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Duration of Ciprofloxacin Use Is Important in the Development of Abdominal Aortic Aneurysm in a Rat Model

ORIGINAL INVESTIGATION

ABSTRACT

Background: Recent findings suggest that fluoroquinolones, most prescribed antibiotic to treat various infections, have increased abdominal aortic aneurysm formation. We aimed to investigate the relation of the development of abdominal aortic aneurysm and the duration of ciprofloxacin use.

Methods: Male Sprague–Dawley rats were divided into 2 groups to administer saline to the control groups and $CaCl_2$ to the aneurysm groups. These groups were then divided into 3 subgroups: intraperitoneal saline, ciprofloxacin for 2 weeks, and ciprofloxacin for 4 weeks. At the end of 4 weeks, the diameter of abdominal aorta was determined by ultrasonography and animals were sacrificed to obtain abdominal aorta specimens. Elastic fiber fracture, tunica media layer thickness, and aortic tissue damage were evaluated histologically.

Results: Aortic diameter of control-saline (2.15 mm ± 0.06), control-2 weeks (2.25 mm ± 0.06), and control-4 weeks (3.31 mm ± 0.09) ciprofloxacin groups was significantly different ($P < .0001$). Also, aortic diameter of aneurysm-saline (2.07 mm ± 0.02), aneurysm-2 weeks ciprofloxacin (3.33 mm ± 0.64), and aneurysm-4 weeks ciprofloxacin (8.55 mm ± 1.70) groups showed significant increase in aortic diameter with increasing duration of ciprofloxacin use ($P < .01$). A significant difference was found between the control-saline (0.00 ± 0.00), control-2 weeks (1.50 ± 0.33), and control-4 weeks ciprofloxacin groups (1.57 ± 0.20) in the histological aneurysm scores ($P < .001$). Aortic tunica media thickness did not change between control-saline and control-ciprofloxacin groups ($P > .05$).

Conclusion: The study showed that ciprofloxacin caused injury in the aortic wall but not a significant change in the thickness of the aortic tunica media layer. The duration of ciprofloxacin use was important in the development of aneurysm and aneurysm severity.

Keywords: Ciprofloxacin, aneurysm, aorta

Yekta Çulpan [1#]
İrem Keçeci [2#]
İrem Sandıkçı [2#]
Şeyda Gökçe [2#]
Hande Göker [2#]
Nagehan Özyılmaz Yay [3]
Rabia Ergelen [4]
Dilek Akakın [3]
Rezzan Gülhan [1]

[1]*Department of Medical Pharmacology, Faculty of Medicine, Marmara University, İstanbul, Turkey*
[2]*Faculty of Medicine, Marmara University, İstanbul, Turkey*
[3]*Department of Histology and Embryology, Faculty of Medicine, Marmara University, İstanbul, Turkey*
[4]*Department of Radiology, Faculty of Medicine, Marmara University, İstanbul, Turkey*

Corresponding author:
Yekta Çulpan
✉ yculpan@yahoo.com.tr

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INTRODUCTION

Aortic aneurysm (AA) is an irreversible, progressive, and degenerative disease that occurs with abnormal enlargement of the transverse diameter of the aorta 1.5 times or more than the adjacent normal aortic segment.[1] Abdominal AAs (AAAs) arising from the region between the aortic hiatus in the diaphragm and the iliac bifurcation are the most common among aneurysms observed in the body.[2]

The aortic wall of the aneurysm shows marked changes such as accumulation of inflammatory cells, neovascularization, collagen destruction, loss of elastic fibers, and apoptosis of smooth muscle cells. This process results in serious remodeling of the extracellular matrix of the aortic wall, especially the tunica media and adventitia, which causes hardening and thinning of the wall.[3] Gradually, the elasticity of the vascular wall decreases. Degeneration of the elastic structures in tunica media weakens the wall of the aorta and facilitates the development of an aneurysm. Elastin is the main load-bearing component against aneurysm formation, whereas collagen plays the most critical role in preventing rupture after aneurysm formation. Elastin decreases from the proximal to the distal aorta, explaining the frequency of aneurysms in the infrarenal aorta.[4]

Fluoroquinolones are one of the most commonly prescribed antibiotic groups worldwide, especially for urinary and respiratory system infections.[5-7] However, observational and clinical studies have shown that fluoroquinolones cause collagen-related adverse effects such as tendon rupture, tendinopathy, retinal detachment, and gastrointestinal perforation.[8] The extracellular matrix of the aortic wall also contains a high amount of collagen. Since fluoroquinolones cause collagen degradation, effects of fluoroquinolones on AA or dissection have also been investigated. Recent studies have shown that fluoroquinolones may cause AA or dissection or may aggravate the existing damage.[7,9-14] It was even stated that the use of fluoroquinolone doubles the risk of aortic dissection and aneurysm in a meta-analysis.[7] Following these studies, the Food and Drug Administration (FDA) added a new warning to the previous ones on December 20, 2018, on the side effects of fluoroquinolones and warned patients and healthcare professionals that fluoroquinolones pose a serious risk for AA and dissection formation.[15]

Although AAA is a process that develops gradually over the years, as shown in both *in vitro* and *in vivo* studies, fluoroquinolones can increase the risk of AA/dissection or worsen the aneurysm that has already existed in a short period by stimulating metalloproteinases and enhancing collagen destruction.[5] A cohort showed that the mean time to diagnose aortic dissection and the AA was 20 days after fluoroquinolone use.[11] In an umbrella review conducted in 2020, the results of 4 different systematic reviews were compiled; it has been reported that the use of fluoroquinolone in individuals over the age of 65 increases the risk of AA or dissection more than twice within 30-60 days after the use of the drug. Although the risk is maximum in the first 30 days, it remained high up to 365 days.[16] In an experimental study published in 2020, it was found that more than half of the fatal aortic ruptures occurred within the first 14 days following the use of ciprofloxacin in Marfan mice.[10]

At this point, new experimental studies are needed to show how ciprofloxacin causes AA in a time-dependent manner in subjects with or without any previous tissue damage. This study aims to examine the effect of the duration of ciprofloxacin use on the development of AAs on the intact and damaged aorta. For this purpose, we induced aneurysm by $CaCl_2$ in rats.

METHODS

Ethical approval of study was obtained from Marmara University Animal Experiments Local Ethics Committee (Ethics approval code: 115.2018.mar). All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals.

Animals Used in the Study

Experimental animals were obtained from Marmara University Experimental Animal Research Center (DEHAMER). Forty-two male Sprague–Dawley rats weighted 200-300 g, approximately 2-4 months old, were randomly divided into 6 groups of equal numbers (n=7). The rats were kept in a room with 12 hours of a light–dark cycle during the experiment, where temperature was 20–22°C and the humidity was automatically adjusted to 45%-50%. All rats were kept in transparent cages and fed with standard rat food and tap water during this period.

Experimental Groups and Experimental Design

$CaCl_2$ model of AAA was used to induce aneurysm. Extraluminal application of $CaCl_2$ to arteries is useful to investigate potential pharmacotherapy in AAA management. $CaCl_2$ generates aneurysm by causing vascular remodeling, decreased insoluble collagen, and enhanced gelatinases activity.[17] In this model, aneurysm formation is observed approximately 2-4 weeks after $CaCl_2$ administration.[17,18] Fifteen minutes of extraluminal application of 0.5 M $CaCl_2$ to aorta is adequate for the development of aneurysm.[19]

Firstly, 42 male Sprague–Dawley rats were divided into 2 groups. Sham operation with application of sterile saline-treated gauze on the infrarenal aorta for 15 minutes was performed on 21 rats. The other 21 rats underwent same experimental surgery with application of $CaCl_2$-treated gauze to the infrarenal aorta for 15 minutes to induce aneurysm development. Each group was further divided into 3 treatment groups of 4 weeks sterile saline, 2 weeks ciprofloxacin, or 4 weeks ciprofloxacin injections. Ciprofloxacin (100 mg/kg) and saline injections were performed intraperitoneally (i.p.) every day. Ciprofloxacin was kindly provided by Neuland Laboratories Limited.

Control (sham operation) + saline group (C/SF)

Control (sham operation) + 2 weeks ciprofloxacin group (C/2w)

Control (sham operation) + 4 weeks ciprofloxacin group (C/4w)

Aneurysm ($CaCl_2$ applied) + saline group (A/SF)

Aneurysm ($CaCl_2$ applied) + 2 weeks ciprofloxacin group (A/2w)

Aneurysm ($CaCl_2$ applied) + 4 weeks ciprofloxacin group (A/4w)

Animals received 4 weeks of saline, 2 weeks of ciprofloxacin, or 4 weeks of ciprofloxacin injections every day, and the experiment was terminated after performing abdominal ultrasonography (USG) on the 28th day. Under ketamine anesthesia, abdominal aorta of the animals was collected for histopathologic examination. The abdominal aorta of anesthetized rats was ligated at the level of renal artery and the aortic bifurcation, and the aorta was cut out at the indicated levels (Figure 1).

HIGHLIGHTS

- Ciprofloxacin caused injury in the aortic wall.
- No significant change was seen in the thickness of the aortic tunica media layer with ciprofloxacin use.
- The duration of ciprofloxacin use was important in the development of aneurysm and aneurysm severity. It is more important if the aortic wall was already damaged or if aneurysm was triggered by other factors.

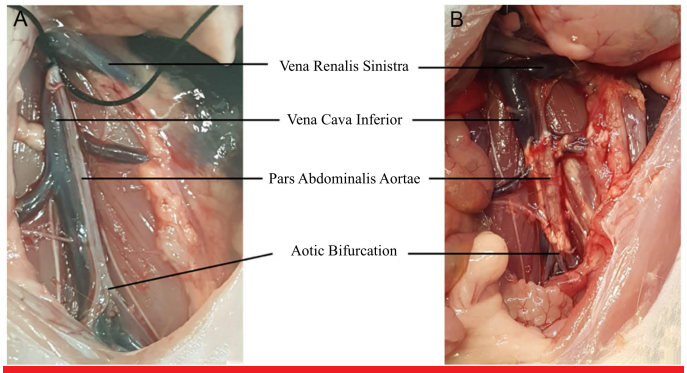

Figure 1. Abdominal aortic tissue at 4 weeks after surgery. (A) An example of the abdominal aorta observed in rats having sham operation with saline application. (B) An example of the abdominal aorta observed in rats having surgery with $CaCl_2$ application to induce abdominal aortic aneurysm.

Surgery

The animals were anesthetized with ketamine (100 mg/kg) and chlorpromazine (30 mg/kg) i.p. A single dose of ketamine was repeated, when necessary, throughout the procedure. A midline median laparotomy was performed. The intestines were deviated to the right with the help of a moist gauze. Then, the infrarenal abdominal aorta was explored by blunt dissection. A sterile gauze (1 × 2 cm^2) treated with 0.5 M $CaCl_2$ or sterile saline was applied on the infrarenal aorta for 15 minutes. Meanwhile, the intestines were kept moist with saline. At the end of 15 minutes, gauze was removed and the abdominal cavity was washed with warm sterile saline. After insertion of the intestines, the laparotomy incision was closed with a 2/0 sharp silk suture. All animals survived after the surgery and were assigned for the treatment groups.

Ultrasonograpy

All animals had abdominal USG examination on the 28th day after the surgery. The infrarenal aorta was imaged with a GE-LOGIQ P6 USG device using 11L linear probe, and the images were recorded with the device's own program. Abdominal aortic diameter measurements were obtained at the level of mid-infrarenal region. The assessor of aortic diameter with USG was blinded to study groups.

Histopathological Examination of Abdominal Aortic Tissue

For light microscopic examination, the abdominal aorta specimens were removed from each animal and immediately fixed in 10% neutral buffered formalin. The tissue samples were dehydrated in ascending alcohol series (70%, 90%, 96%, and 100%), cleared in toluene, and embedded in paraffin. The tissue blocks were then sliced (thickness of 4 µm) and sections were stained with hematoxylin and eosin (H&E) and Verhoeff Van Gieson Elastic stains. The histological aortic tissue damage was graded semiquantitatively from 0 to 4 based on the disruption of medial layer elastic network (0: no disruption, 1: mild disruption, only external elastic lamina disrupted; 2: moderate disruption, external elastic lamina and outer medial layers degraded; 3: high disruption, external elastic lamina and medial elastic layers breakage; 4: severe disruption, all elastic layers breakage, and aortic rupture).[20] The microscopic examination was performed by a blinded manner on aortic cross-sections. Thickness of tunica media layer was measured in 3 different regions of the sections in control groups, and the average value was calculated. Tunica media layer thickness of the aneurysm groups were not measured due to increased damage on the vessel wall.

All sections were examined using an Olympus BX51 light microscope and photographed with a digital camera (Olympus DP72).

Statistical Analysis

All data are expressed as mean ± standard error of the mean. Groups were compared with analysis of variance (ANOVA) followed by Tukey's multiple comparisons tests, and $P < .05$ was considered statistically significant. One-way ANOVA followed by Tukey's multiple comparisons test was performed using GraphPad Prism version 9.2.0 for Mac OS X, GraphPad Software, San Diego, Calif, USA; www.graphpad.com.

RESULTS

Ultrasonography

Abdominal aortic diameters of all animals were measured with USG (Figure 2). Ciprofloxacin treatment produced a significant increase in the diameter of abdominal aorta in both sham operation and aneurysm groups in a time-dependent manner (saline applied: C/SF, C/2w, and C/4w, $P < .0001$; $CaCl_2$ applied: A/SF, A/2w, and A/4w, $P < .01$).

In multiple comparisons, there was a significant difference in diameter of abdominal aorta between C/SF (2.15 ± 0.06 mm) and C/4w (3.31 ± 0.09 mm) groups ($P < .0001$) but not between C/SF and C/2w groups. Also, abdominal aorta diameter of C/4w group was greater than the C/2w (2.25 ± 0.06 mm) group ($P < .0001$) (Figure 3). In the aneurysm group, there was a significant difference in diameter of abdominal aorta between A/SF (2.07 ± 0.02 mm) and A/4w (8.55 ± 1.70 mm) groups ($P < .01$). Also, abdominal aorta diameter of A/4w group was greater than the A/2w (3.33 ± 0.64 mm) group ($P < .05$). However, the difference between A/SF and A/2w groups was not significant ($P > .05$) (Figure 3).

Histopathological Examination

Histologically, in the control groups (sham operation with a saline-gauze applied on the aorta), ciprofloxacin treatment produced a significant damage compared to daily intraperitoneal saline treatment ($P < .001$). In multiple comparisons, there was no damage in the C/SF group, and the damage score of the C/SF group (0.00 ± 0.00) was significantly less than the C/2w (1.50±0.33) and C/4w (1.57 ± 0.20) groups ($P < .001$) (Figure 4). Damage score of ciprofloxacin treatment groups was similar ($P > .05$). In the aneurysm groups ($CaCl_2$-gauze applied on the aorta), the effect of $CaCl_2$ was very significant and it produced grade 3-4 damage in all animals. Damage scores of the aneurysm groups were comparable to each other ($P > .05$) (Figure 4). Among the control groups, there was no significant difference in tunica media layer thickness (C/SF, 94.00 ± 9.12 µm; C/2w, 79.80 ± 8.52 µm; and C/4w, 94.10 ± 3.42µm; $P > .05$) (Figure 5).

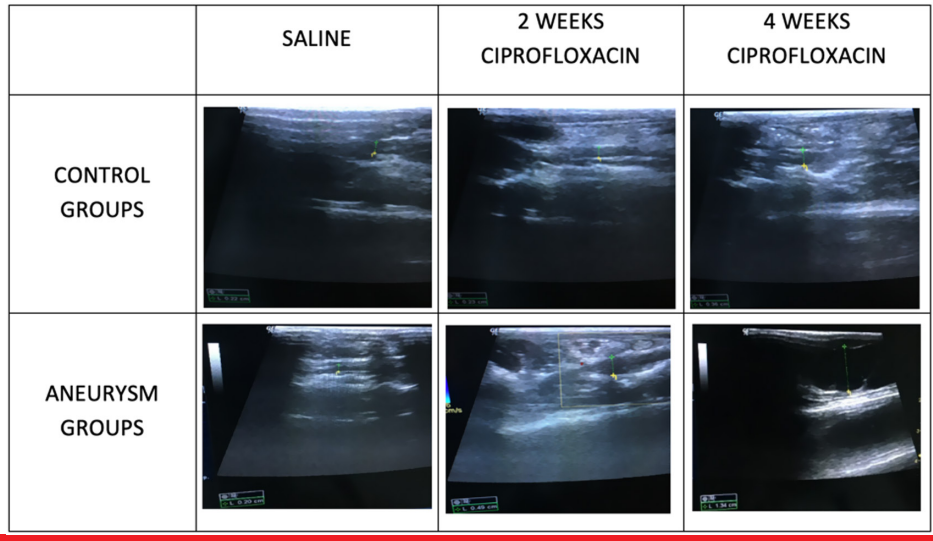

Figure 2. Representative USG images of rat abdominal aorta at the end of 4th week (the part between the green and yellow plus signs shows the abdominal aorta). USG, ultrasonography.

Representative images of the rat abdominal aorta sections stained with H&E are presented in Figure 6 and Verhoeff van Gieson stain in Figure 7.

DISCUSSION

In the present study, sham-operated and aneurysm-induced animal groups received saline or ciprofloxacin either for 2 or 4 weeks, and the development of AAA was evaluated by abdominal USG and histopathological staining.

Our results show that the use of ciprofloxacin increased the diameter of the abdominal aorta with or without an underlying triggering factor, and the duration of ciprofloxacin use is an important factor for the diameter of aneurysm. Ciprofloxacin caused inflammatory changes and damage to the aortic wall but did not affect the thickness of the tunica media layer.

Ciprofloxacin is a second-generation fluoroquinolone highly effective against various microorganisms with its excellent antimicrobial activity and pharmacokinetic properties. It exhibits few side effects and is frequently used in clinical practice for the treatment of various bacterial infections for about 30 years.[21] The bactericidal activity of

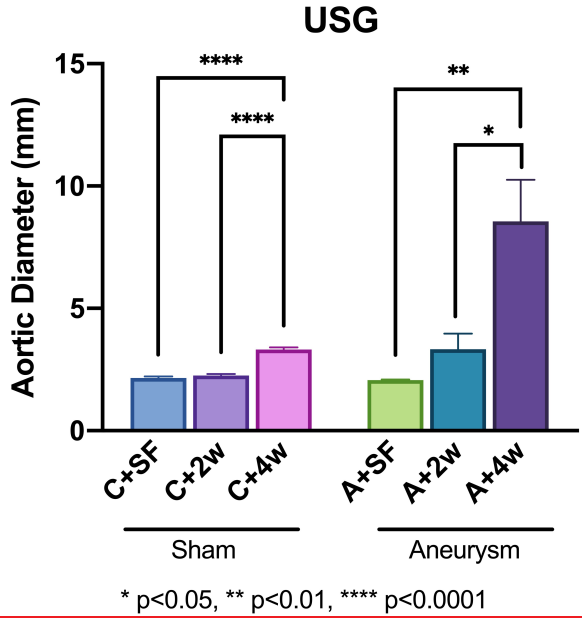

Figure 3. Abdominal aortic diameters of all groups (C/SF, control + saline; C/2w, control + 2 weeks ciprofloxacin; C/4w, control + 4 weeks ciprofloxacin; A/SF, aneurysm + saline; A/2w, aneurysm + 2 weeks ciprofloxacin; A/4w, aneurysm + 4 weeks ciprofloxacin. Each group included 7 rats.

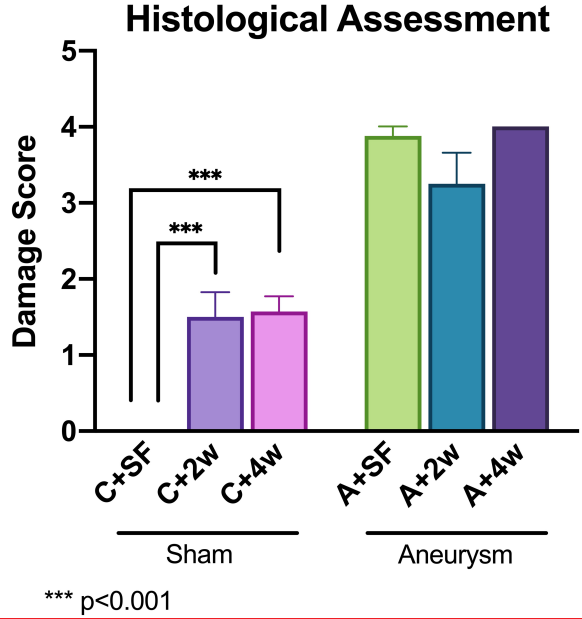

Figure 4. Histological assessment of all groups (C/SF, control + saline; C/2w, control + 2 weeks ciprofloxacin; C/4w, control + 4 weeks ciprofloxacin; A/SF, aneurysm + saline; A/2w, aneurysm + 2 weeks ciprofloxacin; A/4w, aneurysm + 4 weeks ciprofloxacin. Each group included 7 rats).

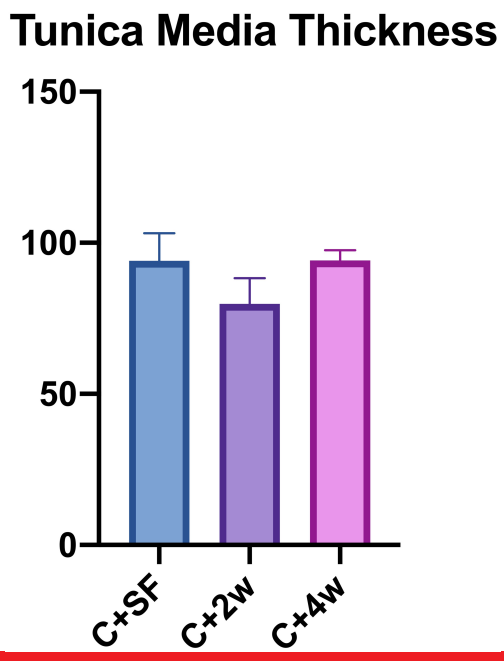

Figure 5. Histological assessment of tunica media thickness of sham-operated groups (C/SF, control + saline; C/2w, control + 2 weeks ciprofloxacin; C/4w, control + 4 weeks ciprofloxacin. Each group included 7 rats).

fluoroquinolones is associated with the combination of DNA fragmentation, reactive oxygen species (ROS) generation, and programmed cell death systems.[22] They also cause a decrease in type 1 and type 3 collagen levels, tropoelastin, and lysyl oxidase activity which is important for elastic fiber stabilization in the smooth muscle cells of the aorta.[10] The FDA made a "Black Box" warning for fluoroquinolones in May 2018 and stated that although it has not been proven, drugs in this group can potentially cause an AA.[23] Nevertheless, even when it was stated that the risk of AA is 9/100 000 in general and 300/100 000 in high-risk groups such as the elderly, aneurysm risk was not seen as very high, but this warning has increased the studies on fluoroquinolones.[24]

In a recent study, using a mouse model of moderate sporadic AA disease induced by exposure to high-fat diet and angiotensin II infusion, researchers have shown that ciprofloxacin increased the incidence and severity of challenge-induced aortic degeneration, dissection, and rupture, thereby establishing a causal effect of ciprofloxacin on sporadic aortic disease development.[25] The mice also showed decreased lysyl oxidase expression and activity, increased matrix metalloproteinase (MMP) levels, and increased elastic fiber degradation and cell damage. Although ciprofloxacin alone caused mild aortic elastic fiber breakage, it did not spontaneously trigger AA and dissection in the absence of external stress. These results suggested that the use of ciprofloxacin may increase susceptibility to AA and dissection formation and fatal rupture.[25] In a following study, researchers used another model and demonstrated that ciprofloxacin significantly accelerated aortic root enlargement and increased aortic dissection and rupture in Marfan mice.[10] The histologic analysis with H&E staining and elastin staining showed significant destruction in the aortas of mice that received ciprofloxacin. Additionally, compared with aortic tissues from Marfan

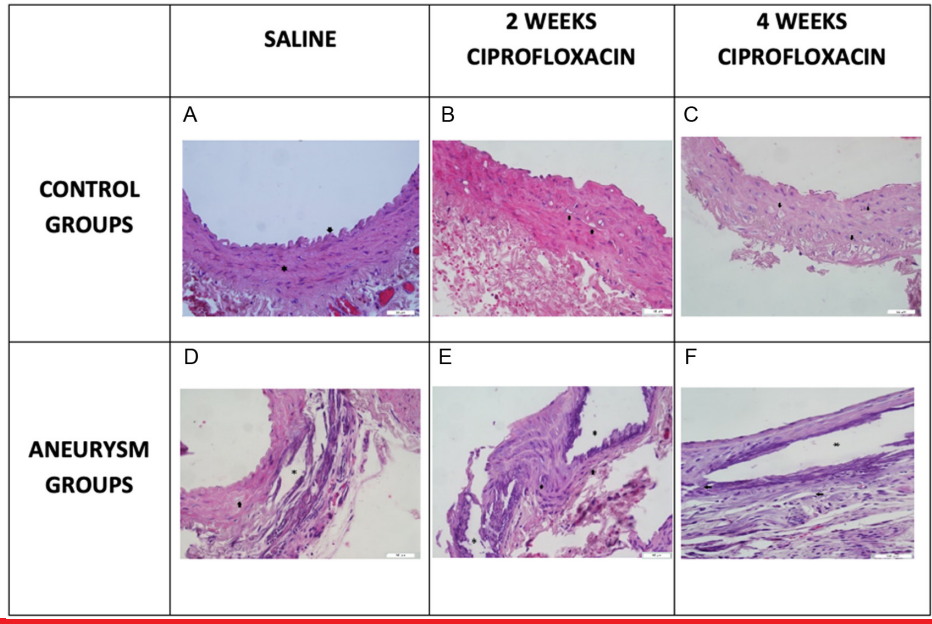

Figure 6. Representative images of rat abdominal aorta stained with hematoxylin and eosin in experimental groups. (A) Regular layout of intima (arrow) and media layers (*) of the saline group rats. (B) Slight separation (arrows) between smooth muscle cells in the tunica media layer of C/2w group rats. (C) Separation (arrows) between smooth muscle cells in the tunica media layer of C/4w group rats. (D) Destruction of normal tunica media architecture with large dissection area (*) and separation (arrow) between smooth muscle cells in the A/SF group rats. (E) Separation (arrows) between smooth muscle cells and large dissection area (*) in the destructed tunica media layer A/2w group rats. (F) Separation (arrows) between smooth muscle cells in the tunica media layer and large dissection area due to dissecting aortic aneurysm (*) in the A/4w group rats. Scale bars: 50 μm.

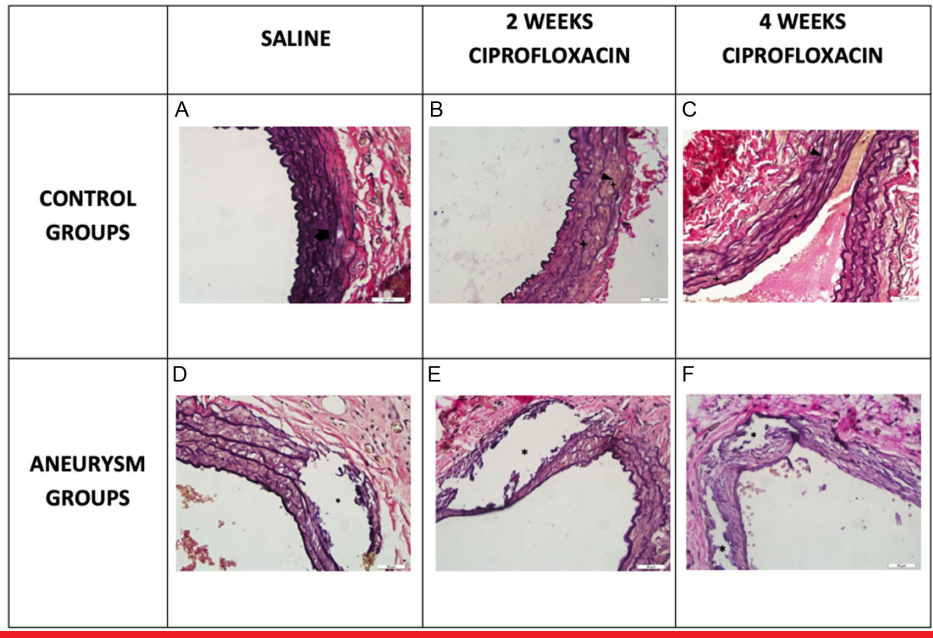

Figure 7. Representative images of rat abdominal aorta stained with Verhoeff van Gieson stain in experimental groups. (A) Regular elastic fiber arrangement (arrow) in the tunica media layer in the saline group. (B) Irregular appearance (►) and ruptures (+) in elastic fibers of tunica media layer in C/2w group. (C) Flattened (►) and ruptured (+) elastic fibers in some areas of the tunica media layer in C/4w group. (D) Large dissection area (*) due to aneurysm in the tunica media layer and deep medial tear in the A/SF group rats. (E) Large dissection area (*) and medial tear, rupture of elastic fibers in the tunica media layer in the A/2w group rats. (F) Rupture of elastic fibers, large dissection area (*) with detachments in the tunica media layer of A/4w group rats. Scale bars: 50 μm.

control mice, aortas from ciprofloxacin-treated Marfan mice showed more compromised elastic fiber structure and severe destruction accompanied by increased levels of MMPs and apoptosis.[10] However, these effects of ciprofloxacin were not investigated depending on the dose or duration of drug use. The results of our study showed that the use of ciprofloxacin in rats in which aneurysm formation was induced by $CaCl_2$ administration or not induced increased the aortic diameter according to saline. In addition, the use of ciprofloxacin for 4 weeks produced significantly larger aortic diameters compared to the use of ciprofloxacin for 2 weeks in the control and aneurysm groups. This finding implies that the duration of ciprofloxacin use besides ciprofloxacin use can play an essential role in the development of an aneurysm.

In humans, 4 systematic reviews investigating the triggering effect of quinolones and the risk of AA/dissection showed consistent findings by emphasizing that the use of fluoroquinolones in adults >65 years of age more than doubled the risk of AA/dissection within 30-60 days following exposure. Furthermore, the risk was greater within 30 days after fluoroquinolone use and remained significant up to 365 days.[16] According to a cohort study conducted with 360 088 patients who had previously used ciprofloxacin or amoxicillin between 2006 and 2013 in Sweden, the use of fluoroquinolone increased the risk of AA or dissection, while this risk was highest in the first 10 days of antibiotic treatment in the study.[26] The risk for Achilles tendon disorders was highest in the first 15-30 days following the start of fluoroquinolone treatment.[27] A meta-analysis showed that the use of fluoroquinolones in adults more than doubles the risk of AA or aortic dissection within 60 days following the exposure.[7] Furthermore, according to a pooled duration response analysis, as the duration of fluoroquinolone therapy increased from 3 to 14 days to greater than 14 days, there was an increased risk of AA or dissection.[7]

While studies provide data on the development of aneurysm due to ciprofloxacin being higher in women, the incidence of aneurysm development in the general population is higher in men than in women.[7,9,28] In a study, subgroup analysis showed that female and older patients are more susceptible to fluoroquinolone-associated AA or dissection than males and younger patients, respectively. Early exposure to fluoroquinolones, female gender, and elderly patients contribute to fluoroquinolone-associated AA or dissection.[7] Another study showed that the reported higher risk of collagen-associate adverse effect in fluoroquinolones-related cases was found in patients at the age of over 18 years old. Most of these studies were designed as case–control studies, and the subjects were patients older than 18 years old.[13] In our experiment, we do not study these factors. Other reported risk factors for fluoroquinolone-associated AA or dissection, including concurrent older age and female gender, may need to be studied.

Abdominal aortic aneurysm is an intense inflammatory response with destruction of the elastin matrix of the media, and reduced presence of medial smooth muscle cells. The vascular structure will get thinner as AAA progresses, and the vessel diameter will get bigger. Preservation of elastin

tissue has a protective effect on the development of AAA. Moreover, collagen is important in preventing rupture after aneurysm formation. Aortic preparations obtained in our study were stained with Verhoef Von Gieson stain, and the number of elastic fibers and the damage to these fibers has been examined. Flattening of the elastin fibers in the aortic tunica media layer, opening between them, and ruptures were observed in the sham operation groups exposed to ciprofloxacin for 2 or 4 weeks. However, aneurysm ($CaCl_2$ applied) groups had large dissection areas in the tunica media layers, probably due to the damage caused by $CaCl_2$. The concentration of $CaCl_2$ and application time were probably high so that histologically it produced severe damage by itself and masked the damage of ciprofloxacin. All these results show us that the use of ciprofloxacin alone does not cause dissection in a short time, but it can cause irregularities and deformations in the elastic fiber tissue and may cause serious complications such as aortic dissection in the presence of an underlying disease or insult. The study conducted on Marfan mice by LeMaire et al[10] can also be a good example of this situation associated with underlying diseases.

In line with these studies, our results show that ciprofloxacin causes damage in the aortic wall, yet tunica media thickness of aorta does not change. Meanwhile, the longer duration of ciprofloxacin use increases the severity of the aneurysm. Induction of aneurysm by other factors exacerbates the increase in aortic diameter associated with prolonged ciprofloxacin use.

In the study by LeMaire et al,[25] ciprofloxacin in healthy mice causes aortic damage and dilatation which is not statistically significant. However, in our study, histological damage score in the sham-operated group is significantly higher in the ciprofloxacin treatment groups compared to the saline treatment. There is no significant difference in the thickness of the tunica media layer between the sham operation groups treated with saline, ciprofloxacin for 2 weeks, and ciprofloxacin for 4 weeks. In accordance with LeMaire et al[25] study, the use of ciprofloxacin has lesser risk in cases where the aneurysm is not triggered than the conditions in which an aneurysm is triggered.

A recent meta-analysis study assessed that all 3 fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin) are associated with an increased risk of AA, and levofloxacin is also associated with an increased risk of aortic dissection. The risk of an AA is higher than the aortic dissection. With regards to the administration route, oral administration is more likely to be associated with either AA or dissection. Levofloxacin is associated with the most significant risk, followed by moxifloxacin and ciprofloxacin.[29]

It is found that there was an increase in the rate of AA/aortic dissection in patients with pneumonia treated with fluoroquinolones vs. azithromycin but no evidence of an increased rate when fluoroquinolones were compared with combined trimethoprim and sulfamethoxazole in patients with a urinary tract infection. It is also observed that there was an increased rate of AA/aortic dissection with fluoroquinolones when amoxicillin was used as the comparator group; however, this association was attenuated after accounting for recent imaging that could have detected existing AAs or aortic dissections.[5]

Most of these studies support the histological and ultrasonographic findings we looked at in our study. Parameters such as MMP and tissue inhibitor of metalloproteinase-1, the amount of collagen and fibroblasts, and examining the inflammatory cell responses and ROS accumulation will be useful in terms of obtaining more precise results.

CONCLUSION

The effect of i.p. saline, ciprofloxacin for 2 weeks, or ciprofloxacin for 4 weeks on the development of AAA was examined by USG imaging and histopathological staining in aneurysm-induced ($CaCl_2$ applied) groups and control (sham operation) groups.

In both control groups and aneurysm groups, treatment with ciprofloxacin for 4 weeks produced a statistically significant difference in aortic diameter measured by USG compared with saline and ciprofloxacin for 2 weeks.

It was found that the use of ciprofloxacin in rats increased the aortic diameter, and the aortic diameter was significantly larger in rats treated with ciprofloxacin for 4 weeks than in those treated with ciprofloxacin for 2 weeks. The duration of ciprofloxacin use appears to play an important role in the development of aneurysm. Additionally, in both saline and aneurysm groups, an increase in aortic diameter was observed in the 2-week ciprofloxacin treatment compared to the saline treatment although this was not statistically significant. This indicates that even the short-term use of ciprofloxacin contributes to aneurysm formation.

In terms of aortic wall layers, no significant difference was found in the tunica media layer thicknesses between the control groups treated with saline, ciprofloxacin for 2 weeks, and ciprofloxacin for 4 weeks. On the other hand, histological damage score in ciprofloxacin treatment for 2 or 4 weeks was significantly different from the saline treatment.

As the use of ciprofloxacin is a factor in the development of aneurysm, its prolonged use becomes even more important, especially if the aortic wall is already damaged or the aneurysm is triggered by other factors.

Ethics Committee Approval: Ethical committee approval was received from the Marmara University Animal Experiments Local Ethics Committee (Approval No:115.2018.mar).

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