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In vitro evaluation of gentamicin and teicoplanin release from cancellous human bone

İnsan süngerimsi kemiğinden gentamisin ve teikoplanin salınımının in vitro değerlendirilmesi

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Amaç: Bu çalışmada teikoplanin veya gentamisin emdirilen insan süngerimsi kemiğinin lokal antibiyotik taşıyıcısı olarak *in vitro* antibakteriyel etkinliği araştırıldı.

Çalışma planı: Çalışmada parsiyel veya total kalça protezi uygulanan yedi hastanın eksize edilen femur başlarındaki süngerimsi kemikten elde edilen örnekler kullanıldı. Kemik örnekleri iki gruba ayrılarak bir grup gentamisin, diğer grup ise tekoplanin içinde bir saat bekletildi. Kontrol amaçlı olarak, steril salin solüsyonu içinde bekletilmiş kemik örnekleri kullanıldı. Kemik örneklerinin antibiyotik salgılama özellikleri disk difüzyon yöntemiyle, her bir günde yedişer örnekte olmak üzere, 1, 3, 7, 10, 14, 18 ve 21. günlerde *in vitro* olarak değerlendirildi. Gentamisin için *E. coli* ATCC 25922, teikoplanin için *S. aureus* ATCC 25923 suşları kullanıldı ve sırasıyla ≥15 mm ve ≥14 mm zon çapları *in vitro* etkinlik olarak kabul edildi.

Sonuçlar: Test edilen günlerde ölçülen agar disk çapları incelendiğinde, teikoplanin + kemik (12-18 gün) yapısının gentamisin + kemik yapısına (7-10 gün) oranla daha uzun süreli salınım oluşturduğu izlendi. Kontrol grubunda inhibisyon oluşmadı. Teikoplanin ve gentamisin arasında agar disk çapları ortalamaları açısından birinci ve üçüncü günlerde anlamlı farklılık bulunmazken, yedinci (p=0.008) ve 10. günlerde (p=0.003) teikoplanin örneklerinin inhibisyon alanı anlamlı derecede fazla bulundu.

Çıkarımlar: Bulgularımız, insan süngerimsi kemiğinin uygun koşullarda teikoplanin ile önemli miktarda birleşme oluşturduğunu ve yaklaşık iki hafta boyunca etkin düzeyde antibiyotik salınımı sağladığını göstermektedir.

Anahtar sözcükler: Antibakteriyel ajan; kemik transplantasyonu; ilaç taşıyıcısı; gentamisin/terapötik kullanım; stafilokok enfeksiyonu/önleme ve kontrol; teikoplanin/terapötik kullanım.

Objectives: This study was designed to determine the *in vitro* antibacterial activity of gentamicin- or teicoplanin-impregnated human cancellous bone as a local antibiotic carrier.

Methods: The study samples were obtained from human cancellous bone within the femur head in seven patients who underwent partial or total hip arthroplasty. Bone specimens were processed and incubated with gentamicin or teicoplanin for an hour. Control bone specimens were soaked in sterile saline solution for the same duration. Antibiotic release of bone specimens was assessed by the disc diffusion technique after 1, 3, 7, 10, 14. 18, and 21 days of antibiotic impregnation, with seven samples in each group. The test strains were *E. coli* ATCC 25922 for gentamicin, and *S. aureus* ATCC 25923 for teicoplanin. *In vitro* antibiotic efficacy was defined as an inhibition zone diameter of ≥15 mm for gentamicin, and ≥14 mm for teicoplanin.

Results: Evaluation of inhibition zone diameters showed that bone + teicoplanin complexes had a longer duration of antibiotic release than that of bone + gentamicin complexes (12 to 18 days *vs* 7 to 10 days). There was no inhibition in the control group. There were no significant differences in inhibition zone diameters of teicoplanin- and gentamicin-treated specimens on the first and third days; however, teicoplanin exhibited significantly greater zone diameters on the seventh (p=0.008) and tenth (p=0.003) days.

Conclusion: Our data show that, under appropriate conditions, human cancellous bone incorporates a considerable amount of teicoplanin and exhibits effective antibiotic release for approximately two weeks.

Key words: Anti-bacterial agents; bone transplantation; drug carriers; gentamicins/therapeutic use; staphylococcal infections/prevention & control; teicoplanin/therapeutic use.

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The treatment of bone infection and bone defects occurring during infection is troublesome. As the penetration of antibiotics to the bone is very low in musculoskeletal infection, systemic antibiotic treatment alone is not enough for bacterial eradication. In addition, long term high dose systemic antibiotic therapy is inconvenient because of its serious side effects.[1-4] Deep infection occurring after the surgery or with open fractures increases the duration of hospitalisation, treatment cost and mortality seriously. Moreover the gradual increasing prevalence of infections with resistant microorganisims such as metisilin resistant S. Aereus (MRSA) and ESBL forming E.Coli strains increase the unsuccessful treatment ratio. [2-5]

Antibiotic impregnated polymethylmethacrylate (PMMA) beads is capable of delivering local antibiotic to the infection field with high concentrations and long duration. The PMMA beads are in widespread use for the treatment of chronic osteomyelitis and postoperative deep infection. The main disadvantage of this method is the need of an additional surgery for the removal of PMMA beads.[6-8] Developing biodegradable polymers as local antibiotic delivery system has been popularised in recent years. Many of the polymers can able to elute antibiotics efficiently for long duration. However the use of polymers is disadvantageous for some reasons such as foreign body reaction and incapability of filling bone defects.[9,10] Human bone is easily applicable at the surgery field and has superior osteoinductive and osteoconductive properties from other bone substitutes. Therefore if human bone can be used as an local antibiotic carrier many of the problems may be reduced or eliminated. Few studies have been concerned human bone as an antibiotic carrier.[6,8,11] These studies claimed that both cancellous and cortical human bone can able to elute antibiotics efficiently for long duration.[5-8] Therefore some authors have suggested that human bone can be be used as an efficient antibiotic carrier in the treatment of deep infection and chronic osteomyelitis. [7,11,12] In these studies antibiotics like tobramisin and gentamisin which are effective against gram negatif organisims are generally concerned.[5,6,13] These antibiotics are not the agents of choice for infections with gram positive strains. Teicoplanin is an effective antibiotic against infections with MRSA. To our knowledge there is no any study concerning the impregnation of teicoplanin to human cancellous bone.

The hypothesis of this study was that human cancellous bone and teicoplanin is capeable of forming antibiotic bone complex which elutes antibiotic with sufficient concentration and time, if proceeded properly. Our study is a prior study which investigate the duration of effective antibiotic release from human cancellous bone after being impregnated with gentamicin and teicoplanin antibiotics for one hour.

Material and methots

Samples of human cancellous bone were obtained from the excised femoral heads of seven patients who underwent partiel or total hip arthroplasty at our clinic during 2007 (female n=5, male n=2). Initially the bone specimens were cleaned. Fat, bone marrow and soft tissues were removed as possible in order to increase the antibiotic impregnation through the bone canaliculies. Thereafter cancellous bones were morsellized in a bone mill (Aesculap, Coarse, HARRİS, GB44) under sterile conditions. Morsellized bone specimens were then washed again and dried before the impregnation process. After that bone samples of each patient were divided into two groups. One group was impregnated with gentamicin (Gentreks ® 80 mg ampul Bilim İlaç) and the other group with teicoplanin (Targocid ® 400mg steril toz Aventis). Briefly 20g of morselized bone was impregnated with 20ml of antibiotic solution (antibiotic concentration for each solution was 40mg/ml) for one hour at room temperature. After the fluid was cleared, bone samples were compressed into a wire mesh cylinders. The mean weight of bone in each cylinder was adjusted briefly to 1.00 g with a sensetive balance.

After being vortexed with 5ml %0.9 Nacl solution for 15 seconds the bone specimens were placed in an elution tube (15 x 100mm steril glass tube) containing 5ml phosphate-buffered saline solution. Thereafter the bone specimens were incubated for 24 hours at 37°C temperature. Cylinders containing bone impegrated with saline instead of antibiotic were used as a control group. The in-vitro evaluation of the antibiotic release was carried out with modified disc diffusion technique. Escherichia coli ATCC 25922 strain was used for gentamicin and Staphylococcus aureus ATCC 25923 strain was used for teicoplanin. The overnight culture of the bacterias were isoleted and diluted in phosphate buffered saline to yield bacterial concentrations to 2x105 CFU/mL and then were incubated onto a Muller-Hington Agar. Gentamicin/ 10μg (oxoid, England) discs were used in the Escherichia coli incubated Agar plates and Teikoplanin/ 30

μg (oxoid, England) discs were used in the S. Aereus incubated Agar plates.

On test days the discs were impregnated with 10 μg elution solution. Thereafter the discs were incubated at 37°C for one night. After that the inhibition zone diameters of standart discs and test discs were measured with a Vernier Caliper. Zone diameters ≥15 mm for gentamisin and ≥ 14 mm for teicoplanin were evaluated as sensitive.[14] The in-vitro effectivity of antibiotic release were evaluated with regard to mean inhibition zones at 1., 3., 7.,10.,14.,18 ve 21. test days.

Results

The mean inhibition zones (Table 1-2) for gentamicin with respect to test days showed that gentamicin+bone complex exerted inhibition for about a week (7-10 days) whereas, teicoplanin+bone complexs exerted longer and steadier inhibitory effect which continued about two weeks (12-18 days) (Figure 1). At the 21. test day all antibiotic+bone complexes lost their inhibitory effect and the study was ended. There was no inhibitory effect in the control group. Figure 2 presents the inhibitory zone diameters of two groups at test days. There was no statistically important difference on 1. and 3. test days between two groups with regard of zone inhibition effect. On 7. and 10. test days the inhibitory effects of two group showed significant importance (P<0.05) (table 3).

Statistics

Statistical differences were analyzed by Mann-Whitney U test. All the parameters were summarized by mean %95 CI, graphics and tables. Significance was set at p< 0.05. SPSS software 11.5 was used for statistical analysis.

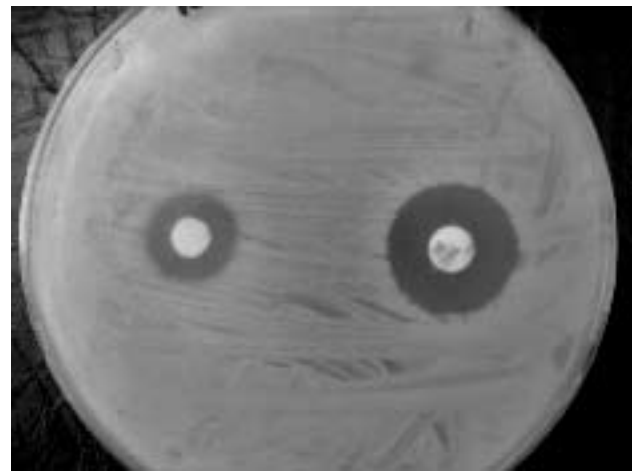

Figure 1. The zone inhibition of a disc embeded in teicoplanin and a test disc at 14. test day.

Discussion

In this study cancellous human bone impregnated with teicoplanin showed longer duration of antibiotic release. The duration of antibiotic release was shorter with gentamicin. The amount of antibiotic released from a antibiotic delivery system depends on many factors, such as the type and the amount of antibiotic used, surface area and structural properties of the carrier.[15] As the shape, surface area, surface structure of the carrier and the amount of each antibiotic was controlled in our study, we think that the antibiotic release was directly releated with the type and solubility of the antibiotics. Local administration of antibiotics provide high concentrations at the site of the infection besides protecting the patient from systemic adverse effects.[15] Because of that many materials have been investigated as local antibiotic deli-

Table 1. Zone inhibitions (mm) of Teicoplanin and Gentamicin discs at test days.

	1. day		3. day		7. day		10. day		14. day		18. day		21. day	
	T	G	T	G	T	G	T	G	T	G	T	G	T	G
Sample 1	28	22	17	16	16	8	15	R	14	R	R	R	R	R
Sample 2	30	28	24	22	20	16	18	15	16	R	15	R	R	R
Sample 3	36	34	30	23	22	15	18	R	16	R	R	R	R	R
Sample 4	40	30	28	25	25	15	17	R	14	R	R	R	R	R
Sample 5	40	32	31	24	24	17	21	15	16	R	14	R	R	R
Sample 6	26	22	18	17	16	16	15	R	R	R	R	R	R	R
Sample 7	34	30	23	21	20	15	17	R	16	R	R	R	R	R
Mean	33.4	28.3	24.4	21.1	20.4	14.6	17.3	4.3	13.1	0.0	4.1	0.0	0.0	0.0
SD	5.6	4.7	5.6	3.4	3.6	3.0	2.1	7.3	5.9	0.0	7.1	0.0	0.0	0.0
p	0.138		0.2		0.008		0.003							

R: Resistant

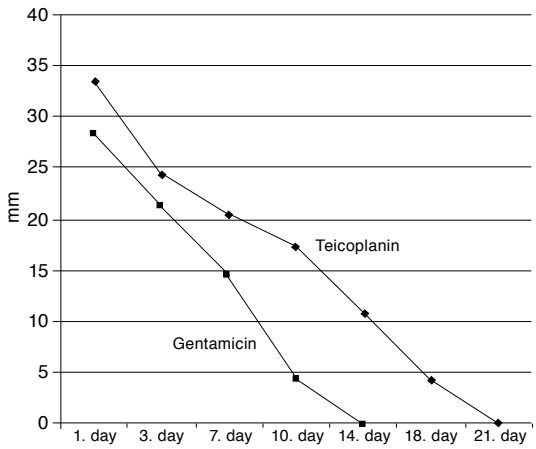

Figure 2. Demonstration of zone of inhibitions (mean) of two groups at test days.

very system. Among these, Polymethylmethacrylate (PMMA) is the most widely used implant material in orthopaedics. After the introduction by Insall for two stage reconstruction of infected total knee prothesis, antibiotic impregnated PMMA use became widespread.[17] Many in-vitro and in-vivo studies have suggested that Antibiotic impregnated PMMA beads are very effective against eradication of infection.[16-19] However antibiotic impregnated PMMA beads has certain disadvantages. After the eradication of infection residual bone defects should be restored. In addition antibiotic impregnated PMMA beads become a foreign substance suitable for bacterial colonization after the antibiotic has been depleted. Therefore antibiotic impregnated PMMA beads must be removed and bone defects must be filled with autologous or allograft bone with an additional surgery. To overcome these disadvantages many bioresorbable materials including hydroxyapatite,[20-23] calcium sulfate pellets, [24,25] chitosan,[26] fibrin-glue,[27] polymers, [6,28] collegen sponges [29] and xenografts [30] have been investigated as a local antibiotic carrier. The releated literature have revealed contradictory results about the effectiveness of these bioabsorbable materials. Moreover, most of these materials are incapable of mechanical loading and eventhough their in-vitro effectivity they have diffuculties in the in-vivo application at the operative field. Zelken stated in his in-vivo study that the poor handling properties of hydroxyapatite paste prevent its in-vivo effectivity againts infection.[21] Additionaly some of these materials have only osteoinductive (hydroxyapatite) properties whereas, some of them have only osteoconductive (calcium sulfate) properties. The antigenic properties of allogenic bone reduces during sterilisation. This sterilisation process have negative effects on both the mechanical strength and biologic activity of the graft material compared with autogenous bone.[16] Also in-vivo application of some osteoinductive materials may result with foregin body reaction.[24] Winkler et al stated in his study that both human and bovine bone grafts are suitable as local antibiotic carrier.[5]

This study is a prior study that has shown that considerable amounts of teicoplanin can be bound in human cancellous bone. The resulting antibiotic bone complex releases antibiotic into the surrounding medium, reaching high concentration initially and then decreasing and becaoming steadier for approximately two weeks. This seems to fulfill the conditions needed for bone infection treatment. During the treatment of deep infection, the initial antibiotic concentrations should be high enough for bacterial eradication. Thereafter decreased but steady antibiotic concentrations which prevents recontamination and enables the antibiotic to reach more distant parts of the operative field is important.[1,4,5] The autogenous corticospongios bone grafts obtained during surgery is the most valuable bone restoration material which can fill the bone defects, capable of mechanichal loading and promote bone healing. The osteogenic, osteoinductive and osteoconductive properties of autogenous bone cannot be provided by any known bone graft substitute. Corticospongious allografts which are the most commercially available bone graft material have only osteoconductive property. Autogenous bone is the most suitable graft material for bone healing however much it loses most of its ostegenic property during the process of antibiotic impregnation. Eventhough the donor site morbitity and the limitation of the bone stock, the agreeable time required for the antibiotic impregnation (1 hour) allows the procedure to be done during the surgery.

The main limitation of this study is that the real antibiotic concentrations could not be measured and a semiquantitative method (Modified disc diffusion technique) was used for the evaluation of antibiotic release. However Bayston and Miller have stated that modified disc diffusion technique is a sensitive method which can reveal long duration and lower concentrations of antibiotic release. The other limitation is the inavability to measure the variabilities such as pH changes, impregnation times and antibiotic concentrations.

Unfortunately very few studies have concerned the release of antibiotics from human cancellous bone. Therefore the pharmacokinetics information about the in-vivo release of antibiotics from human cancellous bone is limited. More studies concering pH differences, immun response and the effects of antibiotic solutions on neo-osteogenesis should be done.

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