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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

TAVŞAN OMURGASINDA ANTERİOR BODY KAFES BORON UYGULAMASININ KEMİK FÜZYONUNA VE FÜZYON KALİTESİNDE ETKİSİ

THE EFFECT OF ANTERIOR INTERBODY CAGE BORON APPLICATION IN RABBIT VERTEBRAE ON BONE FUSION AND FUSION QUALITY

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ÖZET

AMAÇ: Bu çalışmadaki amacımız, Anterior Lombar Interbody Fusion (ALIF) kullanılan operasyonlarda hidroksiapatit (HA) ve Boron bileşiklerinin etkinliğini göstermektir.

GEREÇ VE YÖNTEM: Çalışmada 2.5-3 kg ağırlıkta 18 adet Yeni Zelanda ırkı erkek tavşan kullanıldı. Sol tarafa yatırılan hayvanlarda cerrahi temizlik sonrası flank kesi yapıldı. Lomber retroperitoneal bölgeden lomber 5-6 mesafesine ulaşıldı. Anterior diskektomiyi takiben; kontrol grubuna PEEK (polietilen eter keton) kafesi (Grup I), PEEK kafesi ve 50 mg/kg boron (boraks pentahidrat) (Grup II) yerleştirildi ve HA grubuna PEEK kafes ve 50 mg/kg toz ProOsteon (Grup III) kullanılarak ALIF yapıldı. ALIF operasyonu tüm gruplara uygulanmıştır. Altı hafta sonra, hayvanlara bilgisayarlı tomografi (BT) taraması yapıldı. Daha sonra sakrifiye edilen hayvanların diskektominin yapıldığı ve kafesin yerleştirildiği mesafenin alt ve üst omurları çıkarılarak patoloji için ayrıldı. Bir gece tamponlu %10 formaldehit ile mikroskobik olarak tespit edildikten sonra rutin takip edilen örnek dokulardan hazırlanan kesitler hematoksin-eozin ile boyandı ve ışık mikroskobu ile histopatolojik olarak incelendi.

BULGULAR: Bor grubunun %83.3' ünde osteoklast skoru 1 iken %16.6' sında 2 olarak saptandı. Aynı grupta osteoblast skoru % 50' sinde 1, % 50'sinde 2 olarak bulundu. HA grubunun osteoklast skoru %50' sinde 1, %50' sinde 2 saptanırken, osteoblast skoru %50, 2' sinde 1, %16,6' sında 2 ve %33,4' ünde 3 bulundu. HA grubunda Boron ve kontrol grubuna göre daha yüksek osteoblast ve osteoklast skorları gözlendi. Bor grubunda kontrol grubuna göre yüksek HA grubuna göre daha düşük osteoblast ve osteoklast skoru olduğu görüldü.

SONUÇ: Boron ve Hidroksiapatitin aynı büyüklükteki taşları oluşturduğu gösterilmiştir. Morfolojik olarak boron hidroksiapatit kadar olmasa da histopatolojik inceleme ile kontrol grubuna göre daha iyi miktarda füzyon oluşturduğu görülmüştür. Osteoblastik aktivite en sık hidroksiapatit grubunda görüldü.

ANAHTAR KELİMELER: Füzyon, Boron, Hidroksiapatit, Lomber vertebra

ABSTRACT

OBJECTIVE: Our aim in this study was to demonstrate the effectiveness of hydroxyapatite (HA) and Boron compounds in operations using an Anterior Lumbar Interbody Fusion (ALIF).

MATERIAL AND METHODS: In the study, 18 male rabbits of New Zealand breed weighing 2.5-3 kg were used. The animals who were hospitalized on the left side underwent flank incision after surgical cleaning. Lumbar 5-6 distance was reached from the lumbar retroperitoneal region. Following anterior discectomy; ALIF was performed using PEEK (polyethylene ether ketone) cage (Group I) in the control group, PEEK cage and 50 mg/kg boron (borax pentahydrate) (Group II) in the boron group, PEEK cage and 50 mg/kg powder ProOsteon (Group III) in the HA group. The ALIF operation has been applied to all groups. Six weeks later, the animals underwent a computed tomography (CT) scan. Then the lower and upper vertebrae of the sacrificed animals at the distance at which discectomy was performed and the cage was placed were removed, separated for pathology. After being microscopically detected with buffered 10% formaldehyde overnight, the sections prepared from the sample tissues that were routinely followed were stained with hematoxin-eosin and examined histopathologically with light microscopy.

RESULTS: While the osteoclast score was 1 in 83.3% of the boron group, it was 2 in 16.6%. In the same group, the osteoblast score was found to be 1 in 50% and 2 in 50%. The osteoclast score of the HA group was 1 in 50%, 2 in 50%, while the osteoblast score was found in 50%, 1 in 2, 2 in 16.6%, and 3 in 33.4%. Higher osteoblast and osteoclast scores were observed in HA group compared to Boron and control groups. It was observed that the boron group had higher osteoblast and osteoclast scores than the control group and lower than the HA group.

CONCLUSIONS: Boron and Hydroxyapatite have been shown to form stones of the same size. Although morphologically not as much as boron, hydroxyapatite, histopathological examination showed that it formed a better amount of fusion compared to the control group. The osteoblastic activity was most common in the hydroxyapatite group.

KEYWORDS: Fusion, Boron, Hydroxyapatite, Lumbar vertebrae

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INTRODUCTION

The treatment of defects caused by column vertebrates due to trauma, infection, fractures, tumor resection, congenital anomalies continues to be one of the main problems of neurosurgeons despite today's wide possibilities. Anterior interbody cage and fusion are commonly used in most spinal diseases in the treatment of spinal diseases. Various biomaterials were used in interbody fusion in collaboration with bone grafts. In this regard, autologous bone grafts have been accepted as the gold standard and used frequently. Autologous bone intake may cause conditions that increase morbid ability such as additional incisiveness, prolonged operation, postoperative pain and infection. Due to such complications and comorbid situations, it is aimed to use synthetic bone grafts instead of autologous bone grafts (1). Synthetic bone graft and fusion applications applied then applied increasingly frequently and pseudoarthrosis rates ranging from 3% and 36% were reported (2). Problems such as deterioration of the shape of the spinal colon, instability, pain, and potential neurological deficits may occur in patients who develop pseudoarthrosis (3, 4). Over the years, neighboring segment disease, reassuring disorders, inadequate fusion, and morbidity brought on by surgery appear as important problems (5). So far, various bone grafts have been tried, but no perfect graft material has been found yet.

MATERIALS AND METHODS

In the study, 2.5-3 kg of body weight, 18 male New Zealand breed rabbits were used. The animals were kept in single cages in heat, light, and air-controlled environments, fed for two weeks with standard feed and fountain water. In the study, the subjects were divided into three groups.

General anesthesia was applied to the subjects with 100 mg/kg intramuscular Ketalar via injection. The subjects who were admitted to the left side were made flank include after surgical cleaning. Lumbar 5-6 distance reached from the retroperitoneal area. Following anterior discectomy; ALIF was performed using PEEK (polyethylene ether ketone) cage (Group I) in the control group, PEEK cage and 50 mg/kg boron (borax pentahydrate) (Group II) in the boron group, PEEK cage and 50 mg/kg powder ProOsteon (Group III) in the HA group. The ALIF operation has been applied to all groups.

Since boron rabbit spine was not applied locally before, 2, 5, and 10 hydroxyl group boron materials were looked at as in vivo how thawed they were in the water. It was seen that the boron composition with 2 hydroxyl groups quickly and completely dissolved, the boron composition with 10 hydroxyl groups did not dissolve, and the boron composition with 5 hydroxyl groups slowly dissolved in the water and remained a concentrated solution. Due to this feature, the borax pentahydrate compound with 5 hydroxyl groups was used. Since there were not enough subjects to dosing, the highest dose of boron was used that would not have a toxic effect (6, 7). A vertical PEEK Interbody cage was used in all groups. Cage dimensions were 3x4x2 mm in size.

During the postoperative period, subjects were given standard feed and fountain water and observed for six weeks. Six weeks later, the animals underrated a spiral CT scan. Then the lower and upper vertebrae of the distance where the discectomy of the sacrificed animals was carried out and the cage was placed were removed and separated for pathology.

In our research, the toxic properties of the pipe were not evaluated.

After being detected microscopically with 10% formaldehyde with tampons one night, the sections prepared from the sample tissues that followed routinely were painted with hemotoxin-eosin and histopathologically examined with the light microscope.

Ethical Committe

This study was approved by Afyon Kocatepe University Animal Ethical Committee 04.05.2009 / B.30.02.AKÜ.0.8Z.00.00/52 and informed consent were obtained from all the participants.

Statistical Analysis

Were performed using IBM SPSS Statistics, version 22.0 (IBM Corp., New York, USA). Associations between all assessed variables were analyzed using Student T and Mann-Whitney methods.

RESULTS

Surgical intervention was performed on 18 subjects. Cage was placed on 6 of the animals, cage, and Boron on 6 and cage and HA in the remaining 6. In the control group, a postoperative left leg was temporarily paralyzed. In the boron group, the wound site of two animals was opened and debrided.

All animals were anesthetized with Ketalar IM and tomography was performed with Philips Brillance 4-row multislice tomography device. During tomography, images were taken with 1 mm sections. All subjects showed bone callus forming and their size was calculated. In the boron group, changes were seen in the callus, giving the appearance like septa. In **Figures 1**, pictures of computed tomography of the Boron and HA group were put in place.

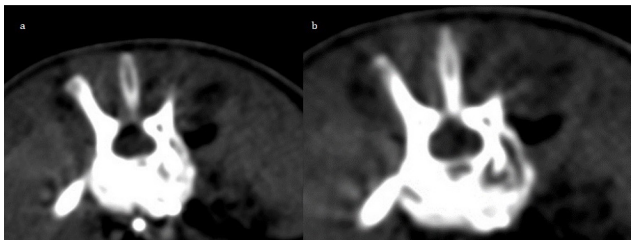
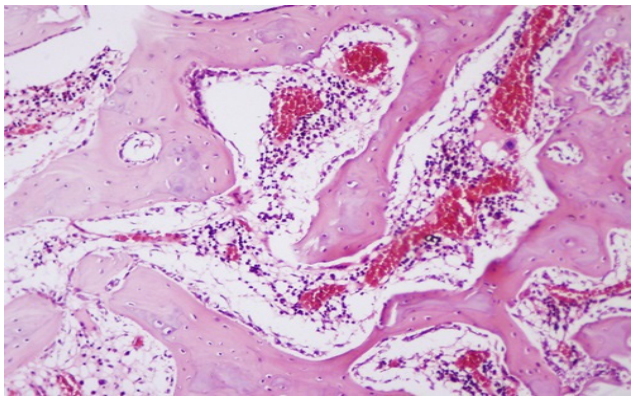
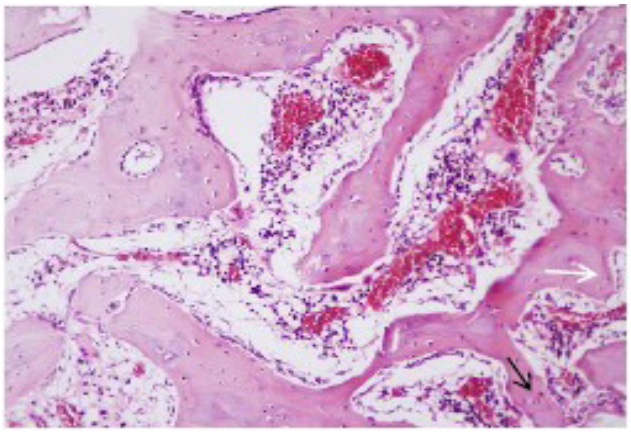


Figure 1: a) Tomography image of HA group, **b)** Tomography image of the boron group

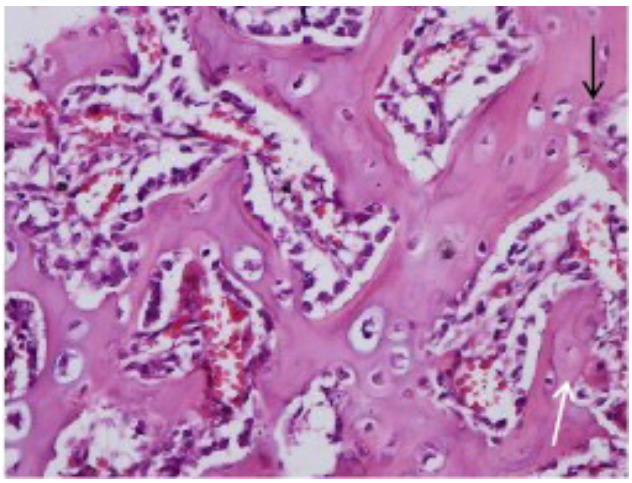
Although macroscopically the callus was seen in all groups, large remains were seen in Boron and HA groups. The remains of the boron group were attached to the surrounding soft tissue. After being detected with 10% formaldehyde with tampons during one night, the sections prepared from the sample tissues followed routinely were painted with hemotoxin-eosin and histopathologically examined with the light microscope **(Picture 1, 2, 3)**.



Picture 1: Score rated 0 on a cross-section of the control group, most bone tracheas have no osteoblast/osteoclast activity (H&E, x100)



Picture 2: Score rated 1, osteoblast/ osteoclast (H&E, x200) Black arrow osteoblast, white arrow osteoclast surrounding less than 5% of the bone surface in the most bone trabecula



Picture 3: Score rated 3, osteoblast/ osteoclast (H&E, x200) Black arrow osteoblast, white arrow osteoclast surrounding 25-50% of the bone surface in most bone tracheas

The samples were evaluated with the osteoblast-osteoclast evaluation scale. In the evaluation of osteoclastic activity and osteoblastic activity severity in pathology sections, scoring was done using the following criteria (8) The variation in osteoblast score was thought to be due to surgical technique and biological variations (8)

Osteoblast Score:

0. Most bone tracheas do not have osteoblasts
1. In most bone tracheas, the osteoblast surrounds less than 5% of the bone surface
2. Osteoblast surrounding 5-25% of the bone surface in the most bone trachea
3. Osteoblast surrounding 25-50% of the bone surface in the most bone trachea
4. In most bone tracheas, the osteoblast surrounds more than 50% of the bone surface

Osteoclast Score:

0. Most bone tracheas do not have osteoclast
1. Osteoclast surrounding less than 5% of the bone surface in the most bone trachea

2. Osteoclast surrounding 5-25% of the bone surface in the most bone trachea
3. Osteoclast surrounding 25-50% of the bone surface in the most bone trachea
4. Osteoclast surrounding more than 50% of the bone surface in the most bone trachea

When the pathological data were examined, it was seen that the osteoclast score was 0 point in 66.6% of the control group, 1 point in 33.4%, and the osteoblast score was 0 point in 50% and 1 point in 50% of the control group. In the boron group, osteoclast score was 1 point in 83.3%, 2 points in 16.6%, osteoblast score was 1 point in 50% and 2 points in 50%. In the HA group, the osteoclast score was 1 point in 50%, 2 points in 50%, 1 point in 50%, 2 points in 16.6% and 3 points in 33.4%. Higher osteoblast and osteoclast scores were observed in the HA group compared to the Boron and control groups. It was observed that the boron group had higher osteoblast and osteoclast scores than the control group. Osteoclast and osteoblast score percentages are given in **Table 1** and **Table 2**.

Table1: Osteoclast score percentages of groups

Osteoclast Score	0	1	2	3	4
Control	% 66,6	% 33,4	0	0	0
Boron	0	% 83,3	% 16,7	0	0
HA	0	% 50	% 50	0	0

Table 2: Osteoblast scoring percentages of groups

Osteoblast Scoru	0	1	2	3	4
Control	% 50	% 50	0	0	0
Boron	0	% 50	% 50	0	0
HA	0	% 50	% 16,6	% 33,4	0

DISCUSSION

Anterior lumbar interbody fusion (ALIF) is one of the important surgical procedures used in degenerative disc hernia. Especially in pathologies caused by lumbar intervertebral foraminal stenosis, it was reported that ALIF increased the intervertebral foraminal distance and decompressed the spinal root at the outlet. In traditional ALIF surgery, graft material is placed at a distance after the disc is emptied by the anterior way (9). It is planned to maintain the height of the disc distance with the cage placed at the disc distance until fusion is formed.

A wide range of graft materials was used in ALIF. Autologous bone grafts are widely used in fusion, but unwanted resorption may occur. Complications may develop in the reed area (10). Complications such as infection, pain, hemorrhage, and nerve damage were reported in areas where autologous grafts were taken between 8-25% (11, 12).

Autogenous bone grafts are considered the gold standard for cases requiring the repair of bone defects (13). However, there are disadvantages such as the need for a second operation, increased operation time and trauma, morbidity, and limited graft amount. Autogenous bone grafts cause problems such as increased blood loss, infection, delayed return to normal life, and prolonged hospital stay. The problems that can be observed secondary to the use of autogenous bone grafts have led to the development of bioimplants that can replace these grafts (14). Bone substitute materials such as natural and synthetic hydroxyapatites, tricalcium phosphate, collagen, ceramics, demineralized bone matrices, polymers, allogeneic or xenogeneic implants are used quite frequently.

Although there are many implant materials with different origins and structures that have been used, each material has its advantages and disadvantages. Today, studies on ideal bone graftmaterials continue (15).

Ideal bone graft is expected to show osteoconductive and osteoinductive properties, as in autologous bone graft. These grafts should be easy to apply, cost-effective, and non-immunogenic. Our aim in this study was to show the efficacy of HA and Boron compounds in operations using ALIF (14).

HA is a suitable biomaterial for bone tissue due to its biocompatibility and osteoconductivity. The chemical composition of hydroxyapatite graft materials is similar to the natural structure of human bone. Although the porous structure and particle size of these grafts affect the degree of resorption, they do not prevent them from forming a connection with the bone. The particle sizes of the grafts affect the active resorption mechanism. Results of previous studies have shown that HA has excellent osteoconductivity and biodegradability and can promote bone repair. In this study, the superior osteoconductivity of HA was also confirmed when grown in ALIF (16 - 18).

The use of HA has been found to increase bone metabolism, total protein, and collagen synthesis. It was determined that the cavities in the bioactive glass and all cage materials using HA graft were covered with an osteoid layer, the defects were filled with new bone tissue and bone healing was sufficient. HA is highly effective in repairing bone defects (19).

In our study, it was determined that HA graft and discuss intervertebral provide the formation of callus and fusion around it. Bone tissue was found to form around the cage, which was placed by filling the graft. A thicker formation of callus was observed compared to boron and control groups. It was found to increase osteoblastic and osteoclastic activity more than the control group.

Boron is one of the trace elements in the human body that plays important role in many life processes, including embryogenesis, bone growth and maintenance, immune function, and psychomotor skills. Especially in postmenopausal women, boron can stimulate hormones and thus mimic the effects of estrogen by stimulating estrogen production. Currently, estrogen therapy is one of the most effective methods of preventing postmenopausal bone loss, which can lead to osteoporosis and debilitating fractures. Therefore, the introduction of boron traces in bioactive materials for bone health and bone regeneration is of particular interest (20, 21).

Boron is found in the body in the figure of insoluble boric acid. The boron taken into the body is stored in body tissues in this form. The amount of pipe in the dryness of many soft tissues varies between 0.5-1.5 mg/kg, while the amount in the bones is several times more. As the amount of boron consumed increases, its concentration increases (22).

While the addition of boron to nutrients does not affect the boron level of fat tissue and especially testicles, it has increased the boron level of soft tissues and plasma such as liver, kidneys, muscle, but the highest increase is in bone tissue. Boron is also found at high levels in nails, hair, and teeth (22).

The effects of boron on fusion were demonstrated by comparing boron-containing mesoporous bioactive glass [B-MBG) and p(N-isopropylacrylamide-co-butyl methacrylate) (PIB) in the study of Chen et al. on mice. This study shows that PIB nanogels/B-MBG composites show greater improvement in mechanical strength compared to PIB nanogels alone. In vivo, hematoxylin and eosin staining revealed significantly more newly formed bone in defects involving PIB nanogels/B-MBG composite scaffolds compared to PIB nanogels alone. Tartrate-resistant acid phosphatase positive staining showed that both scaffolds deteriorated over time and bone remodeling occurred in the surrounding bone defect even 4 weeks after implantation (23).

In our study, although a callus formation was not as thick as the HA group in the Boron group, it was found that a statistically significant callus tissue was formed compared to the control group. Osteoblast and osteoclast activities were observed in the formed callus.

In our study, not the oral effect of the pipe, but the effect of localized fusion were evaluated. It was observed that the effect of local use of the pipe, which has been shown to affect bone metabolism when used systematically, was positive on fusion, but not as effective as HA rich in the hydroxyl group. Although the effect of boron on fusion is positive, we believe that it will be useful to keep our subject count to a minimum and to perform more and wide-series studies to investigate the effects of boron dosing studies on fusion in local use.

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