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THE EFFECT OF POSTOPERATIVE ADMINISTRATION OF LOW-MOLECULAR-WEIGHT-HEPARIN (TINZAPARIN) ON ARTERIAL THROMBOSIS IN AN EXPERIMENTAL RAT MODEL

DENEYSEL BİR RAT MODELİNDE POSTOPERATİF DÜŞÜK MOLEKÜL AĞIRLIKLI HEPARİN (TİNZAPARİN) UYGULAMASININ ARTERİYEL TROMBOZ ÜZERİNDEKI ETKİSİ

YILDIRIM GÜLTEKİN[1] ALİ BOLAT[2] MERİH ÖZBAYBURTLU[1] HATİCE KELEŞ[2] ATİKE TEKELİ KUNT[2]

[1]Eskişehir City Hospital, Cardiovascular Surgery Clinic, Eskişehir, Türkiye
[2]Department Of Cardiovascular Surgery, Faculty Of Medicine, Kirikkale University, Kirikkale, Türkiye
[3]Department Of Internal Medicine, Faculty Of Medicine, Kirikkale University, Kirikkale, Türkiye

ABSTRACT

Introduction: Low molecular weight heparins are licensed in the prophylaxis of venous thromboembolism but not of arterial thrombosis. This study aimed to evaluate the effect of postoperative administration of low molecular weight heparin, tinzaparin, on the rate of arterial thrombosis in a rat model.

Methods: The right femoral arteries of sixteen male Wistar Albino rats were incised transversely and then were repaired with a continuous suture. Eight rats were given 175 IU/kg/day tinzaparin postoperatively for three days, while the remaining eight rats were kept free of tinzaparin. All rats were controlled daily for vascular circulation, bleeding, and hematoma until the reoperation on the fourth postoperative day. Reoperation was performed to explore vascular patency and excise a sample of vascular tissue from the repaired femoral artery for histopathological examination. A blood sample was also withdrawn for the detection of anti-factor Xa activity to show the efficacy of tinzaparin.

Results: During the postoperative three-day follow-up period, while vascular circulation disorder was detected in none of the tinzaparin-treated rats, it was detected in two rats not treated with tinzaparin (25%). None of the rats in either group developed bleeding or hematoma at the surgical site. Anti-factor Xa activity in the rats treated with tinzaparin postoperatively was found to be significantly higher than in the rats not treated with tinzaparin ($p<0.001$). Histopathological examination revealed thrombus and fibrin formation at the femoral artery incision line in only one rat (12.5%) treated with tinzaparin, and in seven rats (87.5%) not treated with tinzaparin ($p<0.001$). Intimal hyperplasia was not detected in any group, but mixed-type inflammatory cell infiltration and endothelial and fibroblastic activity around the sutures were noted in both.

Conclusion: The postoperative subcutaneous administration of 175 IU/kg/day tinzaparin effectively attenuates the rate of arterial thrombosis in arterial surgical interventions in a rat model.

Keywords: Arterial thrombosis, low molecular weight heparin, tinzaparin

ÖZET

Giriş: Düşük molekül ağırlıklı heparinler, venöz tromboembolizmin profilaksisinde ruhsatlıdır ancak arteriyel trombozun profilaksisinde yoktur. Bu çalışmada sıçan modelinde postoperatif düşük molekül ağırlıklı heparin tinzaparinin uygulanmasının arteriyel tromboz oranına etkisinin değerlendirilmesi amaçlandı.

Yöntemler: On altı erkek Wistar Albino sıçanın sağ femoral arterleri transvers olarak kesildi ve daha sonra sürekli dikişle onarıldı. Sekiz sıçana ameliyat sonrası üç gün boyunca 175 IU/kg/gün tinzaparin verilirken, geri kalan sekiz sıçana tinzaparin verilmedi. Tüm sıçanlar ameliyat sonrası dördüncü günde tekrar ameliyata alınana kadar damar dolaşımı, kanama ve hematom açısından günlük olarak kontrol edildi. Vasküler açıklığı araştırmak ve histopatolojik inceleme için onarılan femoral arterden bir vasküler doku örneğini çıkarmak için yeniden ameliyat yapıldı. Tinzaparinin etkinliğini göstermek amacıyla anti-faktör Xa aktivitesinin saptanması için de bir kan örneği alındı.

Bulgular: Postoperatif üç günlük takipte tinzaparin uygulanan sıçanların hiçbirinde damar dolaşım bozukluğu saptanmazken, tinzaparin tedavisi uygulanmayan iki sıçanda (%25) damar dolaşım bozukluğu tespit edildi. Her iki gruptaki sıçanların hiçbirinde cerrahi bölgede kanama veya hematom gelişmedi. Postoperatif dönemde tinzaparin uygulanan sıçanlarda anti-faktör Xa aktivitesinin, tinzaparin uygulanmayan sıçanlara göre anlamlı derecede yüksek olduğu görüldü ($p<0,001$). Histopatolojik incelemede tinzaparin tedavisi gören yalnızca bir sıçanda (%12,5) ve tinzaparin tedavisi uygulanmayan yedi sıçanda (%87,5) femoral arter insizyon hattında trombüs ve fibrin oluşumu saptandı ($p<0,001$). Hiçbir grupta intimal hiperplazi saptanmadı ancak her iki grupta da karışık tipte inflamatuar hücre infiltrasyonu ve sütür çevresinde endotelyal ve fibroblastik aktivite dikkat çekti.

Sonuç: Sıçan modelinde postoperatif subkutan 175 IU/kg/gün tinzaparin uygulamasının arteriyel cerrahi girişimlerde arteriyel tromboz oranını etkili bir şekilde azalttığı görüldü.

Anahtar Kelimeler: Arteriyel tromboz, düşük molekül ağırlıklı heparin, tinzaparin

INTRODUCTION

Functional insufficiency of vessels or grafts after cardiovascular interventions creates serious problems. Thrombosis is the most important complication that may require surgical operation in such interventions (1). The cause of arterial thrombosis is vascular endothelial damage and disruption of its integrity, and the thrombosis continues to be stimulated until this integrity is achieved

Corresponding author: Yıldırım GÜLTEKİN, MD, Eskişehir Şehir Hastanesi, Kalp ve Damar Cerrahisi Kliniği, Odunpazarı, Eskişehir, Türkiye.
E-mail: dr.ygultekin@hotmail.com
ORCID: https://orcid.org/0000-0002-9384-0556
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(2). Classical anticoagulants (heparin, warfarin), new generation oral anticoagulants (rivaroxaban, dabigatran) and antiaggregant drugs (acetylsalicylic acid, clopidogrel) are commonly used to suppress this process, but the use of unfractionated heparin is at the forefront in the prophylaxis and treatment of perioperative thrombosis. However, there is still no agreement on a particular preparation (3).

Heparin, a sulfated polysaccharide which is widely used as an anticoagulant to prevent and treat thromboembolic events, shows its effect by binding reversibly to antithrombin-III (AT-III) and greatly accelerates the rate at which AT-III inactivates coagulation enzymes thrombin (factor IIa) and factor Xa. In this way, the intermediate and final stages of coagulation are blocked (4). Although highly effective for its intended use, heparin has been associated with paradoxical thrombosis and its use requires continuous intravenous administration with routine monitoring of coagulation via activated partial thromboplastin time (aPTT) (5). Besides its desired effects in the case of thrombosis, problems in determining the effective dose and thus the need for biological testing, its potential to cause bleeding, and the possibility of causing, albeit rare, paradoxical thrombosis that may occur at a rate of 5% are the limitations of the use of heparin (6). There is also a risk of heparin-induced thrombocytopenia (HIT) which increases as the duration of heparin use prolongs (7).

Antithrombotic effect of heparin is represented by anti-factor Xa activity, while hemostatic activity is represented by anti-factor IIa activity. Activity of these two parameters is related to the molecular weight of heparin. As the molecular weight of heparin decreases anti-factor IIa activity decreases, but anti-factor Xa activity does not change. This means that while the antithrombotic effect is maintained, the anticoagulant effect is reduced, and bleeding problems are not dealt with (8).

Low molecular weight heparins (LMWHs), which are used in the prophylaxis and treatment of venous thromboembolism (9), exert their anticoagulant effect mainly by inhibiting factor Xa. Their anti-factor IIa activity is lower than that of heparin. Among LMWHs tinzaparin has the highest rate of neutralization with protamine sulfate in case of bleeding (10). Compared with heparin, it has lower risk of thrombocytopenia and bleeding, and its once daily subcutaneous administration is an important advantage. Like any other low molecular heparin, tinzaparin does not require test control in determining the effective dose while using. Except for special conditions, it can be applied in doses suitable for weight, and can also be used safely in pregnant women in case of possible thromboembolism (11). While these provide less trauma to the patient, outpatient use of tinzaparin reduces the length of hospital stay and lowers the cost of the treatment (12).

Low-molecular-weight heparins are licensed in the prophylaxis of venous thromboembolism but not of arterial thrombosis. In this study, we aimed to evaluate the effect of postoperative administration of a particular low molecular weight heparin, tinzaparin, on arterial thrombosis in a rat model.

METHODS

This experimental study protocol was approved by Kırıkkale University Animal Experiments Local Ethics Committee (No: 18/06, Date: 31.01.2018). The animals used in the study were approached according to the criteria specified in the Guide for the Care and Use of Laboratory Animals. The animals were supplied from Saki Yenilli Experimental Animal Production and Application Laboratory and all experimental stages were carried out in Kırıkkale University Hüseyin Aytemiz Experimental Research and Application Laboratory.

The animals and preparation for the experiment

This experimental study included a total of 16 male Wistar Albino rats, three to four months old, weighing an average of 350-400 grams. A maximum of seven or eight rats were placed per cage, all were kept at 21±1 °C and 50-55% humidity and were maintained on a 12-hour light and 12-hour dark cycle. Water and feed were provided as needed but all animals fasted overnight prior to experimentation.

Rats were randomly allocated into two groups, each containing 8 animals, by using a random numbers table. Group I received 175 IU/kg tinzaparin (Innohep®, Leo Pharma, Ballerup, Denmark) subcutaneously just before the operation and once daily for the following three days. Group II served as a control group and did not receive tinzaparin at all before or after the operation.

Anesthesia and operation

Animals were applied anesthesia with 50 mg/kg ketamine hydrochloride (Ketalar®, Eczacıbaşı, İstanbul, Turkey) and 10 mg/kg xylazine HCl (Alfazyme®, Alfasan International BV, Woerden, Holland) intraperitoneally throughout the operation.

In this experimental study, the effect of tinzaparin on the formation of postsurgical arterial thrombosis was studied in the rat femoral artery model. The right inguinal hair of 16 male Wistar Albino rats was shaved and they were placed on their backs for the operation. Preparative phase of surgical intervention was completed by disinfecting the surgical field with polyvinylpyrrolidoneiodine solution. Surgery of all animals was performed by the same operator and 2.5x magnification loupe glasses were used to enlarge the surgical field. As a standard procedure, an incision was made in the right inguinal region of each rat, passing the skin and subcutaneous tissues, and the femoral artery was reached and suspended (Figure 1a). Bulldog clamps were placed distal and proximal to the artery and an arteriotomy incision transverse to the femoral artery, containing 50% of

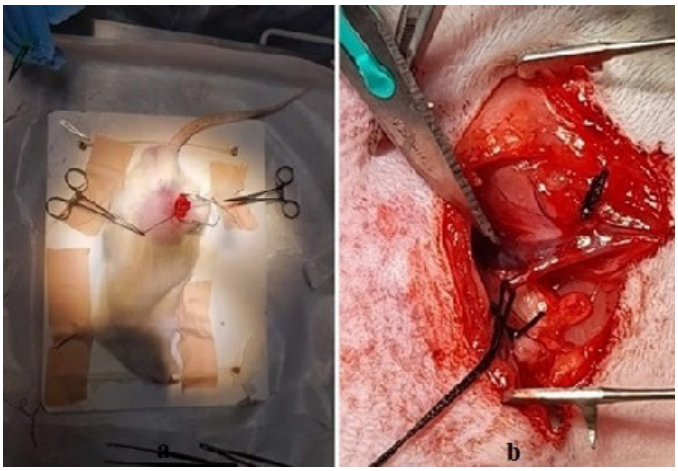

Figure 1: Demonstration of surgical procedure of incisional injury and its repair in the femoral artery of a Wistar Albino rat. (a): Exposure and suspension of the femoral artery to create incisional injury at the beginning of the operation. (b): Repair of incision with a continuous suture technique using a 10/0 polypropylene suture.

its lumen, was made with a coronary scalpel. This incision was then repaired with a continuous suture technique using a 10/0 polypropylene suture (Ethicon prolene®, Johnson & Johnson, New Brunswick, New Jersey, USA), (Figure 1b). Bulldog vessel clamps were then opened, and blood flow was ensured. After bleeding control, the skin layers were closed one by one using 4/0 silk suture. Then the operation was terminated by dressing with polyvinylpyrrolidone iodine solution.

Daily monitoring of temperature, color and motor function was performed to evaluate the vascular circulation in the operated extremities of the rats for three days. Surgical field was also followed up for the formation of hematoma. At the end of the 3rd day, the silk sutures on the skin were removed and femoral artery suture lines were inspected macroscopically, and after circulation control, 8-12 mm long femoral artery segment including the suture line was excised transversely for the histopathological examination. All biopsy materials were fixed in 10% formaldehyde solution. Blood samples for anti-factor Xa activity were also taken from the abdominal aorta to show the efficacy of tinzaparin, just before the rats were sacrificed with carbon dioxide inhalation method.

Histopathological examination

Sections from femoral artery biopsy materials of the rats were cut semi-perpendicular to the long axis of the tissue samples for routine tissue processing, after which samples were embedded in paraffin. Then, 3-4 µm thick sections were prepared from the paraffin blocks and stained with Hematoxylin Eosin (H&E) and Elastic Van Gieson (Bio - Optica, Milan, Italy). All images were digitized using a light microscope (Olympus AX80; Olympus Optical, Tokyo, Japan).

Biochemical analysis of blood

The blood samples withdrawn from abdominal aorta just before sacrification of the rats were kept in citrate anticoagulant treated tubes at +4 0C until tested at which time they were centrifuged at 3000g for 15 minutes to obtain platelet-poor plasma. Plasma anti-factor Xa activity levels were then measured with a chromogenic assay (Anti-Xa STA-Liquid; STAGO, Asnieres-sur-Seine, France) (13).

Statistical analysis

The analysis of the data obtained from this study was performed using the SPSS for Windows 21.0 package program (SPSS Inc. Chicago, IL, USA). Categorical variables were expressed as percentage (%) and continuous variables as mean±standard deviation (mean±SD). The Kolmogorov-Smirnov test was employed to evaluate the normal distribution of the data obtained from the study. Student's t-test and Chi-square test were used for comparison of parametric data and nonparametric data,

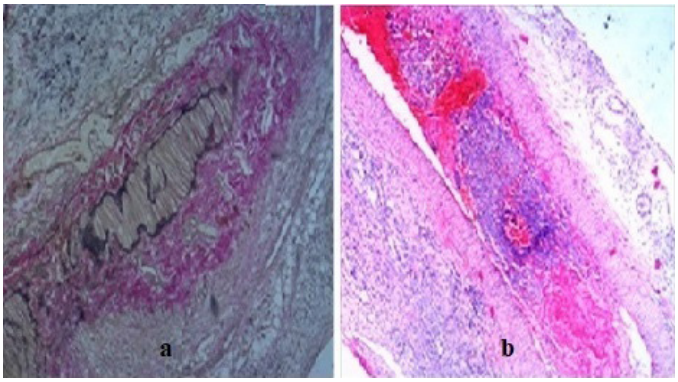

Figure 2: The histopathological image on the fourth day of a repaired incisional injury in the femoral artery of a Wistar Albino rat that was not treated with tinzaparin after the operation. Fibrin network and thrombus formation within the lumen of the femoral artery at the suture line is noted. Mixed type inflammatory cell infiltration, and endothelial and fibroblastic activity around the suture are also noted. (a): Elastic Van Gieson stain x100, (b): Hematoxilen Eosin stain x100.

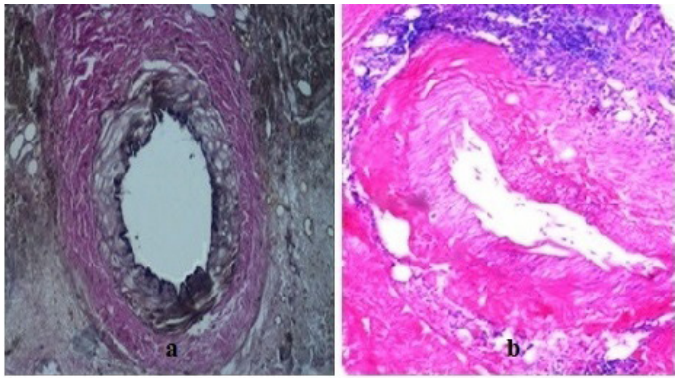

Figure 3: The histopathological image on the fourth day of a repaired incisional injury in the femoral artery of a Wistar Albino rat that was not treated with tinzaparin after the operation. Fibrin network and thrombus formation within the lumen of the femoral artery at the suture line is noted. Mixed type inflammatory cell infiltration, and endothelial and fibroblastic activity around the suture are also noted. (a): Elastic Van Gieson stain x100, (b): Hematoxilen Eosin stain x100.

respectively. 95% confidence interval and a p value less than 0.05 were considered statistically significant.

RESULTS

Anti-factor Xa activity in male Wistar Albino rats treated with tinzaparin postoperatively was found to be significantly higher than the rats not treated with tinzaparin (1.21±0.09 IU/ml vs 0.5±0.03 IU/ml, p<0.001).

During the three-day follow-up period postoperatively, none of the rats developed bleeding or hematoma at the surgical site and there was no death in either group. Vascular circulation disorder was detected in two rats (25%) not treated with tinzaparin, however, in none of the rats treated with tinzaparin postoperatively (p<0.001). During surgical exploration, thrombus was detected in the incision line in only one rat (12.5%) in postoperative tinzaparin treated group but in seven rats (87.5%) in tinzaparin free group (p<0.001). Histopathological examination of the excisional material from those seven rats not treated with postoperative tinzaparin revealed fibrin mesh/thrombus in the vessel lumen of the femoral artery at the incision line (Figure 2). On the other hand, the vessel lumen was open, and thrombus (dense fibrin, erythrocytes, and degenerated leukocyte aggregates) was not observed on histopathological examination of the biopsy specimens from seven rats treated with tinzaparin postoperatively (Figure 3). While intimal hyperplasia was not detected in any group, mixed type inflammatory cell infiltration and endothelial and fibroblastic activity were noted around the sutures (Figure 2 and Figure 3).

DISCUSSION

The data obtained from this experimental study showed that prophylactic subcutaneous administration of tinzaparin once a day protected from arterial thrombosis after an intimal damage/repair in the rat femoral artery.

Thrombus can develop in either the arterial or venous system. Arterial thrombosis may develop due to vascular injury from many causes such as traumatic, iatrogenic, and direct vascular surgical interventions (14). Thrombus formation at the vascular repair region is one of the most severe complications in arterial surgical operations. Platelet and fibrin constitute the main structure of a thrombus, but while arterial thrombus is rich in platelet and poor in fibrin and erythrocyte, venous thrombus is poor in platelet and rich in fibrin and erythrocyte. The main cause of arterial thrombosis is the deterioration of the integrity of the vascular endothelium, and thrombosis continues to be stimulated until this integrity is achieved (15). Disruption of the vascular endothelium for various reasons results in platelet-tissue interaction that leads to platelet adhesion to the vessel wall that has lost its endothelial integrity. Patelets then become activated to release the content of their cytoplasmic granules including adenosine diphosphate and thromboxane A2 being very important in platelet aggregation. They increase thrombin production from prothrombin by activating platelets in the region of vascular injury. Thrombin is a potent platelet activator that induces arachidonic acid metabolism and the release of granular content (16). Blood flow in the arterial system is higher compared to the venous system, and the thrombi in arterial system either may not grow and be resorbed with the intrinsic fibrinolytic system or may lead to devastating complications up to extremity loss by causing vascular occlusion (17).

To obtain a satisfactory treatment result in vascular surgeries, it is aimed to prevent coagulation in the arteriotomy or repair site of the vessel, not to cause bleeding and vasoconstriction, and to keep blood volume and viscosity within normal limits. Various groups of drugs such as anticoagulants, antiaggregant agents, vasodilators, and drugs that increase blood volume are used prophylactically to achieve this goal (18). Anticoagulant heparin, which was discovered in 1917 and started to be used clinically in 1930, has played a very important role in the prevention of thrombosis after vascular surgeries. Anticoagulation with heparin may reduce the possibility of thrombotic events and improve functional outcomes (19). Arterial microsurgery applications, which started to develop at the beginning of the century, have reached the highest level of success in arterial surgery today with the development of microscope and suture technology (20). However, the use of heparin for more than three days increases the risk of HIT and the risk of bleeding as the duration of use is prolonged; both are undesirable events during thrombosis prophylaxis. Paradoxical embolic events may also occur. For heparin to be effective in thrombosis prophylaxis, close aPTT follow-up is required to adjust its titer in blood (21).

Disruption of endothelium in vascular anastomoses or repair sites increases platelet aggregation and activates coagulation. The endothelial healing process takes 2-3 days, thus at least during this period, platelet aggregation should be prevented, and coagulation should be suppressed (22). However, this suppression should not lead to bleeding diathesis, as the blood can leak out of the vessel through the sutures, collect around the vessel and form a hematoma, causing external pressure on the lumen (23).

Currently, in addition to heparin, acetylsalicylic acid, LMWHs and thrombolytic agents are used in the prophylactic treatment to prevent thrombosis in vascular surgery, and research are going on their effects (24). LMWHs are obtained by breaking down heparin by different chemical or enzymatic methods. The factor Xa inhibitory effect of LMWHs is the same, but the effect on AT-III is weaker. That's why they are mostly used in the prophylaxis of venous thromboembolism (25). The fact that the inhibition of platelet aggregation becomes more evident as the molecular weight of heparin decreases, LMWHs also

reduce the risk of thrombosis, which is rarely (5%) seen in the use of classical heparin (4,6). Moreover, studies on their use in the prevention and treatment of vascular disorders, especially deep vein thrombosis and pulmonary embolism, have shown that LMWHs are superior to unfractionated heparin in terms of both efficacy and safety (26). LMWHs are licenced in the prophylaxis of venous thromboembolism in patients undergoing intermediate and high-risk surgery, especially those undergoing orthopedic or cancer surgery, in the prevention of pulmonary embolism, deep vein thrombosis, and thrombosis in the extracorporeal circulation during hemodialysis, in the prophylaxis of venous thromboembolism in patients with reduced mobility at high risk of venous thromboembolism, and in the case of acute myocardial infarction with ST-elevation to be used together with an antiaggregant. They are not used in the prophylaxis and treatment of thromboembolism that may occur following arterial intimal damage (27,28).

LMWHs including tinzaparin have been accepted as standard therapy for thromboprophylaxis as well as treatment in deep vein thromboembolism (DVT). Tinzaparin has greater bioavailability and a longer duration of action than unfractionated heparin, and its once daily subcutaneous injection at a dose of 175 IU/kg is sufficient for both prophylaxis and treatment (29). It has also a higher ratio of anti-factor Xa/anti-factor IIa activity than unfractionated heparin, providing the theoretical advantage of similar antithrombotic efficacy and a reduced risk of hemorrhagic complications. The current study which was aimed to show the efficacy of postoperative use of tinzaparin in the prevention of arterial thrombosis in an experimental rat model revealed that postoperative anti-factor Xa activity was significantly higher in rats receiving tinzaparin compared to those not treated with tinzaparin.

In clinical studies thrombocytopenia was detected in 1% of patients treated with tinzaparin, and the bleeding rate was reported to be 1.5%, the incidence of major bleeding being very low (30). In our study, no bleeding or hematoma that would compress the artery was detected in surgical region of any rats treated with tinzaparin. Unlike heparin, the anti-factor Xa activity of tinzaparin cannot be completely neutralized with a single dose of protamine sulfate, as has been observed with other LMWHs. However, as an important feature, the fact that tinzaparin can be neutralized by 70% with protamine sulfate makes it stand out compared to other LMWHs in case of bleeding (31).

Review of the current literature revealed that there is no study showing the effect of once-a-day subcutaneous administration of tinzaparin on thromboembolism in case of intimal damage/repair in the arterial wall. Therefore, our study was compared with studies conducted with other LMWHs. Hadlock et al. investigated the effect of a LMWH, namely enoxaparin, in a thrombosis model on the rat femoral artery and detected comparable rates of thrombosis in both groups of rats treated with and without enoxaparin (6 of 26 vessels and 6 of 24 vessels, respectively, $p>0.05$) (32). Another study performed by Emerick et al. in a thrombosis model on the mouse femoral vein, showed that dalteparin, another LMWH, application did not make a difference in the rates of thrombosis between the rat groups treated with and without dalteparin (18 of 30 vessels and 14 of 28 vessels, respectively, $p>0.05$) (33). In a study conducted by Chen et al. on a rat femoral artery model, investigators created injury by crushing the artery and applied topical irrigation with heparin, enoxaparin, or streptokinase before anastomosis to the femoral artery and found that enoxaparin is an antithrombotic agent as effective as standard heparin when applied topically during microvascular anastomoses (34). In another study Korompilas et al. evaluated the effect of enoxaparin dose and administration methods on patency rate of crushed rat femoral arteries after anastomosis. Following a 25 kg impact crush applied to a 2 mm section of the femoral artery, they performed anastomosis and observed that postoperative use of enoxaparin was potentially beneficial for increasing the patency rate in at-risk microvessels (35). Gürbüz et al. studied the effect of postoperative enoxaparin in the prevention of thrombosis in microsurgical vascular repairs on rabbit femoral arteries and concluded that enoxaparin can be preferred for the thrombosis prophylaxis in arterial reconstructions, as it does not cause bleeding while providing the desired antithrombotic effect due to its low anti-hemostatic activity and high anti-thrombotic activity depending on the dose (36).

The results of our study investigating the effect of 3-day-postoperative use of tinzaparin for prevention of arterial thrombosis in a model of rat femoral artery were consistent with the previous data suggesting that LMWHs are effective in the prevention of thrombosis. Postoperative use of daily single dose tinzaparin prevented the development of circulatory disturbance in extremities of rats after the repair of femoral arteries. Following 3-day-use of tinzaparin postoperatively, histopathological examination of the biopsies taken from the damaged/repaired rat femoral arteries revealed that treatment with tinzaparin provided less fibrin and thrombus formation in incision line and reduced number of degenerated leukocytes and erythrocytes; thrombosis was observed only in 12.5% of rats that were treated with tinzaparin, but in 87.5% of rats that were not treated with tinzaparin.

Tinzaparin is more cost effective than unfractionated heparin in the treatment of established thromboembolic disease. In addition, outpatient treatment with tinzaparin may provide more cost advantages than hospital-based treatment (37). The drug is well tolerated in elderly patients and those with renal insufficiency, including those on long-term therapy. Tinzaparin does not cross the placenta and provides satisfactory antithrombotic protection and can also be used safely in pregnant women (38). The ease of

its once-daily subcutaneous use, less risk of bleeding, no need for follow-up with blood tests are other advantages of tinzaparin. Therefore, tinzaparin stands out as a valuable LMWH in the prophylaxis and management of thromboembolic disease.

CONCLUSION

In conclusion, we suggest that subcutaneous administration of 175 IU/kg/day tinzaparin in postoperative period significantly reduces the rate of arterial thrombosis following the arterial interventions. Further clinical studies for thromboembolism prophylaxis with tinzaparin after arterial surgeries on human are needed to be conducted.

Additional information: This study was presented as an oral presentation on 05/11/2021 at the 17th International Congress of Innovations in Cardiology and Cardiovascular Surgery held online between 5-7 November 2021.

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Ethics Committee Approval: The study protocol was approved by the Kırıkkale University Animal Experiments Local Ethics Committee (no: 18/06, date: 31.01.2018).

Authorship Contributions: Idea/Concept: YG, AB, ATK, Design: MÖ, HK, YG, AB, Supervision: YG, AB, ATK, Data Collection or Processing: YG, AB, MÖ, HK, Analysis or Interpretation: HK, MÖ, YG, AB, Literature Search: YG, AB, ATK, MÖ, Writing: YG, MÖ, ATK, HK, Critical Review: YG, AB, HK, ATK, MÖ, References And Fundings: YG, AB, MÖ, ATK, HK, Materials: YG, AB, ATK.

Conflict of Interest: No conflict of interest was declared by the authors.

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