edentulous atrophic ridge can adversely affect the patient's speech as well as their ability to masticate. Currently, the dental atrophies caused by surgical resection, dental pathologies or trauma can withhold patients of satisfactory tissue support for dental prosthesis and make the placement of dental implants much more difficult. Dental implants treatment may be performed only when the alveolar ridge is adequate in quantity and quality [1, 2].

The lack of teeth and alveolar bone often leads to further resorption, which leads to vertical and horizontal atrophies of the maxillary bones. The presence of teeth preserves the alveolar processes of the maxilla and mandible, and natural bone resorption begins following tooth loss. A severe resorption may prohibit implant placement in the prosthetic position, necessitating a ridge augmentation procedure to obtain a sufficient amount of bone available for implant placement [3]. Numerous bone-grafting procedures have been developed to replace jaw bony atrophies with different success rate. Some of the more common technique includes autogenous bone harvested from the patient's extra oral sites like iliac crest, tibia, or intra oral areas by using posterior mandible, or maxillary tuberosity [4].

Even if the application of autogenous bone grafting techniques is considered the gold standard, these techniques have strong limitations including possible surgical complications, biological cost, and patient morbidity associated with harvesting site [5, 6].

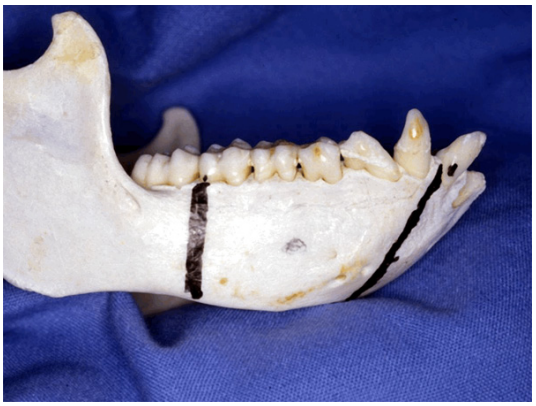

Figure 1. Model of the monkey mandible involved in the study. The skull of the monkey mandible was underlined in order to create a bone defect for then placing the distractor osteogenesis device.

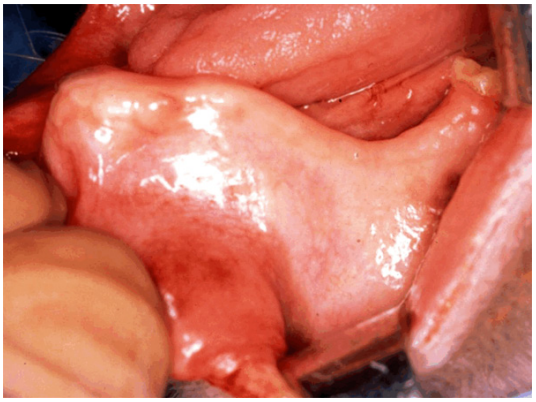

Figure 2. Clinical view of the mandible monkey just at the time of the healing after the teeth extraction and before the second regenerative surgery.

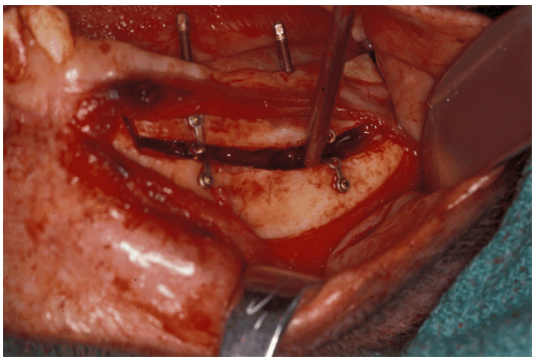

Figure 3. Distractor osteogenesis device have been applied in the non-human mandible monkey after the creation of a large bone defect.

Several surgical procedures using graft materials from a variety of autologous, heterologous, or synthetic sources have been developed in order to recreate ideal clinical condition for the subsequent dental implants positioning [7, 8].

The most common methods include grafting procedures, with or without coverage by a barrier membrane (guided bone regeneration (GBR)) [9, 10]. Horizontal ridge augmentation with autogenous block grafts, covered with no resorbable expanded polytetrafluoroethylene (ePTFE) membrane, is well documented and it results in a predictable and successful clinical results [11]. Other methodologies can related with autologous or homologous bone grafts, ridge splitting, sub-periosteal membrane-guided regeneration, alveolar osteotomies/sandwich grafts, distraction osteogenesis, and the use of growth factors [12-14]. Distraction osteogenesis is an alternative technique for managing large bone defects [15]. This surgical procedure can be defined as a procedure of new bone apposition between the surfaces of bone portions or fragments that are gradually differentiated in response to incremental traction. This technique has reliable results in large bone mandibular defects [16]. As well documented, the growth factors and specifically rhBMP2 have been used in numerous oral surgery procedures giving the patients the possibility of reducing the quantity of harvested bone, having final regenerated bone of high quality and quantity [17, 18]. An ideal bone regenerative procedure should give a quick healing with no contracture of soft tissue or granulation tissue formation. At the same time it should favour homeostasis, give no infection, and reduce the patient discomfort by avoiding a second surgical site [19].

The idea that animal study, particularly that relating to pharmaceuticals and environmental agents, may be a not valid predictor of human experience is not new [20]. Animal studies should always follow the rules of the three R's; Reduction, Replacement and Refinement suggested by Russell and Burch in a paper published on 1959 [21]. The number of animals used should be directed to reduce to the minimum required to achieve a valid statistically significant result. Wherever possible the use of animals should be substituted by other means, such as computer simulation or in vitro testing, and the investigation must refined or altered in any way possible so as to decrease potential for suffering for all involved animals. The present study has been developed on Macaca

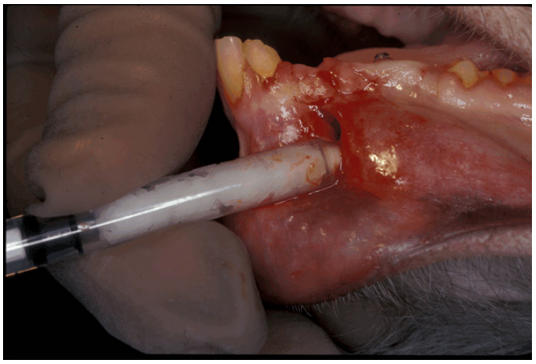

Figure 4. The BMP-2 have been added in the half mandible selected in the study group.

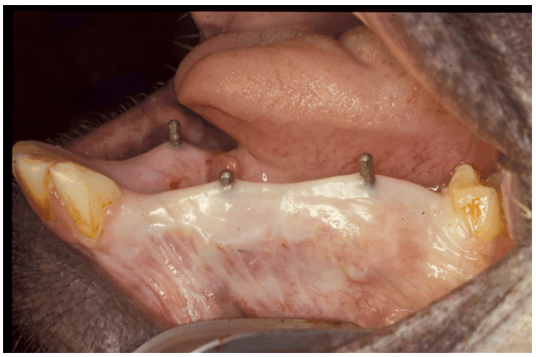

Figure 5. The hard and soft tissue healing of both half mandibles. Healthy hard and soft tissue can be underlined for no signs of inflammation presence.

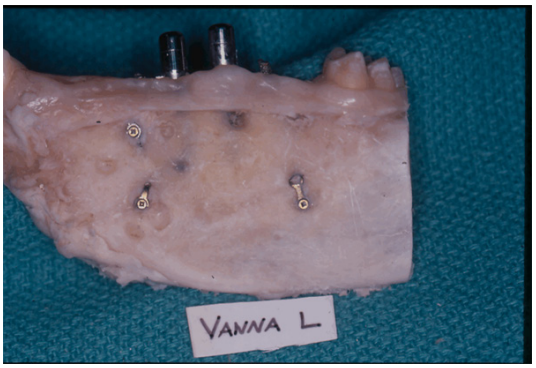

Figure 6. After the Monkey euthanasia the pieces have been prepared for histological evaluation. In this slice, the distractor is completely involved in the newly formed bone.

Fascicularis for their affinity to the human species [22].

The purpose of this animal study is to evaluate by histological analysis the effectiveness of rhBMP2 application on the newly formed bone quality on created defect from distraction osteogenesis in nonhuman primates mandibles. The excellent regenerative action of rhBMP2 factor as a chemo-attractant and morhogenic, along with its ability to promote angiogenesis, indicate it as a key for having final high quality regenerated bone tissue.

Materials and methods

Note: The Loma Linda University Ethical Committee has approved this study.

Six adult Macaca Fascicularis underwent a surgical procedure to create bilateral mandibular defects, for a total of 12 defects. The created defects reflected the Class V or VI of Cawood and Howell's classification [23], having vertical and horizontal deficiencies of bone tissue. A healing period time of 12 weeks after the first surgery was performed. Each defects was about 40 mm × 30 mm, carried out on the monkey's posterior mandible. Each monkey had its mouth divided into two halves; each half had a test and the control group assigned to it. Three months after the first surgery, the monkeys were scheduled for the second reconstructive surgery by the application of the distractor. A collagen matrix was applied to cover the bone graft and the distractor, then the soft tissue was closed.

During the reconstructive surgery, a horizontal supra-crestal muco peri-osteal flap was elevated in the mandibular mucosa, extending to the periosteum overlying the defect. The incision was carried out on the alveolar ridge of the defect. Following visualization of the defect, a surgical stent was used to remove bone and a standardized 40 mm × 30 mm defect was created.

A custom fabricated internal distractor was placed after corticotomy of both the buccal and lingual cortex of the both mandible and all the defects were covered by absorbable collagen matrix membrane in order to help the soft tissue healing due to the possible tissue-stretch effect during the distraction osteogenesis. One of the osteogenesis distraction point is the possibility of increasing both soft and hard tissues.

Distraction was started on the 9 weeks after the first surgery and continued at the rate of

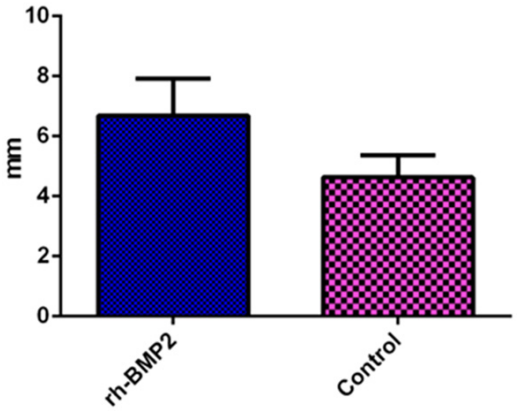

Figure 7. Statistical evaluation of bone quantity during the distraction. The BMPs group reveals better healing on final volume bone tissue obtained.

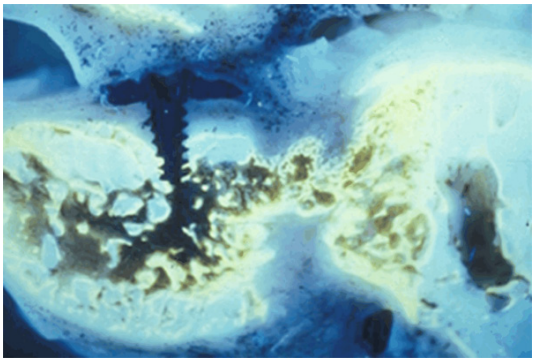

Figure 8. The No BMP-2 half mandible have been characterized by some area of no bone formations.

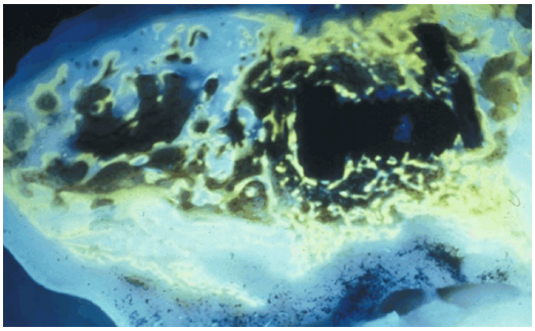

Figure 9. The BMP-2 group have been characterized by a more consistent and significant newly bone tissue formation.

0.5 mm 12 hourly till completion of the calculated deficiency and was left to consolidate for a period of 8-10 weeks. After 2 weeks of distraction rhBMP2 was added to the selected monkeys mandibles and the surgical site was closed.

The recombinant human Bone Morhogeneic Protein type 2 was then mixed with absorbable collagen sponge and then fixed into the distractor in the group A enclosing the right mouth of each monkey while in the each left side just the distractors have been applied (**Figures 1**-**3**).

It is well documented how the growth factor application also increased tissue repair process, favored soft tissue and bony wound healing and when delivered exogenously, and initiated callous formation [24].

Following 3 months of healing, the monkeys were humanely euthanized. Histologic sections of bone and soft tissue were prepared and analyzed (**Figures 4**-**9**). The specimens were fixed in neutral buffered 10% formalin, dehydrated and infiltrated in resin, and then embedded and polymerized in resin blocks. The blocks were cut and ground using the Exakt-cutting-grinding system to a thickness of 50 μm and stained with Mayer's hematoxylin and eosin or Masson's Trichrome stain. Histological evaluation included searching for any residual matrix as well as any evidence of inflammation. The quantity of the grafted bone was also evaluated. Qualitative and quantitative histological evaluations of soft-tissue ingrowth and bone regeneration were performed on non-decalcified grounded sections. For statistical evaluation, data have been analyzed using GraphPad Prism software 6.00 (GraphPad Prism Software, San Diego, CA, USA). Statistical significance between different groups was determined with Wilcoxon test, a level of P-value < 0.05 was considered as statistically significant. (**Figure 7**).

Results

Histometric analysis of regenerated bone height

The amount of bone formation and the newly formed tissue quality have been recorded (**Figure 7**). The areas of regenerated tissue were randomly selected per section. The height of new bone was measured in separate sections. The height was reported as an average by measuring the distance from the non-grafted bone to the crest of the regenerated ridge. The test group showed a greater amount of bone regenerated compared to the control group. The mean value for the rh-BMP2 group was 6.69 mm with a standard deviation of 1.22, while the osteodistraction alone group showed

a mean value of regenerated bone of 4.62 mm with a standard deviation of 0.74. The comparison with Wilcoxon test revealed a significant difference between two group with a *p*-value of 0.0156.

Clinical and histological observations

During the healing period some clinical differences were noted regarding the two groups. In the rh-BMP2 group there was membrane exposure in the 62.5% (5/8) of cases, while in the control group the exposure was present in all (100%; 8/8) the cases. (**Figures 8**, **9**).

Discussion

Many research have been performed in the recent years involved the use of autologous/homologous/xenogeneic block grafts for bone regeneration [25, 26]. It is known that in the 1st year after performing reconstructive surgery, the bone graft resorption is significant and may progress in the following years [27]. Some studies have demonstrated, that the augmentation procedures of the alveolar ridge, using the positioning of a corticocancellous bone graft over the mandibular buccal cortex is a non-predictable methodology to increase the vertical size of the edentulous ridge [28].

Cawood and Howell classes V and VI [23] alveolar defects require bone grafting prior to dental implants placement, in order to provide a sufficient amount of bone available for the implant placement after the bone healing. When guided bone regeneration (GBR) is performed in this clinical situations, the insufficient amount of soft tissues available lead to the the risk of graft exposure to oral cavity and the subsequent resorption and failure of regenerative therapy [29-31]. Although using autograft bone is considered the 'gold standard' of choice for bone reconstruction, the advantages for vertical alveolar increment are small due to limited tissue resources and donor morbidity. Alveolar distraction osteogenesis (ADO) represent a valid option to avoid bone graft techniques in these types of defect [32]. ADO is characterized by some advantages since it avoids donor site morbidity and determines predictable gain of hard and soft tissues. It also showed: low infection rate, decreased bone resorption, and a short bone healing period prior to implant insertion [33-35]. Kim et al. [36] directly compared ADO and autogenous onlay bone graft for vertical augmentation after a follow-up period of 12 years. They reported a similar vertical bone gain and implants survival with this two procedure, the cumulative survival rates was 97.3%, in the ADO group and 94.1% in the bone graft group. These results are in accord with a prospective study by Chiapasco et al. [37], who reported a similar survival rate after implant placement in bone regenerated with these two procedures. However, some complication are associated with ADO technique like: oral displacement of the transport vector, inadequate soft tissue extension after distraction and fracture of distractor device [38]. In order to improve the amount of regenerated bone and decrease the possibility of alveolar bone exposure during the ADO therapy, we performed this study on Macaca Fascicularis evaluating if the addition of the recombinant human bone morphogenetic protein type 2 (rhBMP 2) to a collagen matrix applied on a distraction osteogenesis device would help healing in the middle new formed bone. A major area of research in recent years is represented by the use of growth factors for bone regeneration. It has been demonstrated that growth factors induce normal autogenous bone in clinically relevant defects in the craniofacial skeleton favoring the healing of hard and soft tissues [39, 40]. The newly forme bone assumes the characteristics of the adjacent resident bone and allows placement, osseointegration, and functional loading of dental implant [41-45]. Factors like bone morphogenic proteins (BMPs) play an important role in chemotaxis and cell proliferation [47]. BMPs regulate the controls for healing and regeneration of the bone tissue by repairing the tissue in case of bone fracture [47]. The results of this study, along with other recent investigations into the application of growth factors in bone regeneration techniques clearly underlined how the cytokine implanted on the carrier can accelerate the healing process [48]. Moreover, collagen carriers may improve soft tissue volume over the graft by inducing less incidence of bone graft exposure [49-51]. Many biomaterials have been used as a biological barrier in the past to cover the grafts, allowing growth of host epithelial cells beneath the bone. Kim et al. [52] reported that a double layer collagen membrane positioned over the bone graft is helpful for the integration of the onlay block bone graft. A recent research pub-

lished by Thoma, et al. [46] analyzed the effectiveness of a synthetic, biodegradable matrix made of polyethylene glycol. In that investigation, the placing of the absorbable membrane successfully prevented collapse of the covering soft tissues protecting the grafted material. The collagen used in this research (Mucograft®) is a bio-resorbable, bilayer matrix collagen used in the place of soft tissue. Recent clinical studies have demonstrated how this collagen matrix® can be applied to increase both keratinized and non-keratinized mucosa with rapid degradation and healing process [44, 53].

This study results showed that the addition of rh-BMP2 to the sites of distraction osteogenesis improves soft tissue healing, and reduced graft exposure and protecting the bone tissue healing. The exposure rate was lower in the test group (62.5%) compared to the control group (100%) demonstrating the healing properties of rhBMP on soft tissues. Results related to the amount of vertical bone regeneration are strongly in favor to the use rh-BMP2 in the sites of distraction osteogenesis. The mean amount of vertical bone gain was 6.69 mm compared to 4.62 mm in the control group (*P*-value = 0,0156), indicating a need for studies on human model to confirm these results. The histological observations of the present investigation confirmed the previous animal studies in which growth factor have been used in order to increase the bone quality and quantities.

Disclosure of conflict of interest

None.

Address correspondence to: Marco Cicciù, Department of Human Pathology, School of Dentistry, University of Messina, Policlinico G. Martino, Via Consolare Valeria, Messina 98100, Italy. Tel: +3909-02216920; E-mail: acromarco@yahoo.it

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