The effect of locally administered organic silicon on calvarial bone defects

Lokal olarak uygulanan organik silikonun kalvaryal kemik defektleri üzerindeki etkisi

Effect of organic silicon on bone formation

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Öz

Amaç: Bu çalışmanın amacı lokal olarak uygulanan organik silikonun otojen kemik grefti uygulanan kritik boyuttaki kalvaryal kemik defekti üzerine etkisini incelemek. Gereç ve Yöntem: 24 adet rat Grup C (pasif kontrol), Grup Au ve Grup Si olmak üzere üç gruba ayrıldı. Hayvanlar kafatasında 5 mm çapında kritik boyutta defektler oluşturuldu. Grup C'de defekt bölgesine steril salin solüsyonu emdirilmiş absorbe olabilen kollajen tampon yerleştirildi. Grup Au'da, steril salin emdirilmiş absorbe olabilen kollajen tampon, otojen greft yerleştirilen defekt alanına uygulandı. Group Si'de, 500 mg organik silikon emdirilmiş absorbe olabilen kollajen tampon otojen greft uygulanan defekt alanına yerleştirildi. Bütün denekler işlemden 28 sonra ötenazi yoluyla sakrifiye edildi. Stereolojik analiz işlemi uygulandı ve yeni kemik oluşumu ve bağ doku hacim ölçümü yapıldı. Bulgular: Stereolojik sonuca göre, Grup Si'de ortalama yeni kemik hacmi 1.79 ± 0.48 mm3 bulundu ve diğer gruplar arasındaki farkın istatistiksel olarak anlamlı olduğu tespit edildi ($p \leq 0.05$). Grup Au ve C 'deki ortalama yeni oluşan kemilkhacmi ise sırasıyla, 1.50 ± 0.51 mm3 and 1.04 ± 0.03 mm3 bulundu. Bağ dokusu hacmi, grup Au'da daha yüksek olmasına rağmen grup Si ile aralarında anlamlı bir fark bulunamadı. Tartışma: Lokal olarak uygulanan organik silikonun otojen greft uygulanmış kritik boyuttaki kalvaryal defektlerde kemik rejenerasyonunu arttırdığı ortaya konulmuştur.

Anahtar Kelimeler

Silikon; Kemik Oluşumu; Kalvarya

Abstract

Aim: The purpose of this study was to investigate the potential of the local administration of organic silicon on autogenous grafted critical-sized cortical bone defects. Material and Method: Twenty-four rats were divided into three groups: group C (passive control), group Au, and group Si. A 5-mm diameter critical-size defect was created in the calvarium of each rat. In group C, only a sterile saline-treated absorbable collagen sponge was applied to the defect area. In group Au, autogenous graft was performed and sterile saline-treated absorbable collagen sponge was applied on the autografted defect area. In group Si, autogenous graft was performed and a 500 mg silicon-treated absorbable collagen sponge was applied to the autografted defect area. All animals were euthanized at 28 days postoperative. Stereologic analyses were performed. New bone area and connective tissue volumes were measured. Results: Stereologic analysis showed that the difference between group Si with a mean bone formation of 1.79 ± 0.48 mm3 and groups Au and C was statistically significant ($p \leq 0.05$), the latter having mean bone formations of 1.50 ± 0.51 mm3 and 1.04 ± 0.03 mm3 respectively ($p \leq 0.05$). Connective tissue volume was larger in group Au than in group Si, but the difference was not statistically significant. Discussion: Locally administered organic silicon enhances bone regeneration in critical-size calvarial rat defects filled with autogenous graft.

Keywords

Silicon; Bone Formation; Rat

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Introduction

Surgical procedures involving bone play an important role in oral and maxillofacial surgery. Various sized bone defects may occur after cysts or tumour excision or orthognathic surgery. Some bone defects can be repaired by the body, but in cases of large defects, physiological regenerative capability may be exceeded [1]. The type of bone defects that will not heal spontaneously during the lifetime are called critical-sized bone defects. Critical-sized bone defects need extra surgery such as a grafting procedure [2]. Today, autogenous graft is regarded as the gold standard. In extensive large defects, different materials can be used to hasten bone formation by stimulating surrounding tissues and making a positive contribution to the graft materials [3].

Bone is the most important structure of the human skeletal system and also plays a role as a connective tissue to support other organs of this system. It consists of cells, intracellular material, and extracellular matrix. Osteoblasts are responsible for matrix formation, ossification, and bone damage repair. Bone extracellular matrix involves minerals, collagens, water, non-collagen proteins, and other organic materials [4]. 70% of organic matrix is formed by collagens. Type I collagen is the most common type. In healthy bones, the rate of Type I collagen is 95%. Type I collagens join to form different tissues, such as bone, dentine, tendon, skin, and cartilage; they are synthesized by fibroblasts, osteoblasts, odontoblasts, and chondroblasts [5]. Basically, bone repair is a kind of connective tissue healing. The steps of the healing process for bone formation are similar to those for soft tissue, except that for soft tissue, osteoblasts and osteoclasts are responsible for the reconstruction and the remodeling of ossifying tissue [6].

Silicon (Si) is one of the most abundant minerals in nature. It can be found in drinking water and some phytonutrients. Silicon participates in the structure of different organs, such as bone, nails, hair, and skin [7]. In the literature, studies have shown the effect of silicon on different bone mechanisms, such as biocalcification [8], osteoblast activity and bone mineralization [9], and bone mineral density [10]. Reffitt et al. [11] reported that Si enhances osteoblastic differentiation and stimulates Type I collagen synthesis.

The aim of this study was to investigate the potential of the local administration of organic silicon on autogenous grafted critical-sized calvarial defects in rats.

Material and Method

The 6- to 8-week-old Wistar rats (n = 24) used in this study were housed in standard cages in rooms with a relative humidity of 40–60% and a temperature of 22 ± 1°C. The illumination system of the room was configured to automatically provide 12 h of light and 12 h of darkness. This study was approved by the Animal Experimentation Committee of Bülent Ecevit University, Zonguldak, Turkey.

Surgical procedure

All surgical procedures were performed under sterile conditions in the surgical suite of an animal laboratory. Each rat was anesthetized by intramuscular injection of 3 mg xylazine hydrochloride/kg (Rompuns; Bayer, Leverkusen, Germany) and 35 mg ketamine hydrochloride/kg (10% [w/v] Ketasol; Richter Pharma AG, Wels, Austria). After anesthesia, the right side of the mandible was shaved and sterilized using conventional methods, and articaine (in a 1:200,000 weight ratio with epinephrine; Ultracain-DS, Hoechst Marion Roussel, Istanbul, Turkey) was injected for hemostatic purposes. Under general anesthesia, the rat calvarium was shaved and the cutaneous surface was disinfected with povidone iodine solution. A semilunar incision was then made and a full-thickness flap was reflected, exposing the parietal and frontal bones. A 5-mm-diameter (critical size) calvarial defect was made with a trephine used in a low-speed hand-piece under continuous sterile saline irrigation. The defect included a portion of the sagittal suture. Care was taken during the surgery to avoid damage to the dura mater. In two groups, defects were filled by autogenous graft. The flap was closed using a reverse-cutting 4/0 needle and undyed, Polyglactin 910, braided absorbable sutures; the skin was closed using 3/0 silk sutures. As prophylaxis against postoperative infection, 10 mg/kg cefazolin sodium (Sefazol; M Nevzat, Istanbul, Turkey) was injected daily for 5 days.

Autogenous bone grafts were harvested from the left tibia of the rats. The medial surface of the left legs of the subjects were shaved and the area was disinfected with povidone iodine solution. The legs were given the flexion position and longitudinal incisions of 20-25 mm were made periosteally in order to reach the medial surfaces of the tibia. The medial surfaces of the tibia were exposed with blunt dissection and soft tissues were excluded. Autogenous bone graft, covering the cortex and medula layers of the bone, was obtained by using a round-tipped, stainless steel drill with a diameter of 3 mm under sterile saline solution.

Experimental groups

After surgery, the rats were divided into three groups of eight and treated as follows:

Group C: Only a sterile saline-treated absorbable collagen sponge (ACS) was applied to the defect area.

Group Au: A sterile saline-treated ACS was applied on the autografted defect area.

Group Si: A 500 mg silicon-treated ACS was applied to the autografted defect area.

The animals were euthanized by lethal anesthetic injection 28 days after surgery. The skin was dissected, and the calvaria of the animals were removed and immediately immersed in 10% (v/v) buffered formaldehyde.

Tissue processing and stereological methods

Our routine histological procedures featured sample fixation in 10% (v/v) formalin for 10 days and decalcification with 5% formic acid for 21 days; the samples were then gradually dehydrated in ethanol (70, 80, 96, and 100%), placed in xylene for clearing, and paraffin-embedded. The tissue blocks were sectioned at 5 µm thickness using a microtome (Leica RM2255, Germany) and stained with hematoxylin-eosin.

Between 16 and 25 slides from each rat were stereologically analyzed. The first section was randomly chosen, followed by every tenth slide (this was, thus, a systematic random sampling strategy). The sections were photographed under a light microscope fitted with a camera at x10 magnification (Zeiss Primostar, Germany), and the Cavalieri method was used to calculate

the volumes of connective tissue and newly formed bone. The dimensions of the point-counting test grid were 80 μM x 80 μM. The Cavalieri method (an unbiased stereological technique) was used to estimate the following parameters: the volume of newly formed bone (Vnb) and the volume of connective tissue (Vct).. We used a point-counting grid for area estimations. The Vnb and Vct were estimated using the following formula:

Volume (V) = t × a/p × ∑p,

where V is the mean volume of the calvaria, t is the mean section thickness, a/p is the inter-point area, and ΣP is the total number of points on entire serial sections of the calvaria. The coefficient of error (CE) and the coefficient of variation (CV) for volume estimations of connective tissue and newly formed bone were confirmed to be within appropriate ranges. We counted the number of points in each segmental compartment. These point counts were used to estimate the volume of each compartment using the following formula:

Volume= t × a/p × P, where t is the section thickness, a/p is the area of each point on the point-counting grid, and P the total number of points touching the surface areas of the sections [12].

Statistical analysis

The Shapiro–Wilk test was used to confirm the normal distribution of the data. The μCT and stereological parameters were analyzed using the Kruskal–Wallis nonparametric test, followed by post-hoc group comparisons with the Bonferroni-adjusted Mann–Whitney U test, after a failed normality test of the data. For the Bonferroni correction, α = 0.05 / 3 = 0.016 was considered to indicate statistical significance. All tests were performed using statistical software (SPSS version 19.0; SPSS Inc., Chicago, IL, USA). P < 0.05 was considered to indicate statistical significance.

Results

All animals tolerated the surgery well and survived the post-surgical period. No wound dehiscence, wound infection, or abscess formation was observed at any surgical site.

Histological evaluation

New bone formation were observed in all groups. In group C, new bone formation was only at defect margins and a thin layer of connective tissue was lying between defect borders. In groups Au and Si, new bone formation was around graft particles and also near the defect margins. In both groups, connective tissue also was between graft particles (Fig-1).

Stereological analyses

In group Si, mean connective tissue volume was 1.56 ± 0.07 mm3, whereas mean values in the Au and C groups were 1.61 ± 0.07 mm3 and 0.89 ± 0.05 mm3, respectively (Fig 2). The differences between group C and the other two groups were statistically significant (p ≤ 0.05). Connective tissue volume was larger in group Au than in group Si, but the difference was not statistically significant.

Mean new bone volume in group Si was 1.79 ± 0.48 mm3, whereas mean volumes in the Au and C groups were 1.50 ± 0.51 mm3 and 1.04 ± 0.03 mm3, were statistically significant (p ≤ 0.05). Also new bone formation in group Si was superior to that in group Au and the difference was statistically significant (p ≤ 0.05) (Fig 3).

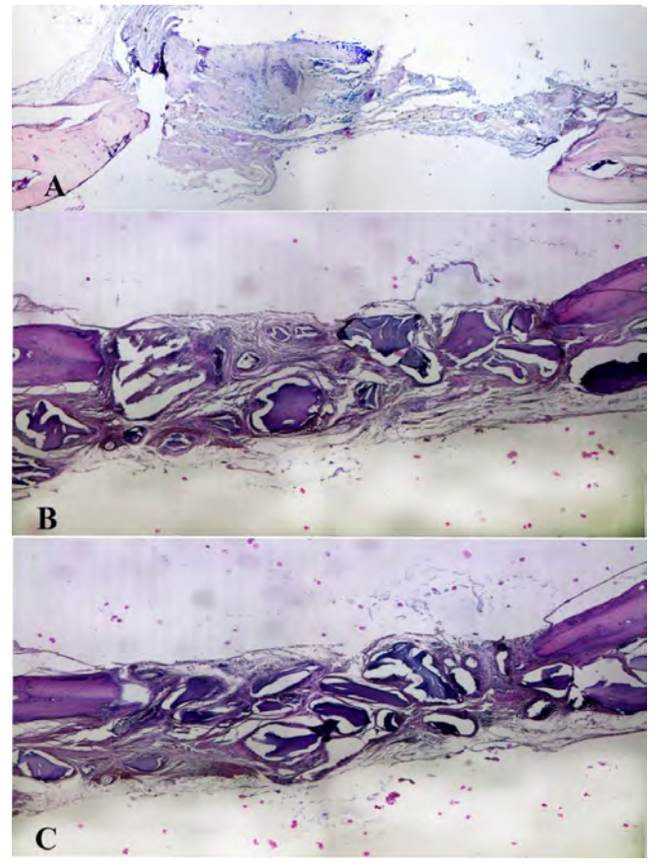


Figure 1. Panoramic views of the defects. (A) Group C; (B) Group Au; (C) Group Si.

Discussion

Silicon is one of the most common elements in nature, also taking part in the structure of many organs. We hypothesized that silicon may increase new bone formation with autogenous graft in rats. To test the hypothesis, 5 mm diameter critical-sized calvarial defects were created in rats; defects were filled by autogenous graft in the non-control groups, and local organic silicon was administered in the organic silicon group. New bone formation and connective tissue volumes were analyzed by stereologic methods.

In our study, rats were used as an experimental animal model. Rats have similar physiological properties as humans and are one of the most common animals used as models for bone formation. Rats are easy to care for and are not expensive [13]. In the literature, there are different calvarial critical defect sizes in rats; diameters can range between 4 and 8 mm. We used 5 mm calvarial defects as a critical-sized defect in our study. There was no defect healing between defect margines in the control group, consistent with the literature [14].

Silicon is one of the most essential elements for the human body. It plays an important role in the skeletal system. Different studies have investigated effects of silicon on bone metabolism and different components of bone. Kim et al. [9] examined the effect of Si on bone mineralization and osteoblast activity at the cellular level. Mature osteoblasts were treated by different doses of Si. They reported that Si affected bone metabolism positively by increasing osteoblast mineralization. Bu et al. [15] investigated the effect of two different doses of silicon on bone

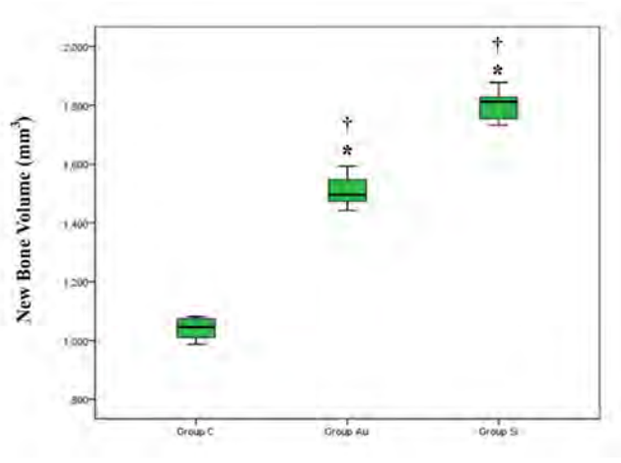


Figure 2. The connective tissue volume (mm^3) from stereological analysis in defects

* Statistically significant difference among groups (Bonferroni-adjusted Mann–Whitney U test)

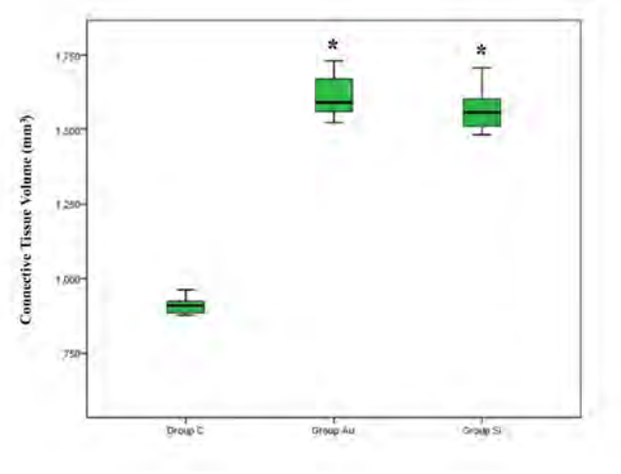


Figure 3. The new bone volume (mm^3) from stereological analysis in defects

* Statistically significant difference among groups (Bonferroni-adjusted Mann–Whitney U test)

metabolism and inflammatory mediators in ovariectomized rats. They stated that silicon did not increase bone mineral density but decreased bone resorption by inhibiting expression of bone resorptive mediators. In another study, the effect of silicon was examined on Type I collagen synthesis in human osteosarcoma cells and primary osteoblast-like cells. According to the study, silicon stimulated Type I collagen synthesis and osteoblastic differentiation [11]. Gereli et al. [16] investigated the effect of locally injected silicon on Achilles tendon healing. Biomechanics, histological, and immunohistochemical analyses were made. They found that silicon injection increased fibroblastic growth factor level and enhanced tendon healing. Although there have been studies about the effect of silicon on various bone mechanism and metabolism, to our knowledge there is no study about the effects of organic silicon on defect treatment. We investigated the effect of local administered organic silicon on bone formation in critical-sized defects. Consistent with the literature, we found that silicon had a positive effect on defect treatment and increased new bone formation.

In our study, new bone formation and connective tissue volume measures were made by stereological analyses. In stereological methods, sections are analyzed in 3D space so it shows more results that are more reliable than in conventional histological analyses [17]. Based on stereological analyses, silicon had a positive effect on bone formation compare to the other groups. Group Au had higher connective tissue volume than group Si, but the difference was not significant.

In conclusion, according to our histological and stereological findings, this experimental study showed that organic silicon could have positive effects on autogenous graft compared with control and autogenous groups. Further studies are needed for establishing the optimal dose to maximize the anabolic actions and to minimize the side effects of organic silicon on bone.

Ethical Responsibilities

All institutional and national guidelines for the care and use of laboratory animals were followed.

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Competing interests

The authors declare that they have no competing interests.

References

1. Mardas N, Dereka X, Donos N, Dard M. Experimental model for bone regeneration in oral and cranio-maxillo-facial surgery. J Invest Surg 2014;27(1):32-49.
2. Aurégan JC, Bégué T. Induced membrane for treatment of critical-sized bone defect: a review of experimental and clinical experiences. Int Orthop 2014;38(9):1971-8.
3. Precheur HV. Bone graft materials. Dent Clin North Am 2007;51(3):729-46
4. Gelse K, Pöschl E, Aigner T. Collagens--structure, function, and biosynthesis. Adv Drug Deliv Rev 2003;55(12):1531-46.
5. Fleischmajer R, MacDonald ED, Perlish JS, Burgeson RE, Fisher LW. Dermal collagen fibrils are hybrids of type I and type III collagen molecules. J Struct Biol 1990;105(1-3):162-9.
6. Amini AR, Laurencin CT, Nukavarapu SP. Bone tissue engineering: recent advances and challenges. Crit Rev Biomed Eng 2012;40(5):363-408.
7.Nielsen FH. Update on the possible nutritional importance of silicon. J Trace Elem Med Biol 2014;28(4):379-82.
8. Matsko NB, Znidaršič N, Letofsky-Papst I, Dittrich M, Grogger W et al. Silicon: The key element in early stages of biocalcification. J Struct Biol 2011;174(1):180-6.
9.Kim EJ, Bu SY, Sung MK, Choi MK. Effects of silicon on osteoblast activity and bone mineralization of MC3T3-E1 cells. Biol Trace Elem Res 2013;152(1):105-12.
10. Kim MH, Bae YJ, Choi MK, Chung YS. Silicon supplementation improves the bone mineral density of calcium-deficient ovariectomized rats by reducing bone resorption. Biol Trace Elem Res 2009;128(3):239-47.
11. Reffitt DM, Ogston N, Jugdaohsingh R, Cheung HF, Evans BA, Thompson RP et al. Orthosilicic acid stimulates collagen type 1 synthesis and osteoblastic differentiation in human osteoblast-like cells in vitro. Bone 2003;32(2):127-35.
12. Gundersen HJ, Jensen EB. The efficiency of systematic sampling in stereology and its prediction. J Microsc 1987;147(Pt 3):229-63.
13. Choi JY, Jung UW, Kim CS, Eom TK, Kang EJ, Cho KS et al. The effects of newly formed synthetic peptide on bone regeneration in rat calvarial defects. J Periodontal Implant Sci 2010;40(1):11-8.
14. Vajgel A, Mardas N, Farias BC, Petrie A, Cimões R, Donos N. A systematic review on the critical size defect model. Clin Oral Implants Res 2014;25(8):879-93.
15. Bu SY, Kim MH, Choi MK. Effect of Silicon Supplementation on Bone Status in Ovariectomized Rats Under Calcium-Replete Condition. Biol Trace Elem Res 2016;171(1):138-44.
16. Gereli A, Akgün U, Uslu S, Ağır İ, Ateş F, Nalbantoğlu U. Sıçanlarda organik silikon enjeksiyonunun Aşil tendonu iyileşmesine etkisi. Acta Orthop Traumatol Turc 2014;48(3):346-354.
17. Novaes RD, Penitente AR, Talvani A, Natali AJ, Neves CA, Maldonado IR. Use of fluorescence in a modified disector method to estimate the number of myocytes in cardiac tissue. Arq Bras Cardiol 2012;98(3):252-8.

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