Türk Göğüs Kalp Damar Cerrahisi Dergisi 2017;25(2):273-281
http://dx.doi.org/doi: 10.5606/tgkdc.dergisi.2017.13748

Experimental Study / Deneysel Çalışma

Cardioprotective effect of zileuton: a 5-lipoxygenase inhibitor against myocardial ischemia/reperfusion injury

5-Lipoksigenaz inhibitörü zileutonun miyokardiyal iskemi/reperfüzyon hasarına karşı kalp koruyucu etkisi

Ersöz Gonca,[1] Figen Barut,[2] Salih Erdem[1]

Institution where the research was done:
Bülent Ecevit University, Faculty of Arts and Sciences, Zonguldak, Turkey

Author Affiliations:
[1]Department of Biology, Bülent Ecevit University, Faculty of Arts and Sciences, Zonguldak, Turkey
[2]Department of Pathology, Medical Faculty of Bülent Ecevit University, Zonguldak, Turkey

ABSTRACT

Background: This study aims to evaluate the effects of zileuton on myocardial ischemia/reperfusion injury and ischemia-induced ventricular arrhythmias and to investigate the role of 5-lipoxygenase pathway in the pathogenesis of myocardial ischemia/reperfusion injury.

Methods: In anesthetized rats, myocardial ischemia was induced by the ligation of the left main coronary artery for 30 min before 120-min reperfusion period. Zileuton was given both at the doses of 3 and 10 mg/kg 15 min before the ligation. During the experiment, electrocardiography, blood pressure, and heart rate were recorded. The duration of arrhythmia types were determined during the ischemic period. To evaluate the ischemia/reperfusion injury in the myocardial tissue, histopathological examination was performed and the infarct size was determined by 2,3,5-triphenyltetrazolium chloride staining.

Results: Zileuton at a dose of 3 mg/kg significantly decreased the infarct size and the tissue injury score obtained using histopathological examinations (infarct size [% of the area at risk]: zileuton 3 mg/kg 36±7% versus control 66±6%, $p<0.05$). Zileuton 10 mg/kg was found to be ineffective. Both 3 and 10 mg/kg doses of zileuton did not shorten the duration of arrhythmias during the ischemic period.

Conclusion: Our study results showed that 3 mg/kg dose of zileuton protected the heart against myocardial ischemia/reperfusion injury. However, it was ineffective to reduce the ischemia-induced ventricular arrhythmias. Based on these results, zileuton may be a promising drug for the treatment of myocardial ischemia/reperfusion injury.

Keywords: Myocardial ischemia/reperfusion injury; rat model; ventricular arrhythmias; zileuton.

ÖZ

Amaç: Bu çalışmada zileuton'un miyokardiyal iskemi/reperfüzyon hasarı ve iskemi ile uyarılan ventriküler aritmiler üzerine olan etkileri değerlendirildi ve 5-lipoksigenaz yolağının miyokardiyal iskemi/reperfüzyon hasarının patofizyolojisindeki rolü araştırıldı.

Çalışma planı: Anestezi altındaki sıçanlarda, miyokardiyal iskemi 120 dakikalık reperfüzyon periyodundan önce sol ana koroner arterin 30 dakikalık ligasyonu ile oluşturuldu. Zileuton 3 ve 10 mg/kg'lik dozlarda ligasyondan 15 dakika önce verildi. Deney süresince elektrokardiyografi, kan basıncı ve kalp atımı kayıt edildi. İskemik periyot boyunca aritmi tiplerinin süreleri tespit edildi. Miyokard dokusunda iskemi/reperfüzyon hasarını değerlendirmek için, histopatolojik inceleme yapıldı ve enfarkt alan 2,3,5-trifenil tetrazolyum klorit boyaması ile tespit edildi.

Bulgular: Zileuton 3 mg/kg dozda enfarkt alan ve histopatolojik incelemeler sonucu elde edilen doku hasarı skorunda anlamlı bir azalmaya neden oldu (enfarkt alan [% risk alanı]: zileuton 3 mg/kg %36±7'ye kıyasla kontrol %66±6; $p<0.05$). 10 mg/kg zileuton etkili bulunmadı. Zileutonun 3 ve 10 mg/kg'lik dozu iskemi periyodunda ventriküler aritmilerin süresini kısaltmadı.

Sonuç: Çalışma sonuçlarımız zileutonun 3 mg/kg'lik dozda kalbi miyokardiyal iskemi/reperfüzyon hasarına karşı koruduğunu gösterdi. Ancak, zileuton iskemi ile uyarılan aritmileri azaltmada etkili değildi. Bu sonuçlara göre, zileuton miyokardiyal iskemi/reperfüzyon hasarının tedavisi için umut verici bir ilaç olabilir.

Anahtar sözcükler: Miyokardiyal iskemi/reperfüzyon hasarı; sıçan modeli; ventriküler aritmi; zileuton.

Available online at
www.tgkdc.dergisi.org
doi: 10.5606/tgkdc.dergisi.2017.13748
QR (Quick Response) Code

Received: July 29, 2016 *Accepted:* October 13, 2016

Correspondence: Ersöz Gonca. Bülent Ecevit Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, 67100 İncivez, Zonguldak, Turkey.
Tel: +90 543 - 639 53 74 e-mail: ersozgonca67@hotmail.com

Despite the tremendous amount of studies conducted to explore the effective therapeutics up to date, ischemic heart disease, secondary to acute myocardial infarction (MI), has been recognized globally as one of the main causes of mortality and morbidity.[1] The sudden cessation of blood supply to some parts of the heart results in myocardial ischemia, thereby, leading to the death of the affected myocardial tissue. The generation of fetal ventricular arrhythmias in ischemic myocardium is the main cause of sudden death following a heart attack.[2] In the in-hospital setting, one of the indispensable goals of the MI therapy is the opening of the occluded vessel and, therefore, to provide the blood supply to previously ischemic tissue (reperfusion), limiting the potential for the occurrence of myocardial injury. Paradoxically, reperfusion, also, itself aggravates the myocardial tissue death (ischemia/reperfusion [I/R] injury) which may cause heart failure in discharging patients.[3]

5-Lipoxygenase (5-LO) enzyme catalyzes the production of leukotriene (LT) eicosanoids from the arachidonic acid.[4] Leukotrienes induce the increment in vascular permeability and leukocyte chemotaxis, leading to more intense inflammatory response and exacerbating the tissue injury.[5-7] Leukotriene B4, in particular, increases in ischemic myocardium.[8,9] These findings imply that 5-LO inhibitors can be used to treat myocardial I/R injury. Similarly, it has been reported that 5-LO inhibitors, nafazatrom, and TZI-41127 are effective in decreasing the canine myocardial I/R injury.[10,11]

Zileuton, which is being currently used as a drug to treat asthma, is a selective inhibitor of 5-LO enzyme.[12] Zileuton has been also found to be a protective agent against renal I/R injury, testicular torsion/detorsion injury, and global brain ischemia in the experimental researches.[13-15] However, there are few studies examining its cardioprotective effect. Kwak et al.[16] revealed that zileuton treatment protected cardiomyocytes against hydrogen peroxide cardiotoxicity. In a recent study conducted by Gonca,[17] zileuton has also been demonstrated to have a strong antiarrhythmic effect against I/R-induced ventricular arrhythmias. Therefore, in the present study, we aimed to investigate the effect of zileuton on myocardial I/R injury and ischemia-induced ventricular arrhythmias.

MATERIALS AND METHODS

Surgery for coronary occlusion

Forty-five male Wistar albino rats weighing 250 to 350 g were used in this study. The subjects were provided by Bülent Ecevit University Experimental Animal Production Center, Zonguldak, Turkey. The subjects were fed with tap water and rat pellet food ad libitum and all were kept in convenient conditions throughout their lifespan (temperature: 21±2 °C, humidity: 40 to 65% and a 12 h light/dark cycle). All experimental procedures were confirmed as being ethically appropriate by Bülent Ecevit University, Animal Experiment Local Ethical Committee (protocol no: 2014-05-05/02).

Surgical procedures performed in our study were previously described by Clark et al.[18] The subjects were anesthetized with urethane (intraperitoneal, 1.2 g/kg) and were placed on an operation table with animal rectal temperature controller (RTC 9404-A, Commat Ltd., Ankara, Turkey) to keep a body temperature of 37±1 °C throughout the experiment. Trachea was cannulated with polyethylene tubing. Blood pressure was recorded and monitored following the cannulation of the left carotid artery. Electrodes were, then, placed to record electrocardiography (ECG) (lead II) (Data acquisition system, MP35 and blood pressure transducer, SS 13 L Biopac Systems, California, USA). The subjects were connected to a ventilator for artificial respiration (60 beats/min, at a tidal volume of 1.5 mL/100 g; SAR 830, Life Science, California, USA). Immediately, left thoracotomy was performed. The rats were allowed to stabilize for 10 min following the placement of a 5/0 silk suture around the left anterior descending (LAD) coronary artery. Myocardial ischemia was performed by the ligation of the LAD coronary artery for 30 min. The ligation was, then, loosened to allow reperfusion of myocardial tissue for two hours.

The exclusion criteria of subjects

In all rats on which were performed successful ligation, the following changes were observed: ST segment elevation on ECG, the decline in mean arterial blood pressure (MABP) values (20-30% in comparison to baseline values). The following changes were also observed for successful reperfusion procedures: disappearance of the ST segment elevation on ECG, the increment in MABP again and the approach of MABP values to the pre-ischemic values. In addition to these changes, in all rats in which the ligation of LAD coronary artery was performed at the correct site (approximately 2-3 mm from its origin), the area at risk values were higher than 40%. As a result, 12 rats were excluded from the experiment.

Experimental groups and drug treatments

A total of 45 rats were divided into four separate experimental groups in the following way: (group 1) sham-operated group, (group 2) vehicle control, (group 3)

zileuton (3 mg/kg), and (group 4) zileuton (10 mg/kg) group. Zileuton was supplied by Sigma Chemical Corp. (St., Louis, MO, USA). Drug containing solutions were prepared daily by dissolving the drug with 1% dimethyl sulfoxide (DMSO): 0.9% NaCl (SF) (1:1). Zileuton was administrated at a dose of both 3 mg/100 µL/kg and 10 mg/100 µL/kg. Zileuton and its solvents were given through the femoral vein 15 min prior to coronary ligation. The dose of zileuton, timing of treatment, and application route used in the present study were based on a previous study completed by Gonca,[17] in which zileuton at a dose of 3 mg/kg exhibited a strong antiarrhythmic effect. Dimethyl sulfoxide and SF (1:1) mixture in a 100 µL/kg volume was given to the rats, both in the sham-operated group (group 1) and control group (group 2) 10 min prior to sham operation and coronary artery occlusion, respectively.

Histomorphometric measurement of infarct size

In the present study, the infarct size and area at risk were measured as in a previous study conducted by Gonca and Kurt.[19] At the end of reperfusion period, the heart was removed. The residual blood remains in coronary vessels was washed out with 10 mL of saline solution at 37 °C. Following the ligation of the LAD coronary artery, the heart was, then, perfused with 2 mL of 96% ethanol to delineate the zone at risk. The zones, which perfused with ethanol appeared as white in color. Other regions remained in original tissue color and, therefore, were determined as zone at risk regions. The ventricle was separated from the atria and stored at -20 °C for 20 min. The frozen ventricle tissues were sliced from the apex to the base into six pieces of 1 to 2 mm slices. The slices were, then, kept at room temperature to allow them to defrost.

One slice taken from the apex region of the myocardium was left as a myocardial tissue specimen for further histopathological evaluation. The area at risk and total area in each of the rest five slices were photographed using a digital camera (Samsung Galaxy Note 3, South Korea).

The at-risk regions of the slices were separated from those that were well perfused with ethanol. Those slices that only consisted of at-risk regions were, then, stained with 1% triphenyl tetrazolium chloride (TTC) (Sigma Chemical Company; St., Louis, MO, USA) and fixed in 10% formaldehyde solution overnight. The living myocardial tissue stained with TTC as deep red in color, while the necrotic tissue was not stained with TTC and appeared as tan in color (Figure 1). The area at risk and the infarct area in each slice were photographed. The photographs were transferred to a computer to measure the area of the left ventricle, area at risk, and infarct size using an image processing program (Image J software, National Institute of Health (NIH), Maryland, USA). The area at risk and the infarct size were measured as a percentage of the total left ventricular area and as a percentage of the area at risk, respectively.

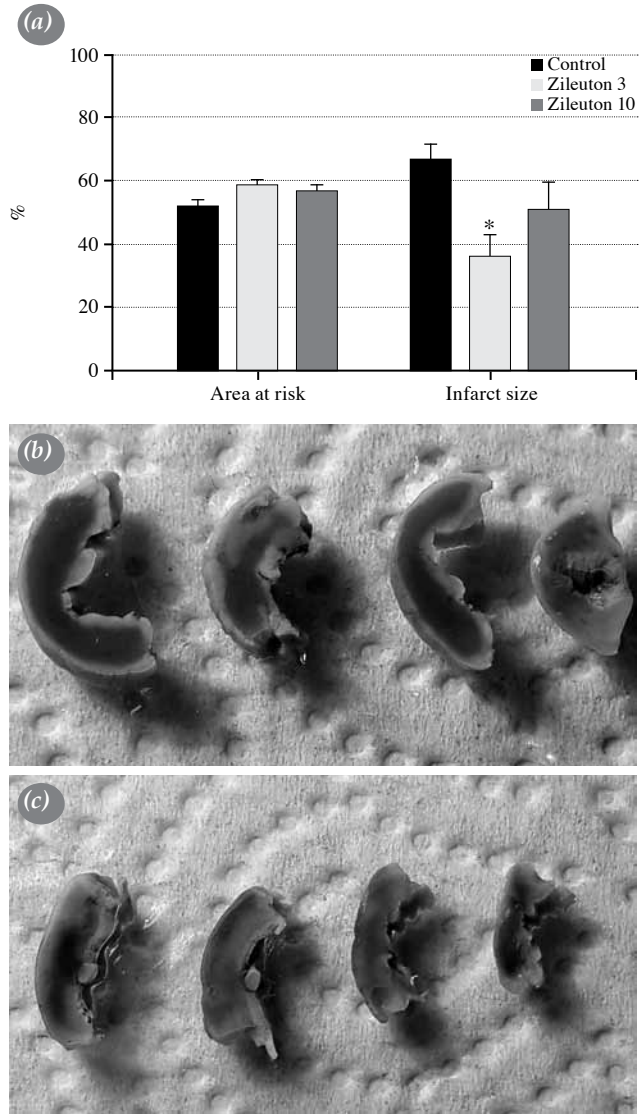

Figure 1. (a) Effects of zileuton on area at risk and infarct size. Area at risk was measured as a percentage of total left ventricular area and infarct size was measured as a percentage of area at risk. **(b and c)** Triphenyl tetrazolium chloride staining in a zileuton (3 mg/kg)-treated and control animal, respectively. Alive tissue is stained red in color and infarct one is white.

* p<0.05: Compared to control. Both sets of data are expressed in mean ± standard deviation.

Histopathological examination

Each heart sample was fixed with 10% formaldehyde immediately following their collection. The heart samples were removed from the solution and, then, embedded in paraffin blocks. Sections were cut with a cryostat at 4-5 µm thickness from the paraffin blocks of each tissue. Specimens were, then, deparaffinized and stained with hematoxylin and eosin (H-E) and Masson's trichrome for histopathological evaluation. The extent of the myocardial I/R injury was scored on a scale of 0-4 by an experienced pathologist who was blinded to all study groups. A light microscope was used to evaluate the I/R injury in myocardial tissue. Histopathological examination of the heart tissue (a minimum of 10 fields for each slide) was based on a scoring system described by Goyal et al.[20] A grade was given according to myonecrosis, inflammatory cell infiltration, and edema in each slide. The scoring system was: (0) no inflammation, edema and necrosis (1) focal areas of inflammation, edema and necrosis; (2) patchy areas of inflammation, edema and necrosis; (3) confluent areas of inflammation, edema and necrosis, and (4) massive areas of inflammation, edema and necrosis.

The analyses of ECG and blood pressure recordings

The MABP and heart rate values were determined from the analysis of blood pressure and ECG recordings, respectively throughout I/R periods (Data acquisition system; MP35, SS 13L, Biopac Systems, California, USA). Arrhythmia types were diagnosed during the ischemic period in accordance with the Lambeth Conventions,[21] which are as follows: ventricular fibrillation (VF), ventricular tachycardia (VT), and ventricular premature contraction (VPC) including salvos, bigeminy and single extrasystoles (Figure 2).

The severity of arrhythmias was evaluated with a scoring system, which was defined by Leprán et al.[22] This system was based on both the fatal degree of the arrhythmia types and the duration of the arrhythmias. The scoring scale was: (0) no arrhythmia; (1) VT and/or VPC ≤10 s, no VF; (2) VT and/or VPC= 11-30 s, no VF; (3) VT and/or VPC=31-90 s, no VF; (4) VT and/or VPC= 91-180 s, and/or VF ≤10 (5), VT and/or VPC >180 s, and/or VF >10; (6) irreversible VF.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5 software (GraphPad Prism, San Diago, CA, USA). The chi-square (χ^2) test was performed to analyze mortality and the incidence of arrhythmias. Descriptive data were expressed in mean ± standard deviation (SD). One-way analysis of variance with the Dunnett's post-hoc test was used to compare baseline and post-occlusion MABP/HR values at 1, 10, and 30 min. As the numbers of subjects were different among the groups, the Kruskal-Wallis test with the Dunn's post-hoc test was used to compare drug-treated groups with the control group for all parameters excluding the mortality and the incidence of arrhythmias. A p value of less than 0.05 was considered statistically significant.

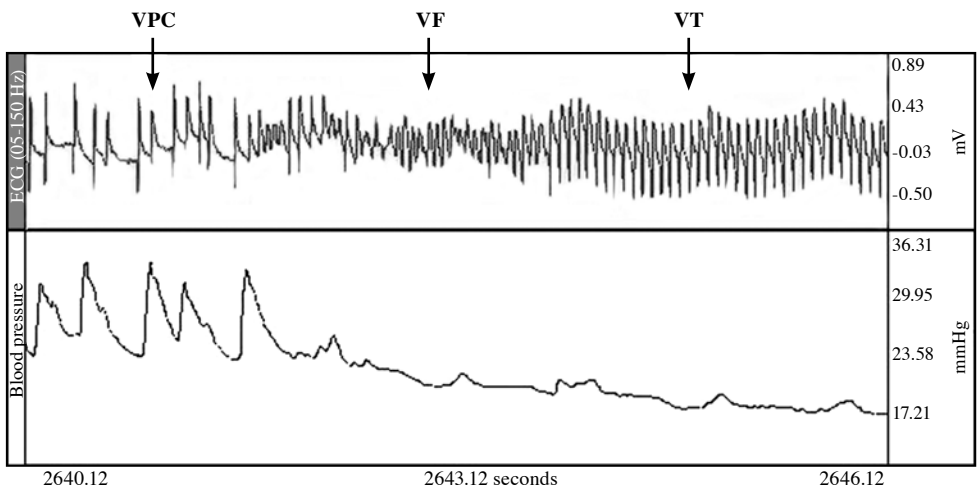

Figure 2. Original electrocardiography and blood pressure tracings from zileuton (10 mg/kg) treated rat.
VPC: Ventricular premature contraction; VF: Ventricular fibrillation; VT: Ventricular tachycardia.

Table 1. The summary of heart rate and mean arterial blood pressure values during ischemia and reperfusion

Time	Control (3 mg/kg)	Zileuton (10 mg/kg)	Zileuton
	Mean±SD	Mean±SD	Mean±SD
MABP (mm Hg)			
0 (Baseline)	71±2	72±3	65±4
1 (Ligation 1 min)	48±4*	54±4*	43±3*
10 (Ligation 10 min)	58±6	62±5	61±8
30 (Ligation 30 min)	69±9	62±4	73±3
35 (Reperfusion 5 min)	59±7	65±4	74±3
90 (Reperfusion 60 min)	61±8	67±5	74±5
150 (Reperfusion 120 min)	61±6	59±5	62±5
Heart rate			
0 (Baseline)	399±13	375±13	365±14
1 (Ligation 1 min)	403±14	382±13	349±11
10 (Ligation 10 min)	352±26	371±25	347±17
30 (Ligation 30 min)	389±30	387±16	396±11
35 (Reperfusion 5 min)	377±27	387±12	377±12
90 (Reperfusion 60 min)	401±14	389±3	396±14
150 (Reperfusion 120 min)	399±13	396±6	410±11

SD: Standard deviation; MABP: Mean arterial blood pressure; * $p<0.05$: Compared to pre-ischemic values. n: 7-9; One-way analysis of variance with Dunnett's post hoc test was used for comparison of the HR and MABP values with basal value. Kruskal-Wallis test with Dunn's post hoc test was used for the comparison between the groups.

RESULTS

Effects of zileuton on hemodynamic variables during I/R period

The MABP significantly decreased at the first min of the ligation in all groups compared to baseline values. However, it showed recovery in the late phase of I/R period. Zileuton treatments did not affect MABP and HR values, compared to the control group at any time point during the I/R periods (Table 1).

Effects of zileuton on ventricular arrhythmias during 30 min ischemia

The weights of the animal and the area at risk values were not significantly different between the groups. The ligation of the LAD coronary artery resulted in the generation of ventricular arrhythmias, the majority occurred as VPC. Both doses of zileuton did not decrease the arrhythmia incidence and durations, compared to the control group in the 30 min of the ischemic period (Table 2).

Effects of zileuton on infarct size

Figure 1 illustrates the effects of zileuton on both areas at risk (percentage of LV) and infarct size (percentage of area at risk). The area at risk did not differ between the groups. Zileuton treatment at a dose of 3 mg/kg significantly decreased infarct size compared to controls (zileuton 3 mg/kg: 36±7% versus control: 66±6%, $p<0.05$), whilst 10 mg/kg dose of zileuton was found to be ineffective at reducing the infarct size.

Effects of zileuton on histopathology of the heart

Histopathological scores, demonstrating myocardial I/R injury on the basis of myonecrosis, inflammatory cell infiltration and edema are summarized in Table 3. In the sham operated group (group 1), intact myocardial histology without any evidence of myocardial I/R injury was observed (Figures 3a and 4a). However, prominent myocardial necrosis, edema, and infiltration of inflammatory cells were observed in the control group, compared to the sham group (Figures 3b and 4b). Rats administered zileuton at a dose of 3 mg/kg showed improved myocardial I/R injury, compared to the control group, with focal and patchy areas of inflammation, edema, and necrosis (Figures 3c and 4c). The rats which were administered zileuton at a dose of 10 mg/kg did not show improved myonecrosis, infiltration of inflammatory cells, or edema. A prominent myocardial I/R injury was observed in this group as was the case with the control group (Figures 3d and 4d).

DISCUSSION

The results from both the histomorphometric and histopathological analyses showed that 3 mg/kg dose

Table 2. The effects of zileuton on the incidence and duration of arrhythmias during 30 min of ischemia

Group	N	Area at risk	Mortality		Arrhythmia incidence						Duration of arrhythmia				Arrhythmia score
					VF		VT		VPC		VF	VT	VPC	*Total*	
		Mean±SD	n*	%	n	%	n	%	n	%	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control	11	51±3	2	18	4	36	4	36	10	91	0.6±0.4	2±1.4	11±3	13±4	1.8±0.3
Zileuton (3 mg)	9	58±2	1	11	1	11	1	11	9	100	0.1±0.1	0.1±0.1	12±3	12±3	1.5±0.2
Zileuton (10 mg)	7	56±2	0	0	0	0	0	0	7	100	0±0	0±0	6±3	6±6	1.3±0.2

N: The number of subjects before the ligation; n*: The number of dead subjects after 30 minutes of ischemia; n: The number of animal experienced arrhythmias; VF: Ventricular fibrillation; VT: Ventricular tachycardia; VPC: Ventricular premature contraction (extrasystoles, salvos and bigeminy); SD: Standard deviation; Chi Square (χ^2) test for arrhythmia incidence, mortality variables; Kruskal-Wallis test with Dunn's post hoc test for the duration of arrhythmias, arrhythmia scores, area at risk variables.

of zileuton protected heart against myocardial I/R injury. Leukotrienes generated from the 5-LO pathway may play a significant role in the pathogenesis of the myocardial I/R injury. They activate neutrophils, leading to the chemotaxis to the site of the ischemic injury. The activated neutrophils release reactive oxygen species, proteases, elastase, myeloperoxidase, all of which exacerbate inflammation and contribute to the tissue injury.[23] Therefore, in the present study, the cardioprotective effect of zileuton, a selective 5-LO inhibitor, might have been mediated via its possible anti-inflammatory effect.[24] However, in contrast to this hypothesis, Hahn et al.[25] reported that LY255283, a LT antagonist, did not limit canine myocardial infarct size.

In a previous report, Kwak et al.[16] reported that zileuton decreased hydrogen peroxide cardiotoxicity. Kwak et al.[16] also showed that the pharmacological inhibition of 5-LO by zileuton resulted in a shunt to cyclooxygenase (COX) pathway. They suggested that the cardioprotective effect of zileuton may be mediated by (COX)-2 via the activation of protein kinase C-delta. The activation of protein kinase C-delta may also lead to the activation of mitochondrial ATP dependent potassium channels (mitoKATP), which has been reported to decrease myocardial I/R injury.[26,27] In the present study, throughout a similar signalling pathway, zileuton may also have activated mitoKATP and the tissue protective effect of it may have depended on this activation.

There is a limited number of data concerning the role of 5-LO on myocardial I/R injury. In canine studies, conflicting results were reported. BW A4C, a 5-LO inhibitor, did not reduce the myocardial infarct size.[28] However, other 5-LO inhibitors such as nafazatrom and TZI-41127 were found to be effective in a canine model.[10,11] In the present study, 3 mg/kg dose of zileuton decreased the infarct size and improved the histopathologic outcomes of the heart specimens.

Table 3. The effect of zileuton on histopathological scores

Group	n	Scores
		Mean±SD
Sham	6	0±0
Control	7	2.4±0.2†
Zileuton (3 mg/kg)	6	1.3±0.2*†
Zileuton (10 mg/kg)	7	2.4±0.3†

SD: Standard deviation; * $p<0.05$: Compared to control and zileuton (10 mg/kg) groups; † $p<0.05$: Compared to sham; *† Kruskal-Wallis test with Dunn's post hoc test.

(a)

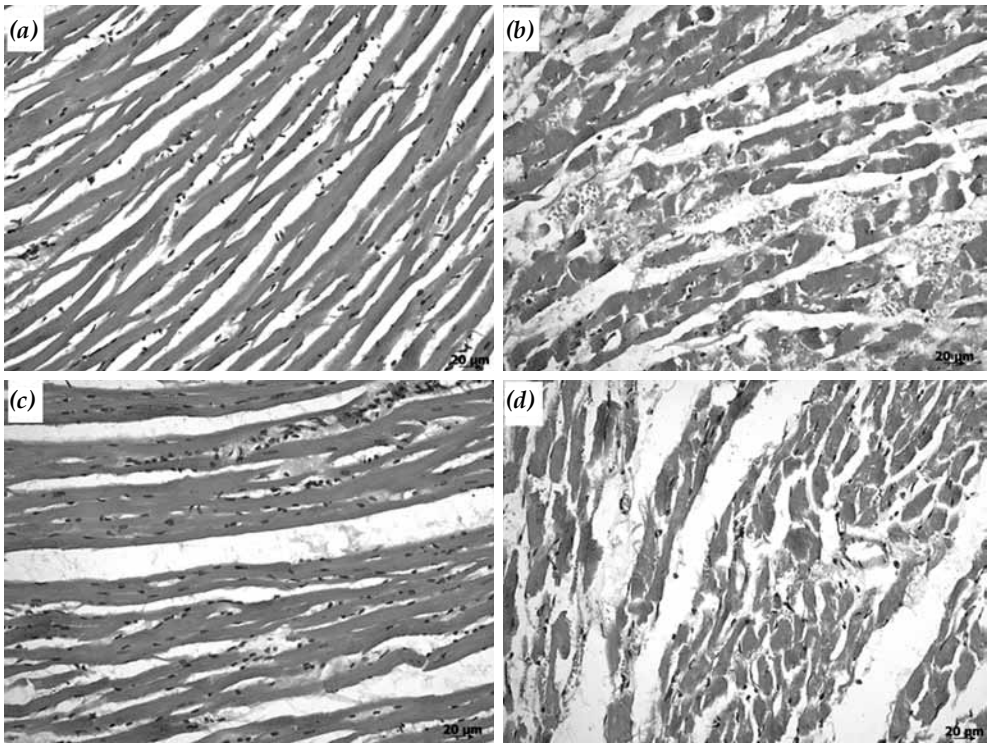

Figure 3. The histopathological changes in the rat myocardium in groups with hematoxylin and eosin **(a)** sham group, intact myocardial tissue with absence of inflammation, edema and necrosis (H-E x 200); **(b)** control group, prominent evidence of myocardial I/R injury with confluent areas of inflammation, edema and necrosis (H-E x 200); **(c)** Zileuton 3 mg/kg treatment group, improved myocardial I/R injury with focal areas of inflammation, edema and necrosis (H-E x 200); **(d)** Zileuton 10 mg/kg treatment group, evident myocardial I/R injury with confluent areas of inflammation, edema and necrosis (H-E x 200).

These findings support the role of 5-LO in myocardial I/R injury in rats. On the other hand, in the present study, we found zileuton 10 mg/kg to be ineffective in decreasing the myocardial I/R injury. To the best of our knowledge, no comparable studies have been conducted to examine the effect of zileuton at a dose of 10 mg/kg on the myocardial I/R injury. A previous study conducted by Adamek et al.,[29] using 5-LO-deficient mice did not show any difference in the infarct area between knockouts and control mice. Surprisingly, however, the authors showed that the inhibition of 5-LO increased the number of infiltrating neutrophils and the expression of an inflammatory cytokine, Tumor necrosis factor-alpha (TNF-α) in the myocardial tissue of 5-LO-deficient mice compared to wild-type mice following I/R. They, accordingly, concluded that the increment in myocardial inflammation in 5-LO-deficient mice might depend on the activation of the COX pathway activated by the elimination of LTs products. Consistent with this finding, Gaulet et al.[30] showed that a COX inhibitor blunted the inflammatory response seen in 5-LO-deficient mice. In the present study, in a similar way, zileuton 10 mg/kg may have caused the complete elimination of LT production, which may increase the substrate availability for the COX pathway. As suggested by Kwak et al.,[16] this may result in a shunt to the COX pathway from 5-LO pathway. The ineffectiveness of zileuton 10 mg/kg may depend on the COX-dependent pathway mediating inflammation. Alternatively, it may also depend on unknown off-target actions of zileuton at this dose.

In a recent comparable study conducted by Gonca,[17] zileuton 3 mg/kg (intravenous) showed a strong antiarrhythmic effect against myocardial I/R-induced ventricular arrhythmias. However, in the present study neither 3 nor 10 mg/kg intravenous doses of zileuton were found effective in reducing the duration of arrhythmias in the ischemic period. The possible explanation of the discrepancy in these results may depend on the role of inflammation; ischemia versus I/R-induced ventricular arrhythmias in different extents. Although the inflammation and the resultant reactive oxygen species production

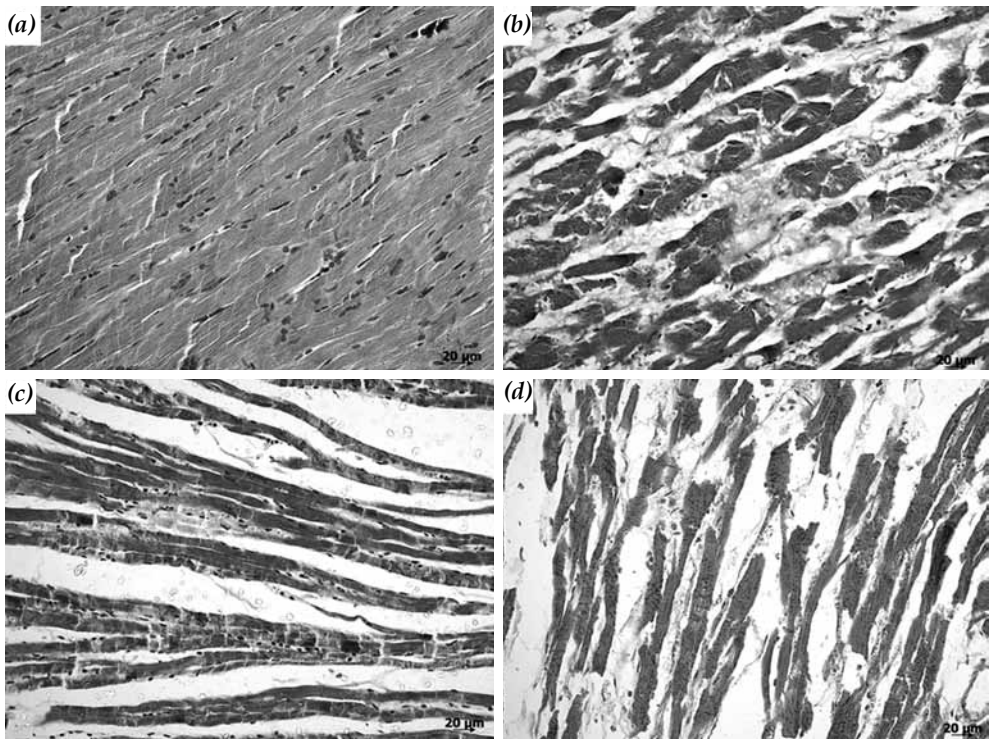

Figure 4. The histopathological changes in the rat myocardium in the groups with Masson's trichrome histochemical staining **(a)** sham group (H-E x 200); **(b)** control group (H-E x 200); **(c)** Zilueton 3 mg/kg treatment group (H-E x 200); **(d)** Zilueton 10 mg/kg treatment group; (Masson's trichrome (H-E x 200).

is responsible for the generation of ventricular arrhythmias during both periods, it is less effective in generating ischemia-induced arrhythmias compared to I/R-induced arrhythmias.[31] Therefore, in the present study, the possible anti-inflammatory properties of zileuton may have not resulted in an antiarrhythmic effect during the ischemic period. Consistent with this suggestion, a study conducted by Gonca and Kurt[19] showed that thymoquinone, which has strong anti-inflammatory and antioxidant properties, did not decrease the ischemia-induced arrhythmia, while it was found to have a strong antiarrhythmic effect against I/R-induced ventricular arrhythmias.

In conclusion, our study results showed that 3 mg/kg dose of zileuton protected the heart against myocardial ischemia/reperfusion injury, while it was ineffective in decreasing ischemia-induced ventricular arrhythmias. Zileuton, which is currently being used for the treatment of asthma, can be also considered as an appropriate drug to treat myocardial ischemia/reperfusion injury in coronary artery patients. Drug treatment in the ischemic period before reperfusion is more applicable to the clinical situation of myocardial ischemia/reperfusion injury than that of pre-ischemic treatment. Therefore, the effect of the zileuton treatment on myocardial ischemia/reperfusion injury during the ischemic state should be further researched. Further studies are also needed to delineate its mechanism of action. However, it can be speculated that the cardioprotective effects of zileuton may be dependent upon its possible anti-inflammatory effects or may involve the activation of mitoK_{ATP}, or both of these outcomes combined.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors disclosed the receipt of research grant from Bülent Ecevit University, Scientific Research Coordinator. No: 2014/84906727-1.

REFERENCES

1. Huffman MD, Lloyd-Jones DM, Ning H, Labarthe DR, Guzman Castillo M, O'Flaherty M, et al. Quantifying options for reducing coronary heart disease mortality by 2020. Circulation 2013;127:2477-84.

2. Arevalo HJ, Vadakkumpadan F, Guallar E, Jebb A, Malamas P, Wu KC, et al. Arrhythmia risk stratification of patients after myocardial infarction using personalized heart models. Nat Commun 2016;7:11437.
3. Gerber Y, Weston SA, Enriquez-Sarano M, Berardi C, Chamberlain AM, Manemann SM, et al. Mortality Associated With Heart Failure After Myocardial Infarction: A Contemporary Community Perspective. Circ Heart Fail 2016;9:2460.
4. Werz O, Steinhilber D. Development of 5-lipoxygenase inhibitors--lessons from cellular enzyme regulation. Biochem Pharmacol 2005;70:327-33.
5. Singh RK, Gupta S, Dastidar S, Ray A. Cysteinyl leukotrienes and their receptors: molecular and functional characteristics. Pharmacology 2010;85:336-49.
6. Carbone F, Crowe LA, Roth A, Burger F, Lenglet S, Braunersreuther V, et al. Treatment with anti-RANKL antibody reduces infarct size and attenuates dysfunction impacting on neutrophil-mediated injury. J Mol Cell Cardiol 2016;94:82-94.
7. Bonaventura A, Montecucco F, Dallegri F. Cellular recruitment in myocardial ischaemia/reperfusion injury. Eur J Clin Invest 2016;46:590-601.
8. Sasaki K, Ueno A, Katori M, Kikawada R. Detection of leukotriene B4 in cardiac tissue and its role in infarct extension through leucocyte migration. Cardiovasc Res 1988;22:142-8.
9. Carry M, Korley V, Willerson JT, Weigelt L, Ford-Hutchinson AW, Tagari P. Increased urinary leukotriene excretion in patients with cardiac ischemia. In vivo evidence for 5-lipoxygenase activation. Circulation 1992;85:230-6.
10. Fiedler VB, Mardin M, Perzborn E, Grützmann R. The effects of nafazatrom in an acute occlusion-reperfusion model of canine myocardial injury. Naunyn Schmiedebergs Arch Pharmacol 1985;331:267-74.
11. Hashimoto H, Miyazawa K, Hagiwara M, Miyasaka K, Nakashima M. Beneficial effects of a new 5-lipoxygenase inhibitor on occlusion- and occlusion-reperfusion-induced myocardial injury. Arzneimittelforschung 1990;40:126-9.
12. Kubavat AH, Khippal N, Tak S, Rijhwani P, Bhargava S, Patel T, et al. A randomized, comparative, multicentric clinical trial to assess the efficacy and safety of zileuton extended-release tablets with montelukast sodium tablets in patients suffering from chronic persistent asthma. Am J Ther 2013;20:154-62.
13. Patel NS, Cuzzocrea S, Chatterjee PK, Di Paola R, Sautebin L, Britti D, et al. Reduction of renal ischemia-reperfusion injury in 5-lipoxygenase knockout mice and by the 5-lipoxygenase inhibitor zileuton. Mol Pharmacol 2004;66:220-7.
14. Isikdemir F, Kurcer Z, Dengiz GO, Sipahi EY, Banoglu ZN, Baba F, et al. Effects of montelukast and zileuton on testicular torsion/detorsion injury in rats. Andrologia 2014;46:59-64.
15. Silva BC, de Miranda AS, Rodrigues FG, Silveira AL, Resende GH, Moraes MF, et al. The 5-lipoxygenase (5-LOX) Inhibitor Zileuton Reduces Inflammation and Infarct Size with Improvement in Neurological Outcome Following Cerebral Ischemia. Curr Neurovasc Res 2015;12:398-403.
16. Kwak HJ, Park KM, Choi HE, Lim HJ, Park JH, Park HY. The cardioprotective effects of zileuton, a 5-lipoxygenase inhibitor, are mediated by COX-2 via activation of PKC delta. Cell Signal 2010;22:80-7.
17. Gonca E. The effects of zileuton and montelukast in reperfusion-induced arrhythmias in anesthetized rats. Curr Ther Res Clin Exp 2013;75:27-32.
18. Clark C, Foreman MI, Kane KA, McDonald FM, Parratt JR. Coronary artery ligation in anesthetized rats as a method for the production of experimental dysrhythmias and for the determination of infarct size. J Pharmacol Methods 1980;3:357-68.
19. Gonca E, Kurt Ç. Cardioprotective effect of Thymoquinone: A constituent of Nigella sativa L., against myocardial ischemia/reperfusion injury and ventricular arrhythmias in anaesthetized rats. Pak J Pharm Sci 2015;28:1267-73.
20. Goyal SN, Sharma C, Mahajan UB, Patil CR, Agrawal YO, Kumari S, et al. Protective Effects of Cardamom in Isoproterenol-Induced Myocardial Infarction in Rats. Int J Mol Sci 2015;16:27457-69.
21. Curtis MJ, Hancox JC, Farkas A, Wainwright CL, Stables CL, Saint DA, et al. The Lambeth Conventions (II): guidelines for the study of animal and human ventricular and supraventricular arrhythmias. Pharmacol Ther 2013;139:213-48.
22. Leprán I, Baczkó I, Varró A, Papp JG. ATP-sensitive potassium channel modulators: both pinacidil and glibenclamide produce antiarrhythmic activity during acute myocardial infarction in conscious rats. J Pharmacol Exp Ther 1996;277:1215-20.
23. Pasnik J and Zeman K. Role of the neutrophil in myocardial ischemia-reperfusion injury. J Organ Dysfunct 2009;5:193-207.
24. Silva BC, de Miranda AS, Rodrigues FG, Silveira AL, Resende GH, Moraes MF, et al. The 5-lipoxygenase (5-LOX) Inhibitor Zileuton Reduces Inflammation and Infarct Size with Improvement in Neurological Outcome Following Cerebral Ischemia. Curr Neurovasc Res 2015;12:398-403.
25. Hahn RA, MacDonald BR, Simpson PJ, Potts BD, Parli CJ. Antagonism of leukotriene B4 receptors does not limit canine myocardial infarct size. J Pharmacol Exp Ther 1990;253:58-66.
26. Coetzee WA. Multiplicity of effectors of the cardioprotective agent, diazoxide. Pharmacol Ther 2013;140:167-75.
27. Wang C, Hu SM, Xie H, Qiao SG, Liu H, Liu CF. Role of mitochondrial ATP-sensitive potassium channel-mediated PKC-ε in delayed protection against myocardial ischemia/reperfusion injury in isolated hearts of sevoflurane-preconditioned rats. Braz J Med Biol Res 2015;48:528-36.
28. Maxwell MP, Marston C, Hadley MR, Salmon JA, Garland LG. Selective 5-lipoxygenase inhibitor BW A4C does not influence progression of tissue injury in a canine model of regional myocardial ischaemia and reperfusion. J Cardiovasc Pharmacol 1991;17:539-45.
29. Adamek A, Jung S, Dienesch C, Laser M, Ertl G, Bauersachs J, et al. Role of 5-lipoxygenase in myocardial ischemia-reperfusion injury in mice. Eur J Pharmacol 2007;571:51-4.
30. Goulet JL, Snouwaert JN, Latour AM, Coffman TM, Koller BH. Altered inflammatory responses in leukotriene-deficient mice. Proc Natl Acad Sci U S A 1994;91:12852-6.
31. Sedlis SP. Mechanisms of ventricular arrhythmias in acute ischemia and reperfusion. Cardiovasc Clin 1992;22:3-18.