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# The effect of fluoxetine on ischemia–reperfusion after aortic surgery in a rat model

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ABSTRACT

*Background:* Aortic ischemia–reperfusion (IR) is an important factor in the development of postoperative acute lung injury after abdominal aortic surgery. The aim of the present study was to examine the effect of fluoxetine (Flx), a selective serotonin reuptake inhibitor widely used as a preoperative anxiolytic, on lung injury induced by abdominal aortic IR in rats.
*Methods:* Wistar rats were randomized into three groups ($n = 7$ per group): (1) control (sham laparotomy); (2) IR without Flx (60-min ischemia and 120-min reperfusion); (3) IR with Flx (Flx + IR) (Flx 20 mg/kg/d, intraperitoneally for 3 d before surgery). Lung tissue samples and bronchoalveolar lavage (BAL) were obtained for biochemical analysis of oxidative status. Ischemia-modified albumin (IMA) level and protein concentrations in BAL and lung wet to dry weight ratios were determined. Histologic evaluation of the lung tissues was also performed.
*Results:* IR without Flx led to significant increase in lipid hydroperoxide, malondialdehyde, and pro-oxidant–antioxidant balance and decrease in superoxide dismutase, glutathione, and ferric reducing antioxidant power activities ($P < 0.05$ *versus* control), whereas Flx was able to restore these parameters ($P > 0.05$ *versus* control) and decrease IMA level ($P < 0.01$ *versus* control) and protein concentration ($P < 0.05$ *versus* control) in BAL and wet to dry lung weight ratio. Histologic evaluation showed that Flx attenuated the morphologic changes associated with lung injury.
*Conclusions:* The results indicate that Flx confers protection against aortic IR-induced lung oxidative stress and cellular integrity. IMA levels in BAL may be used as a follow-up marker for the efficacy of treatment in lung injury.



## 1. Introduction

The aorta at the thoracic or abdominal levels is cross-clamped during aneurysm surgery. Cross-clamping and releasing of the infrarenal abdominal aorta (IAA) leads to ischemia–reperfusion (IR) in the lower extremities and also remote organ injury, such as lung injury. Lung injury is of primary importance after lower limb IR. Although the precise

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pathophysiological mechanisms of remote organ injury are still unknown, the oxidative mediators released after IR are thought to be responsible for the injury [1–3].

Oxidative stress is defined as an imbalance between reactive oxygen species (ROS) and antioxidant defenses. Free radicals are potent oxidizing and reducing agents that directly damage cellular membranes by lipid peroxidation. Overproduction and-or insufficient removal of ROS during IR result in significant damage to cell structure and organ functions [4,5]. ROS mediated tissue injury by means of endothelial activation, cytokine secretion, and leukocyte activation. These activated leukocytes provoke local and systemic reperfusion inflammatory responses [4,5].

Besides the effects of ROS on DNA and lipids, ROS may directly change the three-dimensional conformations of proteins. The overproduction of ROS can temporarily modify the N-terminal region of albumin and cause an increase in the concentration of ischemia-modified albumin (IMA). In the last decade, IMA was thought to be a biomarker of oxidative stress related to IR in different clinical conditions associated with oxidative stress [6]. Elevation of IMA is directly associated with free radicals that form during ischemia [7,8].

It is well known that oxidant production in the lung can lead to acute lung injury (ALI), which often results in disruption of the alveolar-capillary and epithelial barrier, endothelial cell injury, increased microvascular permeability, and interstitial edema [4,5]. Subsequently, respiratory failure and irreversible fibrosis may occur; however, no specific therapies exist for these diseases [4,9].

Preoperative anxiety is one of the major concerns to the anesthesiologists and intensive care physicians [10]. Preoperative anxiety may also be associated with both postoperative pain [11] and disruption of body systems, such as the nervous system [12]. In current practice guidelines, antidepressants are commonly used drugs to decrease preoperative anxiety [13]. Fluoxetine (Flx) (*N*-methyl-3-[4-{trifluoromethyl}phenoxy] benzenepropanamine) is a selective serotonin reuptake inhibitor widely used in the treatment of anxiety because of its tolerability, ease of dosing, and safety [14]. It has been suggested that Flx treatment for 4, 16, or 28 d did not affect cardiovascular basal parameters in rats [15,16]. In contrast, Crestani *et al.* [17] reported the development of mild hypertension after chronic Flx treatment for 21 d in normotensive rats, whereas acute Flx treatment did not affect hemodynamic changes.

This nontricyclic antidepressant has largely replaced the tricyclic antidepressants in terms of efficacy and their more favorable side effect patterns [14]. Although antidepressants restore noradrenergic and serotoninergic neurotransmitter systems, recently antioxidant effects of antidepressants action have been suggested in the treatment of depressive disorder [18,19]. Nonserotonergic effects of Flx, however, have also been reported. Flx is protective against monocrotaline- [20] and chronic hypoxia-induced [21] pulmonary hypertension in the adult rat and mice by suppressing proliferation of pulmonary arterial muscle cells. Additionally, Flx has been shown to exert antioxidant potential *via* reversal of oxidative damage by enhancing *in vivo* antioxidant defenses and improving the cellular antioxidant status after a stress-induced decline [22]. It has also been suggested that Flx is able to prevent melanoma-induced oxidative changes in mice spleen *via* its antioxidant activity [23]. Additionally, Flx may protect neuronal damage after transient ischemia by increasing the levels of brain-derived neurotrophic factor and antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione (GSH) peroxidase [24].

In light of these findings, we tested the hypothesis that short-term pretreatment with Flx, which is already used as a preoperative anxiolytic, would attenuate or prevent lung tissue oxidative stress and cellular injury because of its antioxidant property in rats subjected to abdominal aortic IR. We also hypothesized that the changes in IMA levels in bronchoalveolar lavage (BAL) by Flx would be associated with evidence of reduced oxidative stress in the lung.

## 2. Materials and methods

### 2.1. Animals

Male Wistar rats weighing 350–400 g were used in this study. Animals were housed in individual cages in a temperature-controlled room (23 ± 1°C) and a controlled environment of light–dark cycle (12 h) with free access to food and water. All experimental protocols were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Istanbul University Animal Care and Use Ethics Committee (2012/48).

### 2.2. Surgical procedure

Rats were anesthetized with pentobarbital sodium (60 mg/kg, intraperitoneally [i.p.]). A tracheotomy was performed, and a polyvinylchloride tube was inserted into the trachea to enable spontaneous ventilation. Rats were placed on a heating pad and body temperature was maintained at 37 ± 0.5°C during the entire experiment. The carotid artery was catheterized (22 gauge) for blood pressure recording. The skin was aseptically prepared and a midline laparotomy was performed. Prewarmed physiological saline (10 mL) was instilled into the peritoneal cavity to prevent dehydration of the rats.

The abdominal aorta was exposed by gently deflecting the loops of the intestine to the left with moist gauze swabs. After fine isolation of the infrarenal segment, an atraumatic microvascular clamp (vascu-statts II, midi straight 1001-532; Scanlan Int, St Paul, MN) was placed on IAA for 60 min. The abdomen was then closed and the surgical field was covered with a humidified gauze compress throughout the entire experiment to minimize heat and fluid losses. Before inducing ischemia, each animal received 50 U/kg (total volume 500 μL) intravenous heparin (Nevparin; Mustafa Nevzat Drug Company, Istanbul, Turkey) in saline *via* tail vein injection. The ischemia protocol was applied 5 min after heparin administration.

The microvascular clamp on IAA was then removed and IAA was reperfused for 120 min. Aortic occlusion and reperfusion were confirmed by the loss and reappearance of satisfactory pulsation in the distal aorta. The changes in the recording of systemic arterial blood pressures were also confirmed by this procedure. Blood samples were obtained