IRANIAN JOURNAL OF VETERINARY SURGERY (IJVS)
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Concurrent Use of Greater Omentum with Persian Gulf Coral on Bone Healing in Dog: a Radiological and Histopathological Study

Iraj Karimi[1], Amin Bigham-Sadegh[2], Ahmad Oryan[3], Mansour Dowlat abadi[4]

Abstract

Objective- To evaluate the role of greater omentum incorporation of coral in healing of the long bone defect in dog model.
Design- Experimental in-vivo study.
Animals- Sixteen adult mongrel male dogs weighing 26.2±2.5 kg, free of evident infectious or parasitic illnesses were used in this study.
Procedures- The operative procedure was undertaken under general anesthesia. Radial bone was exposed via a medial approach and a 10 mm transverse bone defect was created at mid-diaphysis with an electrical bone cutting saw. For omental free graft preparation, the abdominal cavity was approached through a 3 cm ventral midline incision, then the free end of the greater omentum was located and exteriorized from the abdominal cavity. A 30x30 mm piece of the omentum was isolated and harvested. In the control group (n=4), the defect was left empty. In the omental group (n=4) the defect was filled with the harvested omentum, in the omental-coral group (n=4) the defect was filled with omentum and a segment of coral. In the coral group (n=4) a segment of coral was implanted into the defected site. Finally, the injured radial bones were fixed with plate and screw. Radiographs of each forelimb were taken postoperatively on 1st day and at 30th and 60th post injury days to evaluate bone formation radiological criteria. The operated radial bones were removed on 60th postoperative day and were histopathologically evaluated.
Results-Compared to the control groups, more advanced bone healing criteria was present in the coral, omental and omental-coral groups at radiological and histopathological evaluation at 60th post-operative day.
Conclusion and clinical relevance- This study demonstrated favorable bone healing with the coral, omental and omental-coral in long bone defects in dog model.
Key words: Coral, Omentum, Bone healing, Dog model.

Introduction

Bone grafting is used to enhance healing in large bone defects resulting from trauma, tumors, osteitis, delayed unions, non unions, ostoectomies, arthrodesis, and multifragmentary fractures. [1-3] Autogenous bone still remains the "gold standard" of bone graft material in all facets of orthopedic surgery and is commonly used as a standard to which allografts and graft substitutes are compared. [4-9] As a graft, autogenous bone is ideal, but harvest of the autografts may be associated with severe donor site pain and morbidity even with new trapdoor harvesting techniques. [10,11] In procedures requiring large amounts of graft, there may not be adequate quantities of autogenous bone available.[11] Various bone graft substitutes including allografts, xenografts, polymers, ceramics and some metal have been employed to promote bone reunion.[12-16] They may provide a source of osteoprogenitor cells (osteogenesis), induce formation of osteoprogenitor cells from the surrounding tissues (osteoinduction), or provide mechanical support for vascular and bone in growth (osteoconduction).[17] Recently, osteoinductive stimulation of bone formation has received increasing interest.[18] Omentum is a very important element to supply vascularization to the implant.[19] Presence of abundant blood vessels in omentum is a good source of nutrients, oxygen, angiogenic and growth factors, and creates a proper microenvironment for tissue induction. [20,21] Appropriate

[1]Department of Veterinary Pathobiology, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
[2]Department of Veterinary Surgery and Radiology, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.
[3]Department of Veterinary Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.
[4]Graduated DVM
Address all correspondence to Dr. Mohammad Heidarpour (DVM, PhD), E-mail: irkarimi@yahoo.com

vascular flow of omentum increases the oxygen concentrations, and results in production of the osteoprogenitor cells from the perivascular mesenchymal cells. [20,22] Free graft of omentum has been used previously to promote healing of the long bone defects in rabbit [23] and dog [24] models and it has been shown that when the defect was grafted with the free transplant of the greater omentum the process of callus formation and its mineralization was much quicker than the control animals.

Certain coral species form a structure that resembles the matrix of bone. Each species builds a structurally and geometrically typical calcium carbonate skeleton. Choice of the appropriate species, therefore, enables a desired and constant implant structure to be achieved. More than 2000 coral species have been described from the intertropical area and, of these, fourteen corals have been studied as possible bone substitutes. The following genera have already been used as bone grafts: Pocillopora, Acropora, Montipora, Porites, Goniopora, Fungia, Polyphyllia, Favites, Acanthastrea, Lobophyllia and Turbinaria. [25] The most prominent species in terms of cover and frequency are *Porites lutea* and *P. compressa* in Persian Gulf and Kish Island.[26,27]

Calcium carbonate (CaCO3) resembles hydroxyapatite in many respects. The material is biocompatible and osteoconductive but, similar to hydroxyapatite, has no osteoinductive properties.[28] The main difference of CaCO3 with hydroxyapatite is its resorption rate. Resorption seems to be clinically unimportant with hydroxyapatite, but the animal experiments have shown resorption rates of only a few weeks, when calcium carbonate has been used.[29] In this study, role of the greater omentum incorporation of calcium carbonate was evaluated in healing of a long bone defect in a dog model. According to the authors' knowledge, this is the first report in which omentum has been used concurrent with calcium carbonate for bone healing promotion.

Material and Methods

Animals

Sixteen adult mongrel male dogs weighing 26.2±2.5 kg, free of evident infectious or parasitic illnesses were used in this study. The experimental protocol was approved by the Animal Care and Experiment Committee of the University, in accordance with the ethics standards of the "Principles of Laboratory Animal Care".[30]

Preparation of coral implants

Coral exoskeleton from Porites sp. (Persian Gulf, Kish Island, Iran) was used in the form of cylindrical blocks of 4 mm in diameter and 10 mm long. The coral implants were sterilized by autoclaving so that the composition remained intact.[31] The implants were prepared as segmented cone shape to allow them to fill the created defects.

Surgical procedure and study design

The dogs were sedated with acepromazine (0.05 mg/kg subcutaneously) and anesthesia was induced with ketamine (10 mg/kg intravenously) and diazepam (0.25 mg/kg intravenously). The animals were intubated and 1% to 2% halothane was used to maintain anesthesia with spontaneous breathing. Saline (10 ml/kg per hour) was substituted through an intravenous catheter. The operative procedure was undertaken under general anesthesia. In all dogs, the left forelimb from the olecranon region to the metacarpal region and ventral abdomen from umbilicus to the pelvic inlet were prepared for aseptic surgery. Radius was exposed via a medial approach and a 10 mm transverse bone defect was created at mid-diaphysis with an electrical bone cutting saw.

For omental free graft preparation, the abdominal cavity was approached through a 3 cm ventral midline incision, midway between the umbilicus and pelvic inlet; then the free end of the greater omentum was located and exteriorized from the abdominal cavity (omentum was exposed and the abdominal cavity was manipulated in all animals even controls). A 30x30 mm piece of the omentum was isolated by two catgut ligatures and cut free from the remaining body of this structure.

In the control group (n=4), the defect was left empty. In the omental group (n=4) the defect was filled with the harvested omentum, in the omental-coral group (n=4), the defect was filled with a segment of coral that covered with harvested omentum. In the coral group (n=4) the defect was filled only with coral segment. Finally, the injured radial bone was fixed with plate and screw.

The surgical sites were closed routinely. All dogs received sodium ampicillin (22 mg/kg, intravenously, every 6 hours), and gentamicin sulfate (4 mg/kg, intravenously, every 24 hours) for five consecutive post-operative days. Tramadol (0.2 mg/kg, IM) was administered every 6 hours after surgery for 24 hours and as needed thereafter to control pain and discomfort.

Post operative evaluations

Radiological evaluation

To evaluate bone formation, union and remodeling of the defect, radiographs of each forelimb was taken postoperatively at 1st and then 30th and 60th post injury days. The results were scored using the modified Lane and Sandhu scoring system (Table 1).[32] When new bone was formed in the defected area, coral and also covered coral were surrounded by new bone that was considered as bone formation criteria in evaluation. Any bone

formation between coral and proximal segment of the radius bone was considered as the proximal union and any bone formation between the coral with the distal segment of the radius bone was considered as the distal union.

Table 1. Modified Lane and Sandhu radiological scoring system

Bone formation	
No evidence of bone formation	0
Bone formation occupying 25% of the defect	1
Bone formation occupying 50% of the defect	2
Bone formation occupying 75% of the defect	3
Bone formation occupying 100% of the defect	4
Union (proximal and distal evaluated separately)	
No union	0
Possible union	1
Radiographic union	2
Remodeling	
No evidence of remodeling	0
Remodeling of medullary canal	1
Full remodeling of cortex	2
Total point possible per category	
Bone formation	4
Proximal union	2
Distal union	2
Remodeling	2
Maximum Score	10

Histopathological evaluation

Sixty days after operation all the dogs were euthanized for histopathological evaluation. The left forelimb was harvested and dissected free of soft tissues. Sagittal sections containing the defect were cut with a slow speed saw. Each slice was then fixed in 10% neutral buffered formalin for twenty days and formalin was changed first after 24 hr and then with ten days interval. The formalin-fixed bone samples were decalcified in 15% buffered formic acid solution for one month and processed for routine histological examination. Two 5 µm in thickness sections were cut from the centers of each specimen and were then stained with Hematoxylin and Eosin and were blindly evaluated and scored by two pathologists according to Heiple's scoring system (Table 2).[33]

Statistical analysis

The radiological and histopathological data were compared by Kruskal-Wallis, non-parametric ANOVA. When *P-values* were found to be less than *0.05*, then pair wise group comparisons was performed by Mann-Whitney U test (SPSS version 17 for windows, SPSS Inc, Chicago, USA)**.**

Table 2. Heiple histopathological scoring system

Union (proximal and distal evaluated separately)	
No evidence of union	0
Fibrous union	1
Osteochondral union	2
Bone union	3
Complete organization of shaft	4
Cancellous bone	
No osseous cellular activity	0
Early apposition of new bone	1
Active apposition of new bone	2
Reorganizing cancellous bone	3
Completely reorganization cancellous bone	4
Cortical bone	
Non	0
Early appearance	1
Formation under way	2
Mostly reorganized	3
Completely formed	4
Marrow	
None is resected area	0
Beginning to appear	1
Present in more than half of the defect	2
Complete colonization by red marrow	3
Mature fatty marrow	4
Total points possible per category	
Proximal union	4
Distal union	4
Cancellous bone	4
Cortical bone	4
Marrow	4
Maximum score	20

Results

There was no intraoperative and postoperative death during the study. None of the dogs sustained a fracture of the radius bone.

Radiological findings

Radiological evaluation showed significant differences ($P<0.05$) between the control group with all other groups at 30^{th} and 60^{th} postoperative days so that the lesion in the control group was significantly inferior to other three groups. There were no significant differences ($P>0.05$) between other three groups throughout the study (Fig.1, Table 3).

Histopathological findings

As shown in table 4 there was significant differences between the lesions of the animals of the control group

with those of all other groups so that those of the control group were inferior to all other groups. As shown in Fig. 2, by the end of 60 days post-surgery, histological examination demonstrated presence of the regenerated bone with typical structure of trabecular bone in the defect site of the omental, coral and omental-coral groups. In contrast, weak osteogenesis activity could be found with fibrous and fibrocartilage tissues in the control defects (Fig.2, Table 4).

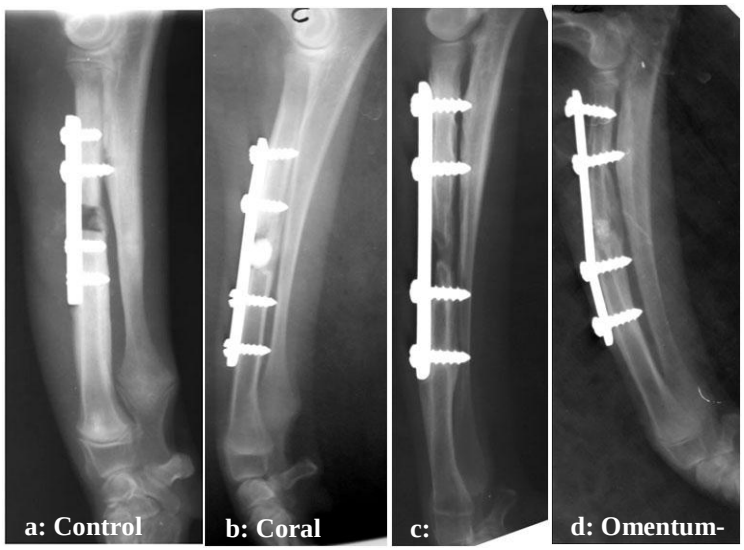

Figure 1. Radiological evaluation on the 60th postoperative day, a) control group, b) coral group, c) omental group d) omentum-coral group

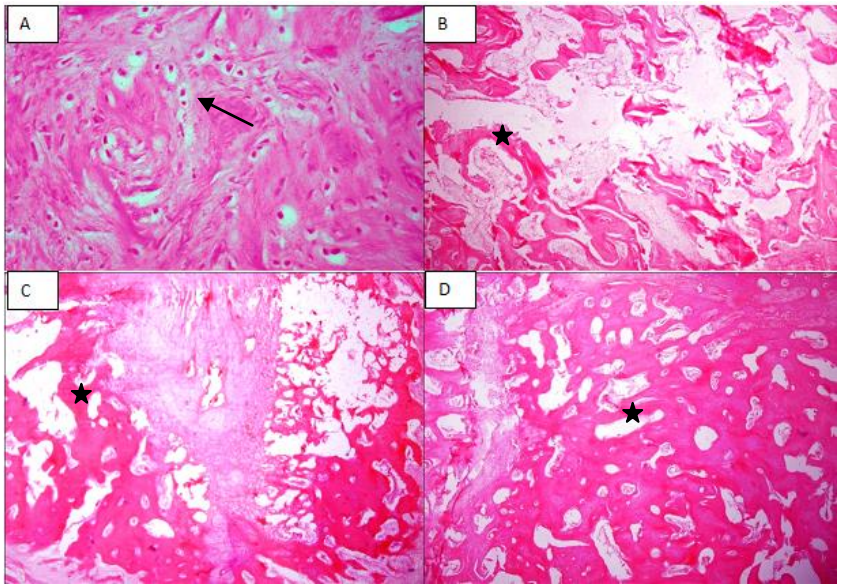

Figure2. At 60th post-operative day, the histological examination demonstrated the regenerated bone with typical structure of the trabecular bone formation (*) in the defect at the experimental side of the omentum (B), omentum-coral (C) and coral (D) groups (H&E staining, 10X). In contrast, weak osteogenesis activity could be found with least bone formation, and high fibrous connective tissue and fibrocartilage (→) at the control defect (A) (H&E staining, 40X)

Table 3. Radiographical scoring for bone healing at various post-operative intervals

Med (min-max)					P[a]
Postoperative days	Control (n=4)	Omentum (n=4)	Coral (n=4)	Omentum-coral (n=4)	
30	1(2-3)[b]	3(2-5)	3(3-6)	3(2-5)	**0.02**
60	4(3-6)[c]	5(4-8)	5(4-8)	5(4-9)	**0.03**

Significant *P-values* are presented in bold face.
[a] Kruskal-Wallis non-parametric ANOVA
[b] There were significant differences between the omentum (*P= 0.03*), coral (*P= 0.01*), omentum-coral (*P=0.03*) groups with the control group and the control group was significantly inferior to other groups.
[c] There were significant differences between the omentum (*P= 0.02*), coral (*P= 0.02*), omentum-coral (*P=0.02*) groups with the control group and the control group was significantly inferior to other groups.

Table 4. Histopathological scoring (sum of histopathological criteria) for bone healing at various groups

Med (min-max)					P[a]
	Control (n=4)	Omentum (n=4)	Coral (n=4)	Omentum-coral (n=4)	
Sum of histopathological criteria	4(4-6)[b]	9(6-11)	10(7-12)	9(6-12)	**0.04**

Significant *P-values* are presented in bold face.
[a] Kruskal-Wallis non-parametric ANOVA
[b] There were significant differences between the omentum (*P= 0.02*), coral (*P= 0.02*), omentum-coral (*P=0.02*) groups with the control group and the control group was significantly inferior to other groups.

Discussion

In this study, coral, omental and omental-coral groups demonstrated favorable osteogenic potential in healing of the radial bone defect in dog model. The radiological and histological findings of the present study indicate a superior bone healing capability in the coral, omental and omental-coral groups, by the end of 60 days post-surgery, in comparison to the control group. Different osteotomy/ectomy models have been described in dogs with good results. The long history of the use of dogs in orthopedic research has resulted in a formidable bank of comparative data, and sometimes the food and drug administration (FDA), or other regulatory agencies may request that efficacy or safety studies to be performed in dogs.[34] In the present study the authors used large animal model such as canine model to evaluate this bone healing model because sufficient amount of adipose tissue from omentum are easily and reliably accessible and have resilience throughout operation and display good postoperative recovery very similar to that observed clinically in humans.

According to this study on radiological and histopathological findings, significant difference was not observed between natural coral and omentum or omentum-coral and all the three treatment regimens led to bone formation in a similar way. It has previously been shown that the natural coral ($CaCO_3$) resembles hydroxyapatite in many aspects. The material is biocompatible and osteoconductive but, similar to hydroxyapatite, has no osteoinductive properties.[35] The main difference of coral and hydroxyapatite is their chemical composition, and while hydroxyapatite is mainly composed of calcium phosphate the coral's chemical composition is calcium carbonate.[28,29,36] The authors proposed that in the present study, the osteoconductive properties of natural coral lead to superior bone formation.

Omentum has been considered as a major source of supplying new vascularization into the implants.[19] Proper vascularization by the omentum provided a valuable source of nutrients, oxygen, and angiogenic and growth factors, and created a proper microenvironment for this graft implantation, further tissue maturation and bone formation.[20,21] A sufficient vascular flow effectively increases the oxygen concentration, and induces production of the osteoprogenitor cells from the perivascular mesenchymal cells.[20,22] During angiogenesis, the vascular endothelial growth factor (VEGF) increases the capillary permeability, supplies hormones and growth factors[37] and maintains high levels of oxygen

concentration, and all these mechanisms[21] have possibly been effective in the ossification process of the graft area of the animals of the present study. The healing response in the bone defects of the omentum and omentum-coral groups, in the present study, were superior to the control group, however, the lesions of the omentum and omentum-coral groups were almost same as the coral group. The authors propose that the coral that has been covered with omentum and the omentum obscured osteoconductive properties of coral, so omentum and omentum-coral act in the same way for bone formation process.

Based on the radiological and histopathological findings of the present study, healing of the defects of the animals of the control group was not very efficient and the defect area was filled with fibrous connective tissues and rarely with cartilage instead of osseous tissue. Barnes et al indicated that the chondrocytes derived from the mesenchymal progenitors proliferate and synthesize cartilaginous matrix until all the fibrous granulation tissue is replaced by cartilage. Where cartilage production is deficient, fibroblasts replace the region with generalized fibrous connective tissue. Discrete cartilaginous regions progressively grow and merge to produce a central fibrocartilaginous plug between the fractured fragments that splints the fracture.[38]

Histopathological evaluation did not show any significant differences after 60 days, in the present study, between the coral, omentum and omentum-coral groups; however, statistical differences between different groups were expected. Probably there have been earlier differences between the histological features of the lesions of the different groups in over the various intervals; however, the authors did not perform histopathological evaluation at the earlier postoperative intervals because of the ethic committee's limitations. Therefore, histopathological studies of such lesions at earlier stages of fracture healing are highly recommended. Such studies could include the inflammatory, proliferative and remodeling phases of fracture healing and investigate the type of inflammatory cell constituents, osteoblasts proliferation and maturation, angiogenesis, total collagen and collagen typing, presence or absence of cartilaginous or osseous material, quantity and quality of mineralization and many other criteria.

Conclusion

In conclusion this study demonstrated favorable bone healing with the coral, omental and omental-coral in long bone defects in dog model.

Acknowledgements

The authors would like to thank the authorities of the Veterinary School, Shahrekord University for their financial support and cooperation.

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نشریه جراحی دامپزشکی ایران
سال ۲۰۱۳، جلد ۸ (شماره ۲)، شماره پیاپی ۱۹

چکیده

استفاده همزمان چادرینه بزرگ و مرجان خلیج فارس بر روی ترمیم نقیصه استخوانی سگ: مطالعه رادیوگرافیکی و هیستوپاتولوژیکی

ایرج کریمی*[۱]، امین بیغم‌صادق[۲]، احمد عریان[۳]، منصور دولت آبادی[۴]

[۱] گروه پاتوبیولوژی، دانشکده دامپزشکی دانشگاه شهرکرد، شهرکرد، ایران.
[۲] گروه علوم درمانگاهی، دانشکده دامپزشکی دانشگاه شهرکرد، شهرکرد ، ایران.
[۳] گروه پاتوبیولوژی، دانشکده دامپزشکی دانشگاه شیراز، شیراز، ایران.
[۴] دانش‌آموخته دامپزشکی، دانشکده دامپزشکی دانشگاه شهرکرد، شهرکرد، ایران.

هدف- ارزیابی استفاده توام مرجان و امنتوم در روند التیام شکستگی استخوان در سگ.
طرح مطالعه- مطالعه تجربی در حیوان زنده.
حیوانات- ۱۶ قلاده سگ نر نژاد مخلوط با وزن ۲۶/۲±۲/۵ کیلوگرم عاری از هرگونه بیماری و عفونت در این مطالعه مورد استفاده قرار گرفت.
روش کار- حیوانات با بیهوشی عمومی بیهوش شده و ناحیه ساعد برش پوست داده شده و یک نقیصه استخوانی به طول ۱۰ میلیمتر درست در وسط استخوان زند زبرین (رادیوس) ایجاد شد. در گروه کنترل (۴ قلاده) نقیصه ایجاد شده خالی رها شد، در گروه امنتوم (۴ قلاده) نقیصه ایجاد شده با قطعه ای از امنتوم که قبلا برداشت شده بود پر گردید، در گروه مرجان نقیصه (۴ قلاده) ایجاد شده با قطعه ای از مرجان استریل شده پر شد و در گروه امنتوم-مرجان (۴ قلاده) نقیصه ایجاد شده با مرجان پوشیده شده با امنتوم پر شد. رادیوگرافها بلافاصله بعد از عمل ، ۱ماه بعد از عمل و ۲ماه بعد از عمل جهت ارزیابی شکل گیری استخوان ، جوش خوردگی و بازآرایی محل نقیصه گرفته شدند. تمامی حیوانات در روز ۶۰ بعد از عمل به روش انسانی معدوم شدند و استخوانهای رادیوس عمل شده خارج گردیده وجهت بررسی هیستوپاتولوژی در فرمالین ۱۰٪ قرار داده شدند.
نتایج- این مطالعه نشان داد از نظر درجه بندی التیام شکستگی در ارزیابی رادیولوژیکی و هم در ارزیابی هیستوپاتولوژیکی گروههای مرجان، امنتوم و مرجان-امنتوم قویتر از گروه کنترل عمل کرده بودند.
نتیجه گیری و کاربرد بالینی- نتایج این مطالعه حاکی از التیام مطلوب استخوان با استفاده از امنتوم،مرجان و مرجان امنتوم بوده است.
کلید واژگان- مرجان، امنتوم، التیام استخوان، مدل حیوانی سگ.