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Characterization of biodegradable chitosan microspheres containing vancomycin and treatment of experimental osteomyelitis caused by methicillin-resistant *Staphylococcus aureus* with prepared microspheres

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Abstract

The biodegradable chitosan microspheres containing vancomycin hydrochloride (VANCO) were prepared by spray drying method with different polymer:drug ratios (1:1, 2:1, 3:1 and 4:1). Thermal behaviour, particle size and distribution, morphological characteristics, drug content, encapsulation efficiency, in vitro release assessments of formulations have been carried out to obtain suitable formulation which shows sustained-release effect when implanted. Sterilized VANCO loaded microspheres were implanted to proximal tibia of rats with methicillin-resistant *Staphylococcus aureus* (MRSA) osteomyelitis. Intramuscular (IM) injection of VANCO for 21 days was applied to another group for comparison. After 3 weeks of treatment, bone samples were analysed with a microbiological assay.

According to the results, encapsulation efficiency and yield of microspheres in all formulations were higher than 98% and 47%, respectively. Particle sizes of microspheres were smaller than 6 μm. All microsphere formulations have shown sustained-release effect. In vitro drug release rate decreased due to the increase in polymer:drug ratio but no significant difference was seen between these results ($p > 0.05$). Based on our in vivo data, rats implanted VANCO-loaded chitosan microspheres and administered IM injection showed 3354 ± 3366 and 52500 ± 25635 colony forming unit of MRSA in 1 g bone samples (CFU/g), respectively.

As a result, implanted VANCO-loaded microspheres were found to be more effective than IM route for the treatment of experimental osteomyelitis.

Keywords: Vancomycin; Biodegradable microspheres; Chitosan; Spray drying method; Methicillin-resistant *Staphylococcus aureus*; Experimental osteomyelitis

1. Introduction

Osteomyelitis being a difficult infection to treat and eradicate creates a challenging clinical problem in orthopaedics (Waldvogel et al., 1970). Increasing incidences of open fractures due to high-energy trauma from gunshot wounds, open injuries of high-speed motor vehicle accidents, bone and joint infection after total joint arthroplasty, antibiotic resistant pathogenic organism and hospital infections and different implants may increase the incidence of osteomyelitis.

The treatment of osteomyelitis can be applied in cases of modalities, surgical debridement, hyperbaric oxygen, soft tissue coverage and various methods of administration of antibiotics. Systemic antimicrobial treatments gave rise to the need of high serum concentration of the antibiotics for extended periods and thereby causing the drawbacks such as higher incidence of side effects, cost and low patient compliance.

Local antibiotic treatment seems to be an ideal method with providing better patient compliance and with avoiding adverse effects such as ototoxicity and nephrotoxicity. Local antibiotics may be given by various ways such as irrigation systems (Compere et al., 1967); venous or arterial perfusion (Finsterbush

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and Weinberg, 1972); implantable antibiotic pumps (Perry et al., 1985, 1986), non-biodegradable systems such as plaster of paris (Mousset et al., 1995), polymethyl methacrylate bone cement and beads (Buchholz et al., 1984; Adams et al., 1992), glass ceramic blocks (Kawanabe et al., 1998), hydroxyapatite blocks (Shinto et al., 1992; Korkusuz et al., 1993; Solberg et al., 1999), and biodegradable systems such as collagen sponges (Stemberger et al., 1997; Kanellakopoulou and Giamarellos-Bourboulis, 2000; Johansson et al., 2001; Mendel et al., 2005), fibrin clots (Tsourvakas et al., 1995), poly (DL-lactide) microspheres (Zhang et al., 1994; Kanellakopoulou et al., 1999, 2000; Castro et al., 2003), poly (DL-lactide-co-glycolide) microspheres (Garvin et al., 1994; Nie et al., 1998) and implants (Lucke et al., 2003, 2005; Mäkinen et al., 2005) and polycaprolactone microspheres (Hendricks et al., 2001; Rutledge et al., 2003).

A biodegradable drug delivery system would have the obvious advantage of eliminating the need for additional surgery to remove the drug carrier. In addition, the drug carrier does not prevent the formation of new bone due to its degradation in implantation area.

VANCO is a broad-spectrum glycopeptide antibiotic which is active against Gram-positive organisms. It is used particularly in the treatment and prophylaxis of staphylococcal infections including those due to MRSA and one of the drugs of choices against pathogens for osteomyelitis (The Extra Pharmacopoeia, 1996). VANCO is currently available in market as the parenteral infusion. It has a plasma half-life of about 6 h and its recommended dosage is 0.5 or 1.0 g two times daily. About 50% of VANCO is bound to plasma proteins. VANCO diffuses into extracellular fluids, including pleural, pericardial, ascetic and synovial fluid, in therapeutic concentrations (The Extra Pharmacopoeia, 1996). Ototoxicity and nephrotoxicity is seen to be associated with VANCO administration by intravenous route (Bailie and Neal, 1989; Gudmundsson and Jensen, 1989; Rybak et al., 1990).

In the literature, orthopaedic (Saito et al., 2002; Wilcox et al., 2003; Rauschmann et al., 2005), topical (Giandalia et al., 2001; Desmond et al., 2003) and ocular (Fleischer et al., 1986; Saitoh et al., 1997; Sotozono et al., 2002; Fukuda et al., 2003; Gavini et al., 2004) route for VANCO and beads (Henry and Galloway, 1995; Lambotte et al., 1998; Ozaki et al., 1998; Lin et al., 1999; Liu et al., 1999, 2002; Sanicola and Albert, 2005; Joosten et al., 2005), microsphere (Giandalia et al., 2001; Le Ray et al., 2005), microcapsule (Ozalp et al., 2001), nanoparticle (Barichello et al., 1999; Lai et al., 2003), liposome (Sanderson and Jones, 1996; Kadry et al., 2004) and implant (Gerhart et al., 1988, Calhoun and Mader, 1997; Thomazeau and Langlais, 1997; Shirtliff et al., 2002; Gitelis and Brebach, 2002) formulations of VANCO have been investigated.

Chitosan is natural, hydrophilic, biocompatible and biodegradable polymer with low toxicity already has been used to prepare microspheres (Giunchedi et al., 1999; Wong et al., 2002; Sinha et al., 2004). It is a cationic polysaccharide, derived by the deacetylation of chitin (Sinha et al., 2004).

The spray drying method is easy, reproducible and suitable method to obtain microspheres. This technique provides high encapsulation efficiencies, narrow size distributions with small particle sizes and low levels of toxic residual organic solvent when compared to other encapsulation methods (Benoit, 1996; Bitz and Doelker, 1996).

In this study, VANCO-loaded microspheres were prepared with chitosan using spray drying method. The surface morphology of the microspheres, the influence of polymer:drug ratio on the formation of microspheres, encapsulation efficiency and particle size were investigated. The formulations were characterized by in vitro release study. The best formulation providing sustained drug release was selected for in vivo study.

The purpose of this study was to prepare biodegradable chitosan microspheres for sustained release of VANCO and to prove its efficacy in an animal experimental osteomyelitis model.

2. Materials and methods

2.1. Materials

Vancomycin hydrochloride was obtained from Eli Lilly (Turkey). Chitosan (medium molecular weight, MW: 190–310 kDa, deacetylation degree: 75–85%) from Aldrich (Germany). Glacial acetic acid (100%) and hydrochloric acid (37%) from Merck (Germany), Mueller–Hinton Agar from Oxoid (UK).

2.2. Preparation of microspheres

VANCO-loaded chitosan microspheres (VMS1-VMS4) were prepared with polymer:drug ratios (w/w) of 1:1, 2:1, 3:1 and 4:1 (Table 1). Briefly, chitosan was dissolved in 1% (v/v) acetic acid solution to obtain a polymer solution at a concentration of 0.5% (w/v). Different amount of VANCO was dissolved in the polymer solution prepared. The prepared solutions (200 mL) were sprayed through the nozzle of a mini spray dryer (Büchi, Model 191, Switzerland). The process conditions were set as follows: Inlet temperature 130 ± 2 °C, outlet temperature 90 ± 2 °C, aspirator setting 100% capacity, pump setting 4 mL/min, spray flow rate 600 NL/h and nozzle diameter 0.5 mm. Microspheres were collected and weighed to determine production yield (PY) (Eq. (1)).

$$\mathrm{PY}(\%) = \frac{\text{Practical mass(microspheres)}}{\text{Theoretical mass(polymer + drug)}} \times 100 \qquad (1)$$

Each formulation was carried out in triplicate. Blank microspheres were prepared for comparison.

2.3. Differential scanning calorimetric (DSC) analysis

Thermal analysis using a DSC method were carried out on VANCO, chitosan, drug-loaded microspheres and blank microspheres, employing differential scanning calorimeter (TA Instruments, Model Q100, USA). Samples (approx. 5 mg) were accurately weighed into aluminium pans and sealed. All samples were run at a heating rate of 10 °C/min over a temperature range 25–350 °C in atmosphere of nitrogen.

Table 1
Production yield, actual drug content, encapsulation efficiency and particle size of VANCO-loaded microspheres ($n = 3$)

Formulation	Polymer	Polymer:drug ratio	Production yield % ± S.D.	Theoretical drug content (%)	Actual drug content % ± S.D.	Encapsulation efficiency % ± SD	Particle size μm ± S.D.
VMS1	Chitosan	1:1	50.16 ± 3.22	50.00	49.33 ± 0.92	98.66 ± 2.12	5.14 ± 0.56
VMS2	Chitosan	2:1	49.73 ± 2.94	33.33	32.96 ± 0.85	98.89 ± 1.98	4.78 ± 0.47
VMS3	Chitosan	3:1	47.01 ± 4.08	25.00	26.67 ± 0.89	106.68 ± 3.63	4.29 ± 0.41
VMS4	Chitosan	4:1	47.89 ± 4.71	20.00	21.06 ± 1.01	105.30 ± 2.85	3.87 ± 0.54

2.4. Size and morphology studies

Particle size analyses were performed on VANCO-loaded microspheres suspended in acetone by Malvern Mastersizer (Malvern Instruments, Mastersizer 2000, UK). The results are the average of three analyses. The d_{50} values for all formulations were expressed as mean particle size range of microspheres. For the investigation of morphology and surface characteristics, prepared microspheres were coated with gold–palladium under an argon atmosphere at room temperature and then the surface morphology of the microspheres were studied by scanning electron microscopy (SEM) using a JEOL JXA 840A (USA).

2.5. Actual drug content and encapsulation efficiency

Weighed samples of VANCO-loaded microspheres (approx. 60 mg) were dissolved in 100 mL 0.1 N HCl with ultrasonication for 4 h at 30 °C. The samples were filtered using 0.2 μm membrane filter and absorbances of samples were read at 281 nm using spectrophotometer. Actual drug content (AC) and encapsulation efficiency (EE) were calculated (Eqs. (2) and (3)). All analyses were carried out in triplicate.

$$\mathrm{AC}(\%) = \frac{M_{\mathrm{act}}}{M_{\mathrm{ms}}} \times 100 \quad (2)$$

$$\mathrm{EE}(\%) = \frac{M_{\mathrm{act}}}{M_{\mathrm{the}}} \times 100 \quad (3)$$

where M_{act} is the actual VANCO content in weighed quantity of microspheres, M_{ms} is the weighed quantity of powder of microspheres and M_{the} is the theoretical amount of VANCO in microspheres calculated from the quantity added in the process.

2.6. In vitro drug release studies

In vitro release profiles of VANCO from chitosan microspheres were examined in pH 7.4 phosphate buffer. The 1.0 mL of dissolution medium was put into eppendorf tube and 40 mg VANCO-loaded microspheres were suspended in. The tubes were put into Forma orbital shaker (Thermo Electron Corporation, USA) thermostated at 37 ± 0.5 °C at 120 ± 1 rpm. At scheduled time intervals, the tubes were taken and centrifuged at 5000 rpm for 5 min; 30 μL samples were withdrawn and replaced with fresh medium. The samples were diluted with same buffer and analysed spectrophotometrically at 281 nm. In vitro release studies for all formulations were done triplicate.

2.7. In vivo studies

Male Wistar albino rats were used for the animal model. All investigations were performed according to the European Community guidelines for animal experimentation (European Commission Directive, 1986). Experimental design and treatment of animals were approved by the Animal Care Committee of Abant Izzet Baysal University, School of Medicine. Osteomyelitis was established in rats using modified Norden's model of experimental osteomyelitis (Norden, 1970; O'Reilly et al., 1999). Sodium morrhuate was not used as a sclerosant agent, foreign body model by using a Kirschner wire was preferred in this modified model. Rats with weight of 200–250 g were fasted during 18 h and weighed before administration of the formulations. 24 rats were randomly divided into three groups of eight.

According to in vitro release experiments, the selected microsphere formulation (VMS4) and blank microspheres were placed in vials, sealed and γ-irradiated (25 kGy) using ^{60}Co as the radiation source at Gamma-Pak Sterilization Company, Turkey. The sterility of the microspheres was checked according to USP 24 "sterility tests" monograph (2000). Sterile microspheres were kept in sealed vials until to experiment.

The proximal tibial metaphysis was exposed through an anteromedial incision below the right knee of rats. A cavity was made using dental burr. 5.0 × 1.0 mm sized Kirschner wire was inserted into this cavity. A 0.2 mL suspension of a patient osteomyelitis strain of *Staphylococcus aureus* resistant to methicillin (MRSA) but sensitive to VANCO (minimum inhibition concentration (MIC) <4 μg/mL) containing 1.0×10^7 CFU/mL was injected into the intramedullary defect. Hole of cavity was sealed by applying a small amount of dental gypsum. The fascia was repaired with a running chromic suture, and the subcutaneous tissue and skin were closed with interrupted sutures. Lateral roentgenogram (Fig. 1) of the tibia was made preoperatively and for two weeks intervals postoperatively for the duration of each experiment. Scan with MDP ^{99}Tc was used. Sedimentation rate and C-reactive protein were also evaluated and experimental osteomyelitis in rats was confirmed (Data was not given).

After six weeks, the rats in all groups were operated and Kirschner wires were extracted. Most of the cases had an encapsulated abscess formation in the subcutaneous area which was excised. The infected area in the bone was also excised and thereafter washed with saline solution. In the first group (VMS4 group), sterile drug-loaded microspheres which are equivalent to intravenous dose of VANCO for 10 days treatment (equivalent to 133 mg VANCO/kg of rats) were inserted to proximal tibia metaphysis cavity of rats infected with MRSA to create

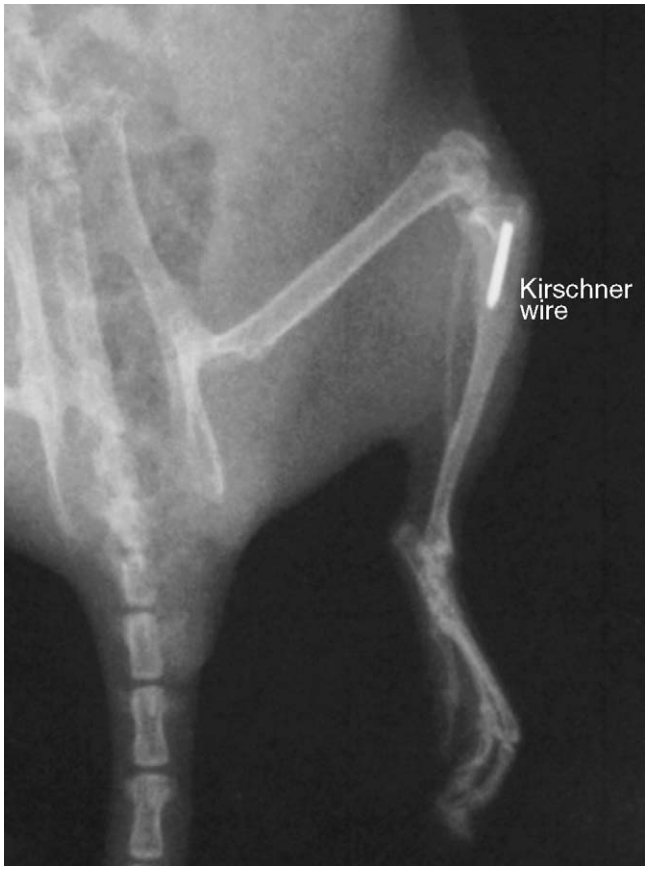

Fig. 1. Postoperative lateral roentgenogram at week 6 shows bone lysis and kirchner wire in the proximal defect in the tibia.

osteomyelitis. IM injection of VANCO (13.3 mg/kg/day) was applied to rats as a comparison for 21 days to the second group. Sterile blank microspheres were also implanted as a control to the third group. All the groups, the subcutaneous tissue and skin were closed with interrupted sutures.

After completion of antimicrobial therapy (after 21 days treatment), the rats were sacrificed with a lethal dose of ether. The infected bones were aseptically removed, weighed, and frozen. The frozen bone samples were grinded, suspended in 1 mL of saline, serially diluted and aliquots (0.1 mL) were plated on Mueller Hinton agar plates and incubated at $37 \pm 0.5\,^{\circ}C$ overnight. The plates were examined for purity and colony morphology. The colonies were counted and CFU/gram of bone was calculated. The results of treatment were expressed as the average CFU/gram of bone for each treatment group.

2.8. Histopathological analysis

Bone samples were removed from osteomyelitis area for histopathological examination, placed in a fixative solution of 10% (v/v) formalin for 1 day, decalcified in a 10% (v/v) nitric acid solution and washed with water and after automatized tissue process, embedded in paraffin. Longitudinal sections 4–5 μm thick were obtained using a standard rotary microtome and fixed onto silanized slides for staining with haematoxylin and eosin. Longitudinal sections of bone samples were investigated under light microscopy. Acute inflammatory cells (polymorphonuclear cells infiltration), chronic inflammatory cells (mononuclear inflammatory cells e.g., lymphocytes, plasmocytes), the presence of giant cells, fibroblast proliferation and the increase in capillaries were evaluated separately. Each item was graded by a single pathologist according to a semi quantitative approach as absent (0), mild (1), moderate (2) and severe (3) without the knowledge of the specimen groups.

2.9. Statistical analysis

In vitro release data obtained from each experiment and the relationships between in vivo results were subjected to statistical analysis using one-way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparisons test. Differences between the groups were tested for significance by the chi-squared test for the histological studies. $p < 0.05$ was considered to be indicative of significance.

3. Results and discussion

3.1. Characteristics of microspheres

VANCO-loaded microspheres (VMS1-VMS4) were successfully prepared in different polymer:drug ratios by spray drying method. Characteristics of microspheres are shown in Table 1. The production yield is relatively low percentage about 47–50% for all formulations and polymer:drug ratio did not affect to yield. This low yields can be attributed to both to small batch size as seen in the study of Giunchedi et al. (2002), and some of the liquid droplets attached inside the wall of main chamber of the spray drying apparatus. Actual drug contents were approximately 49%, 33%, 27% and 21% for the microspheres with polymer:drug ratios 1:1, 2:1, 3:1 and 4:1, respectively. In this study, like the other reports (He et al., 1999; Li et al., 2005), high drug encapsulation efficiency was achieved for all formulations (over 98%).

All microsphere formulations have particle size smaller than 6 μm diameter and mean particle size of microspheres ranged from 3.87 to 5.14 μm and increased with decreasing the polymer:drug ratio. The size distribution of all microsphere formulations was also very narrow and polymer:drug ratio did not significantly affect the size distribution of formulations. In the study of He et al. (1999), cimetidine–chitosan microspheres, which have small particle size (<5 μm) and narrow size distribution, were prepared by spray drying method.

SEM micrograph of VANCO-loaded microspheres (VMS4) is presented in Fig. 3. Microspheres (VMS1-VMS4) were spherical and have porous surface and roughness. Free drug crystals were not seen on the surface of microspheres. The polymer:drug ratio did not affect the morphology of the microspheres.

The physical state of the VANCO inside the microspheres was assessed by thermal analysis. According to the thermograms (Fig. 2), VANCO presented a broad endotherm centered at 105 °C due to drug decomposition by melting. Chitosan showed characteristic exothermic peak at 305 °C. The obtained results are in accordance with the literature (Giandalia et al., 2001;

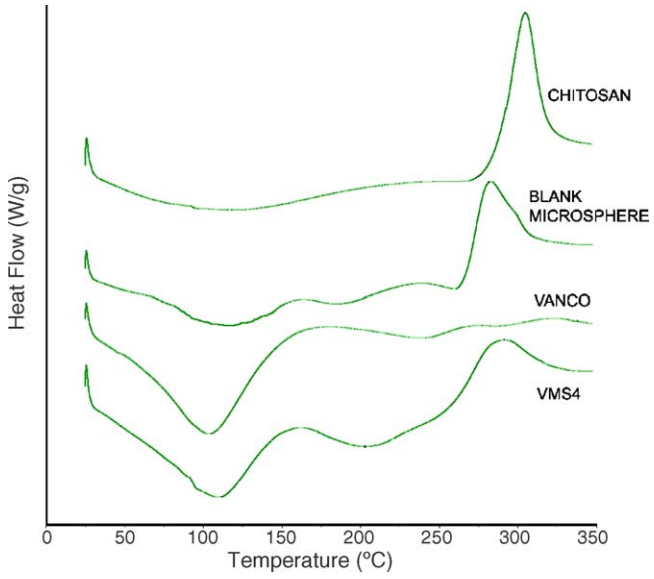

Fig. 2. DSC thermograms of chitosan, blank microspheres, VANCO and VANCO-loaded chitosan microspheres (VMS4).

Giunchedi et al., 2002). In the DSC curves of drug-loaded microspheres (VMS1-VMS4), characteristic peaks of chitosan and VANCO were seen. The thermograms of VANCO-loaded microsphere formulations showed that drug was in its crystalline form and also there was no interaction between VANCO and chitosan (Fig. 2). As a result, spray drying process did not change the nature of the drug in microspheres.

3.2. In vitro drug release

Fig. 4 displays the release profiles of VANCO from chitosan microspheres. The highest dissolution rate was obtained for VMS1, while VMS4 had slowest dissolution profiles. Drug release rate from the microsphere formulations could be arranged as follows: VMS1 > VMS2 > VMS3 > VMS4. In vitro drug release rate from microsphere formulations decreased as polymer:drug ratio increased. While release of the total amount of drug from VMS1 were achieved at 170 h, 73.02%, 62.97% and 58.68% drug release from VMS2, VMS3 and VMS4, respectively, was achieved in the same time. But these differences among the microspheres coded as VMS2, VMS3 and VMS4 were not significant ($p > 0.05$). Based on the in vitro drug release results, VMS4 which has slowest drug release was selected for the in vivo studies. Drug release profile from γ-irradiated microspheres (VMS4) was not changed significantly when compared to non-sterilized microspheres (data was not given).

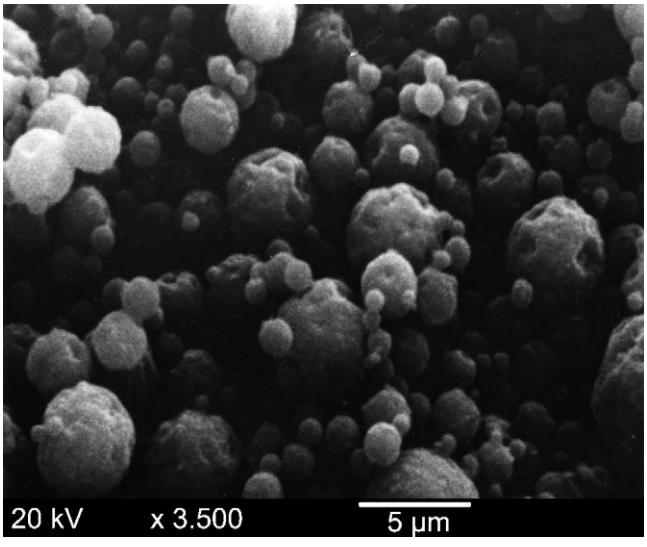

Fig. 3. SEM photography of VANCO-loaded chitosan microspheres (VMS4) (×3500).

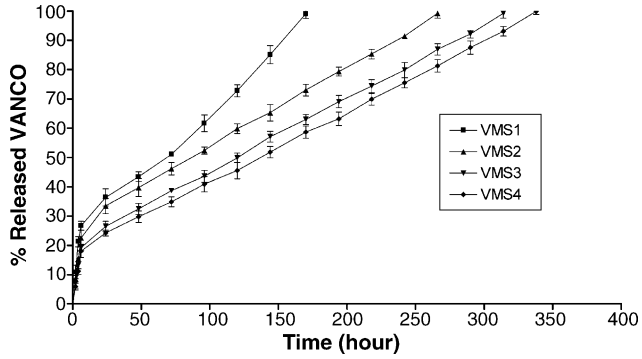

Fig. 4. In vitro release profiles of VANCO from drug-loaded microsphere formulations ($n = 3$).

3.3. In vivo studies

In our in vivo studies, modified Norden's experimental osteomyelitis model was used. The modification of the omission of sodium morrhuate will produce a model with very little dead bone which of course reduces the effectiveness of a local antibiotic delivery system.

In vivo results of treatments are given in Table 2. Average MRSA colonies per gram of bone sample after the local treatment by using chitosan microspheres and IM route are shown in Fig. 5.

Based on in vivo data, counted MRSA number in the bone samples applied VMS4 formulation was 3354 ± 3366 CFU/g, IM group and control group were found 52500 ± 25635 CFU/g

Table 2
MRSA colonies per gram bone sample after the treatment by VANCO-loaded chitosan microspheres (VMS4) and IM injection

Rat	MRSA colonies per gram bone sample (CFU/g)		
	Control group	VMS4 group	IM group
1	65000	5000	80000
2	80000	0	90000
3	90000	5000	30000
4	50000	9000	40000
5	75000	800	30000
6	80000	30	50000
7	40000	0	25000
8	70000	7000	75000
Mean ± S.D.	68750 ± 16636	3354 ± 3366	52500 ± 25635

$P_{VMS\ vs.\ IM} < 0.001$. $P_{VMS\ vs.\ Control} < 0.001$. $P_{IM\ vs.\ Control} > 0.05$.

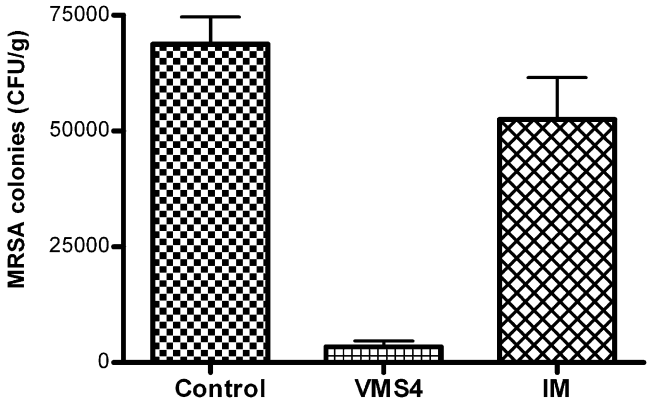

Fig. 5. Average MRSA colonies per gram of bone sample after 21 days treatment ($n = 8$).

and 68750 ± 16637 CFU/g, respectively. The differences between the VMS4 group and other groups were statistically significant ($p < 0.001$). But the difference between IM group and control group was not statistically significant ($p > 0.05$). Two of eight animals treated with VMS4 formulation did not grown MRSA. MRSA was grown from all other tibias. Despite low colony count compared to IM group and control group; we could not eradicate MRSA from all tibias; thus showing the need for further investigation for optimal dosing. In the study of Calhoun and Mader (1997), the systemic vancomycin was to be given at 30 mg/kg body weight every 12 h subcutaneously and the animals in each group were to be treated for 28 days. So our next study will focus on the optimum dosage of this formulation for fully eradication of bacteria without harming the animals.

There are few studies searching the biodegradable delivery systems in animal models of osteomyelitis (Kanellakopoulou et al., 2000; Huneault et al., 2004; Lazarettos et al., 2004).

Biodegradable carriers have an advantage over polymethylmethacrylate beads with bringing benefits like removing surgical application after releasing of antibiotics. On the other hand, the use of heat in the preparation of bone cement limits the use of heat-sensitive active ingredients. Those biodegradable carriers with combination of various antibiotics such as vancomycin achieve longer release at very high concentration and have become essential tools in the surgical management of osteomyelitis (Calhoun and Mader, 1997; Changez et al., 2005; Le Ray et al., 2003, 2005).

3.4. Histopathological analysis

Histopathologic findings are summarized in Table 3. Photomicrographs of trabecular bone in VMS4 group and IM group are shown in Figs. 6 and 7, respectively. The differences between the VMS4 and other groups were significant in terms of the acute inflammation ($p < 0.05$) and chronic inflammation ($p < 0.05$), fibroblast proliferation ($p < 0.05$) and increase in capillaries ($p < 0.05$). VMS4 group has shown less inflammation and fibrosis and increased capillaries compared to IM and control groups. VMS formulation has more pressurized the fibroblast proliferation compared to IM and control groups ($p < 0.05$). Significantly less necrotic bone tissue was seen in the VMS4 group ($p < 0.05$).

Table 3
The histological changes graded from absent to severe in the three groups by number and percentage

Histological alteration	VMS4 group	IM group	Control group	p^1 (X^2)[a]	p^2 (X^2)[a]	p^3 (X^2)[a]
Acute inflammation						
0	4 (50.0)	0 (0.0)	0 (0.0)			
1	4 (50.0)	2 (25.0)	0 (0.0)	0.014	0.001	0.287
2	0 (0.0)	3 (37.5)	3 (37.5)	(10.67)	(16.00)	(2.50)
3	0 (0.0)	3 (37.5)	5 (62.5)			
Chronic inflammation						
0	4 (50.0)	0 (0.0)	0 (0.0)			
1	3 (37.5)	2 (25.0)	1 (12.5)	0.042	0.019	0.788
2	1 (12.5)	3 (37.5)	3 (37.5)	(8.20)	(10.00)	(0.48)
3	0 (0.0)	3 (37.5)	4 (50.0)			
Giant cell						
0	3 (37.5)	2 (25.0)	2 (25.0)			
1	3 (37.5)	3 (37.5)	2 (25.0)	0.912	0.785	0.912
2	1 (12.5)	2 (25.0)	2 (25.0)	(0.53)	(1.07)	(0.53)
3	1 (12.5)	1 (12.5)	2 (25.0)			
Fibroblast proliferation						
0	4 (50.0)	1 (12.5)	0 (0.0)			
1	3 (37.5)	1 (25.0)	1 (12.5)	0.041	0.020	0.221
2	1 (25.0)	6 (62.5)	4 (50.0)	(6.37)	(9.80)	(4.40)
3	0 (0.0)	0 (0.0)	3 (37.5)			
Increase in capillaries						
0	0 (0.0)	2 (25.0)	2 (25.0)			
1	1 (12.5)	3 (37.5)	4 (50.0)	0.075	0.018	0.767
2	1 (12.5)	2 (25.0)	2 (25.0)	(6.91)	(10.13)	(1.14)
3	6 (75.0)	1 (12.5)	0 (0.0)			

[a] Significances between VMS4 and IM injection (p^1); VMS4 and Control (p^2); IM injection and Control (p^3).

Less foreign body type multinuclear giant cells were seen in the reaction area in the VMS4 group compared to IM and control groups, but these results was not statistically significant ($p > 0.05$). Residual microsphere particles were slightly detected in VMS4 and control groups. Histological analysis confirmed that the microspheres are biodegradable and did not impede the formation of new bone growth.

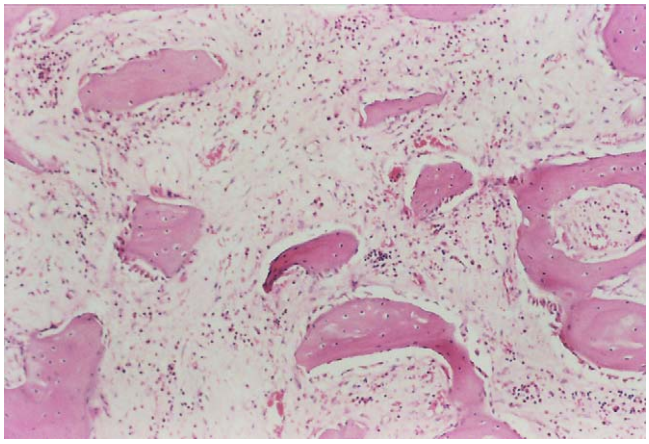

Fig. 6. Less inflammatory infiltrate which involves plasma cells and neutrophils were shown in trabecular bone in VANCO-loaded chitosan microsphere group (VMS4) (haematoxylin & eosin ×100).

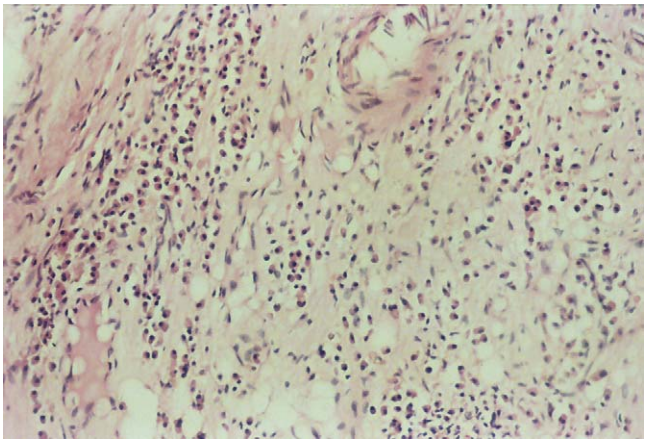

Fig. 7. Severe mix type inflammatory cells which involves plasma cells, lymphocytes and neutrophils were shown in trabecular bone in IM group (haematoxylin & eosin ×200).

4. Conclusion

The current regimen of osteomyelitis therapy involves surgical debridement and prolonged administration of high dose antimicrobial agents by orally or intravenously. Taking into this account, systemic antibiotic therapy, only a small fraction of the antibiotic will ultimately act at the infected site and high dosages may be required to reach effectively to the poorly vascularized area of bone infection. The severity of the disease, more adverse effects of prolonged therapy, low patient compliance and the high rate of failure of systemic therapy requires development of local delivery systems. Controlled and prolonged local release of antibiotics into the infected region play an important role in solving these problems and achieving high local antibiotic concentrations while maintaining low systemic levels (Henry and Galloway, 1995).

Studies have shown that most of the antibiotic is permanently trapped in the cement and cannot be released, though our in vitro release studies and in vivo results supported that biodegradable microspheres are more efficient and cost-effective delivery system compared to non-biodegradable carrier systems. The major disadvantage of the non-biodegradable systems is the need for their surgical removal at the completion of antibiotic release, which usually takes place 4 weeks after their implantation. The biodegradable carriers do not require surgical removal.

The optimal dosage of applied antibiotics; effects on resistance of these delivery systems are still under investigation. Though, its effectiveness, accompanied by the lack of systemic toxicity such as ototoxicity and nephrotoxicity, and the property of biodegradation, which avoids the necessity for surgical removal upon completion of antibiotic release, strongly support the future evolution of such delivery systems of glycopeptide and other appropriate antibiotics for the treatment of implant-related osteomyelitis.

The present study investigated VANCO-loaded chitosan microspheres delivery system for the release of effective concentration of antibiotics for the prolonged period required for eradication of implant-related osteomyelitis due to MRSA. VANCO-loaded chitosan microspheres (VMS4) implanted to proximal tibia of the rats were found to be more effective than IM route ($p < 0.001$) for the treatment of implant-related osteomyelitis.

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