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Restoration of occlusal function using osseointegrated implants in the canine mandible reconstructed by rhBMP-2

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Key words: bone regeneration, loaded condition, oral implants, osseointegration, recombinant human bone morphogenetic protein-2 (rhBMP-2)

Abstract: Bone morphogenetic proteins have been found to be one of the most promising osteoinductive substances and they are expected to be utilized clinically for the reconstruction of defective mandibles. However, newly formed bone induced by recombinant human bone morphogenetic protein-2 (rhBMP-2) has not yet been proven able to withstand the masticatory force applied by oral implants. In this study, we examined the qualitative changes in an rhBMP-2-induced mandible from the functional force of osseointegrated oral implants. Segmental (30 mm) bone defects were created in the mandibles of beagles. A poly D,L-lactic coglycolic acid-coated gelatin sponge impregnated with rhBMP-2 was grafted to the resected canine mandible. The new bone was formed 8 weeks after surgery and the Brånemark system fixtures were implanted into the reconstructed mandible. After another 8 weeks, the prosthesis was placed over the oral implants. The prosthesis was maintained in occlusion with the opposing natural dentition for 0, 4, 12, 24, or 48 weeks before the animal was euthanized. The quality of regenerated bone was then evaluated histologically and the osseointegration ratio between oral implants and the bonemeasured. During the first 4 weeks, the ratio remarkably increased from 48.9% to 64.5%. After 48 weeks, the ratio approached about 74.5%. The bone loaded for 48 weeks had undergone extensive remodeling and consolidation; its quality was better and maturer than that of bone that was not loaded. These results indicated that the newly formed bone induced by rhBMP-2 was able to withstand the masticatory force applied by oral implants and had become as functionally mature as a natural bone.

One of the most difficult procedures in the field of oral rehabilitation is mandibular reconstruction. The goal of this operation is not only the reconstruction of mandibular contour but also the restoration of oral function. Autogenous bone grafting is widely performed on patients after treatment for trauma, tumors and other diseases of the mandible (Foster et al. 1999), and it is considered the gold standard because of its osteoinductive, osteoconductive, and osteogenic benefits (Marx 1993). However, autogenous bone grafting has disadvantages such as limited donor tissue availability, donor site morbidity, and shaping difficulty. To overcome these impediments, bone morphogenetic protein (BMP), one of the most promising osteoinductive substances, is expected to be utilized clinically for bone reconstruction. Considerable interest has been focused on the application of BMPs for therapeutic use (Wozney et al. 1988; Yasko et al. 1992; Cook et al. 1994; Bostrom et al. 1996; Toriumi et al. 1991; Heckman et al. 1991; Mayer et al. 1996; Gerhart et al. 1993; Welch

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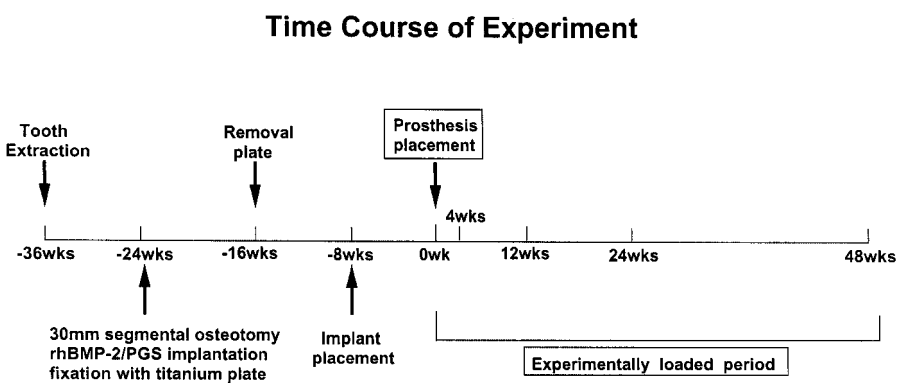

Fig. 1. After a 30-mm full thickness defect was made in the mandible, a titanium plate was fixed across the buccal aspect of the mandibular defect and the rhBMP-2-impregnated PGS was placed in the defect. The plate was removed 8 weeks later.

et al. 1998; Boyne 1996; Cook et al. 1995; Ripamonti et al. 1996; Miyamoto et al. 1993), recombinant human BMP-2, has been used to treat long bone nonunions and segmental mandibular defects in laboratory animals (Cook et al. 1994; Bostrom et al. 1996; Toriumi et al. 1991; Heckman et al. 1991; Mayer et al. 1996; Gerhart et al. 1993; Welch et al. 1998; Boyne 1996; Cook et al. 1995; Itoh et al. 1998; Seto et al. 2001). However, little is known about the medium-term effectiveness of BMP-induced bone. A few reports have describee the relationship between oral implants and induced bone (Sigurdsson et al. 1997; Hanisch et al. 1997; Cochran & Schenk 1999; Cochran et al. 1997, 2000). Furthermore, regenerated bone has not yet been proven to resume a functional matrix form and withstand the masticatory force applied by oral implants. We report here the long-term effectiveness of rhBMP-2-induced bone after the loading of mechanical force by osseointegrated implants.

Material and methods

Animals

Fifteen adult male beagles (1–2 years old, weighing 10–16 kg), without general or oral health problems, were used in this study.

Implant materials

The rhBMP-2 used in this experiment was manufactured by recombinant expression at Genetics Institute (Cambridge, MA, USA) in Chinese hamster ovary cells and purified to >98% purify. Yamanouchi Pharmaceutical Co. Ltd. (Tokyo, Japan) supplied the rhBMP-2 and also the poly D,L-lactic coglycolic acid (PLGA)-coated gelatin sponge (PGS) used as the carrier of rhBMP-2. The PGS had the following properties: 30,000 MW; a 1:1 molar ratio of lactic to glycolic acid; a 4:1 weight ratio of PLGA to gelatin, and approximately 90% porosity. The PGS was soaked in the rhBMP-2 solution (400 μg/ml rhBMP-2, 5 mM sodium glutamate, 2.5% glycine, 0.5% sucrose, and 0.01% Tween 80, pH 4.5) and kept at room temperature for more than 30 min before implantation to allow for incorporation of rhBMP-2. The PGS was shaped to fit in the defect (30 × 15 × 8 mm). Brånemark system implants (Nobel Biocare Japan K.K., Tokyo, Japan) were used to restore the occlusal function. The implants had a diameter of 3.0 mm and a length of either 13 mm or 15 mm. We selected the implant length on the basis of the density and quality of the newly formed bone. Prosthetic superstructures, each to be supported by three implants, were cast in a silver palladium alloy.

Operative procedure

The dogs were anesthetized with an intramuscular injection of medetomidine hydrochloride (40 μg/kg; Domitor™, Meiji Seika, Tokyo, Japan) and an intravenous administration of sodium pentobarbital (15 mg/kg) during the surgery. Three months after extraction of the premolar teeth (P2, P3, P4), a 30-mm section of mandible was resected with a surgical saw (Micro Air, Valencia, CA, USA). A titanium plate (Keisei Instrument, Tokyo, Japan) was fixed across the lingual aspect of the mandibular defect with self-tapping titanium screws; the rhBMP-2-impregnated PGS was placed in the defect and the wound was closed in layers with biodegradable sutures (Fig. 1). Eight weeks after implantation of the rhBMP-2-impregnated PGS, the titanium plate and screws were removed. After another 8 weeks, three Brånemark system implants were inserted into the newly formed bone (Fig. 2). Placement of the implants was performed according to the manufacturer's instructions. Eight weeks after that, the prosthetic superstructure was placed onto the oral implants. The prosthesis was maintained in occlusion with the opposing natural dentition (Fig. 3). Before the placement of the prosthesis, all animals were fed a soft diet of solid dogfood soaked in water. After the placement of the prosthesis, the animals were returned to a diet of solid dogfood. The period of experimental loading was begun with the placement of the prostheses, designated as Week 0. The prosthesis was maintained in occlusion with the opposing natural dentition for 0, 4, 12, 24, or 48 weeks before the animal was euthanized. Cefotetan (25 mg/kg; Yamatetan™, Yamanouchi Pharmaceuticals) was

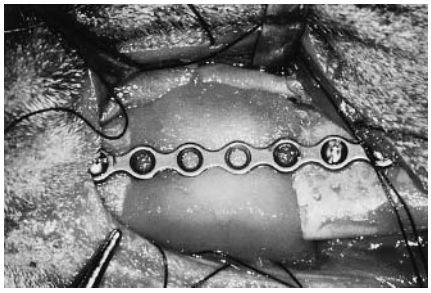

Fig. 2. Eight weeks after the plate was removed, three Brånemark system implants were inserted into the newly formed bone.

Fig. 3. Prosthetic suprastructures, each to be supported by three oral implants, were cast in a silver palladium alloy. Each prosthesis was placed with a gold screw and remained functional under optimal conditions.

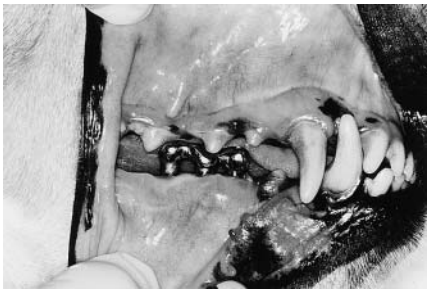

Fig. 4. Time course of the experiment.

administered intramuscularly for a week to control infection. Butorphanol tartrate (0.2 mg/kg; Stadol™, Bristol-Myers Squibb, New York, USA) was administered intramuscularly for a week to control pain. Three dogs were euthanized when the osseointegrated implants were inserted (Week 0), and an additional three dogs were euthanized 4, 12, 24, and 48 weeks after the placement of the prosthesis. The time course of the experimental design is summarized in Fig. 4.

Analysis

After the animals were euthanized, specimens of the newly created bone were harvested and soft radiographs (Sofron 50™, Sofron, Tokyo, Japan) of them were taken. The specimens were then placed in 10% buffered formalin for 2 weeks and dehydrated with a graded ethanol series. Next, the specimens were embedded in histology resin (Technovit 7200VLC, Kulzer, Hanau, Germany). The resin was polymerized by light curing. The resin block was sectioned in 50–60 μm slices; the sections were stained with toluidine blue and examined microscopically. Because the three implants were not inserted in a straight line into the induced bone, it was difficult to take mesio-distal cross-sections of the specimens with the three implants all together. Therefore, each specimen was separated into three parts, with each part containing one implant; sections were prepared at the center of the implant in the bucco-lingual direction. The osseointegration ratio at the interface of the implant and the bone was measured by a quantitative image analyzer (Olympus, Tokyo, Japan) on specimens taken at 0, 4, 12, 24, and 48 weeks of loading. An image of the three portions (cervical, middle, and apical) of the implant was transferred to the computer screen; the fixture surface length (LF) and the bone contact (LB) were measured. The percentage of bone contact length was calculated from the following formula: total LB/total LF of each implant $\times 100$. The osseointegration ratio was expressed as the average of the percentages of the bone contact length of three implants from one animal. The data were expressed as the means $\pm$ SD of three animals at each time point, and were evaluated by analysis of variance (ANOVA). Significance was set at $P < 0.05$.

Results

Postoperative course

No fatalities resulted from infection or complications from anesthesia. All of the dogs showed postoperative swelling at the site of the rhBMP-2-impregnated PGS implantation; the swelling resolved in 4 weeks. Aside from the swelling, no other complications were observed throughout the remaining time of the experiment.

Macroscopic findings

Because the bone was reconstructed beyond the outer edge of the titanium plate, a small amount of bone was cut off with a dental bur when the plate and screws were removed 8 weeks after their placement. The surface of the mandibular bone had become smooth and the contour of the reconstructed mandible approximated that of the mandible at the time of oral implant placement. However, the solidity of the induced bone was not as great as that of the original cortical bone. The regenerated mandibular bone was found to be smoother and harder by the time the specimen was harvested.

Radiological evaluation

Twenty-four weeks after the segmental osteotomy, the radiographs of the specimen showed a complete continuity of the mandible, although the radiopacity was lower than that of the host bone (Fig. 5a, b). The radiopacity increased with time and no bone resorption was evident around the implant either at the time of the prosthesis placement or at the time of euthanasia (Fig. 5c, d).

Histological evaluation

Newly formed bone, induced by rhBMP-2, exhibited a trabecular pattern and was predominantly lamellar under light microscopic observation. If the loading force is excessive, the interface either breaks down or is inhibited during formation. The changes in the histological appearance around oral implants, as a result of this increased loading, have been described. The surface of the oral implant had little bone contact at the bone-implant surface under the nonloaded condition. The trabeculae became thicker and oral implant surface exhibited more bone contact 4 weeks after loading. At 12 weeks, the bone around oral implants started remodeling and the oral

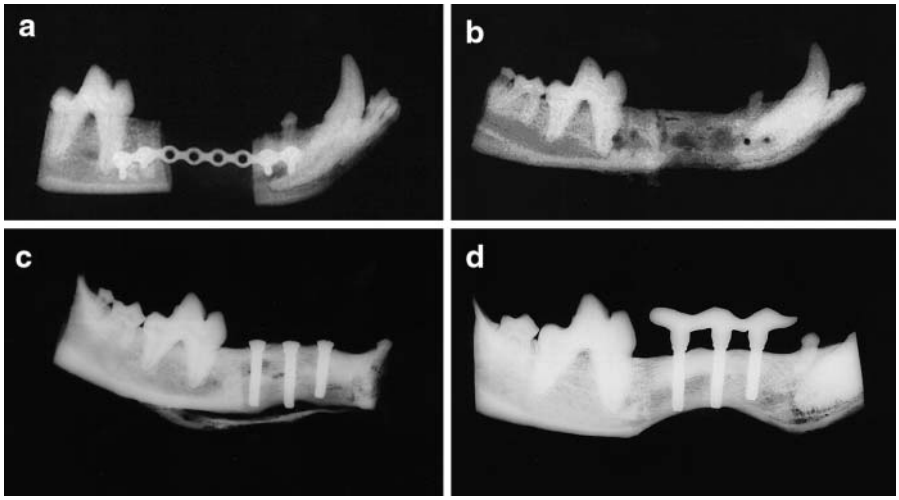

Fig. 5. Soft X-rays taken immediately after (a) the rhBMP-2/PGS complex was grafted to the 30-mm segmental mandibular defect and 16 weeks later (b). The defective mandible was completely reconstructed. Three dental implants were inserted in the regenerated bone in each dog (c). Eight weeks after the insertion of the oral implants, the superstructure was put on the oral implants and the masticatory force was loaded (d).

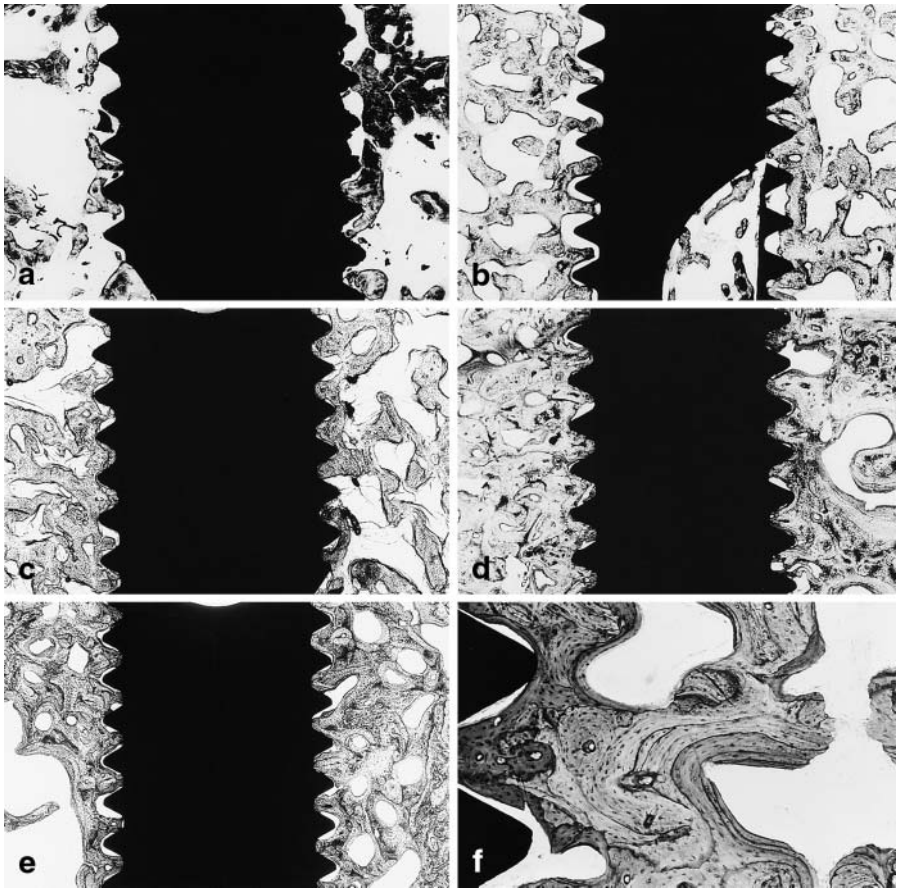

Fig. 6. (a) Onset of loading, 8 weeks after placement of the Brånemark system implants. ×25. (b) After 4 weeks of loading. ×25. (c) After 12 weeks of loading. ×25. (d) After 24 weeks of loading. ×25. (e) After 48 weeks of loading. ×25 (f) After 48 weeks of loading, as seen under high power magnification. ×100.

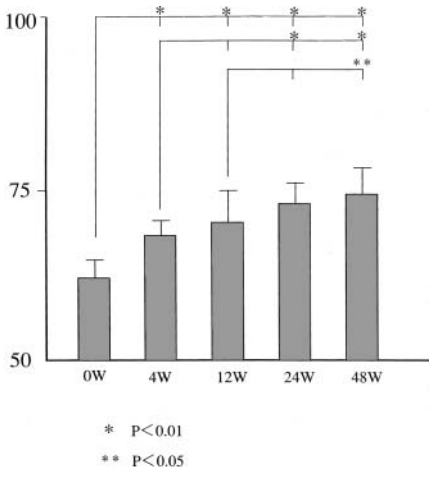

Fig. 7. Percentage of bone-to-implant contact area at 4 weeks (48.9 ± 5.7%), 12 weeks (66.9 ± 8.3%), 24 weeks (73.2 ± 7.1%) and 48 weeks (74.5 ± 7.8%) after loading. The osseointegration ratio increased in a time-dependent manner until it plateaued at 24 weeks.

implant surface had more bone contact. At 48 weeks, the bone showed more active remodeling and there was no apparent difference in quality between the induced bone and the host bone. Havers canals grew within the trabeculae and the bone tissues appeared to promote differentiation. The bone became maturer and further remodeled to support the oral implant (Fig. 6).

Osteointegration ratio evaluation

The osseointegration ratio had increased significantly between 0 week (48.9 ± 5.7%) and 4 weeks (64.5 ± 4.7%) after loading. The ratio increased gradually during the experimental period, although there were no significant differences between 4 weeks and 12 weeks (66.9 ± 8.3%) and between 24 weeks (73.2 ± 7.1%) and 48 weeks (74.5 ± 7.8%). The osseointegration ratio increased in a time-dependent manner until it plateaued 24 weeks after loading (Fig. 7).

Discussion

Many studies have reported that rhBMP-2 can induce ectopic bone formation and the induced bone at no-stress loading site is gradually resorbed and finally disappears. In this study, we wanted to understand the medium-term changes in bone induced by rhBMP-2 under masticatory force from dental implants. Our results showed that, far from being resorbed, induced bone was gradually remodeled. Histologic examination showed that the cellular morphologic appearance of the osteoblasts indicated active bone formation across the mandibular defects. The rhBMP-2 implants probably stimulated the rapid influx and transformation of primitive mesenchymal cells into osteoblasts, resulting in extensive host bone formation at the sites of the defects. The trabeculae then became thicker and more active remodeling was observable after stress loading. The osseointegration ratio increased from 49% at Week 0 to 73% after 48 weeks of loading. Mechanical stress plays an important part in maintaining bone condition and as a result of the superstructure put on the oral implants and the masticatory force transmitted directly to the induced bone in our study, the bone seemed to be remodeled. The role of a matrix carrier is to provide an effective delivery system for rhBMP-2 by delaying diffusion from the local region and by providing a favorable environment for adherence and proliferation of a responsive population of cells. Ideally, the matrix should be biocompatible and biodegradable to minimize local tissue response and it should resorb as new bone begins to form. The poly D,L-lactic coglycolic acid (PLGA) coated gelatin sponge (PGS) has recently been developed as a carrier for bone morphogenetic protein (Kinoshita et al. 1997; Higuchi et al. 1999; Itoh et al. 1998). This carrier has a porous, compressible form, effectively delays diffusion, and has high biocompatibility and biodegradability (Itoh et al. 1998). Remnants of PGS were still recognized in some cases after 4 weeks, but the material was completely absorbed by 8 weeks. Therefore, we removed the titanium Plate 8 weeks after the operation. New bone of substantial mass can be formed only when rhBMP-2 is implanted with a proper carrier material, which means that rhBMP-2 requires a proper delivery system to work at a local site. More

effective carriers for rhBMP-2 are still under investigation. Variations in rhBMP-2 dosage and carrier may influence the osseointegration ratio and should be evaluated.

A few studies have reported that the addition of rhBMP-2 around the oral implants in a beagle peri-implant defect model resulted in substantially greater new bone area and percentage of bone-to-implant contact (Sigurdsson et al. 1997; Cochran & Schenk 1999; Cochran et al. 1997). These results have been already confirmed by the human multicenter study (Cochran et al. 2000). The study showed that the bone induced with rhBMP-2 was stable without resorbing. These studies were made using a peri-implant defect model or a marginal resection model. We believe the present study is the first report on ral implants placed in rhBMP-2-regenerated mandibular bone after segmental defect. The most important findings of this study were that the rhBMP-2-induced bone is clearly strong enough to support mastication in the canine model and that the new bone is remodeled by the mechanical stress transmitted through the oral implants without resorbing. In the future, rhBMP-2 may be one of the most effective materials for reconstructing discontinuous mandibular defects in humans. However, an ideal carrier and an optimum dose must be confirmed before patients can receive the most benefit from rhBMP-2. In conclusion, the present study showed that the rhBMP-2-induced bone was not only clearly strong enough to support mastication but also remodeled to resemble a normal bone.

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Résumé

Des protéines morphogénétiques osseuses ont été définies comme une des substances ostéoinductives les plus prometteuses pouvant vraisemblablement être utilisées cliniquement pour la reconstruction de mandibules réduites. Cependant, l'os néoformé induit par la protéine-2 morphogénétique osseuse humaine recombinante (rhBMP-2) n'a pas encore prouvé qu'il pouvait résister aux forces de mastication appliquées par des implants buccaux. Dans cette étude, les variations qualitatives d'une mandibule induite par rhBMP-2 depuis la force fonctionnelle des implants buccaux ostéointégrés ont été examinées. Des lésions osseuses segmentées (30 mm) ont été créées dans des mandibules de chiens beagle. Une éponge en gélatine recouverte d'acide poly-D, L-lactique co-glycolique imprégnée de rhBMP-2 a été greffée sur les mandibules réséquées. Le nouvel os était présent huit semaines après la chirurgie et des implants *ad modum Brånemark* ont été insérés dans la mandibule reconstruite. Après huit semaines supplémentaires, la prothèse a été placée sur ces implants. Cette dernière était maintenue en occlusion avec la dentition naturelle opposée pour 0, 4, 12, 24 et 48 semaines avant que l'animal ne soit tué. Ensuit, la qualité de l'os régénéré a été évaluée histologiquement et la proportion d'ostéoïntégration mesurée entre les implants et l'os. Durant les quatre premières semaines, la proportion augmentait de 49 à 65 %. Après 48 semaines, la proportion approchait les 75%. L'os chargé pendant 48 semaines avait subi un remodelage important ainsi qu'une consolidation; sa qualité était meilleure et plus mûre par rapport à l'os qui n'avait pas été chargé. Les résultats indiquent que l'os néoformé induit par rhBMP-2 était capable de résister à des forces de mastication appliquées par des implants et était devenu mûr fonctionnellement comme un os naturel.

Zusammenfassung

Man weiss, dass knochenbildende Proteine vermutlich eine der erfolgversprechendsten osteokonduktiven Substantzen sind. Man erwartet von ihnen, dass sie klinisch zum Aufbau von Knochendefekten an Unterkiefern eingesetzt werden können. Ob der von einem synthetisierten menschlichen knochenbildenden Protein-2 (rhBMP-2) neu aufgebaute Knochen aber in der Lage ist, Kaukräfte, die über Zahnimplantate auf ihn einwirken, aufzunehmen, konnte bis anhin noch nicht bewiesen werden. In dieser Studie untersuchten wir die qualitativen Veränderungen, hervorgerufen durch funktionelle Kräfte von osseointegrierten Zahnimplantaten, in eimen mit rhBMP-2 aufgebauten Unterkiefer. Man präparierte am Unterkiefer von Beaglehunden segmentförmige Knochendefekte von 30mm. In die Resektionsstelle des Hundeunterkiefers transplantierte man einen Poly-D, L-lactic co-glycolic und säurebeschichteten Gelatineschwamm, der mit rhBMP-2 imprägniert worden war. Der neue Knochen war 8 wochen nach dem chirurgischen Eingriff fertig ausgebildet und in diesen neu aufgebauten Unterkiefer implantierte man Brånemarkimplantate. Nach weiteren 8 wochen zementierte man Brücken auf die Zahnimplantate. Die Brücken waren mit der Eigenbezahnung des Gegenkiefers während 0, 4, 12, 24 oder 48 wochen in Okklusion, bevor die Tiere geopfert wurden. Dann beurteilten wir histologisch die Qualität des regenerierten Knochens und die Güte der Osseointegration der Implantate. Während den ersten 4 Wochen nahm dieses Verhältnis von 48.9% auf beachtliche 64.5% zu. Nach 48 Wochen erreichte dieses Verhältnis 75.5%. Der Knochen, der 48 Wochen belastet worden war, hatte einen starken Remodellations- und Reifeprozess durchgemacht; seine Quälitat war besser und ausgereifter als bei Knochen, der nicht belastet war. Diese Resultate zeigen, dass neu gebildeter Knochen (induziert von rhBMP-2) in der Lage war, einer funktionallen Belastung durch Zahnimplantate standzuhalten und gleichermassen funktionall ausreift, wie natürlicher Knochen.

Resumen

Se ha descubierto que las proteínas morfogenéticas son una de las sustancias osteoinductivas mas prometedoras y se espera que se utilicen clínicamente para la reconstrucción de mandíbulas defectuosas. De todos modos, no se ha probado que el hueso neoformado inducido por proteína-2 morfogenética recombinante de hueso humano (rhBMP-2) que sea capaz de soportar la fuerza masticatoria aplicada por implantes orales. En este estudio, hemos examinado los cambios cualitativos en la mandíbula inducida por rhBMP-2 de la fuerza funcional de implantes orales osteointegrados. Se crearon defectos óseos segmentales (30mm) en las mandíbulas de beagles. Se injertó una esponja de gelatina cubierta de ácido coglicolico poly D, L-láctico impregnada con rhBMP-2 a la mandíbula resecada. El nuevo hueso se formó 8 semanas tras la cirugía y se implantaron las fijaciones de sistema Brånemark en la mandíbula reconstruida. La prótesis se mantuvo en oclusión con la dentición natural oponente durante 0, 4, 12, 24 o 48 semanas antes de que el animal fuera sacrificado. Entonces evaluamos histológicamente la cantidad de hueso regenerado y medimos la índice de osteointegración entre los implantes orales y el hueso. Durante las primeras 4 semanas, el índice se incrementó del 48.9% al 64.5%. Tras 48 semanas, el índice se aproximó al 74.5%. El hueso cargado durante 48 semanas se sometió a un remodelado extensivo y a consolidación; su calidad mejoró y mas maduro que el hueso sin carga. Estos resultados indican que el hueso neoformado inducido por rhBMP-2 fue capaz de soportar la fuerza masticatoria aplicada por implantes orales y se convirtió en funcionalmente maduro como un hueso natural.

要旨

ヒト遺伝子組み換え骨誘導蛋白（rhBMP-2）は最も有望な骨誘導性材料のひとつであり、口腔外科や整形外科などでの臨床使用が期待されているしかし rhBMP-2 によって誘導された新生骨がはたしてインプラントによる咬合力に耐えうるか否かは未だ証明されていない。本研究では機能的荷重下で rhBMP-2 誘導骨に埋入されたインプラントとその骨性統合の定時的変化を検討した。方法としてビーグル犬の下顎骨に３０ｍｍの区域欠損を作成し、そこへ rhBMP-2 に PLGA ゼラチンスポンジを担体として移植した。術後８週で新生骨を認め、ブローネマルク・インプラントを誘導骨に埋入した。さらに８週後、インプラントに上部構造を装着した。上部構造は対合する天然歯と咬合するよう作製し、０、４、１２、２４、４８週間維持し、その後動物を安楽死させた。誘導骨の組織学的評価は、インプラントと誘導骨間の骨性統合率を測定した。最初の４週間にこの比率は４８．９％から６４．５％へ上昇していた。４８週間荷重した骨では、広範囲な改造とコンソリデーション（凝集）が起こり、その質は荷重しない骨より成熟していた。

これらの結果は、rhBMP-2 誘導骨はインプラントによる咬合力に耐え、天然骨として機能的に成熟したことを示唆している。

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