

Histopathologic evaluation of the Persian Gulf coral powder effect on bone defects

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Received: 05/27/2012

Revised: 12/24/2012

Accepted: 03/06/2013

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Journal of Jahrom University of Medical Sciences, Vol. 11, No. 2, Summer 2013

J Jahrom Univ Med Sci 2013; 11(2): 14-18

Abstract:

Introduction:

The research on the marine products and creatures, in particular coral, has started during the recent decade, confirming the beneficial effects of this material. Therefore, this study aimed to use the coral native to Persian Gulf as oral powder to heal the tibial bone defect in rabbits.

Materials and Methods:

In this experimental study, 18 New Zealander rabbits were randomly categorized into 3 groups (control group, the group which used oral calcium powder and the one which used oral coral powder) of 6 rabbits. For producing the defect, 1/3 of the tibial bone was blunt dissected and the operation site was observed. Using an orthopedic drill, a hole with the depth of 0.6-0.8 mm and diameter of 4 mm was produced and after emptying and washing, the operation site was sutured. The operation was performed on all the 3 groups similarly. The calcium group was treated using 1150 mg calcium powder daily, the coral group received 1220 mg of coral powder on a daily basis, and the control group was kept under standard condition. On days 10, 20, 30, 40, 50, 63, the cases were evaluated and pathological studies were performed.

Results:

In the histopathologic study, fibroblastic proliferation, new bone formation, bone maturation and mineralization were evaluated; the results for the coral group were better than those of the calcium and control groups ($P < 0.05$).

Conclusion:

In conclusion, in the histopathologic evaluation of Persian Gulf coral, it was found to be an osteoconductive biomaterial which act as a scaffold and consequently could be used for healing a segmental tibial defect by increasing the bone formation.

Keywords: Coral, Bone Grafting, New Zealand Rabbit, Biomaterials

Introduction

In recent years, use of corals has attracted huge interest among researchers because they are a rich source of calcium and other minerals. According to the type, species, and volume of holes, corals have obvious biomechanical characteristics. Corals are made up of minerals, but they also contain some amino acids in their structure (1-5).

There are differences between corals and bones. 2/3 of the bone composition consists of minerals, which is essentially in the calcium phosphate form, and the remaining 1/3 is the organic part, which reduces amino acids a little in corals (1, 6). Considering that in all previous studies, corals of the Okinawa region in Japan were used, and that a specific type of

corals is cultured in the laboratory under special echo-system for use as an ideal biocompatible material, due to the presence of a number of coral reefs in the Persian Gulf, it was decided to conduct a preliminary study on the native Iranian corals to assess their potential as a biocompatible material (2).

In studying Persian Gulf corals, it was found that there is 94.73% calcium carbonate in their structure, and that due to the structure and porosity level, there are obvious differences between various species. In the islands of Kish, Nayerbandbay, and Farver, there are over 27 species of corals, and most important ones are *Akrovia*, *Poritida*, and *Favida*. In this study, the *Poritida* species was used in the powder form (7-8).

Given the lack of documented studies on the Persian Gulf corals in oral applications, it was decided to conduct preliminary studies on this species of corals as a biocompatible material to prepare the path for their application in human in future studies (9).

Materials and Methods

A total of 18 heads of white New Zealand rabbits, weighing approximately 2.5-3 kg, were randomly divided into 3 groups of 6, including control, oral calcium, and oral coral powder. All rabbits were coded with numbered tags in the ear for later studies. Occurrence of any unexpected event during anesthesia and surgery, bone fracture during surgery, making a hole both sides of the bone, and enlarged hole diameter were considered as study exclusion criteria.

In this study, after necessary calculations, 1150 mg calcium bicarbonate tablets, and 1220 mg Persian Gulf *Poritida* species of corals in powder form were administered once daily.

Pre-op preparation was made with intramuscular injection of *Aspiromycin* at a rate of 0.5-1 mg for every kilogram weight. Then, the rabbits, were prepared for surgery lying on the side, and

restrained to bed, and after shaving the hairs from top of the knee joint down to the ankle, conventional surgical procedures of disinfection and preparation were carried out. For general anesthesia, 20-50 mg of ketamine, 0.5-1 mg *Aspiromycin*, and 0.1-0.5 mg *Atropine sulfate*, (each calculated according to body weight) were administered IM prior to surgery.

Surgical procedure and creating hole-shape defect in the tibia bone: In the intended groups, the right leg was used for making the defect. First, a 3 cm lateral blunt dissection was made from below the knee joint to mid way down to the tibia, and the tibia bone was exposed without incising muscles, and by pulling the skin aside. The aim of choosing this procedure was to cause fewer traumas in the muscle and prevent bleeding.

Then, with the aid of an orthopedic drill, a hole of 0.6-0.8 mm depth and 4mm diameter was drilled into the upper 1/3 part of the tibia, and after removing bone chippings and washing out the defect, the skin was sutured by 3-0 nylon using a simple interrupted suture pattern. After the surgery, rabbits were coded by numbered tags for later studies (figure 1).

Wide spectrum cephalosporin injections were administered at a dose of 5-15 mg per kilogram body weight every 12 hours for 5 days. In addition, 2 mg *Gentamycin*, based on body weight, was also injected for 5 days.

After the surgery, rabbits in different groups of control, calcium, and coral were separately and individually caged, and fed twice daily with pellet-shaped portions. Water was made constantly available to the rabbits during this period.

On the days 0, 10, 20, 30, 40, 50, and 63, one rabbit was randomly selected and slaughtered according to animal rights protocols (figure 2) in order to separate the tibia bone and prepare suitable samples for histopathological evaluation.

Assessment of bone healing parameters in coral, calcium, and control groups was

performed in pathological slides according to tissue fibrosis (fibroblastic proliferation), new bone formation and maturation, and bone mineralization process. Scoring was expressed for the extensive state as 4, for severe state as 3, moderate as 2, mild as 1, and nothing as 0. The one-way variance analysis and Cheffe tests were used for statistical analysis of data.

Results

Histopathological results of slides for coral, calcium, and control groups on days

0, 10, 20, 30, 40, 50, and 63 are presented in images 3 to 6. It should be noted that, for each of the groups on these days, one H&E stained slide, and one tri-chrome-mason stained slide were provided.

In assessing healing parameters of bone mineralization process, new bone formation, bone maturation, and overall evaluation; coral group showed better results than the other two, and calcium and control groups did not significantly differ from each other on this day. Additionally, fibroblastic proliferation was not observed in any of the groups (tables 1 and 2).

Table 1: Evaluation of bone healing parameters in the coral, calcium, and control groups on day 10

Row	Parameter	Sample number	Treatment group	Fibroblastic proliferation	New bone formation and maturation	Mineralization	Overall evaluation
1-10		10v-	Coral	3.5	1.75	1	2
2-10		10f-	Calcium	2.75	1.25	0.5	1.5
3-10		10k-	Control	3	1.25	0.5	1.5

Table 2: Evaluation of bone healing parameters in the coral, calcium, and control groups on day 63

Row	Parameter	Sample number	Treatment group	Fibroblastic proliferation	New bone formation and maturation	Mineralization	Overall evaluation
1-63		63O-	Coral	0	4	3.5	4
2-63		63G-	Calcium	0	3	3	3
3-63		63L-	Control	0	3	3	3

From the 10th to 63rd day, relative superiority of coral group, compared to the other two was maintained. In statistical analysis of healing parameters, no significant difference was observed between the 3 groups ($P=0.0549$). However, relative superiority of coral group compared to calcium and control

groups was notable (table 3). In general view of the statistical results, the difference in bone healing parameters in the 3 groups of coral group, control, and calcium was insignificant, but relative superiority of coral group, compared to the other two was fully evident.

Table 3: Mean and standard deviation of the 4 pathological healing parameters

Group		Overall evaluation	Fibroblastic proliferation	New bone formation and maturation	Bone mineralization process
Coral	Mean	2.9583	1.125	2.9583	2.42
	Standard deviation	0.92759	1.4642	1.00519	0.970
Calcium	Mean	2.2917	1.000	2.2500	2
	Standard deviation	0.71443	1.1726	0.77460	1.049
Control	Mean	2.2083	0.917	2.1667	1.92
	Standard deviation	0.71443	1.2007	0.76920	1.068
Total	Mean	2.4861	1.014	2.4583	2.11
	Standard deviation	0.82012	1.2111	0.88388	0.993

Discussion

In 1987, Harvey, Zobitz, and Pok, investigated 5 methods to compare calcium-carbonate and calcium-citrate in adsorption based on dosage of calcium (10-11). In this study, calcium-citrate showed better adsorption compared to calcium-carbonate. In a similar study by Brudos, Dominges, Burgh, and Van ducam, similar results were obtained (12-15).

In 1997, Suzuki conducted experiments on rats to discover capabilities of Rioquan species of corals as an alternative to calcium. This coral is formed by calcium and magnesium with a 2:1 ratio. In a 4-week study on rats, he fed the rats with this type of coral, and measured calcium balance in their excrements and urines in the last 3 days. He arrived at the conclusion that Rioquan species of coral as a source of calcium is much more satisfactory than calcium carbonate (9).

In another study, Loko Takora, Konihiko Ishtanio et al. tested calcium carbonate and Rioquan type coral on six men and six women. The 12 were divided into 3 groups of oral coral, oral calcium carbonate, and control. Intestinal adsorption level of calcium derived from coral and calcium derived from calcium carbonate was studied through increased calcium level in urine. In urine, calcium level from coral was evidently higher than calcium from calcium carbonate, which indicates better

intestinal adsorption of calcium from coral compared to calcium from calcium carbonate (16).

In the present study, higher levels of magnesium (36 mg) with coral, and lower levels of magnesium (6 mg) with calcium were used. This showed the 2:1 ratio of calcium from coral, and magnesium is much more effective in coral adsorption than calcium carbonate (16).

Despite the similarity in method, in a study conducted, titled "oral absorption of calcium from Rioquan coral", intestinal absorption of derived calcium is measured through increased calcium level in urine. But, in the present study, coral absorption in the bone was evaluated by measuring histopathological healing parameters. In the present study, comparison method was similar to the study with Rioquan species.

Through study of coral absorption process in the body, it is found that oral method is less used. However, there have been many studies on Akroporida, Poritida, and Favida species planted in long bones, skull, and maxillofacial. Only two studies have used Poritida and Rioquan species in oral form. The interesting and new point that distinguishes this study from others is the investigation of histopathological effect of oral use of Persian Gulf coral, which is unlike other studies. In assessment of results obtained in this study, mineralization, new bone formation,

and bone maturation are observed with passage of time.

Corals are the best form of minerals that preserve alkaline state of the body, and have a significant role in preservation and strength of bones. These minerals are transformed into crystal compositions by the organisms present in the sea called pulp. When pulps die, they leave natural elements including calcium, magnesium, zinc, selenium, and large amounts of minerals (1-2). This organized structure of mineral compositions gives them a unique biological activity. These are the only minerals that can be absorbed in solution form both in water and in fat (1-2). In corals, minerals exist in ionized form, which means that they are rapidly absorbed, and establish alkaline state. Corals absorption is nearly 70%, which is

almost twice as much as calcium absorption (1-2).

Conclusion

Given the results obtained from histopathological investigations in present study, speed and quality of bone formation in tibia bone defect in rabbits with the Persian Gulf corals is indicative of its potential characteristics for use in pharmaceutical products. To achieve this, more comprehensive and accurate studies of different species of corals are required.

Acknowledgements

The authors wish to thank Dr Abbass Vishkini and Dr Reza Sedaghat for their help in different stages of the study.

Conflict of interest

The authors declare no conflict of interest in this study.

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