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ORIGINAL ARTICLE / ÖZGÜN MAKALE

The effect of edoxaban on apoptosis in an abdominal aortic aneurysm model in rats

Edoksabanın sıçanlarda aort anevrizması modelinde apoptoza etkileri

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ABSTRACT

Background: This study aimed to evaluate the effects of edoxaban, which is used in venous thrombosis, systemic embolism, and stroke, on an aortic aneurysm model and to demonstrate the pharmacokinetic and molecular effects of edoxaban through the induction of apoptosis.

Methods: In this double-blind experimental study, 21 Wistar albino male rats (mean weight: 290 g; range, 280 to 300 g) were divided into three groups: the sham group (n=7), the abdominal aortic aneurysm (AAA) group (n=7), and the AAA-edoxaban group (n=7). Edoxaban 10 mg/kg was given to the AAA-edoxaban group by oral gavage daily for 30 days. At the end of 30 days, the aneurysmal aorta was surgically removed and histologically examined. The abdominal aorta was surgically exposed and wrapped with a calcium chloride (0.5 mol/L) sponge for 10 min.

Results: Immunohistochemically, aortic sections were marked with caspase-3 and caspase-9 antibodies. It was observed that the pathways that trigger apoptosis (caspase-3 and caspase-9; $p<0.004$ and $p<0.005$, respectively) were significantly reduced in the AAA-edoxaban group compared to the AAA group. In the AAA-edoxaban group, it was observed that the expansion in aortic diameter and the deterioration in the elastic fibril structure in the aortic aneurysm were decreased as a result of edoxaban treatment. Edoxaban treatment was observed to reduce cell death in both the tunica intima and tunica media.

Conclusion: This study provided strong evidence of the protective effect of edoxaban on aortic aneurysm-related vascular damage by reducing apoptosis and mitophagy.

Keywords: Anticoagulant, aorta, edoxaban, factor Xa.

ÖZ

Amaç: Bu çalışmada venöz tromboz, sistemik embolizasyon ve inme tedavisinde kullanılan edoksabanın aort anevrizması modeli üzerindeki etkileri değerlendirildi ve edoksabanın apoptozu indükleme üzerinden farmakokinetik moleküler etkileri gösterildi.

Çalışma planı: Bu çift kör deneysel çalışmada, 21 Wistar albino erkek sıçan (ort. ağırlık: 290 g; dağılım, 280-300 g) üç gruba ayrıldı: sham grubu (n=7), abdominal aort anevrizması (AAA) grubu (n=7) ve AAA-edoksaban grubu (n=7). Deneyde AAA-edoksaban grubuna 30 gün boyunca oral gavaj yoluyla 10 mg/kg/gün edoksaban verildi. Otuz günün sonunda, anevrizmal aort cerrahi olarak çıkarıldı ve histolojik olarak incelendi. Abdominal aort cerrahi olarak açığa çıkarıldı ve 10 dk süreyle kalsiyum klorürlü (0.5 mol/L) spançla sarıldı.

Bulgular: İmmünohistokimyasal olarak aort kesitleri kaspaz-3 ve kaspaz-9 antikorları ile işaretlendi. AAA-edoksaban grubunda AAA grubuna göre, apoptozu tetikleyen yolakların (kaspaz-3 ve kaspaz-9; sırasıyla $p<0.004$ ve $p<0.005$) anlamlı olarak azaldığı görüldü. Edoksaban tedavisi sonucunda AAA-endoksaban grubunda aortanevrizmasında gözlenen aort çapındaki genişlemenin ve elastik fibril yapısındaki bozulmanın azaldığı gözlendi. Edoksaban tedavisinin hem tunica intima hem de tunica mediada hücre ölümünü azalttığı görüldü.

Sonuç: Bu çalışma, edoksabanın apoptoz ve mitofajiyi azaltarak aort anevrizmasına bağlı damar hasarı üzerinde koruyucu etkisi olduğuna yönelik güçlü kanıtlar sağladı.

Anahtar sözcükler: Antikoagülan, aort, edoksaban, faktör Xa.

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An aortic aneurysm is defined as an expansion of the transverse diameter of any segment of the aorta that exceeds 50% of its normal values.[1] While ascending aortic aneurysms are due to degenerative changes in the elastic layer, descending and abdominal aortic aneurysms (AAAs) are mostly due to atherosclerosis.[1] Important risk factors for aortic aneurysms include age, sex, smoking, hypertension, hyperlipidemia, obesity, chest trauma, genetics (Marfan, Ehlers-Danlos), and family history. It is recommended that males over the age of 65 at risk be screened for AAA with ultrasonography once.[2] Pathological features of AAA include inflammation characterized by increased elastin degradation, vascular smooth muscle cell (VSMC) apoptosis, oxidative stress, and macrophage, lymphocyte, and plasma cell infiltration.[3]

Rivaroxaban, apixaban, and edoxaban, which are direct factor Xa (FXa) inhibitors (non-vitamin K oral anticoagulant), have come into use in 2010.[4] The use of these drugs in the indications of prevention of stroke and systemic embolism in nonvalvular atrial fibrillation, prevention and treatment of venous thromboembolism, and secondary prevention of arterial ischemia in patients with chronic coronary or peripheral artery disease has been approved by the USA Food and Drug Administration.[5] Additionally, it has been stated in the literature that FXa is involved in mitochondrial functions in AAAs and may affect mitophagy-related proteins.[5] Edoxaban may affect aneurysm development through this FXa inhibition.[5,6]

Li et al.[6] established an experimental rat model of acute respiratory distress syndrome. They reported that parkin-dependent mitophagy protects against mitochondria-dependent apoptosis in acute respiratory distress syndrome. Moreover, they showed beneficial antiapoptotic, anti-inflammatory effects. In addition, Sagiv et al.[7] showed that the primary aim of edoxaban treatment in Kawasaki disease large coronary artery aneurysms was to prevent coronary artery thrombosis. In the secondary goal, they were at high risk for developing arterial wall changes in younger children with Kawasaki-associated risk for thrombosis. They suggested that studies are needed to define the safety, effectiveness, and pharmacokinetics of edoxaban.

It is still debated whether direct oral anticoagulant agents provide better results than vitamin K antagonists. Maximum concentration of edoxaban is reached within 1 to 2 h.[8] Half of the absorbed dose is excreted through the kidney.[9,10] A single dose of 30 mg is recommended for those with a creatinine clearance of 15 to 50 mL/min and a weight ≤60 kg.[9,10] The extended plasma half-life is 10 to 14 h.[9] Drug interaction has been noted as minimal (metabolism below 10%). Interaction with food has not been noted. Unlike apixaban and rivaroxaban, edoxaban does not interact with the cytochrome P450 system.[5] As a substrate of P-glycoprotein, a transporter that regulates the entry of substances into cells, edoxaban may be subject to drug-drug interactions.[11] The potential risk of bleeding Cmax may increase with prolonged dosing over 24 h.[9]

Edoxaban, which has been shown to have significant mortality-reducing benefits against thrombosis, may also be beneficial in aortic aneurysm. Edoxaban has not been studied on a calcium model, which is an inflammation-based model. Hence, this study aimed to examine the effects of edoxaban on AAA in a rat model.

MATERIALS AND METHODS

This experimental study was conducted on 21 Wistar albino male rats (mean weight: 290 g; range, 280 to 300 g) obtained from the experimental animal multidisciplinary laboratory of the Dokuz Eylül University Faculty of Medicine. The study was performed in accordance with the National Institutes of Health's Animal Care Guidelines and the principles of the Declaration of Helsinki. The study was started after receiving permission from the Dokuz Eylül University Experimental Animal Ethics Committee with protocol number 07/2022.

In accordance with Laplace's law, significant changes in the structure of the aneurysm aortic wall, such as inflammatory cell activation, oxidative stress damage, neovascularization, calcification, collagen and elastin degradation, and VSMC apoptosis, were reported.[6] The calcium phosphate model is a new modification of the calcium chloride (CaCl2) model, a well-defined method that does not require transgenic rats. The CaCl2 model is one of the three most commonly used aneurysm models.[12] The calcium phosphate model was created by treating CaCl2 with phosphate-buffered saline to provide greater aneurysm expansion in a shorter period of 28 days.[13]

The rats were randomly divided into three groups: the sham group (Group 1), the AAA group (Group 2), and the AAA-edoxaban group (Group 3). In Group 1, a sponge impregnated with 0.09% sodium chloride was applied to the aorta, and the abdomen was closed. The same surgical procedure was repeated

with CaCl2 in the other groups. Edoxaban was given at 10 mg/kg/day to Group 3. In Group 3, the calcium model, which is an inflammation-based model, was studied, and its effects at the end of 30 days were examined in aneurysmal aorta specimens. Hematoxylin-eosin and Masson trichrome staining were performed for the histological evaluation of the vascular sections obtained from the groups. From the obtained sections, the lumen, tunica media area, lumen diameter, and tunica intima thickness were measured with the ImageJ software (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, USA). Tunica intima thickness was calculated by measuring epithelial thickness from 10 different areas for each subject in the groups.

Anesthesia and analgesia were provided with ketamine and xylazine (intramuscular injection of 50 mg/kg ketamine and 5 mg/kg xylazine). Antibiotherapy was administered to the rats preoperatively with intramuscular cefazolin sodium at a dose of 50 mg/kg to prevent infection. To provide better visibility during surgery, the incision areas were shaved and disinfected with 10% povidone. A biological rhythm was maintained with 55 to 60% humidity and a 12-h light-dark cycle. Rats were given standard chow ad libitum. The rectal temperature of the rats was kept at 36.5°C, and the animals were heated with radiant heaters. Observing the loss of corneal reflexes, the rats were fixed to the operating table in a supine position. Spontaneous breathing and oxygen inhalation were continued through the mask at a rate of 3 L/min. For vision during surgical procedures, a ×3.5 extended binocular loop (Design for Vision Inc., Bohemia, NY, USA) was used. Intravenous access to the rat tail vein was performed with a 16-gauge catheter (Bicakcilar, Istanbul, Türkiye).

As a surgical procedure, an abdominal incision was made through a midline laparotomy, and the retroperitoneum and infrarenal abdominal aorta were exposed. The aortic adventitia was stripped, and it was wrapped with a CaCl2 sponge (prepared with a 0.5 mol/L CaCl2 solution) for 10 min. Afterward, a sponge impregnated with phosphate-buffered saline was applied for 5 min, and the abdomen was closed in accordance with the anatomy. In Group 1, after the abdominal incision and exposure of the aorta, the aorta was exposed for 15 min with a sponge impregnated with 0.09% sodium chloride, and the abdomen was closed. Edoxaban at 10 mg/kg/day was administered to Group 3 via gavage.[14] The dosage was given in accordance with the literature.[15] There was no need for blood transfusion during the rat aortic aneurysm experimental model. In the study model, no rats were excluded or died. On the 30th day after the procedure, the rats were sacrificed. The aorta was ligated at the level of the infrarenal artery and the aortic bifurcation and cut at the indicated levels. The aorta was excised, measured, and taken for histological evaluation. The development of AAA was evaluated by immunohistochemistry staining.

Histological evaluation

The aorta specimens were fixed with 10% formalin for 48 to 72 h. Tissues were dehydrated through graded alcohols, cleared with xylene, and embedded in paraffin. Sections were cut at 5 μm using a rotary microtome (RM 2255; Leica Instruments, Nussloch, Germany) and affixed to poly-L-lysine-coated slides. Sections were deparaffinized, rehydrated, and then stained with hematoxylin-eosin and Masson trichrome.[16] The images were analyzed using a computer-assisted image analyzer system consisting of a microscope (BX-51; Olympus, Tokyo, Japan) equipped with a high-resolution video camera (DP-71; Olympus, Tokyo, Japan). The lumen diameters, tunica media areas, and tunica intima thicknesses were all measured using ImageJ. The diameters were compared.

Immunohistochemistry

Determination and evaluation of apoptosis

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 xylene 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 (cat. no. BS-2593R; BossUSA, Woburn, MA, USA), caspase-9 (cat. no. BS-0050R; BossUSA, Woburn, MA, USA), overnight in a humidity chamber with 30 to 60% humidty. The next day, the sections were washed with phosphate-buffered saline and then incubated with biotinylated immunoglobulin G, followed

by streptavidin-peroxidase conjugate (HRP Anti-Polyvalent Lab Pack, cat. no. SHP125; Sensi Tek, Logan, UT, USA). After the sections were washed three times in phosphate-buffered saline, they were incubated with 3,3'-diaminobenzidine (cat. no. 11718096001; Roche Diagnostics, Basel, Switzerland) for 2 min to detect immunoreactivity. Finally, the sections were covered with Entellan (Merck, Darmstadt, Germany) after staining with Mayer's hematoxylin (Sigma Aldrich, St. Louis, MO, USA) for 10 sec.[16]

Scoring of Active Caspase-3 and Active Caspase-9

For quantitative measurement of the number of cells that underwent apoptosis, 100 cells were randomly counted in these different areas for each group. The apoptotic cell percentages were calculated. Examinations were performed by two individuals who were blinded to the study (Table 1).

Statistical analysis

A priori power analysis was conducted using G*Power version 3.1.9.7 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) to determine the minimum sample size required to test the study hypothesis. The required sample size was determined to be 21 to achieve a medium effect size with an alpha of 0.05 and 80% power.

All analyses were performed with IBM SPSS version 23.0 software (IBM Corp., Armonk, NY, USA). The Excel software (Microsoft Corp., Redmond, WA, USA) was used. The Shapiro-Wilk test was applied for the normality test. Since the data was nonnormally distributed, the Mann-Whitney U test was used to compare two independent groups, and the Kruskal-Wallis test was used to compare three groups. Data were presented as mean ± standard error of the mean. A p-value <0.05 was considered statistically significant.

RESULTS

It was observed that intimal hyperplasia significantly increased in Group 2 compared to Groups 1 and 3 (p=0.004 and p=0.006, respectively). However, when Group 3 was compared with Group 1, it was observed that there was no statistically significant difference (p=0.423). The findings were close to normal vascular structure. When the lumen diameter (p=0.004 and p=0.010) and area (p=0.004 and p=0.010) were compared, a statistically significant increase was observed in Group 2 compared to Groups 1 and 3. When Group 3 was compared with Group 2, it was statistically shown that the lumen diameter and area decreased, and there were findings similar to normal vascular structure. Aneurysm diameter measurement was made after sacrification and fixation of vascular tissues. The measurements were evaluated between the groups. The increase in the percentage between Groups 1 and 2 was calculated as 126.43%. There was no statistically significant difference between Groups 1 and 3 (p=0.873, p=0.873). In addition, when the tunica media area was compared, it was observed that there was a correlation between the lumen area measurements and the groups. When Group 2 was compared with Groups 1 and 3, it was observed that the tunica media area increased statistically significantly (p=0.004 and p=0.016, respectively). However, when Group 3 was compared with Group 1, it was observed that there was no statistically significant difference (p=0.337).

Masson trichrome staining was used to show connective tissue and fibrils in histological sections. As a result, fibrils were stained blue and muscle fibers were stained red (Figure 1). It was observed that the elastic fibrils in the tunica media layer in Group 1 had a normal histological structure and showed smooth undulation. However, in Group 2, in which aortic aneurysm was created, it was observed that both the structure of muscle fibers and elastic fibrils were disrupted. Histological deteriorations that negatively altered the elasticity of the aorta were detected in these. Similarly, an increase in collagen fibrils was observed in the tunica intima layer. In Group 3, in which the aortic aneurysm was treated with edoxaban, the undulation of elastic fibrils was close to normal. Likewise, it was observed that collagen fibril accumulation decreased (Figure 2).

Table 1. The aorta tissue sections obtained from the subjects in each group

				Group 1 *vs.* 2	Group 1 *vs.* 3	Group 2 *vs.* 3
	Group 1	Group 2	Group 3	*p*	*p*	*p*
Caspase 3	10.666	53.166	17.666	0.004	0.170	0.004
Caspase 9	12.666	56.333	20.833	0.004	0.012	0.005

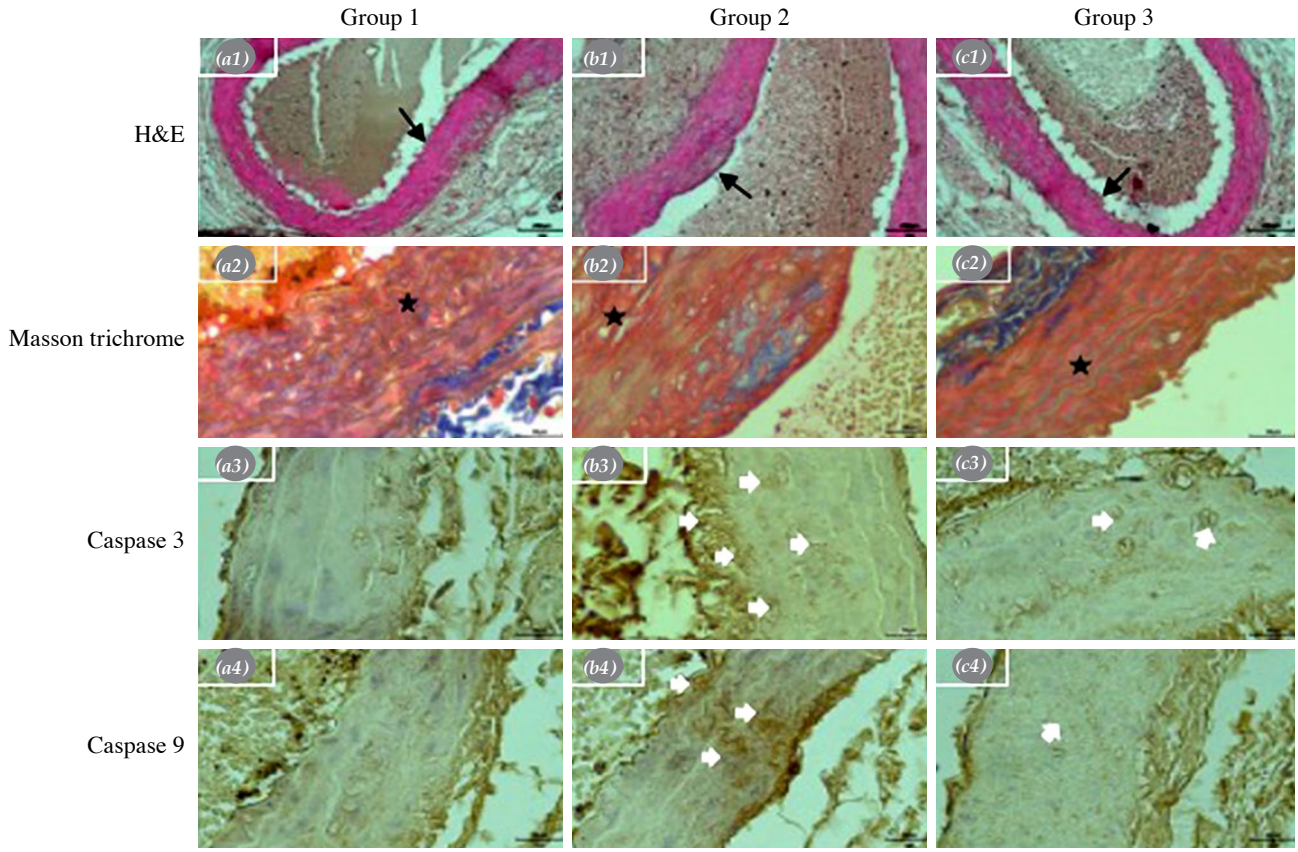

Figure 1. Histological sections of group 1 (a1, a2, c3, c4), group 2 (b1, b2, b3, b4) and group 3 (c1, c2, c3, c4). a1, b1 and c1; hematoxylin + eosin stain (×10): In group 1, normal histomorphological structure is observed in tunica intima and tunica media. In group 2, intimal hyperplasia was observed in the tunica intima (black arrow). In group 3 although intimal hyperplasia (black arrow) was observed in some areas after edoxaban treatment, tunica intima histomorphology was close to normal. Histological sections of groups (caspase-3 and caspase-9 staining, ×40). (a3) group 1, (b3) group 2 and (c3) group 3, caspase-3 staining. (a4) group, (b4) group 2 and (c4) group 3, caspase-9 staining. Caspase 3 and 9 positive cells marked with white arrow.
H&E: Hematoxylin-eosin.

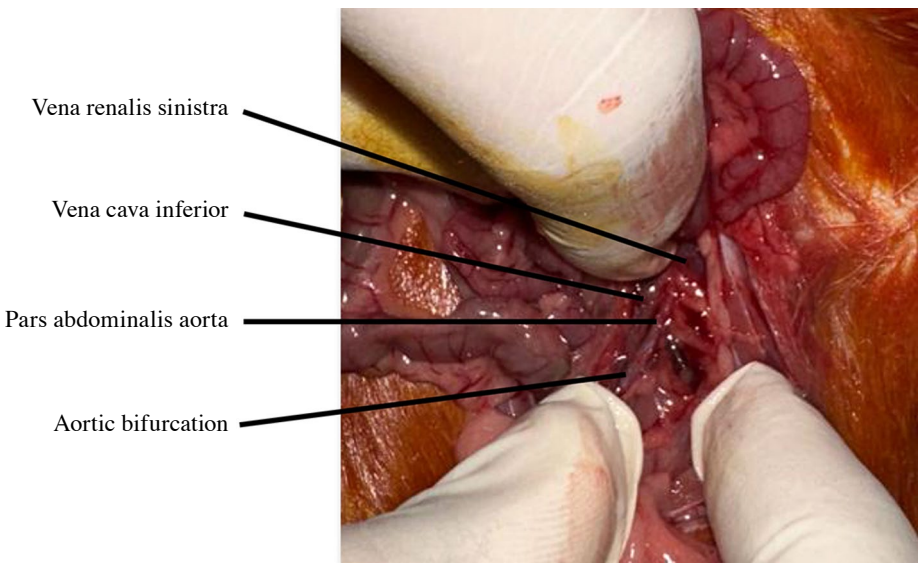

Figure 2. Infrarenal abdominal aorta marked black arrow for aneurysm.

Caspase-3 and caspase-9 immunohistochemical staining was performed to evaluate cell death between groups (Figure 1). It was observed that there was a statistical decrease in the number of cells undergoing apoptosis in Group 3 (Table 1).

As a result of treatment with edoxaban, it was observed that the expansion of the normal aortic diameter and the deterioration of the elastic fibril structure observed in aortic aneurysms regressed. Edoxaban treatment was thought to reduce cell death in both tunica intima and tunica media, thus reducing aortic aneurysm-induced damage.

DISCUSSION

Edoxaban is the newest non-vitamin K oral anticoagulant approved by the USA Food and Drug Administration for once-daily administration.[17] In our rat aneurysm experimental model, periaortic application of CaCl2 to the rat aorta induced AAAs. Specifically, immunohistochemistry determined that edoxaban treatment significantly reduced apoptosis. When Group 3 was compared with Group 2, it was observed that the lumen diameter and area significantly decreased, and there were findings similar to normal vascular structure on the aneurysm model. Therefore, we suggest that edoxaban may have beneficial effects on aortic aneurysms and that it may reduce cell death in both the tunica intima and tunica media by inhibiting apoptosis.

Edoxaban is a FXa inhibitor indicated as an anticoagulant to reduce the risk of venous thromboembolism, systemic embolization, and stroke. Edoxaban was first used in Japan in 2011 for the prevention of venous thrombosis after hip or knee replacement surgery and was approved in 2014 for the prevention of stroke and systemic embolism in patients with atrial fibrillation.[9]

The potential for thrombotic complications has raised the hypothesis of long-term use of oral anticoagulation in patients without a primary indication for anticoagulation. The risk of bleeding has been shown to be high, and the net benefit of combining antiplatelet and oral anticoagulation therapy has not been established.[18] In patients with aortic aneurysms, it is controversial whether anticoagulant therapy has additional protective benefits when oral anticoagulant use is already indicated (e.g., atrial fibrillation).[19] In the study by Ogata et al.,[18] edoxaban was reported to have dose-dependent effects on activated partial thromboplastin time, prothrombin time, international normalized ratio, and anti-FXa activity.[4] There appear to be differences between edoxaban and other anticoagulants in terms of clinical use in some patient populations.[8,10] Although it has been shown to be superior or noninferior to warfarin for the same indications, edoxaban needs to be evaluated separately.[18]

Li et al.[6] created an experimental rat model. They showed that Parkin-dependent mitophagy protects against mitochondria-dependent apoptosis in acute respiratory distress syndrome.[7] Similarly, in our study, the aneurysm-related apoptosis decreased significantly after edoxaban treatment. They mentioned that edoxaban may reduce apoptosis in aneurysms through FXa inhibition, but the mechanism is not yet clear. This is one of the reasons why edoxaban was chosen for the treatment of AAA in this study. As a result, evidence was found that edoxaban reduces oxidative stress and inflammation. In addition, it demonstrated this effect by contributing positively to mitochondrial damage through mitophagy and apoptosis mechanisms. In our study, we aimed to examine the immunohistological effects of edoxaban on apoptosis in the model. It is known that aneurysm development in the CaCl2 model occurs through medial degeneration and leukocyte infiltration.[15] It is also known that intramural thrombus does not occur.[15] For this reason, the elastase model was not preferred.

It was reported that CaCl2 creates an aneurysm model by remodeling the vessels and causing a decrease in insoluble collagen and an increase in gelatinase activity.[20] It is stated that, unlike the elastase model, aneurysm development in the CaCl2 model occurs through medial degeneration and leukocyte infiltration.[15] Moreover, it is presented in the publications that endoluminal and intramural thrombus does not occur.[15] Due to these features, the calcium phosphate model was adopted in our study. The purpose of using the rat model is easy availability, reliability, and high repeatability of the experiment.

Yong et al.[21] reported that FXa protein expression was significantly upregulated in human aortic aneurysms.[6] They also concluded that rivaroxaban reduced the progression of both angiotensin-II and CaCl2-induced aortic aneurysm by inhibiting aortic remodeling and inflammation.[6] Lysgaard Poulsen et al.[20] reported that in human AAAs, rivaroxaban improved mitochondrial functionality associated with changes in apoptosis-related proteins.[6] They also suggested that FXa may modulate the mitochondrial functionality and expression of mitophagy-related

proteins in AAAs.[6] In our study, apoptosis through FXa inhibition was observed less in the edoxaban-treated AAA group. As a result of the activation of caspases, the programmed apoptosis process is activated by intracellular proteases.[22,24] Apoptosis occurs via the cell-extrinsic pathway and the response-to-cell-intrinsic pathway, both of which depend on caspase activation and, consequently, the release of cytochrome C and other proapoptotic factors from the mitochondria.[24] This mitochondrial pathway, which is disrupted in various pathological conditions, is the same pathway that is activated in response to intracellular damage.[24] Our results showed that edoxaban significantly inhibited the pathways that trigger apoptosis (caspase-3 and caspase-9) in the model. In the edoxaban group, the vascular lumen diameter and area were found to be lower or closer to normal.

In addition, no thrombus, atherosclerosis, or rupture was observed in our model. Although findings from animal models of aortic aneurysms do not directly resemble human aneurysms. Identification and awareness of animal models will inform further research and insight into aneurysms in humans. This causes the wall to thin in accordance with Laplace's law. In experimental studies, it was determined that the infrarenal aortic diameters increased by 42 to 60% following CaCl2 administration.[12] This study aimed to investigate the effect of edoxaban on the calcium phosphate-induced aortic aneurysm model in rats. Histological examination of the aorta after CaCl2 administration showed that aortic dilatation was accompanied by VSMC death, elastin degradation, and lymphocyte and macrophage infiltration.[12] Large proinflammatory cytokine and matrix metalloproteinase concentrations have also been noted in the dilated aorta.[25] Autophagy, mitophagy, and apoptosis play an important role in maintaining intracellular homeostasis with vascular origin, including endothelial and arterial smooth muscle cells.[26,27] Similarly, apoptosis is an autophagic response that specifically targets damaged and potentially mitochondria-toxic conditions, such as aneurysms. Apoptosis is particularly important for the homeostasis of cardiovascular diseases.[13] In aortic aneurysm, mitophagy responses promote adaptation to stress and support cellular survival. Mitophagy also regulates specific autophagic transformation of mitochondria and represents an important mechanism in the protection of the aorta. Caspase family and Apaf-1 (apoptotic protease activating factor 1) molecules induce the cellular mitochondrial apoptosis mechanism.[28] Our experimental model was based on this.

We believe that this rat model will be a good aneurysm model because it is easily reproducible. The infrarenal aorta was used in the experiment because it is the most common aortic location for aneurysm in humans.[12] Oxidative stress is an important risk factor of human AAA.[15] Additionally, it has been shown that free radical damage by hydrogen peroxide and hydroxyl radicals can activate matrix metalloproteinases in human VSMCs.[12] According to the results obtained in caspase-3 and caspace-9 staining, it was observed that apoptosis in the edoxaban-treated AAA group decreased significantly ($p<0.004$ and $p<0.005$). In this study, we demonstrated that edoxaban may protect against vascular damage caused by aortic aneurysm. This experimental study supports the idea that edoxaban can reduce oxidative stress and inflammation via apoptosis, which are known to contribute to mitochondrial damage.

The primary limitation of our study was the limited biomarker data at the molecular level. Moreover, the pharmacokinetic effects of edoxaban observed in rats may differ from those in humans. Despite their ease of reproducibility, experimental aortic aneurysm models have limitations, as they do not fully replicate the pathological conditions in human aortic aneurysms. Further studies investigating thrombus-preventive indications for edoxaban are needed.

In conclusion, edoxaban treatment appears to be an acceptable strategy in patients with aortic aneurysms. In aortic aneurysms, the costs and risks of surgical repair may be reduced with anticoagulant, antiapoptotic, and anti-inflammatory medical treatments. Further studies are required to clarify the pharmacokinetic effects of edoxaban.

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

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REFERENCES

1. Ozdemir BA, Karthikesalingam A, Sinha S, Poloniecki JD, Vidal-Diez A, Hinchliffe RJ, et al. Association of hospital structures with mortality from ruptured abdominal aortic aneurysm. Br J Surg 2015;102:516-24. doi: 10.1002/bjs.9759.
2. Haque K, Bhargava P. Abdominal aortic aneurysm. Am Fam Physician 2022;106:165-72.
3. Nordon IM, Hinchliffe RJ, Loftus IM, Thompson MM. Pathophysiology and epidemiology of abdominal aortic aneurysms. Nat Rev Cardiol 2011;8:92-102. doi: 10.1038/nrcardio.2010.180.
4. Steffel J, Collins R, Antz M, Cornu P, Desteghe L, Haeusler KGet 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.
5. de Groot JR, Weiss TW, Kelly P, Monteiro P, Deharo JC, de Asmundis C, et al. Edoxaban for stroke prevention in atrial fibrillation in routine clinical care: 1-year follow-up of the prospective observational ETNA-AF-Europe study. Eur Heart J Cardiovasc Pharmacother 2021;7:f30-9. doi: 10.1093/ehjcvp/pvaa079.
6. 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.
7. Sagiv E, Newland DM, Slee A, Olson AK, Portman MA. Real-world experience with edoxaban for anticoagulation in children at risk for coronary artery thrombosis. Cardiol Young 2024;34:870-5. doi: 10.1017/S1047951123003761.
8. Poulakos M, Walker JN, Baig U, David T. Edoxaban: A direct oral anticoagulant. Am J Health Syst Pharm 2017;74:117-29. doi: 10.2146/ajhp150821.
9. Peixoto de Miranda ÉJF, Takahashi T, Iwamoto F, Yamashiro S, Samano E, Macedo AVS, et al. Drug-drug interactions of 257 antineoplastic and supportive care agents with 7 anticoagulants: A comprehensive review of interactions and mechanisms. Clin Appl Thromb Hemost 2020;26:1076029620936325. doi: 10.1177/1076029620936325.
10. Gencpinar T, Bilen C, Kemahli B, Kacar K, Akokay P, Bayrak S, et al. Effects of rivaroxaban on myocardial mitophagy in the rat heart. Turk Gogus Kalp Damar Cerrahisi Derg 2023;31:301-8. doi: 10.5606/tgkdc.dergisi.2023.24385.
11. Wang Y, Krishna S, Golledge J. The calcium chloride-induced rodent model of abdominal aortic aneurysm. Atherosclerosis 2013;226:29-39. doi: 10.1016/j.atherosclerosis.2012.09.010.
12. Yamanouchi D, Morgan S, Stair C, Seedial S, Lengfeld J, Kent KC, et al. Accelerated aneurysmal dilation associated with apoptosis and inflammation in a newly developed calcium phosphate rodent abdominal aortic aneurysm model. J Vasc Surg 2012;56:455-61. doi: 10.1016/j.jvs.2012.01.038.
13. Çulpan Y, Keçeci İ, Sandıkçı İ, Gökçe Ş, Göker H, Özyılmaz Yay N, et al. Duration of ciprofloxacin use is important in the development of abdominal aortic aneurysm in a rat model. Anatol J Cardiol 2022;26:810-7. doi: 10.5152/AnatolJCardiol.2022.1939.
14. Gencpinar T, Bayrak S, Bilen C, Kemahli B, Akokay P, Baris M, et al. Effect of bivalirudin on neointimal hyperplasia and endothelial proliferation in rabbit. Gen Thorac Cardiovasc Surg 2021;69:425-33. doi: 10.1007/s11748-020-01454-8.
15. Nagata N, Kawasumi M, Fujimura A, Ando H. Edoxaban dosing time affects blood coagulation inhibition in rats. TH Open 2021;5:e107-12. doi: 10.1055/s-0041-1725041.
16. Bliden KP, Chaudhary R, Mohammed N, Muresan AA, Lopez-Espina CG, Cohen E, et al. Determination of Non-Vitamin K Oral Anticoagulant (NOAC) effects using a new-generation thrombelastography TEG 6s system. J Thromb Thrombolysis 2017;43:437-45. doi: 10.1007/s11239-017-1477-1.
17. Budak AB, Gencpinar T, Dogan E, Totan S, Akay HT. Edoxaban in cardiovascular medicine from scientific evidence to clinical practicey. Turk J Vasc Surg 2022;31:195-206. doi: 10.9739/tjvs.2022.09.019.
18. Ogata K, Mendell-Harary J, Tachibana M, Masumoto H, Oguma T, Kojima M, et al. Clinical safety, tolerability, pharmacokinetics, and pharmacodynamics of the novel factor Xa inhibitor edoxaban in healthy volunteers. J Clin Pharmacol 2010;50:743-53. doi: 10.1177/0091270009351883.
19. 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.
20. Lysgaard Poulsen J, Stubbe J, Lindholt JS. Animal models used to explore abdominal aortic aneurysms: A systematic review. Eur J Vasc Endovasc Surg 2016;52:487-99. doi: 10.1016/j.ejvs.2016.07.004.
21. Yong C, Boyle A. Factor Xa inhibitors in acute coronary syndromes and venous thromboembolism. Curr Vasc Pharmacol 2010;8:5-11. doi: 10.2174/157016110790226688.
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. 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.
24. 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.
25. Longo GM, Xiong W, Greiner TC, Zhao Y, Fiotti N, Baxter BT. Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms. J Clin Invest 2002;110:625-32. doi: 10.1172/JCI15334.

26. 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.
27. 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.
28. 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.