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Original Article

The effect of a new oral anticoagulant (Rivaroxaban) on implants pull-out strength. An experimental study in rats

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Abstract

Objectives: Thromboprophylaxis reduces the risk of surgery related deep venous thrombosis and pulmonary embolism. The classical anticoagulants (heparin and LWMH) were associated with systemic osteoporosis, poor bone healing and materials' osseointegration. There is a lack of data concerning the effect of the new orally administered anticoagulants on osseointegration. The aim of this study is to investigate the possible effect of rivaroxaban, a direct anti-Xa factor, on osseointegration. **Methods:** Twenty eight white, male, Wistar rats were divided into two groups: Group A, study group (n=14) and group B, control group (n=14). In all animals under general anesthesia one screw was inserted on the right tibia. For twenty eight days the animals of group A received intraperitoneal rivaroxaban injections 5mgr/kgr every day. The animals of group B received intraperitoneal equal amount of normal saline injections. At the end of the four weeks all animals were sacrificed and their right tibias were excised and underwent the pull-out test. **Results:** The mean values of pull-out test were 92,10±19,12N for the control group and 95,46±21,02N for the study group. The statistical analysis using t-test showed no significant difference (p=0,665) for the pull-out test. **Conclusions:** These results indicate that Rivaroxaban hasn't got any deleterious effect on the osseointegration of implants on rats.

Keywords: Anticoagulation, Osseointegration, Oral, Pull-out, Rivaroxaban

Introduction

Thromboprophylaxis reduces the risk of surgery related deep vein thrombosis and pulmonary embolism[1,2]. The usual anticoagulants (heparin and low molecular weight heparins) where associated with systemic osteoporosis[3-6], a known risk factor for poor bone heeling and possibly implants' osseointegration[5,6].

Rivaroxaban (Xarelto, Bayer) is a novel oral anticoagulant with specific ability to inhibit factor Xa, a serine endopeptidase which plays a key role in coagulation.

There is strong evidence for the beneficial effect of Rivaroxaban in thromboprophylaxis as well in the increased compliance of the patients[7-9] and therefore it is in wide use postoperatively in orthopaedic operations, mainly in elderly patients with osteoporosis where implants (screws, plates, prosthesis) are applied.

The aim of this study was to investigate the possible effect of Rivaroxaban on osseointegration of the screw, the most common material used in every orthopaedic surgery.

Materials and methods

Animals and diet

All experimental designs and procedures have received approval of the Animals Ethics Committee of Aristotle University of Thessaloniki and the Veterinarian Authorities.

Twenty eight white, male Wistar rats of about the same weight (318±17 gr) and age (3 months) were divided into two groups[10]. Group A was the study group (n=14) and group B, the control group (n=14). The animals were acclimatized to the study conditions for a period of fourteen days before

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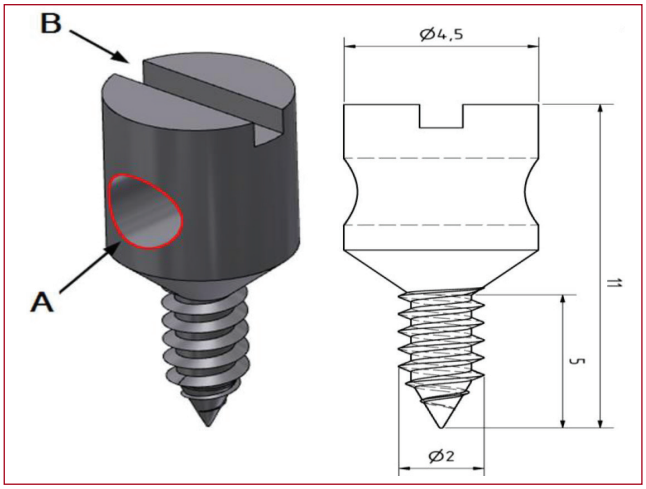
	Type 316 %	Type 316L %
Carbon	0.08 max.	0.03 max.
Manganese	2.00 max.	2.00 max.
Phosphorus	0.045 max.	0.045 max.
Sulfur	0.030 max.	0.03 max.
Silicon	0.75 max.	0.75 max.
Chromium	16.00 - 18.00	16.00 - 18.00
Nickel	10.00 - 14.00	10.00 - 14.00
Molybdenum	2.00 - 3.00	2.00 - 3.00
Nitrogen	0.10 max.	0.10 max
Iron	Balance	Balance

Figure 1. Chemical composition of the screw.



Figures 2. Orthopaedic screw used for the estimation of pull-out strength. In the head of the screw we can see the hole (A) used to fix the screw to the test rings and the cut (B) for the screw driver. The dimensions of the custom-made screw on the right side.

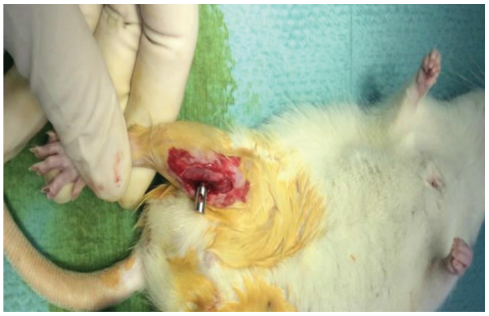


Figure 3. The incision and the screw on the right tibia before the skin sutures.

the implantation of the screws and were housed in special cages with automatic food and water administration, under stable conditions of temperature (22±2°C) and humidity (50±5%).

For a period of four weeks following implantation the animals of group A received intraperitoneal Rivaroxaban at a dose 5 mg/kg every day. This dose is the maximum dose for the human according to previous studies[11,12] and the solution was prepared after pulverization of the tablets, dialysis with 99,5% DMSO (dimethyl-sulphoxide) stirring in Vortex apparatus and centrifugal force at 15000g for 5min. The animals of group B received intraperitoneal equal amount of normal saline every day.

Every day the animals were examined for their general health, feeding, weight, any reactions, wound situation and healing, and the doses of the administered drugs were adjusted accordingly.

Implants design and preparation

The material used for the construction of the screws was stainless steel 316 L with the following chemical composition (Figure 1).

This material is the simplest and most common orthopaedic material in use. These custom made screws were 2 mm cortex screws with thread diameter 2,0 mm, core diameter 1,4 mm, diameter of head 4,5 mm with thread length 5,0 mm. This screw is special because of its head as it has a hole which allows the contact with the machine for the pull-out strength and gives stability. It has also a top cut for the screw driver (Figures 2, 3).

Implantation technique

The animals were anesthetized by abdominal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). The skin of the right tibia was saved and cleaned with 70% ethanol. Under aseptic conditions an anterior approach with a 10 mm incision was made to gain access to the proximal medial aspect of the tibia metaphysis. A 1 mm diameter hole was drilled and the implant was inserted, strictly perpendicular to the surface of the cortical bone on the medial part of the proximal tibia, with about the same entry point and the same depth. After insertion of the implant the skin was sutured using 3-0 absorbable polyagalactic sutures (vicryl) (Figure 3).

Biomechanical test (Pull-out test)

The tibias were excised immediately after the sacrifice of the animals and individually wrapped in saline soaked gauze and frozen at -20°C in plastic bags.

Twenty eight right tibias (14 of group A and 14 of group B) underwent the pull-out test. The experimental device "PROCRUSTIS" was used for the pull-out experiments. It was studied, designed and manufactured by the Laboratory

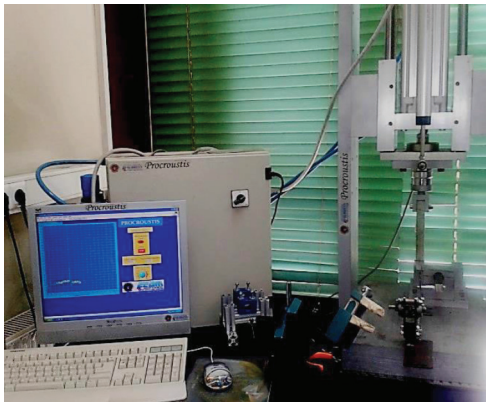


Figure 4. The experimental device "PROCRUSTIS" was used for the pull-out experiments.

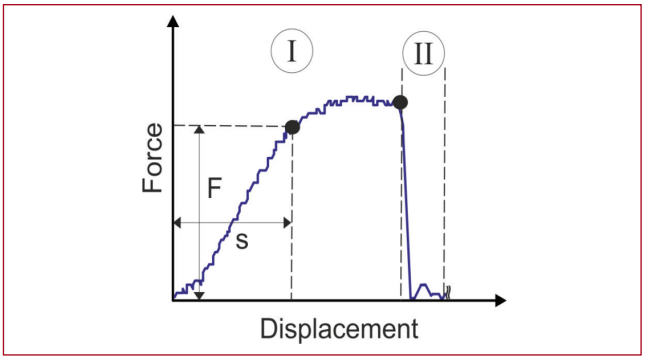


Figure 5. A typical result (Force-Displacement) is shown in the figure. When the pull-out experiment begins, the puller gains contact with the screw, the force raises linearly (Region I) until the screw is pulled out and the force turns to zero value (Region II).

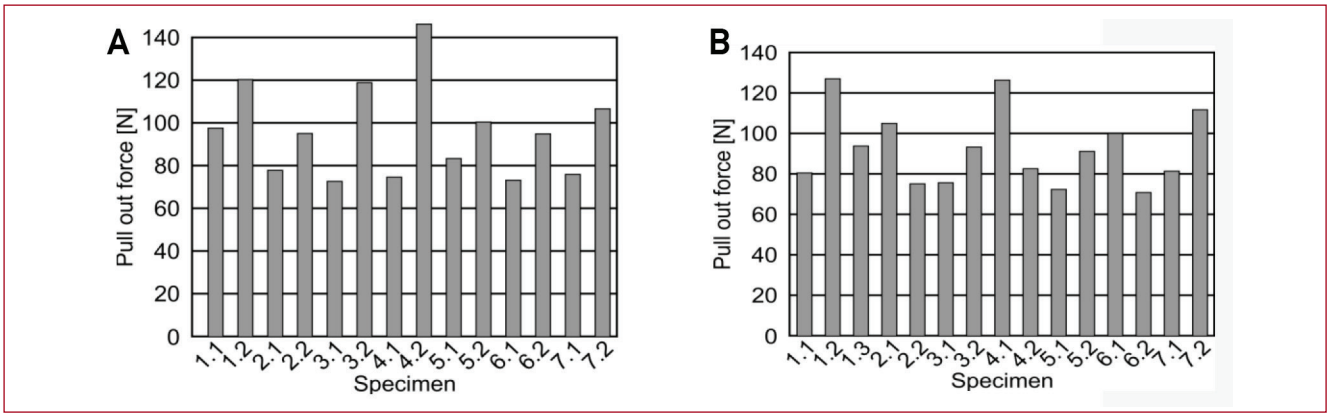


Figure 6. The prices of A) forces for each specimen on the study group from pull-out experiments, B) pull-out forces for each specimen for the control group.

for Machine Tools and Manufacturing Engineering (EEDM) of Polytechnic School of Aristotle University (Figure 4).

It uses advanced software and adjustments so that its operation to be fully automated. The experimental device consists of two basic mechanisms. The first one is the mechanism which holds the bone in a stable position. The second one consists of a piston that uses air pressure in order to move the puller. Between the piston and the puller a piezoelectric measurement device was set in order to record the force and the displacement. The analogue signal of the measurement device was turned into digital through an AT converter. The digital control, the data processing, the recording and the results presentation were achieved through a program based on "Labriew" software. A typical result (Force-Displacement) is shown in the figure. When the pull-out experiment begins, the puller gains contact with the screw, the force raises linearly (Region I) until the screw is pulled out and the force turns to zero value (Region II) (Figure 5).

Results

The prices of forces for each specimen of both groups from pull-out experiments is shown in Figure 6. The mean values of pull-out test were 92,10±19,12 N for the control group and 95,46±21,02 N for the study group.

Statistical analysis

SPSS 24 for Windows was used. Normality of distributions was determined using Shapiro-Wilk test. Comparisons between the two groups were determined by T-Test. Significance level was fixed at $p<0.05$. After statistical analysis using t-test ($p=0,664$) the statistical difference was not significant.

Discussion

Osseointegration refers to a direct bone to metal interface without interposition of non-bone tissue[13]. This concept has been described by Branemark as direct structural and functional connection between the bone and the surface of a load carrying implant[14,15]. Therefore the term osseointegration describes a clinical state that provides a long-term stability of an implant (prosthesis or screws). Further improvement of osseointegration's definition consists of multiple levels such as clinically, anatomically, histologically and ultrastructurally[16,17]. Osseointegration which is the connection of "one living" with "one dead" tissue is very important for the survival of the implant, a very serious matter in every day practice. This procedure of osseointegration is a very complicated and multifaceted "phenomenon" since we have the tissue response to implantation, the peri-implant osteogenesis, the peri-implant bone remodeling and mainly numerous factors (local, general, internal and external) affecting osseointegration[13,18]. Factors enhancing osseointegration include implant related factors (design, chemical composition, shape, coatings, etc), the status of the lost bone bed and its intrinsic healing potential, the use of adjuvant treatments such as bone grafting, osteogenetic biological coatings and pharmacological agents such as simvastatin, biphosphonates and teriparatide.

Factors inhibiting osseointegration include excessive implant mobility, radiation therapy and pharmacological agents such as cyclosporine A, methotrexate, warfarin, low molecular weight heparins, non-steroid anti-inflammatory drugs and patients' related factors such as osteoporosis, rheumatoid arthritis, advanced age, smoking, renal insufficiency etc[17,19].

Thromboprophylaxis is necessary postoperatively in the majority of the Orthopaedic patients and the main and most common weapon for this is still LMWH[20]. Unfortunately there is evidence for these drugs that they have deleterious effect on bone healing and osseointegration procedures[19,20]. Although the exact mechanism for this effect of LMWH on bone biology is not clear, it seems that LMWH reduce cancellous bone volume, affecting osteoblasts and human mesenchymal stromal cells in the early stages of bone healing[21,22]. In addition, LMWH seem to bind the vascular endothelium which disrupts callus vascular assembly and also increase the hematoma and cytotoxic effects on cells of the medullary callus[12,23].

The new orally administrated anticoagulant drugs are equally effective and safe with LMWH but there are also some indications that they do not affect bone healing[23,24]. Kluter et al in an experimental study found that Rivaroxaban does not impair fracture healing in a rat femur fracture model after analysis of callus by histology and the quality of the callus by CT scans[12]. Recent work from Israel[11] showed no delay in bone healing as well as no affection of cell mineralization after the administration of high doses of Rivaroxaban. This phenomenon can be explained by the effect of the drug being transient or by an adaptation of the cells to the inhibiting effect and compensation by some other mechanism[12,25]. It seems that the role of hematoma (post-traumatic or post-surgical) is essential in fracture healing as well as in osseointegration. So, increased fracture side hematoma volume as well as lacking hematoma may provide deleterious effects on physiological cascade of fracture healing[23]. A study of the effect of Enoxaparin, Fondaparinux and Rivaroxaban in fracture healing found no difference on fracture healing for the three drugs[26,27] but they used very small dose of Rivaroxaban (0,2 mg/kg) and for short period. Since Heparin and LWMH effects are dose dependent we used the maximum dose in order to have clear and obvious results. Resent study[28] for the effects of Heparin and Rivaroxaban on bone microstructure and metabolism (bone quality and quantity) found that Rivaroxaban leads to fewer adverse effects on bone.

The present study demonstrates that Rivaroxaban, a novel oral anticoagulant, doesn't at least influence the osseointegration of stainless steel screws at a dose leading to blood Rivaroxaban concentration close to the human maximum exposure after a therapeutic dose in everyday practice. This was illustrated by the small (non-statistically significant) increased force that it is necessary to loosen completely the implant. The evaluation of the test allows us to estimate the functional implant osseointegration into the surrounding bone which depends on several factors including bone mass amount and microarchitecture of the bone (quality). Here we demonstrate, for the first time, that Rivaroxaban, an oral anticoagulant widely used in orthopaedic surgery, hasn't got at least any deleterious effect on osseointegration of implanted materials. These results are only indicative and future studies are needed to confirm and investigate the possible mechanisms of Rivaroxaban in osseointegration's phenomenon.

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References

1. Prevention and treatment of venous thromboembolism. International consensus statement (Guidelines according to scientific advice). Int Angiol 2006;25:101-61.
2. National Institute for Health and Clinical Excellence. Reducing the risk of venous thromboembolism in patients undergoing surgery. NICE Clinical Guidelines No 46; 1-160 Apr 2007 http://www.nice.org.uk/Guidance/CG46.
3. Griffilh CC, Nichols G, Asher JD, Flanagan B. Heparin osteoporosis.

JAMA 1965;193:85.
4. Pettila V, Leinonen P, Mavkkola A, Hiilesmaa V, Kaaja R. Postpartum BMD in women treated for thromboprophylaxis with unfractionated heparin or LMW heparin. Thromb Haemost 2002;87(2):182-6.
5. Street JT, Mc Grath M, O'Regan K, Nakai A, Mc Guinness A, Redmond HP. Thromboprophylaxis using a low molecular weight heparin delays fracture repair. Clin Orthop Rel Res 2000;381:278.
6. Folwarczna J, Janiec W, Sliwinski L. Effects of heparin and low molecular weight heparins on bone mechanical properties in rats. Thromb Haemost 2004;92:940.
7. Eriksson BI, Borris LG, Friedman RJ, Haas S, Huisman MV, Bandel TJ et al. Rivaroxaban versus Enoxaparin for thromboprophylaxis after Hip Arthroplasty. N Engl J Med 2008;358:2765.
8. Kakkar AK, Brenner B, Dahl OE, Eriksson BI, Mouret P, Soglian AG et al. Extended duration rivaroxaban versus short-term enoxaparin for the prevention of venous thromboembolism after total hip arthroplasty, a double-blind, randomized controlled trial. Lancet 2008;372:31.
9. Bauersachs R, Berkowitz SD, Brenner B, Buller HR, Decousus H, Gallus AS, et al. Oral Rivaroxaban for symptomatic venous thromboembolism. N Enlgl J Med 2010;363:2499.
10. Skripitz R, Aspenberg P. Implant fixation enhanced by intermittent treatment with parathyroid hormone. J Bone Joint Surg 2001; 83B:437-440.
11. Dolkart O, Gigi R, Steinberg E, Amar E, Somjen D, Salai M. The effects of Direct Factor Xa inhibitor (Rivaroxaban) on Bone Healing: an in vitro and in vivo study. In Press.
12. Kluter T, Wenster M, Bruggemann S, Menzdorf L, Fitscen-Oestern S, Steubesand N et al. Rivaroxaban does not impair fracture healing in rat femur fracture model: an experimental study. BMC Musculoskeletal Disorders 2015;16:79.
13. Maurogenis AF, Dimitriou R, Parvizi J, Babis G. Biology of implant osseointegration. J Musculoskelet Neuronal Inter 2009;9(2):61-71.
14. Branemark PI. Vital microscopy of bone marrow in rabbit. Scand J Clin Lab Invest 1959;11:1-82.
15. Branemark PI. Osseointegration and its experimental studies. J Prosthet Dent 1983;50:399-410.
16. Stanford CM, Keller JC. The concept of osseointegration and bone matrix expression. Crit Rev Oral Biol Med 1991;2:83-101.
17. Linder L, Obrant K, Boivin G. Osseointegration of metallic implants. Transmission electron microscopy in the rabbit. Acta Orthop Scand 1989;60:135.
18. Matziolis G, Perka C, Disch A, Zippel H. Effects of fondaparinoux compared with dalteparin, enoxaparin and unfractionated heparin on human osteoblasts. Calcif Tissue Int 2003;73:370.
19. Callahan BC, Lisecki EJ, Banks RE, Dalton JE, Cook SD, Wolff JD. The effect of warfarin on the attachment of bone to hydroxyapatite-coated and uncoated porous implants. J Bone Joint Surg 2004; 77:225-30.
20. Kapetanakis S, Nastoulis E, Demesticha Th, Demetriou Th. The effects of Low Molecular Weight Heparins on fracture healing. The Open Orthop J 2015;9:226-236.
21. Monreal M, Vinas L, Monreal L, Lavin S, Lafoz E, Angles AM. Heparin related osteoporosis in rats. A comperative study between unfractioned heparin and low molecular weight heparin. Haemostasis 1990;20:204.
22. Kock HJ, Handsschin AE. Osteoblast growth inhibition by unfractionated heparin and by low molecular weight heparins: an in vitro investigation. Clin Appl Thromb Hemost 2002;8:251-5.
23. Prodinger PM, Burgkart R, Kreutzer K, Liska F, Pilge H, Schmitt A et al. Does anticoagulant Medication alter Fracture Healing? A morphological and biochemical evaluation of the possible effects of Rivaroxaban and Enoxaparin using a rat closed fracture model. PLOS ONE/DOI: 10. 1371/ journal.pone.0159669, July 25, 2016.
24. Pilge H, Frobel J, Prodinger PM, Mrotzek SJ, Fischer JC, Zilkens C et al. Enoxaparin and Rivaroxaban have different effects on human mesechymal stromal cells in the early stages in bone healing. Bone Joint Research 2016;5:95-100.
25. Gigi R, Salai M, Dolkart O, Chechic O, Katzburg S, Stern N, Somjen D. The Effects of Direct Factor Xa Inhibitor (Rivaroxaban) on the Human Osteoblastic Cell Line SaOS2. Connect Tissue Res 2012;53:446.
26. Papathanasopoulos A, Kouroupis D, Henskaw K, McGonagle D, Jones EA, Giannoudis PV. Effects on antithrombotic drugs fondaparinoux and tinzaparin on in vitro proliferation and osteogenic and chondrogenic differentiation of bone-derived mesechymal stem cells. J Orthop Res 2011;29:1327.
27. Demirtas A, Asboy I, Bulut M, Ucar B, Alabalic U, Necmioglou N. Enoxaparin, Fondaparinux and Rivaroxaban used in thromboembolism prophylaxis on fracture healing in rats. European Review Medical and Pharmacological Sciences 2013;17:1850-56.
28. Xia J, Zhang Z, Wang J, Zu J, Wang N, Wang D. Comparison of the effect of Heparin and Direct factor Xa inhibitor Rivaroxaban on bone microstructure and metabolism in adult rats. Connect Tissue Res 2015;56:477-82.