Bone grafts as a vancomycine carrier for local therapy of resistant infections

Melicherčík Pavel[1], Jahoda David[1]*, Nyč Otakar[3], Klapková Eva[2], Barták Vladislav[1],Landor Ivan[1], Pokorný David[1], Krůta Tomáš[1], Sosna Antonín[1]

[1] I. ortopedická klinika UK 1.LF a FN Motol, head: Prof. MUDr. Antonín Sosna, DrSc.
V Úvalu 84, 150 06 Praha 5
[2] Ústav klinické biochemie a patobiochemie UK 2. LF a FN Motol (ÚKBP), head: Prof. MUDr. Richard Průša, CSc.
V Úvalu 84, 150 06 Praha 5
[3] Ústav lékařské mikrobiologie UK 2. LF a FN Motol, head: MUDr. Otakar Nyč, Ph.D.
V Úvalu 84, 150 06 Praha 5

Address for correspondence:
Doc. MUDr.David Jahoda CSc
l.Ortopedická klinika l.LF UK
V Úvalu 84, Praha 5
Fax: 24433920
670715/1759
Email : david.jahoda@post.cz

ABSTRACT. Infections of locomotor apparatus are among major complications in the orthopaedics and traumatology. They are often localized deep and require long-term therapy. The aim of an *in vitro* experiment was to find out to what extent vancomycine fixed to the bone grafts is potentially usable for the therapy of important infections.
Antibiotic concentration in mg/l was measured by high-efficiency fluid chromatography from 20 samples in certain intervals for the period of 16 days.
Concentration of released vancomycine was ranging highly above MIC for VISA for the whole period of observation. We have measured maximum mean antibiotic concentrations between 2nd and 4th day. Antibiotic concentration that is able to inhibit causing agent at the infectious location is a fundamental condition of successful antibiotic therapy. Local application of an antibiotic can in certain situations compensate limits of systemic administration and reduce general systemic therapy.
Vancomycine levels released from bone grafts highly exceeded MIC for methicillin-resistant strains of *Staphylococcus aureus* and coagulase-negative staphylococci for the whole period of observation.

Different results are published in the Witso´s work, who reports in his in vitro trial about lower vancomycine levels than MIC as early as after two weeks.
The experiment performed in laboratory conditions proved efficiency of bone grafts as vancomycine carrier. Measured concentrations highly exceeded MIC to vancomycine sensitive staphylococci and VISA during 16 days.

Infections of locomotor apparatus belong to major complications in the orthopaedics and traumatology. They are often localized deep and require long-term therapy. Eradication of their bacterial causers does not have to be always safe, which is related to frequently poor accessibility of systemic antibiotics into an infection location and to presence of bacteria in biofilm. This problem is currently even complicated by a significant rise of bacterial resistance to antibiotics. Staphylococci that are most frequent causers of infectious complications in the orthopaedics and traumatology are an example of these unfavourable trends.(Boucher et al. 2010, Bahrs et al. 2006, Džupa et al. 2008a, Džupa et al. 2008b, Gallo et al. 2006, Gallo et al. 2009, Jahoda et al 2006a, Jahoda et al. 2006b, Jahoda et al. 2007, Jahoda et al. 2008a, Jahoda et al. 2008b, Jahoda et al. 2008c, Meani et al. 2008, Nejedlý et al. 2007, Krbec et al. 2004). Methicillin resistant strains of *Staphylococcus aureus*(MRSA) and coagulase negative staphylococci (MRCoNS) thus present considerable limitations in selection of effective antibiotics either for prophylaxis or treatment. (Parvizi et al. 2009). It is known, that resistance to methicillin and related resistance to all beta-lactam antibiotics is e.g. in endemic clones of MRSA connected with resistance to all other groups of antibiotics (macrolides, lincosamides, fluoroquinolones) (Melte et al. 2004)

One of the chances how to increase therapeutical potential and reduce impacts of unfavourable development of resistance to antibiotics, there is a use of local application of antibiotic, that has already had a long tradition in the orthopaedics. This way, in case of properly chosen strategy, brings number of advantages – high local antibiotic concentration, reduction of undesirable effects related to systemic administration and reduction of risk of creation of resistance (Campoccia et al. 2010). Antibiotics considered, thanks to its stability, as proper ones for local therapy are particularly glycopeptides, aminoglycosides, macrolides and lincosamides (Randelli et al. 2009). Vancomycine as a representative of glycopeptide group is among preparations that currently belong to fundamental medicaments used for the treatment of heavy infections caused by MRSA and MRCoNS. Its disadvantage can be, in case of systemic administration, relatively frequent incidence of undesirable effects and nephrotoxicity hazards, especially in cases where high doses are applied for the purpose of reaching sufficient inhibition concentration in the infectious location (Cunha 2008). Therefore optimally selected local use of vancomycine, where it is possible, presents i.a. epidemiologically more safe variant regarding risks of onset of resistance due to low (subinhibition) concentrations, that can not be excluded in systemic, especially long-term therapy (Campoccia et al. 2010).

The goal of an experiment was to find out to what extent vancomycine is potentially usable for local therapy of osteomyelitis. There is number of local antibiotic carriers utilizable in the orthopaedics and traumatology. They can be divided e.g. by structure on systemic polymers, natural polymers, ceramics, composites and bone grafts. Polymethylmetacrylate (bone cement) is the most frequent carrier of antibiotics. Compared to other types of carriers it is rigid, and it has to be finally extracted. Collagen sponge with gentamycine of vancomycine is another frequently used carrier. We have chosen for our experiment bone grafts for their suitable qualities, e.g. biocompatibility, ability of osteoinduction, osteointegration and re-shaping by actual local needs of operated infected bone and also for their relatively easy availability, costs and generally positive experiences reported in the literature and in our department (Buttaro et al. 2003, Buttaro et al. 2005a, Buttaro et al. 2005b, Buttaro et al. 2007, Jahoda et al. 2008b, Winkler et al. 2000, Winkler et al. 2006, Winkler et al. 2008, Witso et al. 1999, Witso et al. 2000, Witso et al. 2005). If using vancomycine for local application by bone grafts, there are sufficient antibiotic concentrations, according to available literature, for the period of about two weeks (Buttaro et al. 2003, Meani et al. 2008, Witso et al. 1999). Therefore we have chosen 16-day interval of observation.

Material and methods

We have chosen morselized cancellous bone grafts impregnated by vancomycine (Edicin®) as a local antibiotic carrier for an *in vitro* experiment. Each from 20 samples was prepared in sterile conditions. We have obtained all bone grafts from femoral heads of patients undergoing hip joint replacement after their written agreement. Fibrous tissue, cartilage and cortical layer were removed from bone grafts gained within surgery. We have milled cancellous bone in a standard bone mill (ProSpon, 4,5 mm inlet of a shredder). Grinded bone grafts were impregnated by vancomycine powder by thorough mixing up for 15 minutes in a ratio 10g of bone grafts to 0,1 g of Edicin®. The ratio of 10g of bone grafts and 0,1 g of Edicin® was chosen to minimize an error when setting vancomycine concentration by preliminary measuring. We have weighted the mass of bone grafts and antibiotic by analytical scales. We took away 10 one-gram samples from bone grafts impregnated by vancomycine. Single samples of bone grafts were inserted into sterile gauze and tied up firmly. Then the samples with gauze were put into sterile 50ml test tubes, that were filled by 20ml of phosphate buffer (pH 7,4). Test tubes were placed into thermoregulator (37 °C) for 16 days. In above mentioned periods 100 µl of buffer was taken away from each test tube and then it was refilled by the same amount of fresh buffer. We picked this way of the level monitoring, because we assume, it corresponds better to the organism environment. Mass concentration of vancomycine released from bone grafts into a buffer was determined by high-efficiency fluid chromatography with UV detection in 230nm (Agilent 1200, Agilent Technologies, USA). Vancomycine levels were measured in laboratory temperature of 21 °C, on the colony of Purosphere RP-18e (125x3,5 mm, 5 µm, Merck), in a 0,5 ml/min flow rate of mobile

phase (acetonitrile, distilled water, phosphoric acid, pH 2,8). All used chemicals fulfilled HPLC quality.

Method was at first validated for measuring of an exact form of vancomycine from phosphate buffer.

Results were statistically compiled with the aid of Statistica 6.0 programme. Statistical compilation of results is demonstrated as an average and a standard deviation from 20 independent measurations. We used t test for comparing a significance of concentration difference between particular time intervals.

Results

During the first hour of measuring we recorded vancomycine levels highly exceeding MIC for VRSA, when almost 33% of impregnated vancomycine of all amount got released. After that, between first and fourth hours, elution is slower, but the quotient of released vancomycine in given interval is almost equal. From the 5th hour after carrier application, the pace of antibiotic releasing gradually slows down. After 12 hours from implantation we measured mean concentrations of released vancomycine - 420,98 mg/l, which corresponds with 84,2 % from highest possible vancomycine concentration in one sample. We measured maximum mean concentrations of released vancomycine, and at the same time its entire amount in surrounding buffer, between 2nd and 4th days (table 1), whereas concentration in this period can be regarded as steady, because there is no statistically significant difference between these two values ($p>0,005$). After reaching maximum mean concentration (2nd to 4th days) we recorded decrease of vancomycine concentration until the end of measurement, i.e. 16th day, by 167,42 mg/l.

Single mean vancomycine concentrations in mg/l (Mean), potency of T – test (T) and significance (p value) between time periods are documented in a table 1. Graphic demonstration of dynamics of vancomycine release from bone grafts in it´s mean concentrations is depicted in graph 1.

Discussion

Concentration of antibiotic capable to inhibit etiology agent in infectious location is a basic premise for successful antibiotic therapy. In case of osteomyelitis it is not always easy to meet this condition. If generally administered, many antibiotics have only limited penetration into bone tissue, for instance in case of beta-lactam antibiotics it is only 10 to 20 % of serum concentrations (Fraimow 2009). Probability of treatment failure then rises if there is existing lower sensitivity of microorganism, or apparent resistance to administered antibiotic.

It is not always possible to prove a causer in a laboratory and empirical use of antibiotic results from a hypothesis that the most likely causal organism is *S.aureus.* (Fraimow et al. 2009).

Expectation of MRSA incidence is regionally different, in case of not knowing the progenitor of particular infection, the choice of antistaphylococci antibiotic must respect local epidemiological

situation. Regarding a fact that this infection requires administration of maximum doses for a period of at least one month, probability of incidence of undesirable effects and toxic damage increases (Faden and Faden 2009).

Local antibiotic application can in certain situations compensate limits of systemic administration and reduce duration of general systemic treatment.

There are many different kinds of antibiotics and local carriers available in the orthopaedics and traumatology. Costs, mode and pace of impregnation by antibiotic, biocompatibility and spectrum of sensitivity of used antibiotic are among major problems. We consider Vancomycine as a suitable antibiotic both by spectrum of effect, covering all possible gram positive causal organisms including MRSA, and relatively low price and for other features and also for long-term experience with it´s local application (Buttaro et al. 2005, 2007, Witso et al. 1999, Winkler et al. 2006, 2008). In addition, this antibiotic in local concentration up to 1000 mg/l has none or minimal effect on osteoblast replication (Edin et al. 1996). HPLC method was selected for determining of antibiotic concentration in buffer solution for its high specificity and sensitivity (Dorothy et al. 1998). We chose an amount of extracted buffer sample (100 µl) for setting the concentration of released vancomycine because this way of extraction corresponds better with organism model in comparison with extraction of a buffer from sample. Moreover this way of extraction revealed long-term changes in vancomycine concentrations eliminated into partially permeable space.

Witso´s work similarly deals with vancomycine releasing from bone grafts. In his in vitro trial he reports of highest speed of increasing of antibiotic concentration during the first observation too, i.e. in the first day. After two weeks vancomycine levels were already under MIC (Witso et al. 1999). In another study, performed *in vivo* by Buttar, vancomycine concentration was derived from drainage fluid. He measured maximum antibiotic levels after 5 to 9 hours from application of bone graft with antibiotic (Buttaro et al 2005b). Witso took entire amount of buffer away from test tubes, and measured antibiotic concentration by immunochemical method (bioassay). Difference in volumes of extracted buffer in comparison with our experiment most probably results that Witso recorded vancomycine levels under MIC already after two weeks.

It was possible to expect the differences between results and the study performed *in vivo* by Buttaro. Amount of released vancomycine was measured in his drainage fluid, i.e. from closed compartment as in *in vitro* study. And Buttaro applied vancomycine into osteomyelitic site. The principle of correspondence of Buttaro and Witso´s results with ours lies in a fact that most of the antibiotic was released during the first measurement. It means that in *in vivo* situation the highest levels of vancomycine are reached within first hours, then the pace of antibiotic elusion slows down. According to our study levels of vancomycine would range above MIC, even after 16th day, inhibiting not only sensitive staphylococci, MRSA and MRCoNS (MIC < 2 mg/l), but even staphylococci with limited sensitivity to vancomycine (vancomycin-intermediate), or medium sensitive strains of

Staphyloccocus aureus (VISA, MIC: 4-8 mg/l). (Appelbaum 2007, Bert et al. 2009, Chang et al. 2003).

Regarding minimal dilution within extraction of buffers from samples, we recorded such a decrease of vancomycine levels after reaching its maximum values, that should theoretically never happen without outside effects. Only explanation for this could be thermic degradation of vancomycine into crystalliform degradation products (CDP-1), which amount increases and concentration of its active form (factor B) decreases. Thermic degradation of vancomycine happens already in temperature over 20 ºC . Degradation products of vancomycine lack antibacterial efficiency (Somerville et al. 1999). In our experiment we have determined only exact vancomycine without separation its active form and degradation products. In addition if we would note decrease of active form of vancomycine, then antibiotic concentration at the 2nd day would be 45mg/l according to Sommerville´s study. This concentration would still exceed MIC for VRSA. It remains question why concentration of an exact vancomycine decreases from 2nd to 4th day from the end of measuring. There are several hypotheses. Vancomycine is more likely consumed as a substrate by fungi or bacteria, that contaminated samples. It can be also gradually decomposed by enzymes present in bone tissue or a molecule constitutes, different from CDP-1 and factor B, and therefore not identifiable as exact vancomycine by used method. Single valued answer has not been not known yet. It is clear, from the performed trial, that in spite of vancomycine´s feature to create degradation products depending on surrounding temperature and duration of its action, its subsequent gradual fall of effective concentration levels is not caused only by its thermic degradation. For clinical practice it is though important, that even in spite of vancomycine´s feature to create degradation products depending on surrounding temperature and duration of its action, effective concentration exceeding MIC to vancomycine sensitive staphylococci and VISA stays preserved for certain period.

Performed experiment in laboratory conditions proved usability of bone grafts as carriers for vancomycine. During the period of 16 days, the measured concentrations highly exceeded MIC to vancomycine sensitive staphylococci and VISA. These concentrations are thus undoubtedly therapeutically effective and its long-term persistence is valuable in terms of prevention of creation of resistance to this antibiotic, eventually to all glycopeptide group.

Work came into being with support of VZ MZO 0064203-6604.

References

1. Appelbaum P.C: Reduced glycopeptide susceptibility in methicillin-resistant Staphylococcus aureus (MRSA). Int J Antimicrob Agents. **30**. 398-408. (2007).

2. Bahrs Ch., Marschal M., Weise K., Lingenfelter E., Dietz K., Heeg P., Eingartner CH: Acute Musculoskeletal Infection: Comparison of Different Methods for Intraoperative Bacterial Identification. Acta Chir. orthop. Traum. čech. **73**. 237. (2006).

3. Bert F., Leflon-Guibout V., Le Grand J., Bourdon N., Nicolas.Chanoine M.H: Emergence of vancomycin-dependent enterococci following glycopeptide therapy: case report and review. Pathol Biol (Paris). **57**. 56-60. (2009).
4. Boucher H., Miller G.L., Ranozabler R.: Serious Infection Caused by Methicillin – Resistant *Staphylococcus aureus.* Clin Infect Dis. **51** (S2). 184-197 (2010).
5. Buttaro M., González Della, Valle AM., Pineiro L., Mocetti E., Morandi AA., Piccaluga F: Incorporation of vancomycin-supplemented bone incorporation of vancomycin-supplemented bone allografts: radiographical, histopathological and immunohistochemical study in pigs. Acta Orthop Scand. **74**. 505-13. (2003).
6. Buttaro M., Pusso R., Piccaluga F: Vancomycin-supplemented impacted bone allografts in revision total hip arthroplasty. Two stage revision results. J bone Joint Surg (Br). **87**. 314-319. (2005).
7. Buttaro M., Gimenez M.I., Greco G., Barcan L., Piccaluga F: High local levels of vancomycin without nephrotoxicity released from impacted bone allograft in 20 revision hip arthroplasties. Acta Orthopeadica. **76**. 336-340. (2005).
8. Buttaro M., Comba F., Piccaluga F: Vancomycin-supplemented cancellous bone allografts in hip revision surgery. Clin Orthop Relat Res. **461**. 74-80. (2007).
9. Campoccia D, Montanaro L, Speziale P, Arciola CR. Antibiotic-loaded biomaterials and the risks for the spread of antibiotic resistance following their prophylactic and therapeutic clinical use. Biomaterials. **31**. 6363-77. (2010).
10. Cunha BA. Vancomycin revisited: a reappraisal of clinical use. Crit Care Clin. **24**(2). 393-420. (2008).
11. Dorothy W., Backes Hoda I., Aboleneen Janice A., Simpson: Quantitation of Vancomycin and its Crystalline Degradation Product (CDP-1) in Human Serum by High Performance Liquid Chromatography. Journal of Pharmaceutical and Biomedical Analysis. **16**. 1281-1287. (1998).
12. Džupa V., Nejedlý A., Čech O: Rekurentní osteomyelitida tibie po transportu kosti a její radikální interdisciplinární léčba. Acta Chir. orthop. Traum. čech. **75**. 387 – 391. (2008).
13. Džupa V., Ryantová V., Skála-Rosenbaum J., Vyhnánek F., Fric M., Grill R., Horák L., Pavelka T: Infekční komplikace operační léčby zlomenin pánve. Acta Chir. orthop. Traum. čech. **75**. 293 – 296. (2008).
14. Edin M.L., Miclau T., Lester G.E., Lindsey R.W., Dahners L.E: Effect of cefazolin and vancomycin on osteoblasts in vitro. Clin Orthp Relat Res. **333**. 245-251. (1996).
15. Faden D, Faden HS. The high rate of adverse drug events in children receiving prolonged outpatient parenteral antibiotic therapy for osteomyelitis. Pediatr Infect Dis J. **28**. 539-41. (2009).
16. Fraimow HS.: Systemic antimicrobial therapy in osteomyelitis. Semin Plast Surg. **23**. 90-9. (2009).
17. Gallo J., Sauer P., Dendis M., Lovečková Y., Kolář M., Zapletalová J., Janout V: Molekulární diagnostika infekcí kloubních náhrad. Acta Chir. orthop. Traum. čech. **73**. 85. (2006).
18. Gallo J., Smižanský M., Radová L., Potomková J: Porovnání léčebných postupů používaných v terapii infekce kloubních náhrad kyčle a kolena. Acta Chir. orthop. Traum. čech. **76**. 302-309. (2009).
19. Chang, S., Sievert D.M., Hageman J.C., Boulton M.L., Tenover F.C., Downes F.P., Shah S., Rudrik J.T., Pupp G.R., Brown W.J., Cardo D., Fridkin S.K: Infection with vancomycin-resistant *Staphylococcus aureus* containing the vanA resistance gene. N Engl J Med. **348**. 1342-7. (2003).
20. Jahoda D., Nyč O., Pokorný D., Landor I., Sosna A: Antibiotika v prevenci infekčních komplikací u operací kloubních náhrad. Acta Chir. orthop. Traum. čech. **73**. 108. (2006).
21. Jahoda D., Nyč O., Pokorný D., Landor I., Sosna A: Linezolid v léčbě rezistentních grampozitivních infekcí pohybového aparátu. Acta Chir. orthop. Traum. čech. **73**. 329. (2006).
22. Jahoda D., Nyč O., Šimša J., Kučera E., Hanek P., Chrz P., Pokorný D., Tawa N., Landor I., Sosna A.: Výskyt pozdní hematogenní infekce kloubních náhrad v našem souboru a návrh systému prevence. Acta Chir. ortop. Traum. čech. **74**. 397. (2007).
23. Jahoda D., Nyč O., Šimša J., Kučera E., Hanek P., Chrz P., Pokorný D., Tawa N., Landor I., Sosna A.: Pozdní hematogenní infekce kloubních náhrad. Acta Chir. ortop. Traum. čech.. **75**. 88 – 92. (2008).

24. Jahoda D., Sosna A., Nyč O., et al: Infekční komplikace kloubních náhrad. Praha, Triton (2008).
25. Jahoda D., Pokorný D., Nyč O., Barták V., Hrohádka R., Landor I., Sosna A: Infectious complications of total shoulder arthroplasty. Acta Chir. ortop. Traum. čech. **75.** 422-8. (2008).
26. Krbec M., Čech O., Džupa V., Pacovský V., Klézl Z.: Infekční komplikace TEP kyčelního kloubu. Acta Chir. ortop. Traum. čech. **71.** 179-188. (2004).
27. Meani E., Romano C., Crosby L., Hofmann G: Infection and Local Treatment in Orthopedic Surgery. US, Springer (2008).
28. Melter O, Aires de Sousa M, Laskafeldová K, Urbásková P, Wünschová M, deLencastre H. Delineation of the endemic and sporadic methicillin-resistant Staphylococcus aureus clones in a Czech hospital. Microb Drug Resist. **10**(3). 218-23. (2004).
29. Nejedlý A., Džupa V., Záhorka J., Tvrdek M: Využití muskulárního laloku při léčení infikovaných zlomenin a chronické osteomyelitidy v oblasti bérce a hlezna. Acta Chir. orthop. Traum. čech.. **74.** 162. (2007).
30. Parvizi J, Azzam K, Ghanem E, Austin MS, Rothman RH. Periprosthetic infection due to resistant staphylococci: serious problems on the horizon. Clin Orthop Relat Res. **467**. 1732-9. (2009).
31. Randelli P, Evola FR, Cabitza P, Polli L, Denti M, Vaienti L. Prophylactic use of antibiotic-loaded bone cement in primary total knee replacement. Knee Surg Sports Traumatol Arthrosc.**18**(2). 181-6. (2009).
32. Somerville A.L., Wright D.H., Rotschafer J.C : Implications of Vancomycin Degradation Products on Therapeutic Drug Monitoring in Patiens with End-Stage Renal Disease. Pharmacotherapy. **19**. 702-707. (1999).
33. Winkler H., Janata O., Berger C., Wein W., Georgopoulos A: In vitro release of vancomycin and tobramycin from impregnated human and bovine bone grafts. J Antimicrob Chemother. **46**. 423-8. (2000).
34. Winkler H., Kaudela K., Stoiber A., Menschik F: Bone grafts impregnated with antibiotics as a tool for treating infected implants in orthopedic surgery - one stage revision results. Cell Tissue Bank. **7**. 319-23. (2006).
35. Winkler H., Stobier A., Kaudela K., Winter F., Menschik F.: One stage uncemented revision of infected total hip replacement using cancellous allograft bone impregnated with antibiotics. J Bone Joint Surg Br. **90**(12). 1580-4. (2008).
36. Witso E., Persen L., Loseth K., Bergh K: Adsorbtion and release of antibiotics from morsolized cancellous bone. Acta Orthop Scand. **70**. 298-304. (1999).
37. Witso E., Persen L., Loseth K., Benum P., Bergh K: Cancellous bone as an antibiotic carrier. Acta Orthop Scand. **71**. 80-4. (2000).
38. Witso E., Persen L., Benum P., Bergh K: Cortical allograft as a vehicle for antibiotic delivery. Acta Orthop. **76**. 481-6. (2005).

Table 1. Single average vancomycine concentrations in mg/l (Mean), potency of T – test (T) and significance (p value) between time intervals. Statistical significance is set by value p> 0,005.

Time (h)	Number of samples (N)	Average X±SD	Range	T test	p value
1	20	165,1 ± 34,6	116,6 – 265,3		
				8,532	0,000
2	20	228,3 ± 38,1	185,3 – 311,7		
				8,059	0,000
3	20	283,7 ± 44,6	211,3 – 360,5		
				0,479	0,637
4	20	289,3 ± 38,4	238,6 – 362,8		
				2,621	0,017
5	20	323,5 ± 50,5	250,6 – 419,6		
				4,510	0,000
6	20	343,2 ± 60,5	263,2 – 458,8		
				27,472	0,000
12	20	420,9 ± 53,1	340,1 – 524,6		
				13,866	0,000
24	20	472,9 ± 43,8	412,4 – 563,6		
				4,602	0,000
48	20	499,7 ± 36,2	456,7 – 571,2		
				0,987	0,336
96	20	494,6 ± 31,9	446,3 – 537,1		
				6,439	0,000
144	20	463,8 ± 35,9	403,3 – 533,9		
				1,905	0,072
192	20	441,6 ± 40,3	358,0 – 538,3		
				3,067	0,006
240	20	401,2 ± 61,8	240,7 – 456,5		
				1,434	0,167
288	20	380,4 ± 40,5	320,2 – 436,4		
				0,252	0,803
336	20	377,8 ± 25,7	322,9 – 427,1		
				4,209	0,000
384	20	332,3 ± 48,8	227,5 – 405,7		

Graph 1 Graphic depiction of dynamics of vancomycine release from bone grafts in it´s mean concentrations during given time intervals.