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RESEARCH ARTICLE

THE EFFECTS OF TICAGRELORON THE IMPROVEMENTS OF SKIN FLAP VIABILITY IN RATS

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ABSTRACT

Objective: Concession and endothelial defect caused skin flap failure by thrombus. Our study aimed to support flap survive by an anticoagulant agent.
Materials and Methods: On this study was used 14 Wistar Albino female rats who are separated control (n=7) and ticagrelor (n=7) groups. Dorsal skin flaps were elevated and sutured same incision area. Ticagrelor was infused 20 mg/kg before 24 hour and 10 mg/kg after operation, each 12 hour, 10 days. % 0.9 NaCl infused for control group. After 10 days, flaps were evaluated topographicly and histochemicaly.
Results: Flap survival rate on control group % 62,84(43,40-69,83) and on ticagrelor group % 66,77(57,88-72,68) evaluated. Histopathologycal flap necrosis determined 1/3 distal and proximal parts were same but, central 1/3 part of flap on ticagrelor group has less necrosis than control group. That difference was supported statistical ($p<0.05$).
Conclusion: Conclusion of study; Ticagrelor would increase flap viability and we could use ticagrelor as an alternative drug for flap survive.

INTRODUCTION

The tissue which is transferred to the site with a skin defect in the body by retaining its own blood supply is referred to as flap. The flap survival rate depends on the vascularization status of the flap tissue. (Daniel *et al*., 1990). The skinblood flow is regulated by local and systemic factors, such as neurogenic, humoral, metabolic and physical ones. As for the factors affecting the flap blood flow; flap suppression, flap regression and folding of the flap can be considered among the other factors in addition to the factors that affect the skin blood flow (Daniel *et al*., 1990; Fisher *et al*., 1997; Luce, 1995; Smith *et al*., 2000; Chang *et al*., 2000; Kerrigan, 1983). In the random pattern skin flap, stasis and endothelial damage that occur in the course of the partial blockage of the vascular source lead to thrombosis/thrombus formation. (Vedder *et al*., 2006; Cotran *et al*., 1994). Thrombus gives rise to the development of necrosis by inhibiting the flap blood flow partially or completely (Tonks and Rees, 1995; Senderoff *et al*., 1993; Khouri *et al*., 1993; Arnljots *et al*., 1994). Ticagrelor is a pharmacological agent which decreases theformation of thrombosis/thrombus over the thromboses (blood platelets). The superiority of Ticagrelorto similar drugs is that its thrombus-decreasing effect stops the pharmacological agent intake and the elimination of its hemorrhagic/bleeding effect is faster than the other antiplatelets (Wallentin *et al*., 2009).

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With this pharmacological agent, we aim to minimize the necrotic complications in the flaps by preventing the thrombus that may occur in the wake of the flap surgery.

MATERIALS AND METHODS

This study was conducted in Fırat University, Experimental Research Center (FUERC). 12-week-old 14 Wistar albino type of female rats weighing 210-270 grams were used during the study. The guinea pigs/test subjects were divided into two groups as the control group (n=7) and the study group (n=7).
The dorsal flaps were removed from the test subjects of the study and the control groups and were, later, sutured/ stitchedon their original locations. A maximale tolerable dose of 20 mg/kg Ticagrelorpharmacological agent was administered through a naso-gastric catheter 24 hours before the operation. Starting from this loading dose on, 10 mg/kg maintenance doses were followed on every 12 hours for 10 days. On the other hand, the test subjects in the control group, instead of receiving Ticagrelor pharmacological agent, were administered the same amount of 0.9% NaCl in the same way as the other subjects in the study group.

Surgical Procedure

After the test subjects/ guinea pigs were anaesthetized, their dorsal parts were shaved/trimmed. The rectangular skin flap with a 2x7 frontal base was removed from the dorsal part in compliance with sterilization. After the flap had been removed,

it was adapted back to its original place by means of a 5/0 non-absorbable suture with a sharp needle by suturing at equal distances. No electrocauteryor hemostatic agent was used for hemorrhage/bleeding control.

The Evaluation of the Rates of Viable and Necrotic Regions in Flaps

The topographical and histological evaluation of flap survival was made on the postoperative 10th day. In the topographical analysis, the boundary between the viable parts of the flaps removed from the dorsals of the rats and those that had necrosis was drawn/marked with an acetate pen. The pictures of these were taken by a digital camera and were transferred into the computer in a digital format. The calculations were made by using Adobe Illustrater CS 11.0.0 graphic program.
In the histopathological evaluation of the flaps, on the other hand, the flaps were divided into 3 equal parts as proximal, medial and distal and were, then, put into 10% formaldehyde. Following the routine tissue follow-up procedures, they were evaluated under the light microscope by using hematoxylin-eosin (H&E) stain.

Statistical Method

In this study, the topographical evaluation of the flaps were calculated as median (min-max) via the SPSS computer program (SPSS Inc, USA). The compliance of the data with the normal distribution was performed through Shapiro Wilk test. With the data obtained from the proximal, medial and distal parts of the flap, the histopathological evaluation of the necrosis in the flaps was performed through SPSS 11.5 package program. Mann-Whitney U test was used for the topographical and histopathological comparison of the flaps in both of the groups. The P< 0,05values were regarded as statistically significant.

RESULTS

No rat mortality or wound infection was determined throughout the experiment. In the topographical evaluation of the flaps, the proportion/ratio of the viable flap areas in the control group to the total flap area was found to be 62,84% (43,40-69,83), whereas this proportion/ratio proved to be 66,77% (57,88-72,68) in the study group (Table 1, 2). When the topographical findings of the flaps in the control and study groups were compared, the viability of the flap in the study group administered Ticagrelor was statistically determined to have increased. (p<0,05).

Table 1. The topographical findings of the flaps in the control group

Test Subject No.	The areas viable in flaps (%)	The necrotic areas in flaps (%)
1	69,83	30,17
2	56,98	43,02
3	5430	45,70
4	60,31	39,69
5	68,04	31,96
6	67,82	32,18
7	43,40	56,60
Median (Min-Max)	67,82(50,28-69,83)	39,69 (30,17-56,60)

The % Values of the viable and necrotic areas of the rat flaps in the control group

Table 2. The topographical findings of the flaps in the study group

Test Subject No.	The areas viable in flaps (%)	The necrotic areas in flaps (%)
1	80,49	19,51
2	7,38	32,62
3	76,86	23,14
4	66,03	33,97
5	66,94	33,06
6	61,03	38,97
7	62,89	37,11
Median (Min-Max)	66,94 (61,03-80,49)	33,06 (19,51-38,97)

The % Values of the viable and necrotic areas of the rat flaps in the study group

The Histopathological Evaluation of the Necrosis in the Proximal, Medial and Distal Parts of the Flaps taken from the Rats (Table 3,4).

Table 3. The histopathological findings of the flaps in the control and study groups

	Median	1. quarter	3. quarter	min	max
Control G.					
Flap proximal	0,00	0,00	0,00	0,00	0,00
Flap medial	2,00	2,00	3,00	1,00	3,00
Flap distal	3,00	3,00	3,00	3,00	3,00
Study G					
Flap proximal	0,00	0,00	0,00	0,00	0,00
Flap medial	2,00	1,00	0,00	2,00	0,00
Flap distal	3,00	3,00	2,00	3,00	3,00

The Distribution of the Necrosis Values in 1/3 proximal, medial and distal parts of the flaps in the control and study groups

Table 4. The Statistical Values of the flaps in the control and study groups

	Median	Sequence	U	P	Average Total
Control G.					
Flap proximal	0,00	7,50	52,50	24,500	1000
Flap medial	2,00	10,00	70,00	7,000	0,019
Flap distal	3,00	8,50	59,50	17,500	0,141
Study G					
Flap proximal	0,00	7,50	52,50	24,500	1000
Flap medial	1,00	5,00	35,00	7,000	0,019
Flap distal	3,00	6,50	45,20	17,500	0,141

The Distribution of the Necrosis Values in 1/3 proximal, medial and distal parts of the flaps in the control and study groups (Mann Whitney U Test). When we evaluated the necroses in the flaps histopathologically, no statistically significant difference could be determined between the proximal and distal values of the flaps in the control and study groups (p>0,05). Yet, there was a statistically significant difference between the medial/medium values of the flaps (p<0,05). While the flap proximal sequence average of the necrosis in the control group was 7,50 and the median value was 0,00, the sequence average value of the study group was 7,50 and the median value was 0,0. On the other hand, the flap medium sequence value of necrosis in the control group was 10,00and the median value was 2,00, whereas the sequence average value of the study group was 5,00 and the median value was 1,00. Whereas the flap distal sequence average

value of the necrosis in the control group was 8,50and the median value was 3,00, the sequence average value of the study group was 6,50 and the median value was 3,00.

DISCUSSION

In our study aiming at increasing the random skin flap viability in rats, the rats in the study group were administered the Ticagrelor pharmacological agent that reduces the formation of a blood clot (thrombosis), while the control group was given 0.9% NaCl. In the macroscopic and histopathological evaluation made in the wake of the experiment, there was no necrosis observed in 1/3 of the proximal parts of the flaps in both of the groups, whereas a great number of necroses were seen in 1/3 of the distal parts of them. Nevertheless, in the evaluation of the 1/3 of the medial parts of the flaps, on the other hand, it was statistically determined that the flap necroses in the group administered Ticagrelor were fewer in comparison to those in the control group. ($p<0,05$). In order to be able to prevent the thrombus that occurs after the reduction in the blood flow in the flaps and in the wake of the damage in the blood vessels, various researches have been carried out with anticoagulant agents, and several articles reporting that they increased the flap viability (Tonks and Rees,1995; Senderoff *et al*., 1993; Khouri *et al*., 1993; Arnljots *et al*., 1994; Maeda *et al*., 1990) and those stating that they had no impact on the flap viability (Wallmichrath *et al*.,2014; Ashjian *et al*., 2007; Kroll *et al*., 1995; Bashir *et al*., 2014) were released.

In a study conducted on 517 flaps, Kroll *et al*. (1995) stated that there was no significant difference between the group administered high and low doses of heparin and the one that received no heparin, when they were compared (Kroll *et al*., 1995). Bashir *et al*. (2014) in a study they conducted on 38 patients by using free flaps, stated that there was no statistical difference between the group with heparin and the one without heparin (Bashir *et al*., 2014). We assume that the possible reason why there was no difference between the groups in increasing the flap viability with the help of anticoagulant pharmacological agents may be due to the fact that no blood clot (thrombosis) occurs since no stasis occurs in the blood flow of the flaps planned by taking into consideration the vascular structure that provides the blood flow of the flaps. Indeed, in our study, the fact that no necrosis has been observed in 1/3 of the proximal parts of the flaps promotes this opinion of ours. Okamoto *et al*. (Okamoto *et al*., 1993), in an experimental study they conducted on rabbits, stated that the antiaggreganagent called prostaglandin E1 (Li *et al*., 2013) had increased the flap viability.

Wallmichrath *et al.* (2014) used heparin for the anti-thrombin activation in venous stases in the free adipocutaneous flaps they performed on rats. As the result of the experiment, they reported that the flap viability in the group administered heparin increased when compared to the group that received no heparin (Wallmichrath *et al*., 2014). Maeda *et al*. (1990) in an experimental study they conducted on rabbits by using epigastric flap, stated that the flap viability increased when the group administered heparin andurokinase was compared with the one that received neither of them (Maeda *et al*., 1990).

Li *et al*. (1993), when the flaps of the control group and those of the experimental/test group which were perfused with ex vivo fluid containing heparin and citrate were compared, remarked that they came to the conclusion that the agents containing heparin and citrate had a preventive effect against the ischemic damage. We are of the opinion that the absence of any necrosis in 1/3 of the proximal parts of the flaps in both groups does not inhibit the blood flow to the extent that it will cause the occurrence of a necrosis, and that the necrosis in 1/3 of the distal parts of the flaps causes insufficient blood flow. We also consider that Ticagrelor maintains the flap blood circulation by avoiding any thrombosis in the tissue where the blood flow decreases and where there is an increase in the flap viability in 1/3 of the medial parts of the flaps in the study group when compared to the control group. As the result of our study, we have reached the conclusion that Ticagrelor is a pharmacological agent which increases flap viability and which can be used in cases where a flap surgery is to be performed and the antiaggregan effect is to be stopped in a short period of time.

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