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Investigation of the Effects of Systemic Anticoagulants on Bone Defect Healing in Rat Tibiae

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Objective: This study aimed to histologically evaluate the effects of different direct oral anticoagulants (apixaban, rivaroxaban, edoxaban, dabigatran) on healing of standardized bone defects in rat tibias.

Materials and Methods: Forty eight adult Sprague-Dawley rats were randomly assigned to six groups: healthy control, sham control, apixaban, rivaroxaban, edoxaban, and dabigatran (n=8 each group). In all experimental groups, a standardized defect measuring 4 mm in diameter and 4 mm in depth was created in the metaphyseal region of the right tibia. No pharmacological agents were given to the control group, while experimental groups received oral gavage of the respective agents for four weeks at predetermined doses (apixaban 5 mg/kg, rivaroxaban 3 mg/kg, edoxaban 3 mg/kg, dabigatran 10 mg/kg). After four weeks, all rats were euthanized, tibias decalcified and stained with hematoxylin–eosin, and defect areas measured longitudinally and vertically.

Results: A significant difference was found among groups for longitudinal defect area (p=0.019). The rivaroxaban group had a significantly smaller mean longitudinal defect area than the control group (p=0.011). Reductions in other experimental groups were not statistically significant. No significant difference occurred among groups for vertical defect area (p=0.06); however, both dabigatran (p=0.019) and rivaroxaban (p=0.005) groups showed significant reductions compared to control. Rivaroxaban markedly enhanced bone healing in both longitudinal and vertical dimensions, while dabigatran significantly improved vertical healing.

Conclussion: These results suggest certain direct oral anticoagulants may exert beneficial effects on bone regeneration.

Key Words: *Bone defect healing, oral anticoagulants, apixaban, rivaroxaban, edoxaban, dabigatran*

Sistemik Antikoagulanların Rat Tibialarında Kemik Defekti İyileşmesine Etkisinin İncelenmesi

Amaç: Bu çalışmanın amacı, doğrudan oral antikoagülanların (apiksaban, rivaroksaban, edoksaban ve dabigatran) rat tibialarında oluşturulan standart kemik defektlerinin iyileşmesi üzerine olan etkilerini histolojik olarak araştırmaktır.

Gereç ve Yöntem: Çalışmada toplam 48 adet erişkin Sprague-Dawley rat altı gruba ayrıldı: sağlıklı kontrol, sham kontrol, apiksaban, rivaroksaban, edoksaban ve dabigatran (n=8 her grupta). Tüm deney gruplarında sağ tibianın metafiziyel bölgesinde 4 mm çapında ve 4 mm derinliğinde standart kemik defekti oluşturuldu. Kontrol grubuna herhangi bir farmakolojik ajan uygulanmazken, deney gruplarına 4 hafta boyunca belirlenen dozlarda (apiksaban 5 mg/kg, rivaroksaban 3 mg/kg, edoksaban 3 mg/kg, dabigatran 10 mg/kg) oral gavaj ile ilaç verildi. Dört haftanın sonunda tüm ratlar ötenazi edildi, tibialar dekalsifiye edilerek hematoksilen-eozin ile boyandı ve defekt alanları longitudinal ve vertikal olarak ölçüldü.

Bulgular: Longitudinal defekt alanı açısından gruplar arasında anlamlı fark bulundu (p=0.019). Rivaroksaban grubunda ortalama longitudinal defekt alanı kontrol grubuna kıyasla anlamlı derecede düşük bulundu (p=0.011). Diğer gruplarda azalma olmasına rağmen istatistiksel anlamlılık sağlanmadı. Vertikal defekt alanında gruplar arası genel fark anlamlı değildi (p=0.06), ancak dabigatran (p=0.019) ve rivaroksaban (p=0.005) gruplarında kontrol grubuna göre anlamlı azalma tespit edildi. Özellikle rivaroksabanın hem longitudinal hem de vertikal ölçümlerde kemik iyileşmesini en belirgin şekilde artırdığını, dabigatranın ise vertikal yönde anlamlı iyileşme sağladığını göstermektedir.

Sonuç: Elde edilen veriler, bazı oral antikoagülanların doğrudan kemik iyileşmesi üzerine olumlu etkiler gösterebileceğini ortaya koymaktadır.

Anahtar Kelimeler*:* *Kemik defekti iyileşmesi, oral antikoagülanlar, apiksaban, rivaroksaban, edoksaban, dabigatran*

Introduction

Bone tissue, with its dynamic structure and capacity for remodeling, is considered one of the most resilient tissues in the human body (1). However, the repair of bone defects resulting from trauma, osteoporosis, or surgical interventions remains a significant clinical challenge (2). Recently, there has been growing interest in biochemical agents that modulate bone healing, and the effects of various pharmacological agents, particularly systemically administered drugs, on bone metabolism have become a focal point of research (3).

Oral anticoagulants are among agents for the prophylaxis of non-valvular atrial fibrillation, venous therapeutic the preferred thromboembolism, and cardiovascular

diseases (4). Their clinical use began with warfarin, a vitamin K antagonist, and later expanded to include a new generation of drugs known as direct oral anticoagulants (DOACs), such as apixaban, rivaroxaban, edoxaban, and dabigatran (5). Atrial fibrillation has been identified as an independent and strong risk factor for stroke, and consequently, anticoagulant therapy has become increasingly common, particularly in elderly populations (6).

In recent years, research has focused not only on the effects of anticoagulant drugs on coagulation mechanisms but also on their influence on the skeletal system. In particular, warfarin, a member of the vitamin K antagonist class, has been reported to impair bone mineralization by inhibiting osteoblast function, thereby increasing the risk of calcification in both bone and vascular tissues (7). Systematic reviews and meta-analyses have shown a significant association between warfarin use and cardiovascular as well as valvular calcification (8). This phenomenon is believed to be linked to warfarin's inhibition of γ-carboxylation of vitamin K–dependent proteins, which play critical roles not only in the coagulation process but also in bone mineralization and the inhibition of vascular matrix calcification (7). In particular, insufficient carboxylation of osteocalcin and matrix Gla protein (MGP), vitamin K dependent proteins found in bone and vascular tissues has been associated with the progression of vascular calcification and a reduction in bone mineral density. Accordingly, long-term use of vitamin K antagonists may have adverse effects not only on the cardiovascular system but also on the skeletal system (9). Animal studies investigating the effects of anticoagulants on bone regeneration are limited. Preliminary findings suggest that DOACs, such as rivaroxaban, may exert significant effects on parameters such as osteoblastic proliferation, bone density, and callus formation (10). However, the underlying mechanisms remain unclear, and it is thought that different DOACs may exert varying degrees of effect.

In this research, the effects of systemically delivered anticoagulants, including apixaban, rivaroxaban, edoxaban, and dabigatran, on bone repair were investigated using a rat tibial defect model and comparative histopathological assessment. The aim was to elucidate the potential effects of these widely prescribed clinical agents on bone regeneration and to contribute experimental evidence to the literature in this field.

Materials and Methods

Research and Publication Ethics: This research was conducted at the Experimental Research Center of Firat University (Elazig, Türkiye) after obtaining approval from the university's Animal Experiments Local Ethics Committee (Approval No: 2024/01-11; January 9, 2024).

Sprague Dawley rats, aged at least 3.5–4 months and weighing approximately 250–300 g each, were used in the study. In cases where female animals were preferred, vaginal smears were performed to synchronize the estrous cycle, and only those in the same stage were included in the experiment.

The animals were randomly divided into six groups:

Healthy control group (n = 8): Group with no defect created

Sham control defect group (n = 8): Surgical bone defect created; no treatment administered.

Apixaban group (n = 8): Bone defect + apixaban at 5 mg/kg, administered three times per week.

Rivaroxaban group (n = 8): Bone defect + rivaroxaban at 3 mg/kg, administered daily.

Edoxaban group (n = 8): Bone defect + edoxaban at 3 mg/kg, administered daily.

Dabigatran group (n = 8): Bone defect + dabigatran at 10 mg/kg, administered daily.

After induction of anesthesia through intraperitoneal administration of ketamine (50 mg/kg) and xylazine (10 mg/kg), the periosteum was elevated, and a 4 mm diameter by 4 mm depth defect was drilled in the right tibia's metaphyseal portion, extending to the corticocancellous structure. The soft tissues were repositioned to their original anatomical position, and primary closure was achieved using 4-0 silk sutures. Postoperatively, tramadol hydrochloride (1 mg/kg) and penicillin (50 mg/kg) were administered intramuscularly for the first three days.

The designated anticoagulants were administered to the respective groups via oral gavage at the specified doses and frequencies for a duration of four weeks. No medication was given to the healthy control or sham groups. All animals were ethically sacrificed at the end of the four-week experimental period. The right tibias were harvested, fixed in 10% neutral formalin for 72 hours, and subsequently decalcified in 10% formic acid for approximately one week. Tissue processing was performed using an automated tissue processor (Leica TP1020, Germany), and samples were embedded in paraffin. Sections with a thickness of 3 µm were prepared using a rotary microtome (Leica RM2125 RTS, Germany) and stained with hematoxylin–eosin. Microscopic evaluations were conducted using a light microscope (Olympus BX42, Japan).

Histological evaluation was performed based on the percentage of new bone formation and the degree of fibrosis within the defect areas. For morphometric analysis, the widest (longitudinal) and deepest (vertical) dimensions of the defects were measured.

The obtained data were analyzed using parametric statistical methods. Comparisons between groups were performed using the one-way analysis of variance (ANOVA), and in cases where significant differences were detected, Tukey's HSD post hoc test was applied. A p-value of <0.05 was considered statistically significant.

Results

Histological examination revealed that in the healthy control group (no defect created), the integrity of the bone structure was preserved, whereas in the sham control group, the defect areas were markedly larger and healing remained limited. In the drug-administered groups, bone regeneration occurred to varying degrees depending on the type of anticoagulant used (Figure 1).

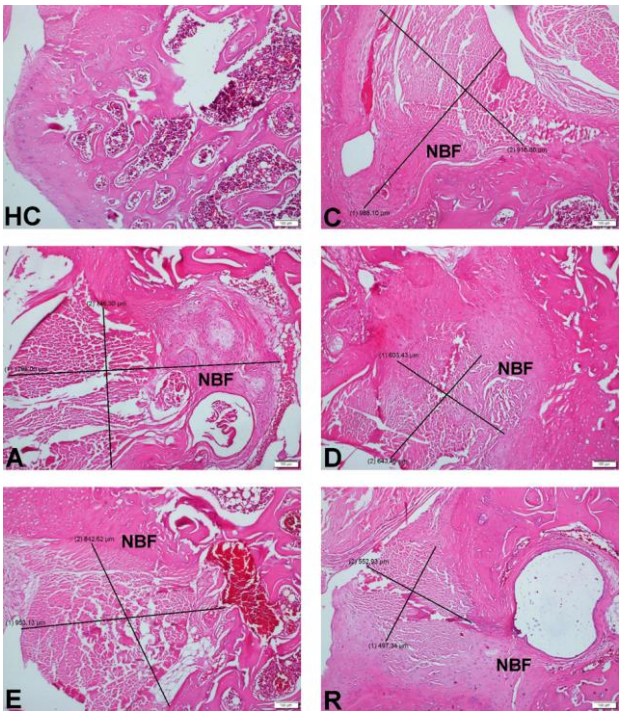

Figure 1. Width measurements of the defect area and new bone formation (NBF) areas in the defect region of the healthy control and experimental groups

According to the vertical bone analysis, the largest defect area was measured in the sham control group (843.25±169.90 µm). A significant reduction was observed in the rivaroxaban (637.75±75.23 µm; p=0.002) and dabigatran (666.00±70.07 µm; p=0.012) groups compared to the sham control group, whereas the reductions observed in the apixaban (717.00±101.42 µm) and edoxaban (742.63±95.90 µm) groups did not reach statistical significance (Table 1).

Longitudinal bone analysis results also showed a statistically significant difference between the groups (p<0.001). The largest defect area was measured in the sham control group (906.00±115.63 µm). In the rivaroxaban group (605.38±92.86 µm), a significantly smaller defect area was detected compared to the sham control (p=0.006). Although the defect areas in the dabigatran (732.38±144.00 µm), apixaban (817.00±257.72 µm), and edoxaban (801.25±198.37 µm) groups were smaller than those in the sham control group, these differences did not reach statistical significance (Table 2).

The findings indicate that systemic rivaroxaban administration significantly improved bone defect healing in both vertical and longitudinal directions, while dabigatran achieved a statistically significant improvement only in the vertical direction. Although apixaban and edoxaban groups showed a trend toward improvement, these changes did not reach statistical significance.

Table 1. Vertical bone analysis of the groups

Groups	n	Mean	Std. Deviation	P*
Control	8	843,25	169,90	0,000
Apixaban	8	717	101,42	
Dabigatran[a1]	8	666	70,07	
Edoxaban	8	742,63	95,90	
Rivaroxaban[a2]	8	637,75	75,23	
Healthy control[a3,b,c,d,e]	8	115,25	30,64	

*One Way Anova Test. a Statistically significantly different compared with the controls [a] Tukey HSD Test (P; [a1]: 0,012, [a2]:0,002 [a3]:0,000). [b] Statistically significantly different compared with the apixaban (P; [b] : 0,000). [c] Statistically significantly different compared with the dabigatran (P; [c] : 0,000). [d] Statistically significantly different compared with the dabigatran (P; [d] : 0,000). [e] Statistically significantly different compared with the rivoraxaban (P; [e] : 0,000). [a,b,c,d,e]: Tukey HSD Test

Table 2. Defect area longitudinal analysis of the groups

Bone Parameter	Groups	n	Mean (μ)	Standart Deviation	*p**
Longitudinal analysis	Control	8	906	115,63	0,000
	Apixaban	8	817	257,72	
	Dabigatran	8	732,38	144	
	Edoxaban	8	801,25	198,37	
	Rivaroxaban[a1]	8	605,38	92,86	
	Healthy controls[a2,b,c,d,e]	8	133,25	35,61	

*One Way Anova Test. [a] Statistically significantly different compared with the controls (*p*: [a1]: 0,006 *p*: [a2]: 0,000). [b] Statistically significantly different compared with the apixaban (*p*; [b] : 0,000). [c] Statistically significantly different compared with the dabigatran (*p*: [c] :0,000). [d] Statistically significantly different compared with the edoxaban (*p*: [d] : 0,000). [e] Statistically significantly different compared with the rivaroxaban (*p*;[e] : 0,000). [a,b,c,d,e]: Tukey HSD Test

Discussion

In this study, the effects of systemic oral anticoagulants (apixaban, rivaroxaban, edoxaban, and dabigatran) on the regenerative process in standardized bone defects created in rat tibias were evaluated. The findings demonstrated that bone healing was more advanced in the groups treated with rivaroxaban and dabigatran.

In the longitudinal analysis, the smallest defect area was observed in the rivaroxaban group ($p = 0.011$). In the vertical measurements, both rivaroxaban and dabigatran groups exhibited significantly smaller defect areas compared to the control group. This indicates that both agents had a positive effect on bone healing.

Although a reduction in defect areas was also observed in the apixaban and edoxaban groups, these decreases, while numerically lower than in the control group, did not reach statistical significance. This suggests that, although these agents may exert some effects on bone tissue, their efficacy might be more limited compared to other DOACs such as rivaroxaban and dabigatran. Pharmacologically, DOACs act as either factor Xa inhibitors (apixaban, edoxaban, rivaroxaban) or thrombin inhibitors (dabigatran), and their impact on the osteoblast/osteoclast balance may differ among agents (11).

Gigi et al. (12) reported that rivaroxaban exerts effects that support osteoblast activity and enhance bone mineralization. Duy Mai et al. (11) stated that dabigatran directly inhibits thrombin, thereby suppressing osteoclast activity, limiting bone resorption, and supporting healing. The positive effects of dabigatran have been confirmed in other studies as well (10, 13). Rocha et al. (14) suggested that dabigatran may inhibit RANKL-mediated osteoclastogenesis, thereby reducing inflammatory mediators and contributing to increased new bone formation. These studies are consistent with the present results regarding the effects of dabigatran and rivaroxaban, supporting our findings. The effects of edoxaban on bone tissue have been addressed in only a limited number of studies, and its role in osteogenesis remains unclear. Zhang et al. (15) have reported similar observations for edoxaban.

The sham group consisted of subjects in which only a surgical defect was created without systemic drug administration. This group represented the natural healing process of bone tissue in response to surgical trauma. The significantly larger defect areas observed in the sham group compared to the rivaroxaban and dabigatran groups suggest that these anticoagulants may accelerate the regenerative process. Similarly, Siltari et al. (16) reported that suppression of surgery-induced local inflammation by systemic anti-inflammatory and anticoagulant effects may positively influence osteogenic cell activity; this finding has also been supported by Shahbazi et al. (17) and other studies.

The present findings indicate that, unlike traditional anticoagulants such as warfarin, DOACs may have the potential to avoid negative effects on bone. Wu et al. (18) reported that warfarin, through vitamin K antagonism, inhibits the carboxylation of bone proteins such as osteocalcin and matrix Gla protein, leading to adverse effects on both the vascular and skeletal systems. Liu et al. (19) stated that DOACs, by not interfering with these mechanisms, may represent a safer option for bone health. Based on the present data and these reports, DOACs may be considered as an alternative to warfarin in order to avoid such negative effects, especially when their beneficial effects on bone are also taken into account.

In this context, the study findings provide important contributions at both the experimental and clinical levels. Our results confirm previously reported positive effects of rivaroxaban and dabigatran on bone regeneration (10, 12, 14). In patients receiving DOAC therapy, the potential contribution of these agents to bone healing should be considered during preoperative planning for surgical interventions.

In conclusion, the findings of this study indicate that certain systemically administered direct oral anticoagulants may positively influence the healing process of bone defects created in rat tibias. In particular, measurements in the rivaroxaban and dabigatran groups revealed significantly smaller defect areas compared to the control group, suggesting that both agents may accelerate bone regeneration and

enhance the healing process. Although reductions in defect area were also observed in the apixaban and edoxaban groups, these changes were not statistically significant.

The obtained data suggest that rivaroxaban and dabigatran may have the potential to promote osteogenic processes and accelerate bone regeneration, whereas the effects of edoxaban and apixaban in this regard may be more limited. These results emphasize the importance of considering the potential influence of anticoagulant selection on the healing process in clinical situations where postoperative bone healing is critical. Further prospective and controlled human studies are required to translate the findings obtained from this experimental animal model into clinical practice.

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