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ORIGINAL ARTICLE / ÖZGÜN MAKALE

Effects of rivaroxaban on myocardial mitophagy in the rat heart

Rivaroksaban'ın sıçan kalbindeki miyokard mitofajisi üzerine etkileri

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ABSTRACT

Background: This study aims to demonstrate the efficacy of rivaroxaban's pharmacokinetic effects on myocardial mitophagy in rats by inducing apoptosis.

Methods: In this double-blind experiment, Wistar albino male rats were randomly divided into three groups for an experimental ischemia model: the sham group (Group 1; n=7), the control group (Group 2; n=7), and the drug group (Group 3; n=7). Rivaroxaban was perorally administered with gavage at 2 mg/kg/day for 28 days in Group 3. The heart was surgically exposed, and ischemia was achieved by compressing the vessel around the proximal part of the left anterior descending coronary artery for 10 min. The heart tissue was then transected, removed, and morphologically and immunohistochemically examined under a light microscope.

Results: Heart sections were immunohistochemically marked with caspase 3, caspase 9, APAF1, and Bcl-2 antibodies. Group 1 was compared to the rivaroxaban-treated group, and the pathways inducing apoptosis was increased (caspase 3, caspase 9, APAF1; $p<0.015$, $p<0.004$, and $p<0.01$, respectively) and Bcl-2, the molecule that inhibits apoptosis, was decreased ($p<0.01$) in Group 3.

Conclusion: The present study provides an evidence that the mitophagy response is less in rivaroxaban-treated rats, showing the protective effect of rivaroxaban against acute ischemia. Rivaroxaban-treated rats may have reduced cell death in cardiomyocytes during myocardial infarction and thus have reduced damage to the heart tissue caused by myocardial infarction.

Keywords: Anticoagulation, apoptosis, cardiac ischemia, mitophagy, rivaroxaban.

ÖZ

Amaç: Bu çalışma, rivaroksabanın sıçanlarda miyokardiyal mitofaji üzerine farmakokinetik moleküler etkilerini, apoptozu indükleme üzerinden göstermeyi amaçlamaktadır.

Çalışma planı: Çift kör deneysel çalışmada Wistar albino erkek sıçanlar, deneysel iskemi modeli için rastgele üç gruba ayrıldı: sham grubu (Grup 1; n=7), kontrol grubu (Grup 2; n=7) ve ilaç grubu (Grup 3; n=7). Rivaroksaban, Grup 3'de 28 gün boyunca 2 mg/kg/gün sonda ile peroral olarak uygulandı. Kalp cerrahi olarak açığa çıkarıldı ve iskemi modeli, sol ön inen koroner arterin proksimal kısmının 10 dk boyunca sıkıştırılmasıyla elde edildi. Kalp dokusu daha sonra transekte edildi, çıkarıldı ve ışık mikroskobu altında morfolojik ve immünohistokimyasal olarak değerlendirildi.

Bulgular: İmmünohistokimyasal olarak kalp dokusu kesitleri kaspaz 3, kaspaz 9, APAF1 ve Bcl-2 antikorları ile işaretlendi. Grup 1, rivaroksaban uygulanan grup ile karşılaştırıldı ve Grup 3 apoptozu indükleyen yollar artmış (kaspaz 3, kaspaz 9, APAF1; sırasıyla $p<0.015$, $p<0.004$ ve $p<0.01$) ve apoptozu inhibe eden molekül Bcl-2 azalmış ($p<0.01$) idi.

Sonuç: Bu çalışma, rivaroksaban uygulanan sıçanlarda mitofaji yanıtının daha az olduğuna dair bir kanıt sunmaktadır ve rivaroksabanın akut iskemiye karşı koruyucu etkisini göstermektedir. Rivaroksaban uygulanan sıçanların kardiyomiyositlerinde miyokard enfarktüsü sırasında hücre ölümü azalabilir ve dolayısıyla kalp dokusunda miyokard enfarktüsünün neden olduğu hasar azalabilir.

Anahtar sözcükler: Antikoagülasyon, apoptoz, kardiyak iskemi, mitofaji, rivaroksaban.

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Acute coronary syndrome (ACS) is a devastating disorder that occurs most frequently due to severe atherosclerosis or coronary embolism and presents a critical health concern that results in high mortality rates. Anticoagulation is a difficult problem in patients undergoing ACS or percutaneous coronary intervention.[1-3] It is important to protect the myocardium against possible thromboembolic ischemic damage. The traditional combination of vitamin K antagonists with dual antiplatelet therapy is associated with an increased risk of severe bleeding.[4-8] Modified antithrombotic strategies are needed that balance the risk of bleeding and the frequency of coronary ischemia.

Myocardial ischemia also causes mitochondrial mitophagy.[6] This process can be visualized by the colocalization of mitochondria with mitophagic proteins. The mitophagy of the mitochondria creates autophagy that is crucial for providing mitochondrial cellular energy, calcium homeostasis, and apoptotic signaling. There are additional pathways that regulate mitophagy, targeting mitochondria to autophagosomes in response to hypoxia or during ischemia development. Mitochondrial DNA (deoxyribonucleic acid) damage, respiratory chain inhibition, loss of membrane potential fragmentation, and mitochondrial unfolded protein or damage all have the potential to indicate mitophagy.[5] Finally, mitophagy resulting from ischemia can be measured as follows: mitochondrial activities including oxygen consumption, regulation of redox state, membrane potential, or release of apoptosis signals.[5]

In 2010, new nonvitamin K oral anticoagulants, or direct oral anticoagulants (DOACs), rivaroxaban, apixaban, dabigatran, and edoxaban were put into use. Rivaroxaban inhibits via factor Xa (FXa) and provides anticoagulation for venous thromboembolism treatment. Recently, rivaroxaban has been reported to potently inhibit platelet aggregation caused by tissue factors.[4,6] Besides, these findings may explain that very low doses of rivaroxaban may reduce cardiovascular events in patients with ACS.[4] Hence, this study aimed to demonstrate the efficacy of rivaroxaban pharmacokinetic effects on myocardial mitophagy in rats.

MATERIALS AND METHODS

Wistar albino male rats weighing over at least 180 g (mean weight: 455; range, 400 to 480 g) provided from the experimental animals laboratory of the Dokuz Eylül University were used for the experimental ischemia model. The rat model was preferred in the myocardial ischemia model due to the high reliability of the findings, the reproducibility of the experiments, and the readiness of the animals. In addition, the rat model has been documented as a suitable model for isolated myocardial mitophagy and autophagy studies in the literature.[2,7] In our experimental study, the choice of animal model as rats was determined in correspondence with the histology department. Rats were randomly divided into three groups: the sham group (Group 1; n=7), the control group (Group 2; n=7), and the drug group (Group 3; n=7). Rivaroxaban (Xarelto; Bayer HealthCare AG, Wuppertal, Germany) was perorally administered with gavage at 2 mg/kg/day, dispersed in 2 mL water for 28 days in Group 3. No procedure or medical application was applied to Group 1. No medication was given to Group 2, but the same surgical model of myocardial ischemia was created. Animals were obtained from the university laboratory animal research center and housed in the same laboratory under standard conditions with a 12-h light-dark cycle and 55 to 60% humidity. The rats were fed with standard rat chow ad libitum. All experimental procedures were also carried out in the center. For the protection against infection, antibiotherapy was applied by preoperative administration of cefazolin at a dose of 50 mg/kg. In addition, the skin of rats was shaved and cleaned with 10% povidone-iodine solution (Poviiodeks; Kimpa İlaç Laboratuvarı Ticaret Ltd Şti, İstanbul, Türkiye) to provide better surgical field visibility. The rectal temperature was kept at an average of 36.5ºC. The rats were heated with radiant heaters during the experiment. No procedure or medical application was applied to Group 1. No medication was given to Group 2, but the same surgical model of myocardial ischemia was created. Rats were sacrificed with 150 mg/kg sodium thiopental, and tissue samples were collected from the heart for histological examinations.

Surgical procedures

All animals (n=21) were anesthetized by an intramuscular injection of 50 mg/kg ketamine hydrochloride (Ketalar; Pfizer, İstanbul, Türkiye) and 5 mg/kg xylazine hydrochloride (Xylazine; Bayer Chemistry, İstanbul, Türkiye). When the corneal reflex disappeared, the animals were fixed on the operating table in the supine position. Spontaneous breathing was maintained and continued at 3 L/min with an oxygen mask. For prophylactic antibiotherapy, 50 mg/kg intramuscular cefazolin sodium was administered. A surgical loop (Design for Vision 3.5× expanded;

Bohemia, NY, USA) was used during surgery and dissection. The tail vein was cannulated with a 16-gauge catheter (Bıçakcılar Company, Istanbul, Türkiye) for intravenous access. The heart was surgically exposed via a middle-line incision in the skin. The mediastinum of the rats was opened, and the heart was exposed. Then, ischemia was induced by compressing the vessel around proximal part of the left anterior descending coronary artery with prolene 6.0 sutures for 10 min. The heart tissue was then transected and removed. No blood transfusion was required for the duration of the experimental model. No animals were excluded from the study. Additionally, none of the rats died in the model.

Light microscopy

Heart tissues dissected from rats were placed in 10% buffered formaldehyde for fixation. After the tissues underwent a routine tissue follow-up procedure, they were blocked by embedding in paraffin. Then, 5 µm sections were taken from the paraffin blocks with a rotary microtome (RM 2255; Leica Instruments, Nussloch, Germany). The sections taken were stained with hematoxylin-eosin stain for histomorphology and morphometric analysis. Images were taken from selected areas using a computer-assisted image analysis system consisting of a microscope

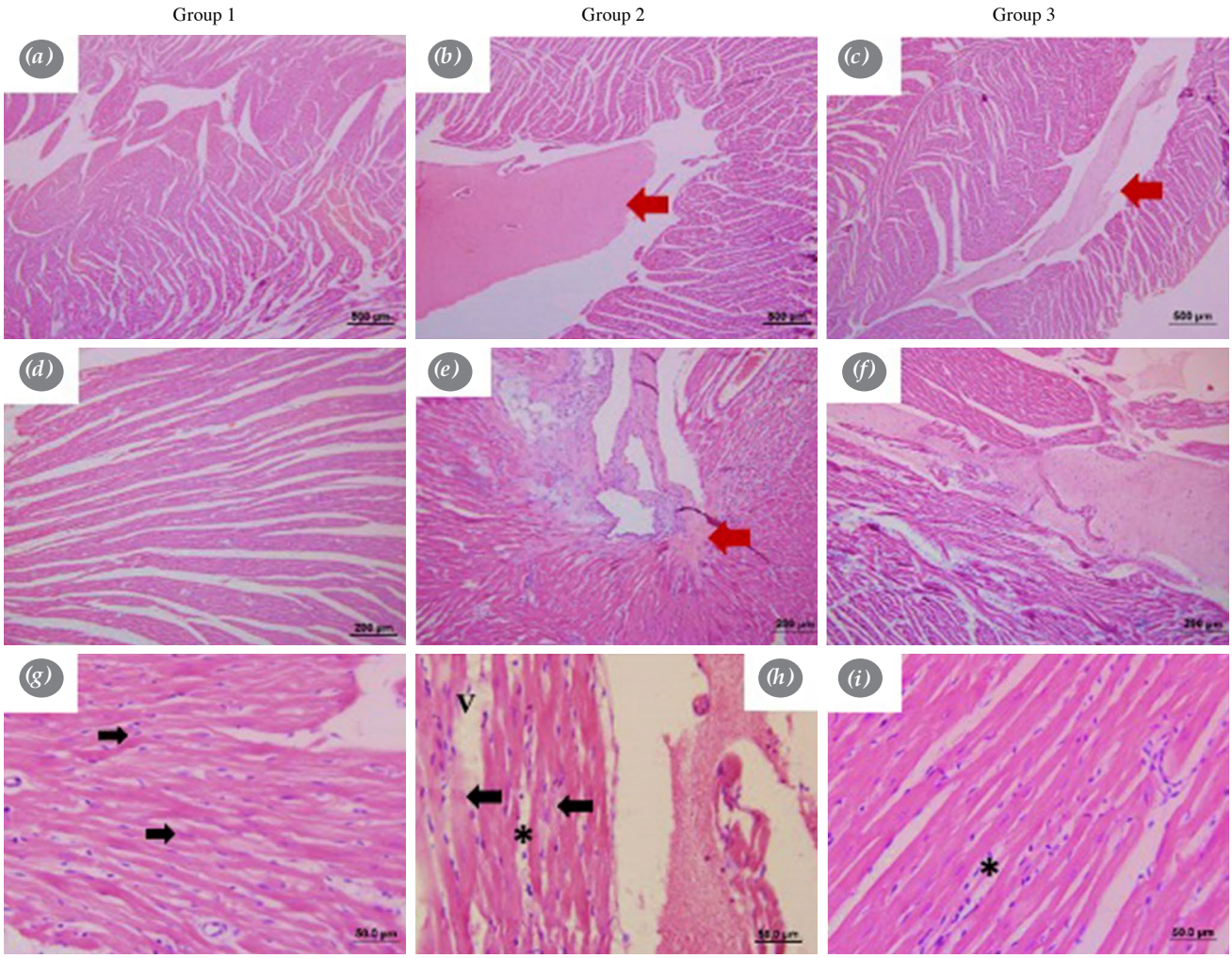

Figure 1. Histological sections of group Group 1 **(a, b, c)** Group 2 **(d, e, f)** and Group 3 **(g, h, i)** (H&E, ×40). In group 1, normal histomorphological structure is observed in cardiomyocytes. In Group 2, fragmentation and fibrotic tissue were observed in the heart muscle fibers. In group 3, fibrotic tissue was observed in some areas after rivaroxaban treatment, but cardiomyocytes were close to normal histomorphology.

(Olympus BX-51; Olympus Tokyo, Japan) equipped with a video camera (Olympus, DP70; Olympus, Tokyo, Japan).

Immunohistochemistry

The streptavidin–biotin method was used for immunohistochemistry. The sections were placed on lysine-coated slides, kept in a 60°C oven overnight, passed through a xylol series, deparaffinized, and then rehydrated through an alcohol series. The sections were treated with 10 mM citrate buffer at 95°C for 5 min to unmask the antigens. The sections were circumscribed using a Dako pen (Dako Aps, Glostrup, Denmark) and incubated in a 37°C oven for 15 min with 3% hydrogen peroxide to inhibit endogenous peroxidase activity. The sections were subsequently incubated with a normal serum-blocking solution for 30 min and incubated with primary antibodies against caspase 3 (BossUSA Woburn, Massachusetts, USA, Caspase 3 Polyclonal Antibody, cat. number: BS-2593R), caspase 9 (BossUSA Caspase-9 Polyclonal Antibody, cat. number: BS-0050R), APAF1 (BossUSA APAF1 Polyclonal Antibody, cat. number: BS-0058R), and Bcl-2 (BossUSA Bcl-2 Polyclonal Antibody, cat. number: BS-4563R) overnight in a humidity chamber of 30 to 60%. The next day, the sections were washed

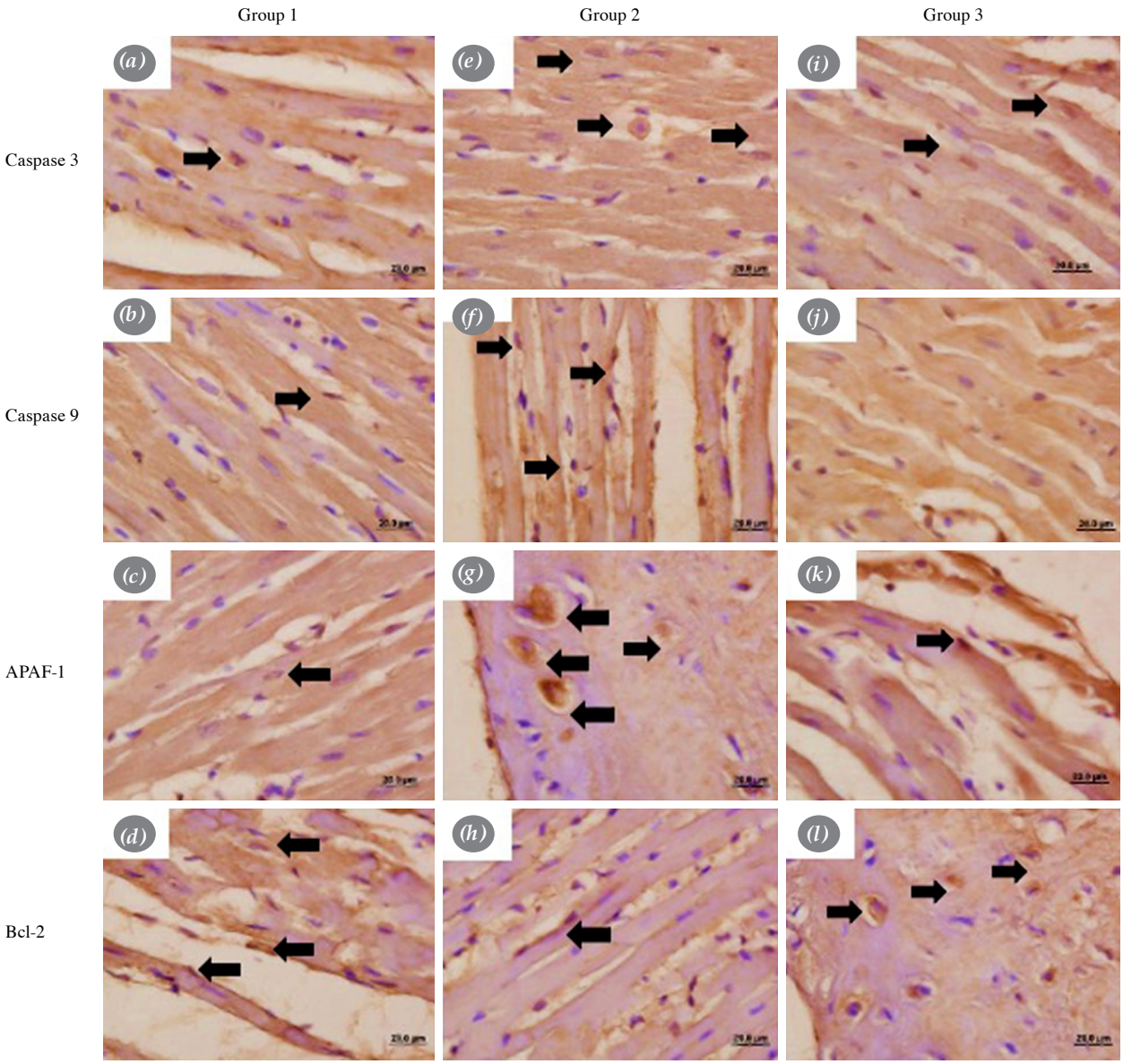

Figure 2. Histological sections of group Group 1 **(a, b, c, d)**, Group 2 **(e, f, g, h)** and Group 3 **(i, j, k, l)** (H&E, ×100).

with phosphate-buffered saline and then incubated with biotinylated immunoglobulin G and then with streptavidin-peroxidase conjugate (SensiTek West Logan, USA, HRP Anti-Polyvalent Lab Pack. cat. number. SHP125). After the sections were washed three times in phosphate-buffered saline, they were incubated with 3,3'-diaminobenzidine (Roche Diagnostics Basel, Switzerland, cat. number. 11718096001) for 2 min to detect immunoreactivity. Finally, the sections were covered with Entellan (Merck, Darmstadt, Germany) after staining with Mayer's hematoxylin (Sigma Aldrich, Ohio, USA) for 10 sec.

Statistical analysis

Data were analyzed using IBM SPSS version 23.0 (IBM Corp., Armonk, NY, USA) and Excel 2011 (Microsoft, Redmond, WA, USA). All data were expressed as mean ± standard deviation or median (min.-max.). Semiquantitative immune scoring variables were expressed as the mean ± standard deviation. Data did not show a normal distribution in the three groups of seven rats; thus, the Mann-Whitney U test and correlation analysis were applied to the data set as nonparametric tests. A p-value <0.05 were accepted as statistically significant.

RESULTS

Histological sections of the rats are shown in Figure 1. In the images obtained, it was observed that the cardiac muscle fibers and cardiomyocytes in Group 1 had a normal histomorphology structure. The nuclei were centrally located and had acidophilic sarcoplasm. In Group 2, fragmented heart muscle fibers, vacuolation, pale cytoplasm, and fibrotic tissue were observed. In Group 3, fibrotic areas were also observed in lesser areas compared to Group 2. Cardiomyocytes were nearly normal in structure, their sarcoplasm was stained acidophilic, and their nuclei were centrally located.

Immunohistochemically, we marked the heart sections with caspase 3, caspase 9, APAF1, and Bcl-2 antibodies (Figure 2). It was observed that the pathways inducing apoptosis (caspase 3, caspase 9, and APAF1) increased ($p<0.015$, $p<0.004$, and $p<0.01$, respectively) and Bcl-2, a molecule that inhibits apoptosis, decreased in Group 3 compared to Group 2 ($p<0.07$). This suggests that rivaroxaban treatment reduces cell death in cardiomyocytes in groups with acute myocardial infarction (MI) and therefore reduces damage to heart tissue caused by ischemia.

The heart tissue sections obtained from the subjects in each group were examined blindly by the histologist. In the semiquantitative scoring, tissues were scored between 0 and 3 according to the density of immune positive cells. Average values were obtained for each group from these scores. Afterwards, statistical evaluation was made between the groups and the *p* values are given in Table 1.

DISCUSSION

Recent publications describe the results of clinical use of rivaroxaban in reducing the risk of cardiovascular death, stroke, and MI.[8-10] This study indicated that rivaroxaban may protect the heart from myocardial damage induced by apoptosis and ischemia. We found that rivaroxaban treatment significantly decreased the levels of mitophagy, which was detected *in vitro* using immunohistochemistry.

Acute coronary syndrome is a devastating disorder that occurs most frequently due to severe atherosclerosis or coronary embolism and results in high mortality rates. It is important to protect the myocardium against possible thromboembolic ischemic damage. Rivaroxaban has been reported to potently inhibit platelet aggregation caused by tissue factors.[4,10] These findings may explain that very low doses of rivaroxaban may reduce cardiovascular

Table 1. Semi-quantitative values of groups according to immune positive cell density and *p* values of statistical comparisons of groups

	Group 1	Group 2	Group 3	Group 1 *vs*. 2 *p* value	Group 1 *vs*. 3 *p* value	Group 2 *vs*. 3 *p* value
Caspase 3	1	2.285714286	1.428571	0.001	0.060	0.015
Caspase 9	1	2.714286	1.428571429	0.001	0.060	0.004
APAF1	1.142857	2.428571429	1.4285714	0.002	0.254	0.010
Bcl-2	2.285714	1.285714286	2	0.006	0.141	0.007

APAF1: Apoptotic protease activating factor 1; Bcl-2: B cell lymphoma 2.

events in patients with ACS. Autophagy is involved in the maintenance of intracellular homeostasis in most cells of cardiovascular origin, including cardiomyocytes, endothelial cells, and arterial smooth muscle cells. Similarly, mitophagy is an autophagic response that targets damaged and potentially mitochondriotoxic conditions, such as ischemia.[11,12] Mitophagy is particularly important for the homeostasis of cardiovascular diseases. In these environments, mitophagy responses promote adaptation to stress and support cellular viability. In addition, mitophagy, which occurs as an ischemia stress response, refers to the specific autophagic transformation of mitochondria and represents an important mechanism in the protection of the myocardium. Adenosine triphosphate (ATP) production decreases in dysfunctional mitochondria and reactive oxygen species are produced as the product of excessive oxidative phosphorylation. They are highly susceptible to reactive oxygen species -mediated damage, and cell death is activated. The best-studied mechanisms of mitophagy in cardiomyocytes are cytosolic E3 ubiquitin ligase Parkin and mitochondrial membrane kinase-induced putative kinase-1 (PINK1).[2,7] When loss of function or damage occurs in mitochondria, Parkin is taken from cytosol into damaged mitochondria, changing their structure by adding ubiquitin to mitochondrial outer membrane proteins such as mitofusin 1, mitofusin 2, and voltage-dependent anion channels.[11,13] In recent studies, it was understood that PINK1-Parkin regulates mitophagy. Most of the known mechanisms are derived from cell culture studies expressing exogenous Parkin.[13] In addition, Bcl-2 family, caspases, molecules such as APAF1, and cellular elements such as mitochondria are involved in the regulation of apoptosis. While Bcl-2 family inhibits apoptosis,[13] caspase family and APAF1 molecules induce apoptosis.[13] Due to the lack of reliable quantitative mitophagy tests, few studies have been conducted to determine the role of the pathophysiological relevant Bcl-2 family in mitophagy under *in vivo* conditions.[11] This experimental model about the mitophagy effects of rivaroxaban was based on this basis. We believe that this rat model will be a good mitophagy model since it is easily reproducible.

Aggarwal et al.[7] found in their study that mitophagy acts as an important modulator of human lung diseases. Li et al.[2] suggested that mitophagy removes dysfunctional mitochondria and is known to play an important role in the pathogenesis of acute respiratory distress syndrome. They created an experimental *in vivo* and *in vitro* mice model. In addition, they suggested that Parkin-dependent mitophagy induced by silencing Parkin and ATG7 genes provides protection against mitochondria-dependent apoptosis in acute respiratory distress syndrome. Similarly, in our study, the ischemia response related to mitochondrial biogenesis was significantly downregulated after rivaroxaban treatment. These data suggest that rivaroxaban reduces the mitophagy by FXa inhibition in ischemia; however, the mechanism remains unclear.

Furthermore, Ding et al.[14] reported that in the human aneurysmal aorta, FXa protein expression is significantly upregulated. Additionally, they emphasized that rivaroxaban attenuates both angiotensin-II and calcium chloride-induced abdominal aortic aneurysm (AAA) progression by inhibiting aortic remodeling and inflammation. Zamorano-Leon et al.[15] reported that in human AAAs, rivaroxaban improved mitochondrial functionality that was associated with changes in proteins related to mitophagy. It has also been noted that FXa can modulate mitochondrial functionality and the expression of mitophagy-related proteins in AAAs. In our study, mitophagy was less common in the rivaroxaban group through FXa inhibition. Moñux et al.[16] have reported that rivaroxaban, an oral FXa inhibitor, could modify the expression of inflammatory and oxidative stress biomarkers in AAA *in vitro*. In another study, rivaroxaban at a dose of 2.5 mg twice daily was not associated with a lower rate of death, MI, or stroke compared to placebo in patients with chronic heart failure.[17]

Anticoagulant therapy should be initiated to protect the myocardium from ischemic damage before ischemia occurs. Rivaroxaban is the only DOAC for ACS that has been studied in phase III trials. Additional experiments are required to elucidate the relative contribution of ischemia damage versus heart failure in cardiovascular disorders. Our study has shown that rivaroxaban is a potential DOAC for acute myocardial ischemia prophylaxis and that rivaroxaban markedly inhibited the pathways inducing apoptosis (caspase 3, caspase 9, APAF1, and Bcl-2 antibodies) in the rat model of myocardial ischemia. Induction of apoptosis is by activation of caspases, a family of intracellular proteases. While these caspases activate proteins directly involved in the apoptotic process, they also inactivate antiapoptotic proteins, such as the Bcl-2 family, responsible for normal homeostasis, leading cells to apoptosis. Apoptosis via extracellular signaling (extrinsic pathway) and in response to

intracellular injury (internal pathway) both depend on caspase activation.[18] Apoptosis in cells is dependent on the release of cytochrome c and other proapoptotic factors from mitochondria in response to caspase activity. This mitochondrial pathway is the same pathway that is activated in the cell in response to intracellular injury. Cytochrome c is released from the intermembrane space of mitochondria and binds with APAF1 along with ATP.[18] In addition, ischemia was associated with antibody cellular translocation that decrease in the Bcl-2 antibodies.[19] Members of the Bcl-2 family play an important role in preventing apoptosis by inhibiting the release of cytochrome c from mitochondria. Bcl-2 binds to the mitochondrial membrane and blocks the release of cytochrome c both *in vivo* and *in vitro*.[19] In cells whose mitochondria are damaged due to various pathological factors, this mechanism may not work properly, and apoptosis may occur because the Bcl-2 family cannot function.[20] Our data suggest that they are inducers of apoptosis via the mitochondrial pathway involving caspase 9 and 3 in myocardial cell death. We suggest that a similar mechanism may be present in patients with ACS syndrome. The results of this study also supported our conclusion.

Mitochondrial disorders can arise from various factors, such as disrupted mitochondrial dynamics, energy depletion, increased oxidative stress, inhibited clearance of damaged mitochondria, and cell apoptosis.[21,22] The maintenance of cardiac metabolism and homeostasis relies heavily on efficient autophagy, including mitophagy. Any impairment in autophagy or mitophagy could lead to heart-related diseases. Mitochondrial dysfunction in the heart may result in several adverse outcomes, such as ischemia/reperfusion injury, heart failure, and arrhythmias. There is currently no known direct relationship between mitophagy and rivaroxaban.[23] However, research has shown that anticoagulants, such as rivaroxaban may have indirect effects on the mitochondria.[23] Studies have suggested that anticoagulants can reduce oxidative stress and inflammation, which are known to contribute to mitochondrial damage.[21,22] Additionally, some studies have suggested that anticoagulants may improve mitochondrial function by increasing the production of ATP, the energy currency of the cell.[21-23]

The primary limitation of our study is that there is limited data at the molecular level. Second, the pharmacokinetic effects of rivaroxaban we found in rats may not necessarily exist in human beings. We plan to conduct future studies with a greater budget to correlate immunohistochemical changes with oxidative stress markers.

In conclusion, while there is currently no direct relationship between mitophagy and rivaroxaban, research suggests that anticoagulants, such as rivaroxaban, may have beneficial effects on mitochondrial health by reducing oxidative stress and inflammation and improving mitochondrial function. However, further research is needed to fully understand the potential implications of these findings.

Ethics Committee Approval: The study protocol was approved by the Dokuz Eylül University Multi-Disciplinary Laboratory Animal Experiments Local Ethics Committee (date: 01.05.2021, no: 05/2021). All experimental procedures were carried out in accordance with the National Institute of Health 'Animal Care Guidelines'.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Concept: P.A., T.G.; Design: T.G., K.K.; Supervision: S.B., K.K., P.A.; Resource: C.B., K.K., T.G.; Materials: T.G., B.K.; Data collection or processing: T.G., C.E.; Analysis: C.E., C.B.; Literature search: T.G., K.K.; C.B.; Writing: T.G., B.K., Critical review: C.B.; Other: P.A., S.B. All authors approved the final version of the manuscript.

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REFERENCES

1. Zhang D, He K, Raghavan N, Wang L, Crain EJ, He B, et al. Metabolism, pharmacokinetics and pharmacodynamics of the factor Xa inhibitor apixaban in rabbits. J Thromb Thrombolysis 2010;29:70-80. doi: 10.1007/s11239-009-0401-8.
2. Li T, Liu Y, Xu W, Dai X, Liu R, Gao Y, et al. Polydatin mediates Parkin-dependent mitophagy and protects against mitochondria-dependent apoptosis in acute respiratory distress syndrome. Lab Invest 2019;99:819-29. doi: 10.1038/s41374-019-0191-3.
3. Chong J, Chen H, Dai D, Wang S, Zhou Q, Liu J, et al. Effects of ticagrelor on the pharmacokinetics of rivaroxaban in rats. Pharm Biol 2020;58:630-5. doi: 10.1080/13880209.2020.1785510.
4. Gibson CM, Mehran R, Bode C, Halperin J, Verheugt FW, Wildgoose P, et al. Prevention of bleeding in patients with atrial fibrillation undergoing PCI. N Engl J Med 2016;375:2423-34. doi: 10.1056/NEJMoa1611594.
5. Zhang J. Teaching the basics of autophagy and mitophagy to redox biologists--mechanisms and experimental approaches. Redox Biol 2015;4:242-59. doi: 10.1016/j.redox.2015.01.003.

6. Steffel J, Collins R, Antz M, Cornu P, Desteghe L, Haeusler KG, et al. 2021 European Heart Rhythm Association practical guide on the use of non-vitamin K antagonist oral anticoagulants in patients with atrial fibrillation. Europace 2021;23:1612-76. doi: 10.1093/europace/euab065.
7. Aggarwal S, Mannam P, Zhang J. Differential regulation of autophagy and mitophagy in pulmonary diseases. Am J Physiol Lung Cell Mol Physiol 2016;311:L433-52. doi: 10.1152/ajplung.00128.2016.
8. Weitz JI, Harenberg J. New developments in anticoagulants: Past, present and future. Thromb Haemost 2017;117:1283-8. doi: 10.1160/TH16-10-0807.
9. Wong PC, Crain EJ, Watson CA, Xin B. Favorable therapeutic index of the direct factor Xa inhibitors, apixaban and rivaroxaban, compared with the thrombin inhibitor dabigatran in rabbits. J Thromb Haemost 2009;7:1313-20. doi: 10.1111/j.1538-7836.2009.03503.x.
10. Hara T, Fukuda D, Tanaka K, Higashikuni Y, Hirata Y, Nishimoto S, et al. Rivaroxaban, a novel oral anticoagulant, attenuates atherosclerotic plaque progression and destabilization in ApoE-deficient mice. Atherosclerosis 2015;242:639-46. doi: 10.1016/j.atherosclerosis.2015.03.023.
11. Wang HT, Lin JH, Yang CH, Haung CH, Weng CW, Maan-Yuh Lin A, et al. Acrolein induces mtDNA damages, mitochondrial fission and mitophagy in human lung cells. Oncotarget 2017;8:70406-21. doi: 10.18632/oncotarget.19710.
12. Bravo-San Pedro JM, Kroemer G, Galluzzi L. Autophagy and mitophagy in cardiovascular disease. Circ Res 2017;120:1812-24. doi: 10.1161/CIRCRESAHA.117.311082.
13. Chang AL, Ulrich A, Suliman HB, Piantadosi CA. Redox regulation of mitophagy in the lung during murine Staphylococcus aureus sepsis. Free Radic Biol Med 2015;78:179-89. doi: 10.1016/j.freeradbiomed.2014.10.582.
14. Ding Y, Li X, Zhou M, Cai L, Tang H, Xie T, et al. Factor Xa inhibitor rivaroxaban suppresses experimental abdominal aortic aneurysm progression via attenuating aortic inflammation. Vascul Pharmacol 2021;136:106818. doi: 10.1016/j.vph.2020.106818.
15. Zamorano-Leon JJ, Serna-Soto M, Moñux G, Freixer G, Zekri-Nechar K, Cabrero-Fernandez M, et al. Factor Xa inhibition by rivaroxaban modified mitochondrial-associated proteins in human abdominal aortic aneurysms. Ann Vasc Surg 2020;67:482-9. doi: 10.1016/j.avsg.2020.02.005.
16. Moñux G, Zamorano-León JJ, Marqués P, Sopeña B, García-García JM, Laich de Koller G, et al. FXa inhibition by rivaroxaban modifies mechanisms associated with the pathogenesis of human abdominal aortic aneurysms. Br J Clin Pharmacol 2017;83:2661-70. doi: 10.1111/bcp.13383.
17. Zannad F, Anker SD, Byra WM, Cleland JGF, Fu M, Gheorghiade M, et al. Rivaroxaban in patients with heart failure, sinus rhythm, and coronary disease. N Engl J Med 2018;379:1332-42. doi: 10.1056/NEJMoa1808848.
18. Steel R, Doherty JP, Buzzard K, Clemons N, Hawkins CJ, Anderson RL. Hsp72 inhibits apoptosis upstream of the mitochondria and not through interactions with Apaf-1. J Biol Chem 2004;279:51490-9. doi: 10.1074/jbc.M401314200.
19. Shiraga S, Adamus G. Mechanism of CAR syndrome: Anti-recoverin antibodies are the inducers of retinal cell apoptotic death via the caspase 9- and caspase 3-dependent pathway. J Neuroimmunol 2002;132:72-82. doi: 10.1016/s0165-5728(02)00314-4.
20. Newmeyer DD, Bossy-Wetzel E, Kluck RM, Wolf BB, Beere HM, Green DR. Bcl-xL does not inhibit the function of Apaf-1. Cell Death Differ 2000;7:402-7. doi: 10.1038/sj.cdd.4400665.
21. Ajoolabady A, Chiong M, Lavandero S, Klionsky DJ, Ren J. Mitophagy in cardiovascular diseases: Molecular mechanisms, pathogenesis, and treatment. Trends Mol Med 2022;28:836-49. doi: 10.1016/j.molmed.2022.06.007.
22. Luan Y, Luan Y, Feng Q, Chen X, Ren KD, Yang Y. Emerging role of mitophagy in the heart: Therapeutic potentials to modulate mitophagy in cardiac diseases. Oxid Med Cell Longev 2021;2021:3259963. doi: 10.1155/2021/3259963.
23. Costopoulos C, Niespialowska-Steuden M, Kukreja N, Gorog DA. Novel oral anticoagulants in acute coronary syndrome. Int J Cardiol 2013;167:2449-55. doi: 10.1016/j.ijcard.2012.08.014.