Iranian Journal of Basic Medical Sciences
ijbms.mums.ac.ir

Aortic ischemia-reperfusion injury and potency of fluoxetine

Mehmet Altan [1], Muhittin Onur Yaman [2], Gülnaz Kervancıoğlu [3], Aysu Kılıç [4], Elif Kervancıoğlu Demirci [5], Sıla Hidayet Bozdoğan Polat [1], Zeliha Karadeniz [6], Ibrahim Güner [7], Nermin Yelmen [1], Gülderen Şahin [8]*

[1] Istanbul University-Cerrahpaşa, Cerrahpasa Medical Faculty, Department of Physiology, Istanbul, Turkey
[2] Istanbul University-Cerrahpaşa, Vocational School of Health Sciences, Istanbul, Turkey
[3] Istanbul Aydın University, Medical Faculty, Department of Histology & Embryology, Istanbul, Turkey
[4] Bezmialem University, Medical Faculty, Department of Physiology, Istanbul, Turkey
[5] Istanbul University, Istanbul Faculty of Medicine, Department of Histology & Embryology, Istanbul, Turkey
[6] Istanbul Aydın University, Medical Faculty, Department of Obstetrics & Gynecology, Istanbul, Turkey
[7] Tekirdağ Namık Kemal University, Medical Faculty, Department of Physiology, Tekirdağ, Turkey
[8] Istanbul Aydın University, Medical Faculty, Department of Physiology, Istanbul, Turkey

ARTICLE INFO

Article type:
Original

Article history:
Received: Jun 8, 2022
Accepted: Dec 7, 2022

Keywords:
Aorta
Apoptosis
Fluoxetine
Inflammation
Oxidative stress
Rats
Reperfusion injury

ABSTRACT

Objective(s): Due to cross-clamping of the aorta during aortic aneurysm surgeries, ischemia-reperfusion (IR) develops, and it may cause damage to the aorta itself or even to remote organs by oxidative stress or inflammation. Fluoxetine (FLX) which might be used in the preoperative period for its tranquilizing effect also has antioxidant effects in short-term use. The purpose of our study is to examine whether FLX protects aorta tissue, against the damage caused by IR.

Materials and Methods: Three groups of Wistar rats were formed randomly. 1) Control group (sham-operated), 2) IR group (60 min ischemia+120 min perfusion), and 3) FLX+IR group (FLX dose was 20 mg/kg for 3 days IP before IR). At the end of each procedure, aorta samples were collected, and oxidant-antioxidant, anti-inflammatory, and anti-apoptotic status of the aorta were evaluated. Histological examinations of the samples were provided.

Results: Levels of LOOH, MDA, ROS, TOS, MPO, TNFα, IL-1β, IL-6, NF-kB, MMP-9, caspase-9, 8-OHdG, NO, and HA were found to be significantly increased in the IR group compared with control ($P<0.05$) and SOD, GSH, TAS, and IL-10 levels were significantly lower ($P<0.05$). FLX significantly decreased LOOH, MDA, ROS, TOS, MPO, TNFα, IL-1β, IL-6, NF-kB, MMP-9, caspase-9, 8-OHdG, NO, and HA levels in the FLX+IR group compared with IR group ($P<0.05$) and increased IL-10, SOD, GSH, and TAS ($P<0.05$). FLX administration prevented the deterioration of aortic tissue damage.

Conclusion: Our study is the first study that demonstrates FLX-mediated suppression of IR injury in the infrarenal abdominal aorta by antioxidant, anti-inflammatory, and anti-apoptotic properties.

▶ Please cite this article as:
Altan M, Yaman MO, Kervancıoğlu G, Kılıç A , Demirci EK, Bozdoğan Polat SH , Karadeniz Z, Güner I, Yelmen N, Şahin G. Aortic ischemia-reperfusion injury and potency of fluoxetine. Iran J Basic Med Sci 2023; 26: 301-307. doi: https://dx.doi.org/10.22038/IJBMS.2023.65974.14508

Introduction

Aortic cross-clamping is widely used to cease the blood flow in the vascular surgery of the aorta (1-3). Pause in blood flow due to clamping and recovery of the flow by removing the clamp causes changes in blood flow dynamics which eventually results in molecular changes at the cellular level. These variations in blood flow dynamics cause IR injury in the tissue that is subjected to ischemia for a while and also in remote organs such as the heart, lung, and kidney (4-6).

Oxidative stress defines the disrupted balance between oxidants and anti-oxidants resulting in accumulation of the oxidants which cause damage to important structural subunits of the cell including membrane lipids, proteins, and DNA. The infiltration of neutrophils, activation of proinflammatory cytokines, disruption of endothelial integrity, and disturbance of ion transport through the cell membrane mediates the damage caused by oxidative stress (7, 8). This cascade of events plays an important role in the formation of ischemia-reperfusion injury (9).

At the onset of IR injury, hypoxia disrupts the integrity of endothelium which takes a major role in neutrophil migration. Hypoxia also activates both transcriptional and non-transcriptional pathways and changes tissue responses eventually causing acute inflammation. When blood supply is reestablished and oxygen delivery is provided, tissue damage both locally and systemically occurs especially due to the activation of proinflammatory cytokines (TNF-α, IL-1β, IL-6, etc.) and rapid release of reactive oxygen species (ROS) from polymorphonuclear leukocytes (PMNs) (10, 11). End products released in IR possibly cause very serious complications called systemic inflammatory response syndrome (SIRS) in surgical repair of abdominal aortic aneurysm repair and may even lead to death by causing multi-organ dysfunction syndrome (MODS). To prevent the injury caused by IR, many methods including leucocyte therapy, ischemic preconditioning, various anti-oxidant therapies, and complement therapy have been used (12).

Fluoxetine (FLX) is a well-known and widely used serotonin reuptake inhibitor. It was first approved for the treatment of depression and then obsessive-compulsive disorder, bulimia nervosa, and many other disorders. It has good tolerability, easy dosing, and is considered safe to use

*Corresponding author: Gülderen Şahin. Florya Yerleşkesi, Besyol Mah. Inonu Cad. No: 38, Sefakoy–Kuçukcekmece,Istanbul, Turkey. Tel: +90 532 393 21 18; Email: emineguldereensahin@aydin.edu.tr

(13). FLX has been shown to have anti-oxidant (14, 15), anti-inflammatory (16), and antiapoptotic effects (17) on various stress conditions. Kirkova *et al*. showed the anti-oxidant effects of FLX on the oxidative stress induced by melanoma in the spleen tissue of mice (18). In addition, we have provided significant results proving FLX alleviates IR injury via its anti-oxidant features in the lung (4) and anti-oxidant and anti-inflammatory features in the kidney and heart in our previous studies (4-6).

To the best of our knowledge, no study has yet studied the effects of FLX on aortic IR-induced aortic tissue injury. Therefore, we examined the possible protective effect of FLX on the aortic tissue damage caused by IR of the infrarenal abdominal aorta (IAA).

Materials and Methods

Animals

Twenty-one male Wistar rats weighing 350–400 g were used in this study. Animals were housed in a controlled room with a 12-hr light/dark cycle and a temperature of 23 ± 1 °C, at 60% humidity. They were fed in individual cages and free access to food and water was provided. Experimental protocols were carried out according to the "Care and Use of Laboratory Animals Guide" provided by the National Institutes of Health. The study was approved by the Istanbul University Animal Care and Use Ethics Committee (2012/48).

Surgical procedure

Anesthesia of the rats was provided by intraperitoneal administration (IP) of pentobarbital sodium (60 mg/kg). Spontaneous ventilation was ensured by a polyvinyl chloride tube inserted through a tracheostomy. All surgical procedures were performed by keeping the body temperature of the rats at 37 ± 0.5 °C, utilizing a heating pad. Blood pressure monitoring was ensured by carotid artery catheterization (22 gauge). A midline laparotomy was implemented after the skin was prepared aseptically. Prewarmed physiologic saline instillation to the peritoneal cavity was ensured to avoid dehydration of rats. Intestinal loops were pulled left through laparotomy by using moisturized gauze swabs to bring out the abdominal aorta. After isolation of the infrarenal abdominal segment, ischemia was induced by placing a microvascular clamp (vascu-statts II, midi flat 1001-532; Scanlan Int, St Paul, MN, USA) on the IAA for 60 min. Humidified gauze compress was applied to the abdominal cavity to avoid heat loss and dehydration. 5 min prior to each ischemia procedure 50 U/kg (total volume 500 ml) IV heparin (Nevparin; Mustafa Nevzat Drug Company, Istanbul, Turkey) in isotonic saline was administered through the tail vein of the rats (4, 19).

At the end of the ischemia, procedure clamps were removed, and reperfusion was maintained for 120 min (20). The efficiency of both occlusion and reperfusion was evaluated by observing the pulsations at the distal portion of the clamped segment of the aorta and blood pressure monitorization was used for the confirmation of the procedure. After the IR procedures, the deep pentobarbital sodium euthanasia (150 mg/kg, IP) technique was used. Samples were taken from the aorta, distal to the microvascular clamp. For histological examination, samples were stored in formalin, and the remaining half of the samples were stored at -80 °C for the assays (21).

Experimental design

Rats were randomly assigned into three groups to have 7 rats in each group: 1) Control group: in addition to sham-operation saline-injected group; II) IR group: saline administered group before IR procedure; III) FLX+IR group: FLX administered group before IR procedure. In the control group, only IAA was dissected following a midline laparotomy. In the IR group, IAA was clamped for 60 min following a midline laparotomy, at the end of the clamping procedure, reperfusion was maintained for 120 min. Dosing in the FLX+IR group was adjusted to 20 mg/kg, IP once daily for 3 days before ischemic surgery (4, 14, 21). The saline injection was performed similarly to the FLX injection. IR procedure is applied 30 min after the injection.

Chemicals

Highest-grade chemicals were obtained from Sigma Chemical Co (St Louis, MO, USA). Deionized water was used in all assays. All reagents were stored following the manufacturer's instructions at +4 °C and their equilibration to room temperature was ensured before using them.

Preparation of aortic homogenates

At the end of the reperfusion procedure of the study, dissection of the aorta was provided immediately, and samples were washed with ice-cold phosphate-buffered saline (PBS, 0.1 mol/l, pH 7.4). Afterward, samples were dried with filter paper and their weight was noted. Following the addition of PBS, the aortic samples were cut into pieces, and homogenization was performed by a high-speed homogenizer (Ultra-Turrax, IKA® Werke, Staufen, Germany) at speed of 22000 rpm. Following the homogenization procedure, centrifugation of the homogenates was performed at 1000 rpm for 10 min at 4 °C to obtain pellets and supernatants (22). All biochemical analyses were performed with supernatant. Firstly, the Bradford method was used to determine the total protein amount (23).

Assays in the aortic tissue homogenates

Measurement of oxidant and anti-oxidant parameters

The levels of Superoxide dismutase (SOD), Glutathione (GSH), Lipid hydroperoxide (LOOH), Malondialdehyde (MDA), Total oxidant status (TOS), Total anti-oxidant status (TAS) and Reactive oxygen species (ROS) were determined by ELISA using commercially available kits from eBioscience (San Diego, CA, USA). Assays were performed according to the manufacturer's instructions.

Measurement of proinflammatory and anti-inflammatory cytokines

Measurement of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), as proinflammatory cytokines, and interleukin-10 (IL-10) as anti-inflammatory cytokine in aorta tissue homogenates were measured with commercial ELISA kits (eBioscience, San Diego, CA, USA). Assays were performed according to the manufacturer's instructions.

Measurement of apoptotic and other parameters

The levels of nuclear factor kappa B (NF-κB), metalloproteinase-9 (MMP-9), caspase-9 (Caps-9), 8-hydroxy-2'-deoxyguanosine (8-OHdG), nitric oxide (nitrite/nitrate), and hyaluronan(HA) were also determined by ELISA methods using commercially available kits (Abcam, Cambridge, MA, USA). Assays were performed

according to the manufacturer's instructions.

All samples were measured in duplicate by an expert who was blind to experimental groups. ELISA kits were equilibrated at room temperature before use. All measurements were presented by dividing the protein content of the respective sample.

Histological evaluation

The aorta samples of all groups were fixed in a neutral buffered formalin solution. The tissues were subsequently embedded in paraffin and 4 µm thick slices were obtained from the paraffin blocks for histological slides. Slides were stained with hematoxylin and eosin for a general overview and Safranelin & Mallory's stain for the evaluation of elastic and collagen fibers. Stained histological sections were examined and photographed by a Leica DMLB light microscope. Photographs from each serial aorta section (n=7 sections per aorta) were examined by an experienced histopathologist blindly.

The difference in aortic damage was semiquantitatively evaluated according to the scoring of Marcela Polachini Prata (3). Changes in the vascular wall were noted. Fragmentation of elastic fibers, loss of the lamellar architecture, disorganization of collagen fibers, mononuclear cellular infiltrate in tunica media, and mononuclear cell infiltration in the tunica adventitia was scored for each feature as follows; 0: normal tissue; 1: mild; 2: moderate; 3: severe; and 4: highly severe. Semiquantitative histological findings are given in Table 1.

Statistical analysis

Means ± SEM values are reported. GraphPad Prism version 8.0 for Windows (Graph Pad Software v5.0, San Diego, CA, USA) was used for statistical analysis. In the comparison, One-Way Analysis of Variance was used; *post hoc* analyses were achieved with Tukey's *post hoc* test, when $P<0.05$ was considered significant for all analyses.

Results

Changes in aortic tissue oxidative stress parameters

IR significantly increased LOOH, MDA, TOS, and ROS levels and significantly decreased SOD, GSH, and TAS levels in aortic tissue samples compared with the control group. MPO level increased significantly by IR ($P<0.05$; control vs IR group) (Table 2).

Pretreatment with FLX prior to IR procedure

Table 1. Semiquantitative histological evaluation of the aorta samples of the rats

	Control group	IR group	FLX+IR group
Fragmentation of elastic fibers	0	4	1
Loss of the lamellar architecture	0	0	0
Disorganization of collagen fibers	0	0	0
Mononuclear cellular infiltration in tunica media	0	4	1
Mononuclear infiltration in the tunica adventitia	0	3	1

IR: Ischemia-reperfusion, FLX: Fluoxetine

Table 2. Results of aortic tissue, aortic tissue results in three experimental groups. Oxidative stress biomarkers, pro and anti-inflammatory cytokines, NF-κB, and specific injury-related markers

Groups	Control group (n=7)	IR group (n=7)	FLX+IR group (n=7)
Oxidant/Anti-oxidant levels			
LOOH (nmol/wet tissue)	2.45 ± 0.50	3.64 ± 0,40 ***	2.40 ± 0.44^{+++}
MDA (nM/100 mg protein)	63.00 ± 2.47	88.74 ± 2.78***	62.5 ± 2.70^{+++}
TOS (nM/100 mg protein)	5.22 ± 0.32	7.74 ± 0.25***	5.59 ± 0.33^{+++}
GSH (µmol/wet tissue)	0.29 ± 0.20	0.18 ± 0.01 ***	0.29 ± 0.02^{+++}
SOD (U/wet tissue)	20.77 ± 1.62	3.39 ± 0.93 ***	18.79 ± 1.28^{+++}
TAS (U/100 mg protein)	23.14 ± 0.69	14.05 ± 1.19 ***	17.52 ± 0.62^{***+++}
ROS (U/100 mg protein)	3908.33 ± 144.77	5519.51 ± 146,41***	4399.12 ± 486^{***+++}
MPO (µU/g tissue)	17.16 ± 0.23	42.11 ± 18.39***	16.57 ± 0.48^{+++}
Pro-and anti-inflammatory cytokines and NF-κB levels			
TNF-α (pg/100 µg protein)	42.54 ± 1,05	72.77 ± 2.34***	44.68 ± 1.54^{+++}
IL-6 (pg/100 µg protein)	102.98 ± 2.05	161.39 ± 2.20 ***	116.34 ± 1.91$^{*** +++}$
IL-1β (pg/100 µg protein)	126.72 ± 2.82	181.63 ± 1.51 ***	188.56 ± 1.54^{***+++}
IL-10 (pg/100 µg protein)	282.6 ± 3.48	182.2 ± 3.48***	285.9 ± 3.48^{+++}
NF-κB (ng/100 µg protein)	1.02 ± 0.06	1.58 ± 0.03***	1.17 ± 0.03 $^{***+++}$
Specific injury-related markers			
MMP-9 (ng/100 mg protein)	0.28 ± 0.03	0.50 ± 0.02***	0.34 ± 0.01^{**+++}
CASP-9 (ng/100 mg protein)	0.82 ± 0.04	1.43 ± 0.02***	0.78 ± 0.01***
8-OhdG (ng/100 mg protein)	4.60 ± 0.24	8.31 ± 8.58***	5.87 ± 0.29^{***+++}
NO (mmol/100 mg protein)	43.63 ±1.02	72.96 ± 1.10***	50.55 ± 1.11^{***+++}
HA (pg/100 mg protein)	43.69 ± 2.56	72.36 ± 0.89***	48.17 ± 2.21^{***+}

LOOH: Lipid hydroperoxide, MDA: Malondialdehyde, TOS: Total oxidant status, GSH: glutathione, SOD: superoxide dismutase, TAS: Total anti-oxidant status, ROS: Reactive oxygen species, MPO: Myeloperoxidase, TNF-α: tumor necrosis factor-alpha, IL-6: Interleukin 6, IL-1β: Interleukin 1β, IL-10: Interleukin 10, NF-κB: nuclear factor-kappa B, MMP-9: Matrix metallopeptidase 9, 8-OhdG: 8-hydroxide oxyguanosine, NO: Nitric oxide, HA: Hyaluronan. Statistical significance and comparisons: * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs control; + $P<0.05$, ++ $P<0.01$, +++ $P<0.001$ vs IR

significantly decreased LOOH, MDA, TOS, and ROS levels while significantly increasing SOD, GSH, and TAS levels in the aortic homogenate (P<0.05; IR vs FLX+ IR group). FLX pretreatment provided a normalization in MPO levels.

Changes in the aortic tissue inflammation parameters

Pro-inflammatory cytokines TNF-α, IL-6, IL-1β, and NF-kB increased significantly in the aortic homogenate of the IR group when compared with the control group (P<0.05). On the other hand, the anti-inflammatory cytokine IL-10 significantly decreased in the IR group compared with the control group (P<0.05) (Table 2).

In the FLX+IR group, pretreatment with FLX before the IR procedure significantly decreased TNF-α, IL-6, IL-1β, and NF-kB levels, and IL-10 significantly increased (P<0.05; IR vs FLX+ IR group) and approached the control group level (Table 2).

***Changes in the aortic tissue-specific injury-related* markers**

IR led to a significant increase in MMP-9, caspase-9, 8-OhdG, NO, and HA levels compared with the control group (P<0.05). FLX preadministration before the IR period reversed the significant increase in MMP-9, caspase-9, 8-OhdG, NO, and HA found in the IR group and levels returned to control group levels (Table 2).

Histological evaluation

The normal structure of the aorta was observed in the histological examination of the control group. Endothelial disruption, subendothelial deterioration, and dissociation of elastic lamina near the intima were in the IR group. Mononuclear infiltration and dissociation areas due to edema were observed between the elastic lamellae in tunica media. Tunica adventitia of the IR group also showed lymphocyte infiltration. A significantly higher injury score was in the IR group. In the FLX+IR group, the endothelial and subendothelial areas were intact. Mononuclear cells were sparsely in the subendothelial space (Figure 1).

Discussion

Pathophysiological mechanisms underlying IR injury emerge in two steps. The first step depends on the lack of oxygen which results in the depletion of energy stores in the cell. Changes in the second step are a consequence of interference between oxidant status, changes in microcirculation, inflammatory responses, and apoptosis. In IR injury, the accumulation of neutrophils, ROS, and cytokines are the inevitable consequence at the tissue level (10, 24) which reaches out to oxidative damage to proteins and DNA (25).

IR generates its damage mainly through the peroxidation of the cell membrane lipids. By reperfusion of the tissue following ischemia, the membrane becomes the target site for oxidative stress. As a matter of fact, in our study, the significantly increased LOOH and MDA results support this finding. FLX preadministration before IR may be useful in terms of eliminating the causes of oxidative stress by decreasing LOOH, MDA, and ROS, and this situation indicates the anti-oxidant effect of FLX.

These findings are comparable with the studies that reported a decrease in ROS levels directly proportional to the dose of FLX (14, 18).

In order to maintain homeostasis in the internal environment, the anti-oxidant enzymatic mechanisms of the cells should rapidly respond to oxidative stress. In this concept, the induction of ROS scavenging enzymes has been shown to have beneficial effects on IR injury by preventing ROS formation (9). SOD and GSH are major anti-oxidant defense system of the cell and exert a key role in the clearance of free radicals (26).

In the present study, the reduction in GSH and SOD in response to IR suggests the consumption of these anti-oxidants during the breakdown of free radicals. Pal & Dandiya (27), have previously shown a similar decrease in brain GSH levels in response to depression induced by stress.

Decreased levels of GSH and SOD, in this study, were prevented by FLX in aortic tissue in IR effectively. By restoring

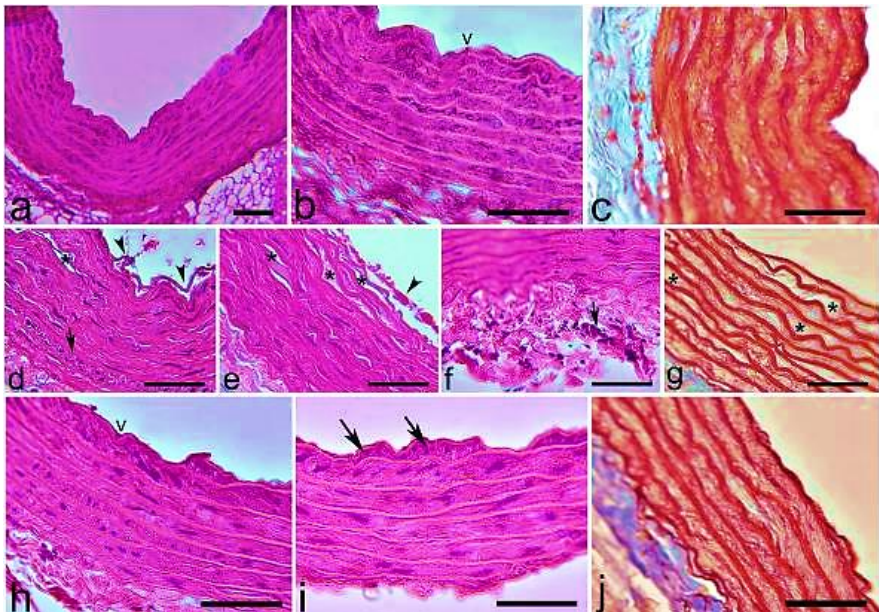

Figure 1. a, b, and c represent the normal general morphology and intact endothelium (v) of the control group; d, e, f, and g represent the IR group, endothelial disruption (arrowhead), dissociation of the elastic lamellae (asterisk), and mononucleolar cells (arrow) in tunica media and adventitia; h, I, and j represent the FLX+IR group, continuous endothelium with sparse subendothelial mononucleolar cells. a, b, d, e, f, h, and i hematoxylin and eosin stain; c, g, and j Safranelin & Mallory's stain. Scale bar respresent 50 µm

impaired GSH pathways, FLX administration may exert the observed anti-oxidant effects via the restoration of critical GSH-related processes such as modulation of cellular redox, free radical scavenging, regulation of cell signaling, etc. (18). A significant increase in SOD, CAT, and GSH activities and a significant decrease in MDA by FLX treatment in restraint stress-induced rats have also been reported (17). Similarly, Kolla *et al.* also showed neuroprotection by FLX pretreatment in cultured pheochromocytoma cells of the rats through an up-regulation in SOD activity (28). Our previous findings were also in line with the present study (4-6). Our results suggest that FLX may reduce oxidative stress-induced injury by enhancing the anti-oxidant response provided by GSH and SOD.

A key cellular pathophysiologic factor in the injury caused by IR is the neutrophils which are the site of production of MPO, an index of neutrophilic accumulation that occurred during IR injury (29). In our study, a reduction observed in the MPO level by the preadministration of FLX prior to IR indicates the anti-inflammatory effect of FLX. In a study similar effect of FLX has been shown in the blood-brain barrier (30). Lim *et al.*'s study also supports our finding (31).

An increase in proinflammatory cytokines in IR suggests that free radicals which develop in the IR process may promote monocytes for the production of proinflammatory cytokines in IR injury. These cytokines stimulate endothelial cells for the further release of various interleukins (8). It is thought that the increase in IL-10 level in the FLX+IR group was caused by decreased suppression of IL-10 because of the FLX's suppressing effect on both acute inflammation and proinflammatory cytokines (32). Similar changes were also found in our previous studies (5, 6). In addition, a study suggested that FLX reduced the levels of pro-inflammatory cytokines (33).

As in our study, increased NF-kβ levels in response to IR have an important role in inflammation because NF-kβ increases gene expression of various proinflammatory cytokines, thereby regulating inflammation (34). NF-kβ is also associated with neutrophilic infiltration and lipid peroxidation into tissue (33). As well as its direct effects on cytokines and chemokines it also mediates the adhesion, proliferation, apoptosis, maturation, and differentiation of cells.

The increase of TNF-α, IL-6, and IL-1β together with NF-kβ in our study suggests that the inflammation of acute aortic tissue damage is increased by NF-kβ mediated proinflammatory cytokines. While FLX suppresses inflammatory mediators, especially TNF-α, and reduces free radicals, it also causes a decrease in NF-kβ.

8-OHdG is widely used to reflect pro-inflammatory cytokines and the reactive oxygen species induced oxidative damage in nuclear and mitochondrial DNA (25). In our study, the increase of 8-OHdG levels in IR-induced aortic tissue injury suggests that nuclear or mitochondrial DNA damage occurred. This DNA damage may initiate the apoptotic pathways (35).

Caspases that are a member of cysteine proteases regulate apoptosis by a cascade of reactions (35). According to our results, the significantly increased levels of caspase-9 and 8-OhDG parameters in the IR group indicate the initiation of the apoptotic process. Although several studies showed anti-apoptotic effects of FLX, Djordjevic *et al.* found FLX-induced apoptotic activity in the rat model of stress-induced liver injury (36). In the present study, a significant decrease in caspase-9 and 8-OhDG in the FLX+IR group indicates the antiapoptotic features of the FLX. A study suggested that FLX treatment either alone or combined with olanzapine has an antiapoptotic effect in the frontal cortex, hippocampus, and striatum nucleus of adult rats (37). The anti-apoptotic effect of FLX may be due to its anti-oxidant and anti-inflammatory properties.

In our study, the increase in NO in the aortic samples of the IR group may be due to the increase in proinflammatory cytokines (31). It has been shown that TNF-α and IL-1β induce an increase in NO production (38). As well as causing nitrosative stress, increased amounts of NO may be involved in the inflammation process. Reduced NO and the proinflammatory cytokine levels in the FLX+IR group may be considered as the anti-inflammatory, and anti-oxidant effects of FLX also reduce the formation of NO.

IR also has harmful effects on the extracellular matrix (ECM). Matrix metalloproteinases (MMPs) take a role in the degradation of various proteins in the ECM. An increase in its activity is accompanied by increased inflammatory mediators, and capillary permeability (39). In our study, the increase in MMP-9 that occurred in the IR group was prevented in the FLX + IR group, and the harmful effects of MMP-9 on the ECM may be reduced by FLX. Lee *et al.* have shown that FLX preserves the function of the blood-spinal barrier by inhibiting the matrix metalloprotease activity (30). Thus, we can say that this reduction is due to the anti-oxidant, anti-inflammatory, and anti-apoptotic effects of FLX.

Mediators released from tissue cells in the ECM during IR induced the inflammatory process to potentiate hyaluronan production (40, 41). The increase in hyaluronan may be referred to as the increase in proinflammatory cytokines as previously explained. In our recent study, it has been shown that hyaluronan levels increased in the kidney tissues of rats exposed to IR (5). On the other hand, increased inflammatory mediators in IR modulate fibroblast activity by increasing hyaluronan (42).

The reduction of both MMP-9 and hyaluronan levels in the FLX-treated group suggests that FLX has a direct preventing effect on ECM remodeling. The hyaluronan level, which was found to be increased in the IR group, suggests that FLX has a direct preventing effect on ECM remodeling.

The limitation of our study is the inability to determine the changes in aortic endothelial biomarkers in IR and the effects of FLX on these changes. In our next research, we plan to investigate this issue.

Conclusion

To the best of our knowledge, our study is the first to evaluate the effect of FLX on IR injury in the infrarenal abdominal aorta. Depending on our findings we may suggest that fluoxetine improves the defense systems of the aorta tissue by giving raise to anti-inflammatory cytokines and anti-oxidant enzymes and by reducing the formation of free radicals in ischemia-reperfusion injury. Also, FLX may be involved in the modulation of cell apoptosis sequence and remodeling of the extracellular matrix.

In line with the findings obtained from the study, FLX can be used as one of the preventive, therapeutic approaches to avoid aorta injury in IR. Short-term use of fluoxetine before aortic surgery may offer beneficial effects in the recovery at least in this study setting, further evaluation and consideration of fluoxetine from a pretreatment perspective

may provide better surgical outcomes.

Acknowledgment

The authors received no financial support for the research, authorship, and/or publication of this article.

Authors' Contributions

MA, MOY, İG and GS designed and performed the experiments; GK and EKD performed histological evaluation of the aorta tissue samples. AK performed the statistical analysis of the findings. SHBP performed biochemical analysis and ZK collected data; MA, MOY, İG, NY, and GS discussed the results and strategy; GS supervised, directed, and managed the study; MA, MOY, and GS approved the final version to be published.

Funding

This study was not supported by any institution, it was performed with the contribution of the researchers.

Data Statement

The data presented in this study are available via the corresponding author's e-mail.

Conflicts of Interest

The authors declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

References

1. Kim GS, Ahn HJ, Kim WH, Kim MJ, Lee SH. Risk factors for postoperative complications after open infrarenal abdominal aortic aneurysm repair in Koreans. Yonsei Med J 2011; 52: 339-346.
2. Zammert M, Gelman S. The pathophysiology of aortic cross-clamping. Best Pract Res Clin Anaesthesiol 2016; 30: 257-269.
3. Prata MP, Jaldin RG, Lourencao P, Sobreira ML, Yoshida RA, Terra SA, *et al.* Acute aortic wall injury caused by aortic cross-clamping: morphological and biomechanical study of the aorta in a swine model of three aortic surgery approaches. J Vasc Bras 2020; 19: e20190025.
4. Guner I, Yaman MO, Aksu U, Uzun D, Erman H, Inceli M, *et al.* The effect of fluoxetine on ischemia-reperfusion after aortic surgery in a rat model. J Surg Res 2014; 189: 96-105.
5. Aksu U, Guner I, Yaman OM, Erman H, Uzun D, Sengezer-Inceli M, *et al.* Fluoxetine ameliorates imbalance of redox homeostasis and inflammation in an acute kidney injury model. Journal of Physiology and Biochemistry 2014; 70: 925-934.
6. Yaman OM, Erman H, Guner I, Tok OE, Pala M, Esrefoglu M, *et al.* Remote myocardial injury: the protective role of fluoxetine. Canadian Journal of Physiology and Pharmacology 2018; 96: 319-327.
7. Ferrari RS, Andrade CF. Oxidative Stress and lung ischemia-reperfusion injury. Oxid Med Cell Longev 2015; 2015: 590987.
8. Wu MY, Yiang GT, Liao WT, Tsai AP, Cheng YL, Cheng PW, *et al.* Current Mechanistic Concepts in Ischemia and Reperfusion Injury. Cell Physiol Biochem 2018; 46: 1650-1667.
9. Yang B, Fung A, Pac-Soo C, Ma D. Vascular surgery-related organ injury and protective strategies: update and future prospects. Br J Anaesth 2016; 117 Suppl 2: ii32-ii43.
10. Eltzschig HK, Collard CD. Vascular ischaemia and reperfusion injury. Br Med Bull 2004; 70: 71-86.
11. Seal JB, Gewertz BL. Vascular dysfunction in ischemia-reperfusion injury. Ann Vasc Surg 2005; 19: 572-584.
12. Halladin NL. Oxidative and inflammatory biomarkers of ischemia and reperfusion injuries. Dan Med J 2015; 62: B5054.
13.Wong DT, Bymaster FP, Engleman EA. Prozac (fluoxetine, Lilly 110140), the first selective serotonin uptake inhibitor and an antidepressant drug: Twenty years since its first publication. Life sciences 1995; 57: 411-441.
14. Zafir A, Ara A, Banu N. *In vivo* anti-oxidant status: A putative target of antidepressant action. Prog Neuropsychopharmacol Biol Psychiatry 2009; 33: 220-228.
15. Behr GA, Moreira JC, Frey BN. Preclinical and clinical evidence of anti-oxidant effects of antidepressant agents: implications for the pathophysiology of major depressive disorder. Oxid Med Cell Longev 2012; 2012: 609421.
16. Alboni S, Poggini S, Garofalo S, Milior G, El Hajj H, Lecours C, *et al.* Fluoxetine treatment affects the inflammatory response and microglial function according to the quality of the living environment. Brain Behav Immun 2016; 58: 261-271.
17. Caiaffo V, Oliveira BD, de Sa FB, Evencio Neto J. Anti-inflammatory, antiapoptotic, and anti-oxidant activity of fluoxetine. Pharmacol Res Perspect 2016; 4: e00231.
18. Kirkova M, Tzvetanova E, Vircheva S, Zamfirova R, Grygier B, Kubera M. Anti-oxidant activity of fluoxetine: studies in mice melanoma model. Cell Biochem Funct 2010; 28: 497-502.
19. Sinay L, Kurthy M, Horvath S, Arato E, Shafiei M, Lantos J, *et al.* Ischaemic postconditioning reduces peroxide formation, cytokine expression and leukocyte activation in reperfusion injury after abdominal aortic surgery in rat model. Clin Hemorheol Microcirc 2008; 40: 133-142.
20. Aksu U, Guner I, Yaman OM, Erman H, Uzun D, Sengezer-Inceli M, *et al.* Fluoxetine ameliorates imbalance of redox homeostasis and inflammation in an acute kidney injury model. J Physiol Biochem 2014; 70: 925-934.
21. Yaman OM, Erman H, Guner I, Tok OE, Pala M, Esrefoglu M, *et al.* Remote myocardial injury: the protective role of fluoxetine. Can J Physiol Pharmacol 2018; 96: 319-327.
22. Li C, Wang Z, Li X, Chen J. Effects of semicarbazide-sensitive amine oxidase inhibitors on morphology of aorta and kidney in diabetic rats. BMC Endocr Disord 2019; 19: 59-Last page.
23. Kruger NJ. The bradford method for protein quantitation. Methods Mol Biol 1994; 32: 9-15.
24. Soares ROS, Losada DM, Jordani MC, Evora P, Castro ESO. Ischemia/reperfusion injury revisited: An overview of the latest pharmacological strategies. Int J Mol Sci 2019; 20: Pages.
25. Nemmar A, Al-Salam S, Beegam S, Yuvaraju P, Ali BH. Aortic Oxidative Stress, Inflammation and DNA Damage Following Pulmonary Exposure to Cerium Oxide Nanoparticles in a Rat Model of Vascular Injury. Biomolecules 2019; 9: Pages.
26. Brigelius-Flohe R, Flohe L. Regulatory phenomena in the glutathione peroxidase superfamily. Anti-oxid Redox Signal 2020; 33: 498-516.
27. Pal SN, Dandiya PC. Glutathione as a cerebral substrate in depressive behavior. Pharmacol Biochem Behav 1994; 48: 845-851.
28. Kolla N, Wei Z, Richardson JS, Li XM. Amitriptyline and fluoxetine protect PC12 cells from cell death induced by hydrogen peroxide. J Psychiatry Neurosci 2005; 30: 196-201.
29. Khan AA, Alsahli MA, Rahmani AH. Myeloperoxidase as an active disease biomarker: Recent biochemical and pathological perspectives. Med Sci (Basel) 2018; 6: Pages.
30. Lee JY, Kim HS, Choi HY, Oh TH, Yune TY. Fluoxetine inhibits matrix metalloprotease activation and prevents disruption of blood-spinal cord barrier after spinal cord injury. Brain 2012; 135: 2375-2389.
31. Lim CM, Kim SW, Park JY, Kim C, Yoon SH, Lee JK. Fluoxetine affords robust neuroprotection in the postischemic brain via its anti-inflammatory effect. J Neurosci Res 2009; 87: 1037-1045.
32. Zhao Y, Shang P, Wang M, Xie M, Liu J. Neuroprotective effects of fluoxetine against chronic stress-induced neural inflammation and apoptosis: Involvement of the p38 activity. Front Physiol 2020; 11: 351-Last page.

33. Liu FY, Cai J, Wang C, Ruan W, Guan GP, Pan HZ, *et al.* Fluoxetine attenuates neuroinflammation in early brain injury after subarachnoid hemorrhage: a possible role for the regulation of TLR4/MyD88/NF-kappaB signaling pathway. J Neuroinflammation 2018; 15: 347-Last page.
34. Liu T, Zhang L, Joo D, Sun SC. NF-kappaB signaling in inflammation. Signal Transduct Target Ther 2017; 2: Pages.
35. Greijer AE, van der Wall E. The role of hypoxia inducible factor 1 (HIF-1) in hypoxia induced apoptosis. J Clin Pathol 2004; 57: 1009-1014.
36. Djordjevic J, Djordjevic A, Adzic M, Elaković I, Matić G, Radojcic MB. Fluoxetine affects anti-oxidant system and promotes apoptotic signaling in Wistar rat liver. European journal of pharmacology 2011; 659: 61-66.
37. Reus GZ, Abelaira HM, Agostinho FR, Ribeiro KF, Vitto MF, Luciano TF, *et al.* The administration of olanzapine and fluoxetine has synergistic effects on intracellular survival pathways in the rat brain. J Psychiatr Res 2012; 46: 1029-1035.
38. Katseni K, Chalkias A, Kotsis T, Dafnios N, Arapoglou V, Kaparos G, *et al.* The Effect of Perioperative Ischemia and Reperfusion on Multiorgan Dysfunction following Abdominal Aortic Aneurysm Repair. Biomed Res Int 2015; 2015: 598980.
39. Wang X, Khalil RA. Matrix Metalloproteinases, Vascular Remodeling, and Vascular Disease. Adv Pharmacol 2018; 81: 241-330.
40. Teder P, Heldin P. Mechanism of impaired local hyaluronan turnover in bleomycin-induced lung injury in rat. Am J Respir Cell Mol Biol 1997; 17: 376-385.
41. Sieve I, Munster-Kuhnel AK, Hilfiker-Kleiner D. Regulation and function of endothelial glycocalyx layer in vascular diseases. Vascul Pharmacol 2018; 100: 26-33.
42. Stair-Nawy S, Csoka AB, Stern R. Hyaluronidase expression in human skin fibroblasts. Biochem Biophys Res Commun 1999; 266: 268-273.