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ARTICLE

The effect of ticagrelor on microarterial thrombosis in an experimental model

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ABSTRACT

Thromboses that form in the pedicle after free flap and/or replantation may result in the loss of the flap and/or limb. Ticagrelor is an adenosine diphosphate (ADP) receptor antagonist antithrombotic that can inhibit ADP-dependent platelet activation and aggregation. It is clinically used in acute coronary syndrome and unstable angina. However, its effect on microarterial anastomoses has not been investigated in the literature. An experimental thrombosis model was developed in both femoral arteries of a total of 40 rats. Twenty rats were randomly selected as the drug-free control group, and 20 rats were randomly selected as the ticagrelor group. The rats in the ticagrelor group were administered a 20 mg/kg loading dose orally by gavage 24 h before the experiment, and a maintenance dose of 2x10 mg/kg ticagrelor for 14 days after surgery. After the experiment, the femoral artery was evaluated for macroscopic and microscopic thrombosis, inflammation, edema, and endothelialization. Macroscopically and microscopically, thrombosis was observed at rates of 73.3% and 33.3% in the control group and the ticagrelor group, respectively. Inflammation in the vessel wall was found as 56.7% in the control group and 16.7% in the ticagrelor group. Edema in the vessel wall was found in 63.3% of the control group and 20% of the ticagrelor group. A statistical difference was found between the two groups in terms of thrombosis, inflammation, and edema. Both groups had similar characteristics in terms of endothelialization. Ticagrelor has a reducing effect on thrombosis in the microarterial tuck model.

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Microsurgery; thrombus; ticagrelor; rat tuck model

Introduction

Microvascular surgery applications have made rapid progress in the last 30 years with the development of surgical microscopes and improvements in the quality of surgical equipment and surgical experience. Despite the development of microsurgery techniques and high success rates, there is a possibility of failure at between 5% and 10% [1]. The most common cause of failure is thrombosis developing in the anastomosis region [2]. Platelets activated by vascular injury induce arachidonic acid metabolism and the release of intragranular products, triggering the clotting mechanism, which results in platelet plug and thrombus formation. In many studies, antithrombotic agents have been used to reduce the development of thrombosis in microvascular anastomoses [3]. An ideal agent should be an easy-to-apply and effective agent with few adverse effects.

Ticagrelor P_2Y_{12} is a new-generation oral antiplatelet agent that acts selectively and reversibly binds ADP receptor antagonists on the ADP-receptor. It prevents platelet activation and aggregation [4]. Due to its reversible binding feature, the fact that platelet functions improve very rapidly with discontinuation of the drug is one of its most significant advantages [5].

In this study, we aimed to investigate the effect of ticagrelor on thrombosis in microvascular anastomoses in the 'tuck' method, which is an experimental thrombosis model.

Materials and methods

Approval for all animal experiments was obtained from the Institutional Animal Care and Use Committee (Protocol # 2014-127). Forty Wistar albino rats with weights varying between 250 and 300 g were used. The rats were randomly divided into two groups of 20, the ticagrelor and control groups. The study was planned to examine the right and left femoral arteries of each rat separately. Ticagrelor was administered to the treatment group, and no antithrombotic agents were administered to the rats in the control group. After the subjects were anesthetized through intramuscular administration of 50 mg/kg 10% ketamine hydrochloride (Ketalar® flacon, Pfizer, Istanbul) and 10 mg/kg xylazine hydrochloride (Rompun®, Bayer, Istanbul), a thrombosis model was created with the 'tuck' procedure previously defined by Stepnick et al. [6].

Surgical technique

The subjects were laid on their backs, and the fur in their right and left inguinal regions was shaved. The surgical site was disinfected with povidone/iodine solution (Batticon, ADEKA, Istanbul), and the surgery was started. Following skin incision, a blunt dissection was used for the identification of the femoral pedicle, and both femoral vessels were isolated and cross-clamped. The femoral artery was placed into a double microvascular clamp, and excess adventitia was trimmed. A 180-degree arteriotomy was formed using microscissors, and the repair was initiated. Afterwards, a 180-degree arteriotomy was formed. A 9-0 Prolene monofilament suture was used for to create a 'tuck' of adventitia into the lumen of the vessel using an operating microscope and microvascular instruments, as specified by Stepnick et al. [6] (Figure 1). The suture was placed through the vessel's distal end

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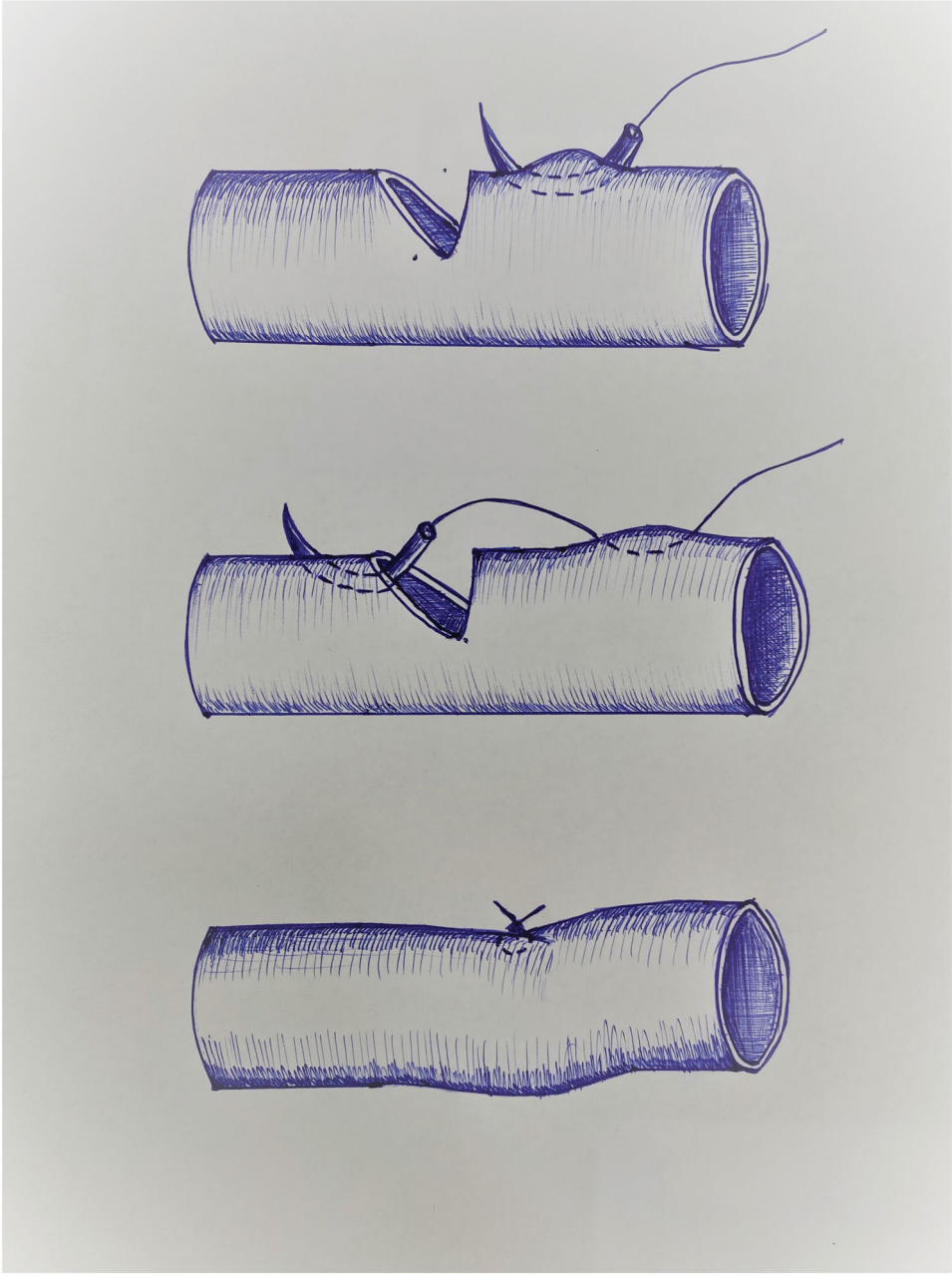

Figure 1. The suture first passed tangentially on adventitia a bit distally from the artery incision, then through the full wall of the proximal vessel, so that the distal part of the edge of the distal vessel can tuck into the lumen. Additional sutures were placed to assure hemostasis as necessary.

and brought out closer to the arteriotomy site, but still on the distal side. Afterwards, the suture was passed from within the lumen of the arteriotomy out through the proximal wall of the vessel. Additional sutures were placed to assure hemostasis. The cross-clamps were removed and blood flow was observed. Any small leaks observed in the suture line were stopped within 1–2 min after a was buffer made using the fat pad. Up-lift and milking tests were performed distal to the suture line. In these tests performed at the first and fifth and minutes after suturing, blood flow through the suture line was detected in all subjects. The surgery was completed by closing the incision with skin sutures.

Experimental protocol

The ticagrelor group ($n = 20$) was administered a loading dose of 20 mg/kg ticagrelor dissolved in isotonic sodium chloride by gavage 24 h before the surgical procedure, and with a 10 mg/kg maintenance dose dissolved in isotonic sodium chloride by gavage 2 h before the surgical procedure. A maintenance dose of 2×10 mg/kg ticagrelor was administered by gavage for 14 days after the surgical procedure. Only isotonic sodium chloride was given to the control group. At the end of the study, the subjects were again anaesthetized using ketamine hydrochloride and xylazine hydrochloride on the 14th day. The femoral artery was dissected by entering through the incision made at the previous incision site in the inguinal region. Femoral artery anastomosis lines were evaluated macroscopically. The presence of flow was checked through distal milking and up-lift tests. The presence of a perivascular hematoma was checked. The presence of flow and the presence of a perivascular hematoma was recorded as 'present' and 'absent'. Following the macroscopic control, a 10–12-millimeter-long femoral artery biopsy was taken, including

the suture line. After the procedure, the rats were sacrificed using carbon dioxide inhalation. Biopsy materials were fixed in a 10% formaldehyde solution. Vertical sections were made to these fixed specimens. Afterwards, 0.4-micron sections were taken and stained using hematoxylin-eosin (H&E) and examined histologically. Histologically, the specimens were examined for thrombosis, inflammation, endothelialization, and edema. The results were recorded as 'present' and 'absent' according to the groups (Figures 2 and 3).

Statistical analysis was performed using the Chi-square (χ^2) test, which is a nonparametric test.

Results

Five rats from each group were excluded from the study due to vascular damage that occurred during the surgical procedure or the loss of rats. A total of 60 femoral arteries, including 30 femoral arteries in each group, were examined. On the 14th postoperative day, macroscopically, there was a passage from the suture line to the distal in eight femoral arteries (26.7%) in the control group, and there was a passage to the distal in 20 femoral arteries (66.7%) in the ticagrelor group. The difference between the two groups was $p = 0.01$, which was found to be statistically significant ($p < 0.05$) (Table 1).

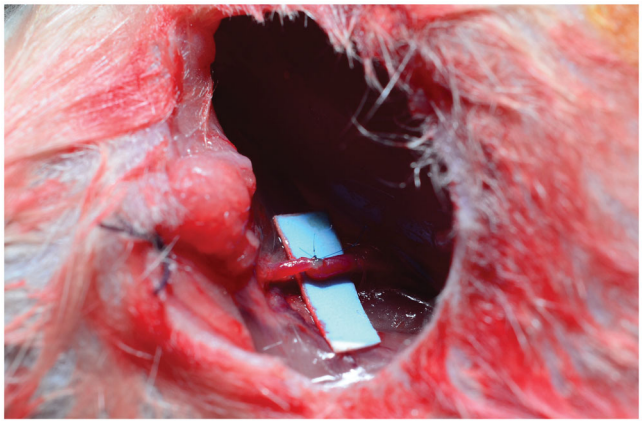

Figure 2. A 9-0 prolene monofilament suture was used to create a 'tuck' of adventitia into the lumen of the vessel using an operating microscope and microvascular instruments as described by Stepnick et al. [6].

When evaluated histologically for thrombosis, there was thrombosis in 22 femoral arteries in the control group (73.3%), and there was thrombosis in 10 femoral arteries in the ticagrelor group (33.3%). The difference between the two groups was $p = 0.01$, which was found to be statistically significant ($p < 0.05$).

The vessel wall and lumen were examined microscopically for inflammation, edema, and endothelization. There was inflammation in 17 femoral arteries in the control group (56.7%), and in five femoral arteries in the ticagrelor group (16.7%). The difference between the two groups was $p < 0.001$, which was found to be statistically significant ($p < 0.05$).

The unstained spaces observed in the vessel wall in the sections were evaluated as edema. It was observed that edema was mostly concentrated in the media layer of the vessel. There was edema in 19 femoral arteries in the control group (63.3%), and six femoral arteries in the ticagrelor group (20%). The difference between the two groups was $p = 0.001$, which was found to be statistically significant ($p < 0.05$).

When evaluated for endothelization, it was determined that endothelization was completed in 13 femoral arteries in the control group (43.3%), and in 18 femoral arteries in the treatment group (60%). This difference was $p = 0.273$, which was not accepted as statistically significant (Table 2).

Discussion

Microvascular anastomoses are commonly performed in many plastic surgery clinics in parallel with the development of microscopes, microsurgery techniques, and instruments. Since the first use of tissue transfer in the 1970s, efforts have been made to optimize perioperative outcomes [7]. In the literature, the failure rates of microsurgery vary between 5 and 10% in free flaps and 15–30% in replantation [8]. Even if a technically successful anastomosis has been performed, thrombosis formation due to endothelial damage in the anastomosis region may result in flap loss or limb loss.

Multiple pharmacologic agents have been used intraoperatively or postoperatively by microvascular surgeons to reduce the rate of thrombosis and flap failure [9]. To minimize thrombosis formed during microvascular surgery, there has been an effort to develop suturing techniques, but even in a perfect anastomosis, thrombosis development was observed due to procedures performed on the vein and needle trauma [10]. Schubert et al.

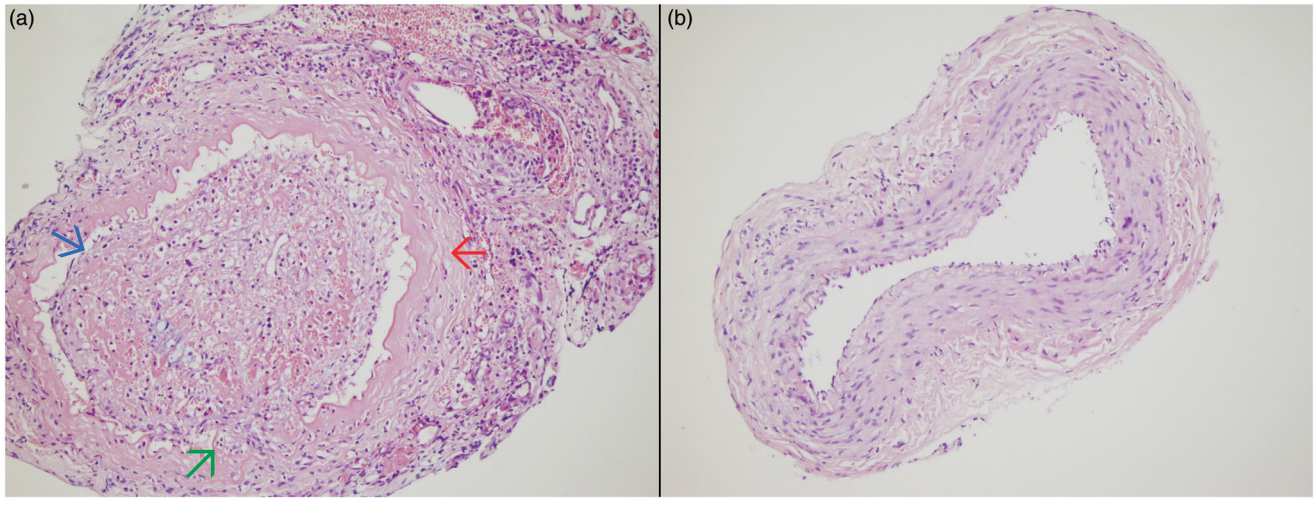

Figure 3. (a) In the control group, an apparent organized thrombus was observed in the vessel lumen (red arrow), and leukocytes (green arrow) and edema (red arrow) were observed in the vessel wall. (b) In the ticagrelor group, sparse inflammatory cells were observed in the vessel wall, but no edema was observed.

Table 1. Blood flow was evaluated macroscopically in each group through distal milking and up-lift on the postoperative the 14th day.

Blood Flow	Control group		Ticagrelor group		
	n	%	*n*	%	*p*
Yes	8	26.7	20	66.7	0.011
No	22	73.3	10	33.3	

Table 2. Evaluation of thrombosis, edema, endothelialization, and inflammation under light microscopy.

	Control group		Ticagrelor group		
	n	%	*n*	%	*p*
Thrombosis					
+	20	66.7	8	26.7	0.011
–	10	33.3	22	73.3	
Inflammation					
–	17	56.7	5	16.7	<0.001
–	13	43.3	25	83.3	
Endothelization					
+	13	43.3	18	60	0.273
–	17	56.7	12	40	
Edema					
+	19	63.3	6	20	0.001
–	11	36.7	24	80	

reported that the trauma created by the needle in the simple intermittent technique and the suture inside the lumen caused vascular wall damage, thrombosis, and intimal hyperplasia [11]. Injury caused by trauma and endothelial damage caused during anastomosis both cause vascular occlusion [12,13]. Antithrombotic agents aiming at platelet aggregation and/or different steps in the clotting cascade have been used. Descriptions of drugs that target platelet aggregation, volume expansion, and fibrin deposition are frequently encountered in the literature. Heparin, low-molecular-weight heparin (LMWH), dextran, and aspirin, as single agents or multidrug regimens are among the most widely used. A problem regarding the use of platelet- or fibrin-targeted antithrombotic pharmacotherapy is an expected increase in the risk of hematoma or hemorrhage in postoperative patients [9]. Dhiwakar et al. determined that hemorrhage and hematoma increased significantly in patients who took perioperative aspirin following the excision of head and neck lesions with a primary repair or local flap closure [14]. Another study performed a retrospective evaluation of hematoma and flap loss rates with heparin in different regimens (intraoperative bolus, low-dose postoperative prophylaxis, and high-dose drips) [15].

Ticagrelor is a potent agent that is effective on the P_2Y_{12} receptor that can prevent ADP-dependent platelet activation and aggregation and causes the inhibition of platelets with a reversible and rapid onset of action [16,17]. It causes the inhibition of all platelets two hours after oral intake. Due to this effect, ticagrelor is used for the prevention of heart attack by reducing thrombus formation and vascular occlusion in acute coronary syndrome (ACS) and patients with non-stable coronary artery disease [18].

Stepnick et al. stated that, in the experimental model in which a thrombogenic intimal flap was created inside the lumen called the 'tuck' model, a thrombus developed in vessels at a rate of 66% within 5–15 min after suturing [6]. In the present study, supporting the study conducted by Steptnick et al., thrombosis was encountered at a rate of 73.3% in the control group and 26.6% in the group in which ticagrelor treatment was initiated, and the difference between the groups was statistically significant. Hadlock et al. [1] reported that they observed thrombosis at a rate of 25% in the control group and 23% in the treatment group with the microvenous 'tuck' model in which they used LMWH. Emerick et al. [19] reported that they encountered thrombosis at a rate of 60%, and no sufficient antithrombotic effect was observed in the microvenous 'tuck' model in which they used LMWH. Nayak et al. [20] reported thrombosis at a rate of 58% in the control group and 19% in the clopidogrel group in a microarterial 'tuck' model. Clopidogrel is an antithrombotic agent that irreversibly inhibits ADP-induced platelet aggregation selectively and strongly [21]. In their experimental study, Akan et al. reported that clopidogrel had positive effects on microvascular anastomosis [3]. Ticagrelor inhibits platelets faster and stronger than clopidogrel, but the fact that a very rapid improvement in platelet functions occurs with the discontinuation of the drug constitutes one of its most significant advantages [5].

An important factor in antithrombotic therapy is unwanted drug-induced bleeding. Birkeland et al. stated that the rate of unwanted bleeding was higher in patients using clopidogrel than in patients using ticagrelor [22]. In this study, no perivascular hematoma was encountered in any rats in the ticagrelor and control groups. The rate of inflammation and edema in the arterial wall was found to be significantly lower in the ticagrelor group compared with the control group. It is thought that thrombus formation narrows the lumen, reduces blood flow through the anastomosis, and increases edema and inflammation. Weisshar et al. [17] reported that ticagrelor reduced ischemia-reperfusion-induced endothelial dysfunction. Although the high cost of ticagrelor compared with other antithrombotic agents used in microsurgery practice is a disadvantage, the rapid and reversible onset of action of the drug is regarded as an advantage [23].

Ticagrelor combined with low-dose aspirin was used for neuroendovascular procedures to prevent thrombosis [24]. Despite the small sample size, the results are encouraging. Dual antiplatelet treatment with ticagrelor and aspirin was found effective in the prevention of thrombosis after coronary artery bypass graft surgery when compared with aspirin monotherapy in a systematic review and network meta-analysis of 21 articles [25]. In these articles, treatment was initiated immediately after surgery, one day after surgery, or a few days after surgery.

When antithrombotic drugs are medicated in the perioperative period, the risk of bleeding should be scrutinized carefully. Some microanastomosis-requiring procedures such as radical and reconstructive surgery for cancer of the head and neck and functional treatment of trauma, and treatment of trauma and cancer excision have a high risk of bleeding [26]. Nevertheless, bleeding that develops after reconstructive microsurgical procedures frequently has the advantage of easy access to the surgical site and lower likelihood of morbidity and mortality than coronary bypass surgery or neuroendovascular interventions. Ticagrelor also has a reversible effect up to a point in comparison with antiplatelet agents such as clopidogrel or aspirin. We consider that immediate ticagrelor or dual antiplatelet therapy with ticagrelor and low-dose aspirin may be useful to prevent thrombosis on reconstructive microsurgical procedures such as replantation or free tissue transfer surgery.

We detected that ticagrelor significantly reduced thrombus formation in a microarterial 'tuck' model. However, more comprehensive experimental studies that compare ticagrelor with other antithrombotic agents frequently used after microsurgical procedures (e.g. heparin, LMWH, acetylsalicylic acid, and clopidogrel) are needed.

Doppler flowmetry or laser Doppler flowmetry could have been used prior to the collection of histologic specimens to quantitatively demonstrate the blood flow on day 14. Flowmetry would

have given more detailed information on the blood flow when compared with the vis-a-vis milking test. This was one of the limitations of this study.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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