



Stereological evaluation of local administered palmatine on bone regeneration

Lokal olarak uygulanan palmatinin kemik rejenerasyonuna etkisinin stereolojik olarak incelenmesi

Palmatine and bone regeneration

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Öz

Amaç: Bu çalışmanın amacı otojen greft yerleştirilmiş kritik boyuttaki kalvaryal defektler de lokal olarak uygulanan palmatinin tedavi potansiyelini araştırmaktır. **Gereç ve Yöntem:** 24 adet rat grup C (pasif kontrol), grup G (aktif kontrol) ve grup G+PLM olmak üzere üç gruba ayrıldı. Deneklerin kalvaryasında 5 mm çapında kritik boyutta defektler oluşturuldu. Grup C'de defekt alanı boş bırakıldı. Grup G'de defekt alanına otojen greft yerleştirildi ve üzerine 300 mg steril salin emdirilmiş absorbe olabilen kolajen tampon uygulandı. Grup G+PLM'de defekt alanına otojen greft yerleştirildi ve üzerine 300 mg palmatin emdirilmiş absorbe olabilen kolajen tampon uygulandı. Bütün denekler operasyondan 28 gün sonra ötenazi yolu ile sakrifiye edildi. Yeni kemik alanı ve bağ doku hacimlerinin ölçümleri, stereolojik analizler kullanılarak yapıldı. **Bulgular:** Stereolojik analizler sonucunda, dördüncü hafta sonunda grup C ile karşılaştırıldığında G ve G+PLM gruplarında anlamlı derecede fazla yeni kemik oluşumu gözlemlendi. Bağ doku hacmi de otojen greft kullanılan gruplarda anlamlı derecede fazla bulundu. G ve G+PLM grupları karşılaştırıldığında yeni kemik ve bağ dokusu hacmi açısından istatistiksel olarak anlamlı bir fark bulunamadı. **Tartışma:** Lokal olarak uygulanan 500 mg palmatinin otojen greft yerleştirilmiş kritik boyuttaki kemik defektlerinde kemik rejenerasyonu üzerine olumlu etkisi olmadığı belirlenmiştir.

Anahtar Kelimeler

Kemik Rejenerasyonu; Palmatine; Rat

Abstract

Aim: The purpose of this study is to investigate the healing potential of the local administration of palmatine on autogenous grafted critical-sized cortical bone defects. **Material and Method:** Twenty-four rats were divided into three groups: Group C (passive control), Group G (active control) and Group G+PLM. A 5-mm diameter critical-size defect was created in the calvarium of each animal. In Group C, the defects were left empty. In Group G defects were filled with only autogenous graft and an absorbable collagen sponge treated with 300 mg sterile saline solution was applied on grafted area. In Group G+PLM defects were filled with autogenous graft, and 300 mg palmatine treated absorbable collagen sponge was applied on grafted area. All animals were euthanized at 28 days postoperative. Stereologic analyses were performed. New bone volume and connective tissue volumes were measured. **Results:** Stereologic analysis showed that Group G and G+PLM significantly had more new bone at four weeks compared with group C. Connective tissue volumes were also significantly higher in autografted groups. New bone and connective tissue volumes' difference were not statistically significant between group G and G+PLM groups. **Discussion:** Locally administered 500 mg palmatine doesn't enhance bone regeneration in critical size calvarial rat defects filled with autologous graft.

Keywords

Bone Regeneration; Palmatine; Rat

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Introduction

Bone tissue has a complex structure, anabolic and catabolic mechanisms work in harmony. Osteoblasts and osteoclasts are main cells that underlie in bone metabolism. Osteoblasts are responsible for new bone formation, on the other hand, osteoclasts play a role in resorption [1]. In certain size bone defects, bone tissue can heal without any support, but healing mechanisms are not sufficient alone in large defects. One of the most effective methods for treating large defects is the grafting procedure. Autogenous grafts are accepted as gold standard in grafting procedure [2].

Treatment of bone defects has an important place in maintaining ideal health of patients or in providing ideal bone formation before prosthetic restorations. Especially in Periodontology and Oral and Maxillofacial Surgery Departments, bone regeneration studies are performed, and effects of different materials on bone formation are examined [3,4].

Palmatine is in class of isoquinoline alkaloids and yellow in color. It is found in some plants and one of the active ingredients of *Coptidis rhizoma*. Palmatine has been used in Chinese alternative medicine for many years in the treatment of many diseases [5]. In the literature, there are studies investigating the different effects of palmatine. In a study, the effect of palmatine on the isometric force and intracellular level was examined in rat arterial muscles. According to study results, palmatine decreased the level of calcium, reduced muscle contraction and caused relaxation in arterial muscles [6]. Another study investigated the effect of different doses of palmatine intake on gastric ulcers in rats. Study results determined that the ulcer area improved in both doses of palmatine [7]. In literature, effect of palmatine on bone metabolism has not been studied much, and the limited number of these studies are related to the antiresorptive effect of palmatine. Ishikawa et al. examined the in vivo and in vitro effect of palmatine. They found that different doses of palmatine cause reduction in OPG and RANKL levels and have antiresorptive effect in ovariectomized rats [8]. In another in vitro study, it was revealed that palmatine may inhibit resorption by acting on osteoclast differentiation [9]. Although the effects of palmatine on bone resorption has been studied, according to our knowledge, there is no study about effect of palmatine on bone formation and healing in literature. The aim of this study is to investigate the effect of locally applied palmatine on calvarial critical size defect filled by autologous grafts in rats.

Material and Method

Experimental Model

The 6- to 8-week-old Wistar rats (n: 24) were used in this study, and they were randomly divided into three groups: group C, group Au, and group PLM. This study was approved by the Animal Experimentation Committee of Bülent Ecevit University, Zonguldak, Turkey.

Surgical Procedures

After anesthesia by intramuscular injection of 3 mg/kg xylazine hydrochloride (Rompuns; Bayer, Leverkusen, Germany) and 35 mg/kg ketamine hydrochloride (10% Ketazol; Richter Pharma AG, Wels, Austria) per kilogram, the surgical procedures were

performed. The hair over the calvarium was removed. The cutaneous surface was disinfected with povidone iodine solution. A semilunar incision was made, and the full thickness flaps were retracted. A 5mm defect was created with a trephine using a low-speed hand-piece. During preparation of the defect, sterile saline was used for irrigation to reduce thermal injury to the bone. Care was also taken in all animals to avoid damaging the dura mater.

In group C, defects were unfilled and were allowed to heal spontaneously without using any grafting material. In group G, defects were filled with only autologous graft, and 300 mg sterile saline treated absorbable collagen sponge was applied on graft area. In group G+PLM, defects were filled with a combination of autologous graft, and 300 mg palmatine treated absorbable collagen sponge was applied on graft area. Palmatine soluted with distilled water, was prepared fresh on the day of the operation for the palmatine-used group. The flaps were sutured after operation with resorbable 4/0 polyglactin 910 sutures.

For postoperative infection control and analgesia, each animal was injected with both 10 mg cefazolin sodium (Sefazol; M Nevzat, Istanbul, Turkey) per kilogram and 200 mg metamizole sodium (Novalgin; Aventis, Istanbul, Turkey) for five days after the operation. The animals were euthanized by overdose anesthesia four weeks after the operation. The calvarias were removed from the scalp, cleaned, and placed in 10% tempered formaldehyde solution.

Histological and stereological analyses

After decalcification process, the sections were selected and stained with hematoxylin-eosin and photographed using a stereology analysis system (Stereoinvestigator 9.0; Microbrightfield, Williston, VT, USA) and a light microscope (M4000 B; Leica Instruments) equipped with a digital color camera (Microbrightfield). The unbiased Cavalieri method was applied to the light microscopy images to stereologically estimate the volume of new bone using point-counting test grids. The point density of the point-counting grids was designed to obtain an appropriate coefficient of error (CE) for the area of interest in the images of the serial sections [10]. The grid, with its systematic array of points, was placed randomly on the image shown on the screen of a personal computer. The volume of each area of interest in each section was estimated with the following formula:

$$\text{Volume} = t \times a/p \times \Sigma p$$

Where t is the section thickness, a/p is the area of each point on the point counting grid, and Σp is the total number of points within the area of interest. The CE and coefficient of variation were estimated according to the formula of Gundersen and Jensen [11].

Statistical Analyses

Statistical analysis was performed using a commercially available software program (SPSS version 19.0; SPSS Inc., Chicago, IL, USA). The Shapiro–Wilk test was used to confirm whether the data were normally distributed. The stereological parameters were analyzed using the Kruskal–Wallis nonparametric test, followed by posthoc group comparisons with the Bonferroni-adjusted Mann–Whitney U test, after

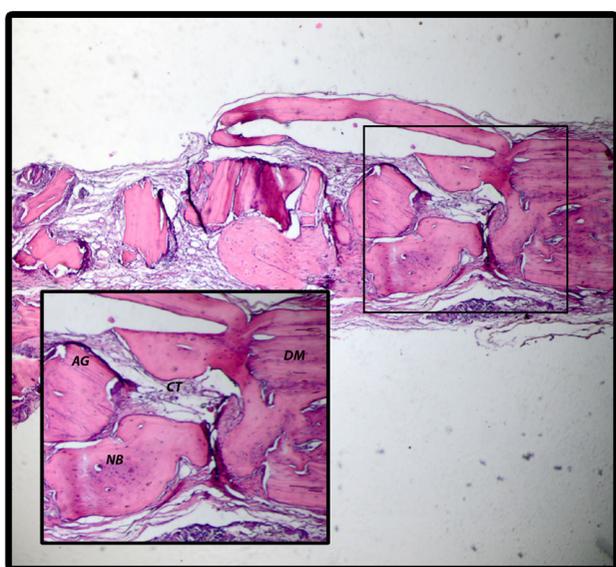
normality assumption of data had been rejected ($P < 0.05$) (the Bonferroni correction, $\alpha = 0.05/3 = 0.016$ was applied to determine statistical significance). $P < 0.05$ was considered to indicate statistical significance.

Results

Histological analyses

In control group, new bone formation was close to the border of defect site. Thin connective tissue layer was reached between defect margins. In group G and G+Plm, new bone formation was observed near the defect margins and also around the graft particles in defect area. There was more connective tissue formation than control group. The histologic view is shown in Figure 1.

Figure 1. Histological view of defect region. CT: Connective tissue, AG: autogenous graft, NB: new bone, DM: defect margin (hematoxylin-eosin, original magnification x 10).



Stereologic Analyses

Table 1 presents the volumes of new bone formation in the defects evaluated by stereological analyses after four weeks. It is shown in the table, after four weeks the palmatine-treated group had formed significantly higher values of bone compared to the control group. Moreover, between the group G and G+Plm, there were no significant differences in bone formation after four weeks.

Connective tissue volumes were significantly higher in autologous grafted groups according to the control groups. Group G had superior connective tissue volume than palmatine treated group but the differences were not statistically significant (Table 1).

Table 1. New bone volume and connective tissue volumes among groups.

GROUPS	NB VOLUME	CT VOLUMES
Group C	0.80±0.08	0.83±0.04
Group G	1.03±0.04 ^a	1.63±0.08 ^a
Group G+PLM	1.12±0.10 ^{a,b}	1.59±0.08 ^{a,b}

Data are expressed as the mean ± SD

NB: New bone, CT: connective tissue, Group C: passive control, Group G: active control, PLM: palmatine

^a significantly different from Group C

^b No significant difference from group G

Discussion

Bone defects that the body cannot heal by itself are called critical defects. The dimension of critical size defects varies between species. In literature, critical size bone defects used for rats range from 4-8 mm. Vajgel et al. screened 257 full texts and analyzed 61 papers that calvarial critical size defects were used in rat models. They stated that 5 mm diameter calvarial defects could be considered as a critical size defects [12].

One of the most used treatment methods for the treatment of critical size bone defects is the grafting procedure. Among the materials that can be used for grafting procedures are autogenous grafts, and this is accepted as the gold standard. Although grafting procedure is frequently preferred in the treatment of bone defects, some studies investigate materials that can be applied to shorten the healing time of the grafted defect area. The main goal of these studies are to improve the comfort of patients by accelerating bone healing [13,14].

Palmatine is an alkaloid derivative that has been used in Chinese alternative medicine for many years to treat different diseases. Palmatine can be found in the content of different plants, and in the literature, some studies examine different effects of palmatine. In a study using different alkaloids obtained from *Coptidis Rhizoma* plant which contained palmatine in its content, antioxidative stress and anti-Alzheimer effects of the related agents were investigated. According to the results, *Coptidis Rhizoma* alkaloids have a positive effect on the protection and treatment of Alzheimer and antioxidative stress-related diseases [15]. Jung et al. [16] showed that palmatine and other protoberberine alkaloids obtained from *Coptidis Rhizoma* has a beneficial effect on preventing complications of diabetes mellitus. According to results of an in-vitro study on anti-resorptive agents, palmatine has an inhibitory effect on osteoclast differentiation and function [17]. In another study examining the effect of palmatine on bone, Ishikawa et al. investigated the effect of different doses of palmatine in the osteoporotic rat model. They stated that palmatine has anti-resorptive effect in ovariectomized rats [8].

When studies in the literature were screened about the effect of palmatine on bone tissue, they are related to osteoclastic activity and bone resorption. To our knowledge, there is no study about effect of palmatine on new bone formation.

In our study, effect of palmatine on new bone formation in rats were examined. Palmatine was locally applied to the autologous grafted calvarial critical size defect area 5 mm in diameter. Bone formation and connective tissue volumes were measured by stereological analyses. Similar to literature, in empty defect group, there was only connective tissue formation between defect margins and it showed us 5 mm diameter calvarial defect can be used as critical size defect. Autologous grafted groups showed more new bone formation according to control group, but there was no statistically significant difference between grafted groups. Same with new bone formation results, connective tissue volumes in grafted groups were higher than control group, but the differences between group G and G+PLM were not statistically significant

As a conclusion, our results showed that locally administered palmatine has no beneficial effects on healing of autografted experimental defects in the calvaria of rat. Further investigations aimed at studying different doses to examine the anabolic actions of palmatine on bone are required.

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Ethical Responsibilities:

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

Conflict of Interest:

No potential conflict of interest relevant to this article was reported.

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References

1. Precheur HV. Bone graft materials. *Dent Clin North Am.* 2007; 51(3): 729-46.
2. Becker W, Urist M, Becker BE, Jackson W, Parry DA, Bartold M, et al. Clinical and histologic observations of sites implanted with intraoral autologous bone grafts or allografts. 15 human case reports. *J Periodontology.* 1996;67(10):1025-33.
3. Messora MR, Nagata MJ, Dornelles RC, Bomfim SR, Furlaneto FA, de Melo LG, et al. Bone healing in critical-size defects treated with platelet-rich plasma activated by two different methods. A histologic and histometric study in rat calvaria. *J Periodontol Res.* 2008;43(6):723-9.
4. Türer A, Türer ÇÇ, Önger ME. Effect of Local Puerarin Administration on Xenograft. *J Clin Anal Med.* 2017; DOI: 10.4328/JCAM.4914.
5. Kim YM, Ha YM, Jin YC, Shi LY, Lee YS, Kim HJ, et al. Palmatine from *Coptidis rhizoma* reduces ischemia-reperfusion-mediated acute myocardial injury in the rat. *Food Chem Toxicol.* 2009;47(8):2097-102.
6. Chang YL, Usami S, Hsieh MT, Jiang MJ. Effects of palmatine on isometric force and intracellular calcium levels of arterial smooth muscle. *Life Sci.* 1999;64(8):597-606.
7. Wang L, Wang X, Zhang SL, Zhu XM, Liu YQ, Song ZJ, et al. Gastroprotective effect of palmatine against acetic acid-induced gastric ulcers in rats. *J Nat Med.* 2017;71(1):257-64.
8. Ishikawa S, Ogawa Y, Tamaki M, Takashima M, Tajika Y, Moue T, et al. Influence of Palmatine on Bone Metabolism in Ovariectomized Mice and Cytokine Secretion of Osteoblasts. *In vivo.* 2015;29(6):671-7.
9. Ishikawa S, Tamaki M, Ogawa Y, Kaneki K, Zhang M, Sunagawa M et al. Inductive Effect of Palmatine on Apoptosis in RAW 264.7 Cells. Evidence-based complementary and alternative medicine. *eCAM* 2016 ;2016: 7262054.
10. Türer A, Türer ÇÇ, Ballı U, Durmuşlar MC, Önger ME, Çelik HH. Effect of Local Rosuvastatin Administration on Calvarial Bone Defects. *J Craniofac Surg.* 2016;27(8):2036-40.
11. Gundersen HJ, Jensen EB. The efficiency of systematic sampling in stereology and its prediction. *J Microsc.* 1987;147(3):229-63.
12. Vajgel A, Mardas N, Farias BC, Petrie A, Címões R, Donos N. A systematic review on the critical size defect model. *Clin Oral Implants Res.* 2014;25(8):879-93.
13. Türer ÇÇ, Türer A, Durmuşlar MC, Önger ME. The Local Effect of Puerarin on Critical-Sized Calvarial Defects. *J Craniofac Surg.* 2017;28(1):143-6.
14. Pradeep AR, Karvekar S, Nagpal K, Patnaik K, Raju A, Singh P. Rosuvastatin 1.2 mg In Situ Gel Combined With 1:1 Mixture of Autologous Platelet-Rich Fibrin and Porous Hydroxyapatite Bone Graft in Surgical Treatment of Mandibular Class II Furcation Defects: A Randomized Clinical Control Trial. *J Periodontology.* 2016;87(1):5-13.
15. Jung HA, Min BS, Yokozawa T, Lee JH, Kim YS, Choi JS. Anti-Alzheimer and antioxidant activities of *Coptidis Rhizoma* alkaloids. *Biol Pharm Bull.* 2009;32(8):1433-8.
16. Jung HA, Yoon NY, Bae HJ, Min BS, Choi JS. Inhibitory activities of the alkaloids from *Coptidis Rhizoma* against aldose reductase. *Arch Pharm Res.* 2008;31(11):1405-12.
17. Lee JW, Mase N, Yonezawa T, Seo HJ, Jeon WB, Cha BY et al. Palmatine attenuates osteoclast differentiation and function through inhibition of receptor activator of nuclear factor- κ B ligand expression in osteoblast cells. *Biol Pharm Bull.* 2010;33(10):1733-9.

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