BioMed Research International

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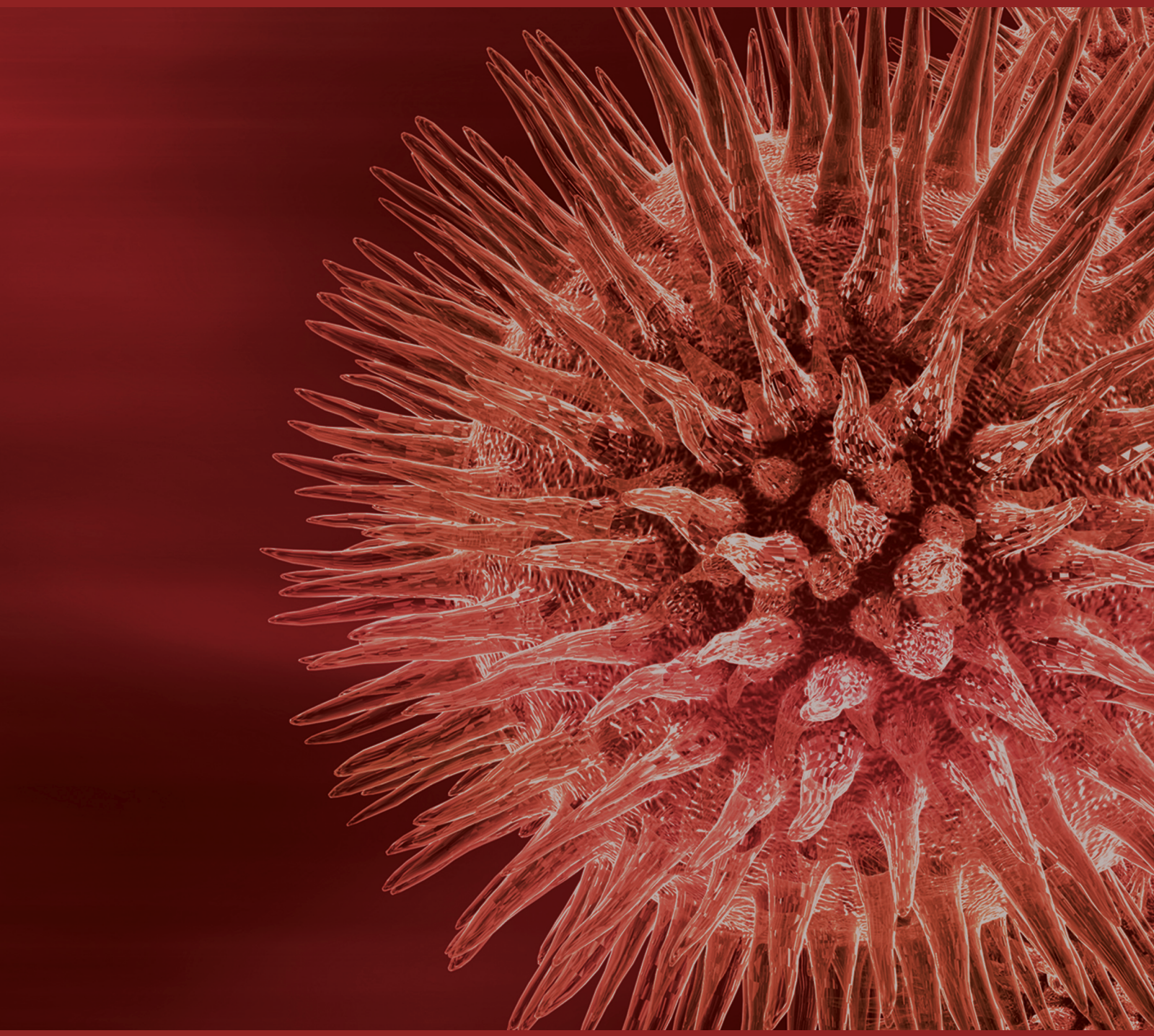
Guest Editors: Ahmet Eroglu, Engin Erturk, Can Ince, and Martin Westphal



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Editorial

Ischemia-Reperfusion Injury and Anesthesia

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Ischemia-reperfusion injury represents a pathological condition characterized by an initial undersupply of blood to an area or organ followed by a restoration of perfusion and concomitant reoxygenation (= reperfusion). Ischemia typically occurs in the presence of embolism or thrombosis but can also be triggered by surgery and transplantation. Anyway, the disturbance in perfusion results in a severe imbalance between metabolic supply and demand, subsequently causing tissue hypoxia [1]. Notably, these initial changes cause time-dependent molecular and structural alterations. In this context, it is also important to consider that all tissues and organs are susceptible to ischemia, but susceptibility to an ischemic insult differs between organ systems. Whereas the brain can endure ischemia only a few minutes, other tissues (e.g., muscle) are able to withstand ischemia for a long time without signs of irreversible damage.

Interestingly, restoration of blood flow and reoxygenation is commonly associated with an exacerbation of tissue injury and a profound inflammatory response ("reperfusion injury") [1, 2]. Ischemia-reperfusion injury contributes to pathology in a wide range of conditions.

For example, myocardial ischemia followed by reperfusion typically manifests in microvascular dysfunction, death of myocytes, and myocardial stunning or dysfunction.

Ischemia-reperfusion injury (IRI) of the lung, for example, following transplantation, is characterized by nonspecific alveolar damage, edema formation, and hypoxemia. The clinical spectrum of pulmonary IRI may range from mild hypoxemia to acute respiratory distress syndrome.

In contrast to other organs, the brain is particularly susceptible to ischemia and irreversible neuronal damage already occurs after only 5 minutes of complete ischemia [3]. For brain ischemia, as occurring in the setting of stroke, reestablishing reperfusion seems to be only beneficial, if carried out within a short time period after the onset of ischemia. Reperfusion of ischemic stroke seems to be very critical, as patients may suffer from cerebral reperfusion injury manifesting in fatal cerebral edema formation and intracranial hemorrhage.

IRI of the kidney may occur in the setting of transplantation and cardiac arrest and during cardiac surgery. Here it is important to note that renal injury is usually associated with a high morbidity and mortality. The cortical-medullary region is the most susceptible region to tubular injury, inflammation, and vascular alterations.

Generally, IRI of a single organ causes the release of different proinflammatory mediators, which may subsequently induce inflammation in other organs, thereby potentially contributing to multiple organ dysfunction or even failure [4].

Different pathological processes contribute to tissue injury secondary to ischemia-reperfusion. During ischemia, limited oxygen availability leads to an impaired endothelial cell barrier function with a concomitant increase in vascular permeability and leakage due to decreases of intracellular

cAMP levels caused by a reduced adenylate cyclase activity [1]. Furthermore, ischemia-reperfusion induces cell death due to apoptosis, necrosis, and autophagy [5]. During the ischemic period, alterations in the transcriptional control of gene expression likewise occur. Another mechanism implicated in the pathophysiology of injury during ischemia is the inhibition of oxygen-sensing prolyl hydroxylase (PHD) enzymes, because they require oxygen as a cofactor. Hypoxia-triggered inhibition of PHD enzymes induces the posttranslational activation of hypoxia and inflammatory signaling cascades, which in turn regulate the stability of the transcription factors, hypoxia-inducible factor (HIF) and nuclear factor-κB (NF-κB) [2].

Reperfusion of ischemic tissue activates a complex inflammatory response without the involvement of pathogenic triggers, a phenomenon also referred to as sterile inflammation. During the initiation of this inflammatory response, endogenous molecules act as alarmins or danger-associated molecular patterns (DAMPs) [6]. The inflammatory process is stimulated through self-antigens, which are functional components of intact cells but become stimulators of innate immunity when released from injured or dying cells [6]. In 1996, Weiser et al. discovered and described a novel mechanism for reperfusion injury that involves antibody deposition and activation of the complement system leading to an acute inflammatory response [7]. One decade later, the concept of innate autoimmunity was introduced, which is based on the discovery that circulating natural antibodies recognize self-antigens and elicit an acute inflammatory response involving the complement system [8]. Although ischemia-reperfusion is typically established in a sterile environment, activation of innate and adaptive immune responses occurs and contributes to injury, including activation of pattern-recognition receptors such as TLRs and inflammatory cell trafficking into the injured organ [9]. During this inflammatory process, the coagulation system is also activated, because the innate immune system and coagulation system are highly interconnected [10]. As ischemia-reperfusion injury is a common clinical problem and is associated with relevant complications, it is important to identify therapeutic approaches which prevent or at least mitigate ischemia-reperfusion-induced organ injury and organ dysfunction.

This special issue is devoted to the modulation of ischemia-reperfusion injury by different measures and contains eight original papers addressing this clinically relevant topic. These papers are accompanied by two review articles dealing with the effects of anesthetics on ischemia-reperfusion injury. Papers from B. U. Togrul et al., D. Dohman et al., and Y. Demirci et al. are focusing on ischemia-reperfusion injury of the liver. In two of these three papers, different therapeutic interventions on hepatic ischemia-reperfusion injury are evaluated, whereas the third paper is a retrospective study in which the authors investigated the efficacy and safety of intermittent portal triad clamping with low central venous pressure during liver resection. In this context, it has been reported that remote ischemic preconditioning and therapeutic interventions can reduce liver damage after inducing ischemia-reperfusion injury. The studies by S. C. Karahan et al., B. Michèle et al., S. C. Karahan et al., D. Dohman et al., and G. Altun et al. elucidate the effects of different anesthetic techniques and drugs on ischemia-reperfusion injury. These eight papers are entitled as follows: "*The effects of remote ischemic preconditioning and N-acetylcysteine with remote ischemic preconditioning in rat hepatic ischemia-reperfusion injury model*" by B. U. Togrul et al., "*The effects of spinal, inhalation, and total intravenous anesthetic techniques on ischemia-reperfusion injury in arthroscopic knee surgery*" by S. C. Karahan et al., "*Efficacy and safety of hepatectomy performed with intermittent portal triad clamping with low central venous pressure*" by D. Dohman et al., "*Adalimumab ameliorates abdominal aorta cross clamping induced liver injury in rats*" by Y. Demirci et al., "*Evidence for the use of isoflurane as a replacement for chloral hydrate anesthesia in experimental stroke: an ethical issue*" by B. Michèle et al., "*The effect of dexmedetomidine on oxidative stress during pneumoperitoneum*" by S. C. Karahan et al., "*The comparison of the effects of sevoflurane inhalation anesthesia and intravenous propofol anesthesia on oxidative stress in one lung ventilation*" by D. Dohman et al., and "*Role of ethyl pyruvate on systemic inflammatory response and lung injury in an experimental model of ruptured abdominal aortic aneurysm*" by G. Altun et al.

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Research Article

Evidence for the Use of Isoflurane as a Replacement for Chloral Hydrate Anesthesia in Experimental Stroke: An Ethical Issue

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Since an ethical issue has been raised regarding the use of the well-known anesthetic agent chloral hydrate, owing to its mutagenic and carcinogenic effects in animals, attention of neuroscientists has turned to finding out an alternative agent able to meet not only potency, safety, and analgesic efficacy, but also reduced neuroprotective effect for stroke research. The aim of this study was to compare the potential of chloral hydrate and isoflurane for both modulating the action of the experimental neuroprotectant MK801 and exerting analgesia. After middle cerebral artery occlusion in rats, no difference was observed in 24 h survival rate, success of ischemia, or infarct volume reduction between both anesthetics. However, isoflurane exerted a more pronounced analgesic effect than chloral hydrate as evidenced by formalin test 3 hours after anesthesia onset, thus encouraging the use of isoflurane in experimental stroke models.

1. Introduction

A cerebrovascular accident is defined by a sudden neurological deficit of vascular origin, as the damage of brain parenchyma arises from infarction (ischemic stroke) or bleeding (hemorrhagic stroke). Stroke is the third cause of death and the leading cause of acquired adult disability in the world, with still no neuroprotectant and few therapeutic options. This situation challenges the current knowledge of stroke pathophysiological events as well as the relevance of experimental models. This led researchers to develop several ischemic stroke models in a variety of species. To simulate human ischemic stroke, with 80% of which being thrombotic or embolic occurring in the territory of the middle cerebral artery (MCA), the intraluminal middle cerebral artery occlusion (MCAO) model has been set up and has been proved to be valuable in the field. This model requires a particularly efficient anesthesia of animals to induce stroke surgically. Indeed, it has both to last enough time for the microsurgery of the brain to be successful and to allow the awakening to be rapid. Moreover, in addition to safety, anesthesia of experimental stroke models has to be free of neuroprotective effect in order to avoid bias in the neuroprotection studies. Finally, for ethical reasons, the ideal anesthetic should provide postoperative analgesia since analgesics cannot be used in stroke model because of their potent neuroprotective action [1, 2].

To date, experimental anesthesia is based on an intraperitoneal injection of chloral hydrate since this method was confirmed as safe and realistic [3], with minimal effects on cardiovascular function or reflexes, as well as absence of synergistic neuroprotection in stroke studies [4]. However, when surgical anesthesia doses are administered, the safety margin is significantly reduced and recovery is prolonged [5]. Chloral hydrate metabolism has received increased attention in recent years as a metabolite of trichloroethylene, a metal-degreasing solvent found to be carcinogen in rodents [6, 7]. Moreover, hepatocarcinogenicity has been associated with

the cardiopathic effect of high dose of chloral hydrate [8]. For these reasons, the French Agency for Safety of Health Products (AFSSAPS) has questioned the benefit/risk ratio of this compound in 2000, leading to possible withdraw from the market [9].

Isoflurane is a volatile halogenated gas that is currently used in stroke studies but also reported to exert neuroprotection [10, 11], the mechanisms of which are however poorly understood. Among numerous mechanisms, isoflurane may antagonize the NR1 subunit of the N-methyl-D-aspartate (NMDA) glutamate receptor, thus preventing its input on synaptic transmission [12, 13]. However, this anesthetic agent has very interesting advantages such as ease of use, titration, and rapid awakening which deserves interest in MCAO model.

To assess the ability of isoflurane and chloral hydrate to interfere with pharmacologically induced neuroprotection, we used MK801, a well-described NMDA receptor antagonist, used as a reference compound for experimental neuroprotection in rodents [14–16]. Since animal pain is centrally considered in the ethical issues of experimental studies, and therefore in the requirements for anesthetics, a pain assessment was included in the study in order to check for the postoperative analgesic potential of both compared anesthetics.

2. Experimental Procedure

2.1. Animals and Drugs Administration. All experiments were performed in accordance with the European Community Legislation (2010/63/UE). The Local Ethics Committee approved the experiments. All rats in this study were adult male Wistar (Janvier SAS, Le Genest-St-Isle, France) weighing 280–330 g according to MCAO model. Animals were housed under controlled laboratory conditions, with a 12-hour dark cycle, a temperature of 21 ± 2°C, and a humidity of 60 to 70%. The animals had *ad libitum* access to standard chow and tap water.

Two experimental protocols were conducted during this study. The first protocol aimed at studying the modulation of infarct volumes by both anesthetics (with or without MK801). The second protocol has examined the analgesic potential of these anesthetics through a pain test, the formalin test. This test was performed on either healthy animals or ischemic animals.

Two modes of anesthesia were tested (see Table 1). MK801 (SIGMA, M107; 0.5 mg/kg in saline) was injected intravenously 3 minutes before MCAO.

One hundred and five animals were included in the study and were randomly assigned to the different groups. Ninety animals have experienced cerebral ischemia (I/R groups): chloral (CHLO), $n = 20$; chloral with MK801 treatment (CHLO + MK), $n = 25$; isoflurane (ISO), $n = 20$; isoflurane with MK801 treatment (ISO + MK), $n = 25$. Ten animals were included in the SHAM group (operated animals without MCAO), $n = 5$ per group (CHLO and ISO). Five animals were included in the Healthy group for formalin test. Healthy animals underwent no anesthesia and no surgery and were considered as controls (Figure 1).

Animals that died at the end of the protocol or survived but without successful ischemia (i.e., without lesion or with only subcortical lesion) were excluded from the study.

2.2. The MCA Occlusion Model. After anesthesia, a rectal probe was inserted and body temperature was maintained at 37 ± 0.5°C with a heating lamp.

The ostium of the right MCA was occluded intraluminally, as previously described [17]. The right carotid arteries were exposed through a midline cervical incision and the common carotid and external carotid arteries were ligated with a silk suture. The pterygopalatine artery was exposed (by developing a plane alongside the internal carotid artery) and then ligated at its origin with a fine silk. An aneurysm clip was placed across internal carotid artery and an arteriotomy was made in the common carotid artery stump, allowing the introduction of a 4/0 monofilament nylon suture with its tip rounded by flame heating. Once the suture was in place, the aneurysm clip on the internal carotid artery was removed. The suture was gently advanced into the internal carotid artery and passed into the intracranial circulatory system to lodge in the narrower lumen of the origin of the MCA. Mild resistance to this advancement indicated that the intraluminal occluder had entered the anterior cerebral artery. After 60 minutes, the suture was carefully removed, until its tip was blocked by a ligature placed on common carotid artery (to allow reperfusion). The animals were placed in a cage to recover from anesthesia at room temperature and were then allowed to eat and drink ad libitum. The sham operation consisted of the same manipulation but without introduction of the monofilament.

2.3. Formalin Test (Pain Test). The formalin test was carried out in a 30 × 30 × 30 cm clear plastic chamber. Behavior was rated for 1 hour from a score calculated for each minute period. 5 rats of each group were tested. Formalin (50 μL) was injected subcutaneously into the plantar surface of the right hind paw while the rat was restrained manually. Behaviors were observed 3 h after the MCAO in order to be sure that animals were awaked and for 1 h. The scored behaviors were those originally described by Dubuisson and Dennis [18]: 0 = normal weight bearing the injected paw; 1 = lameness during locomotion or resting the paw lightly on the floor; 2 = elevation of the injected paw so that at most the nails touch the floor; 3 = licking or biting of the injected paw. For clarity of the results, we grouped the scores every 5 minutes for 1 hour.

2.4. Infarct Volume Measurement. 24 hours after reperfusion, rats were killed with an overdose of pentobarbital (200 mg/kg, ip). Brains were rapidly removed and placed in ice-cold isopentane solution, frozen, and coronally dissected into 50 μm thick slices on a cryostat at 12 levels in 1 mm steps, according to the Paxinos and Watson stereotaxic atlas. Sections were stained with cresyl fast violet. The unstained area of the brain was defined as the infarct zone. Infarcted cortical and subcortical areas and hemispheric areas were calculated separately for each coronal slice using image analysis software (ImageJ) after digitization.

Table 1: Characteristics of anesthesia using chloral hydrate or isoflurane.

Agent	Administration route	Dose	Anesthesia duration	Mechanism of action
Chloral hydrate (CHLO)	Intraperitoneal	300 mg/kg	90 min	GABA A stimulation
Isoflurane (ISO)	Gas mask	Induction: 4% Maintenance: 2% Output: 2 L/min	20 min	Two-pore-domain potassium channels activation

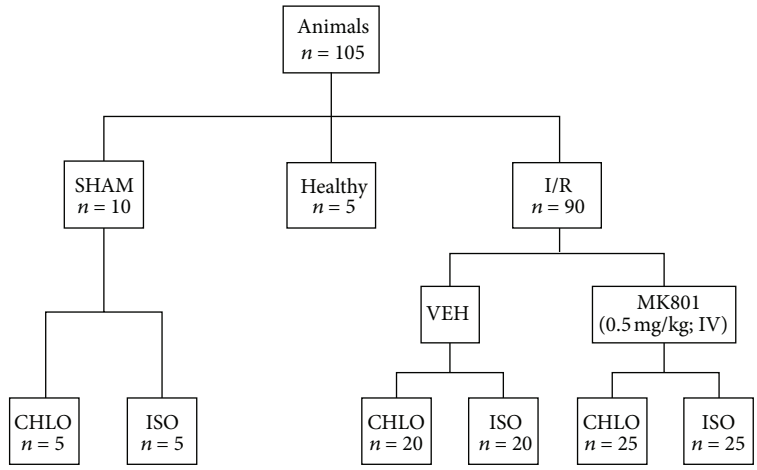


Figure 1: Experimental design and classification of animal groups. I/R: ischemia/reperfusion animals; VEH: vehicle group (saline); ISO: isoflurane anesthesia (4% induction, 2% maintenance); CHLO: chloral hydrate anesthesia (300 mg/kg ip). MK801 group corresponded to animals who received MK801 IV injection (0.5 mg/kg) 3 minutes before MCAO.

Next, infarct volumes (total, cortical, and subcortical) and hemispheric volumes (in mm^3) were calculated by the summing the respective areas for all sections for all animals and the distance between them. Lastly, the infarct volumes were corrected for the brain edema effect using the following equation: corrected infarct volume = total infarct volume − (left hemisphere volume/right hemisphere volume) [17].

2.5. Statistical Analysis. All values were expressed as mean ± standard error of the mean (SEM). Continuous variables were compared with a one-way ANOVA followed by a post hoc Tukey's Multiple Comparison Test if variance analysis was significant or with a t-test analysis. A value of $P < 0.05$ indicated statistical significance.

3. Results

3.1. Effect on 24-Hour Survival and Ischemic Event Rates. Survival of ischemic animals depended on both the type of anesthesia and MK801 treatment. Thus, the death rate reached 31% in animals that were anesthetized with chloral hydrate (14/45) and 33% in anesthetized animals using isoflurane (15/45). The total ischemia rate was similar in the two groups (Figure 2).

3.2. Effects on Infarct Volume. One-hour MCAO was followed by 23 h of reperfusion in rats according to the 2 different modes of anesthesia. The resulting infarct volumes were not different between groups, in term of total infarct volume ($P = 0.98$), cortical infarct volume ($P = 0.75$), or subcortical infarct volume ($P = 0.41$) (total: CHLO, 268.65±13.93 mm^3, ISO, 268.29±14.24 mm^3; cortical: CHLO, 200.13 ± 14.58 mm^3, ISO, 209.51 ± 13.76 mm^3; subcortical: CHLO, 62.27 ± 3.28 mm^3, ISO, 68.51 ± 3.11 mm^3) (Figure 3).

3.3. Compared Abilities to Modulate MK801-Induced Neuroprotection. First of all, we evaluated the neuroprotective effect of MK801 at a dose of 0.5 mg/kg IV 3 minutes before MCAO in animals anesthetized with chloral hydrate. The resulting infarct volumes obtained from animals treated with MK801 (CHLO + MK) decreased significantly in comparison with vehicle treated animals (CHLO) (CHLO: 268.65 ± 13.93 mm^3, versus CHLO + MK: 194.10 ± 13.76 mm^3; $P < 0.05$). Independently, MK801 induced neuroprotection in animals group anesthetized with isoflurane (ISO + MK). A significant decrease in infarct volume was found (ISO: 268.29 ± 14.24 mm^3, versus ISO + MK: 174.98 ± 17.01 mm^3; $P < 0.05$) (Figures 4(a) and 4(b)). To test whether isoflurane modulates MK801-induced neuroprotection, we compared neuroprotection observed in both anesthetics. No difference was observed between ISO + MK and CHLO + MK group ($P > 0.05$).

3.4. Compared Analgesic Abilities after MCAO. To assess the analgesic effect of both anesthetic agents, we used the

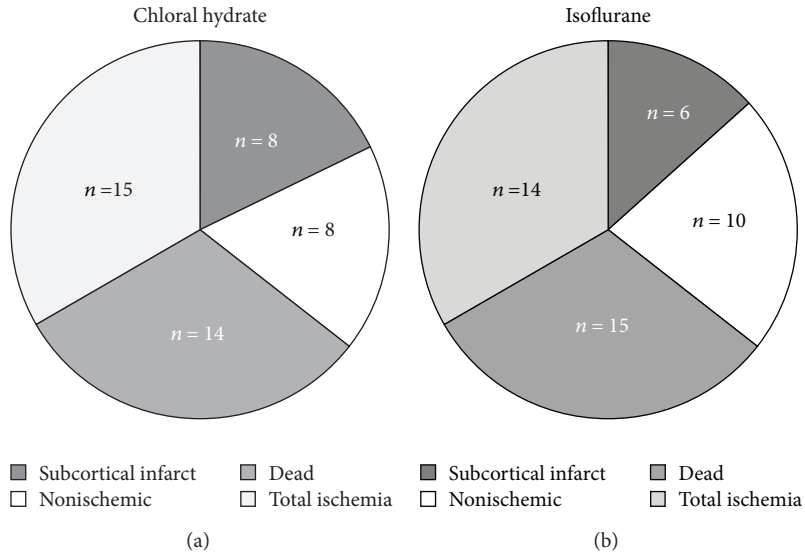


Figure 2: Effects of chloral hydrate and isoflurane on 24 h survival and ischemic events rates after MCAO. Frequency of infarct patterns, subcortical infarct areas and total infarct areas (ischemic), nonischemic and dead animals related to anesthesia type: (a) chloral hydrate; (b) isoflurane. $N = 45$ animals per group. Only ischemic animals were kept in the study.

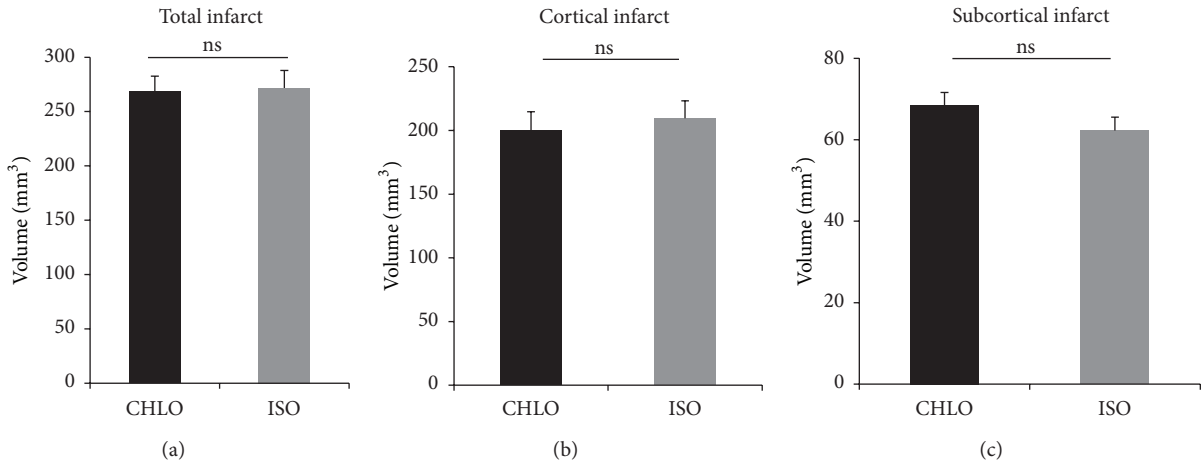
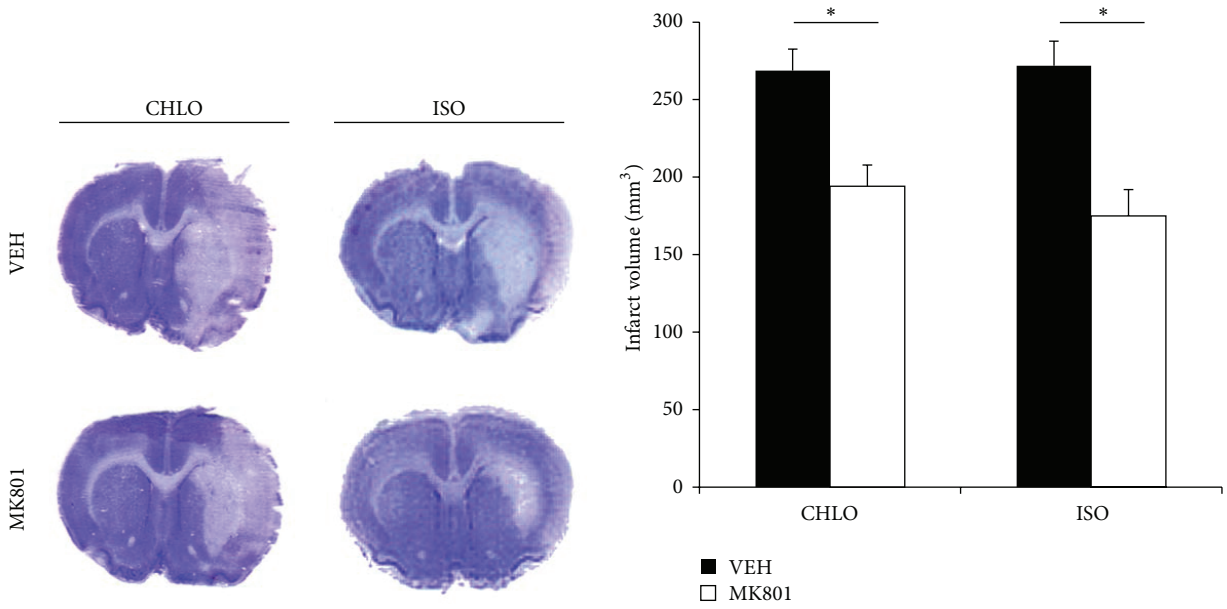


Figure 3: Compared effects of chloral hydrate and isoflurane on infarct volume after MCAO. Volumes are corrected from edema and expressed in mm^3 (mean ± SEM); $n = 8$ animals per group. Differences were not significant on total infarct volume according to t-test analysis ($P = 0.9862$), cortical infarct ($P = 0.7577$), and subcortical infarct ($P = 0.4079$).

formalin test on animals and scored their reaction every minute for 1 hour and pooled the scores every 5 minutes. Healthy animals (HEALTHY) underwent no anesthesia and no surgery and were considered as controls for the experiment. This test was carried out 3 h after induction of anesthesia, to allow for time for all the animals to wake up and to be operational for testing. As a first step, we studied the pain felt after formalin injection for 1 hour in sham animals anesthetized with either chloral hydrate or isoflurane (Figure 5(a)). A significant difference was observed between isoflurane group and healthy animals at 15 min after formalin injection ($P = 0.02$). No significant difference was observed between isoflurane and chloral groups. In a second step, we performed the formalin test on ischemic animals (I/R) anesthetized either with chloral hydrate or isoflurane. In the same way, this test was realized 3 h after induction of anesthesia. A significant difference was observed at 35 min ($P = 0.03$) and at 50 min ($P = 0.005$) after formalin injection between isoflurane group and healthy animals (Figure 5(b)). However, no significant difference was observed between isoflurane and chloral groups.

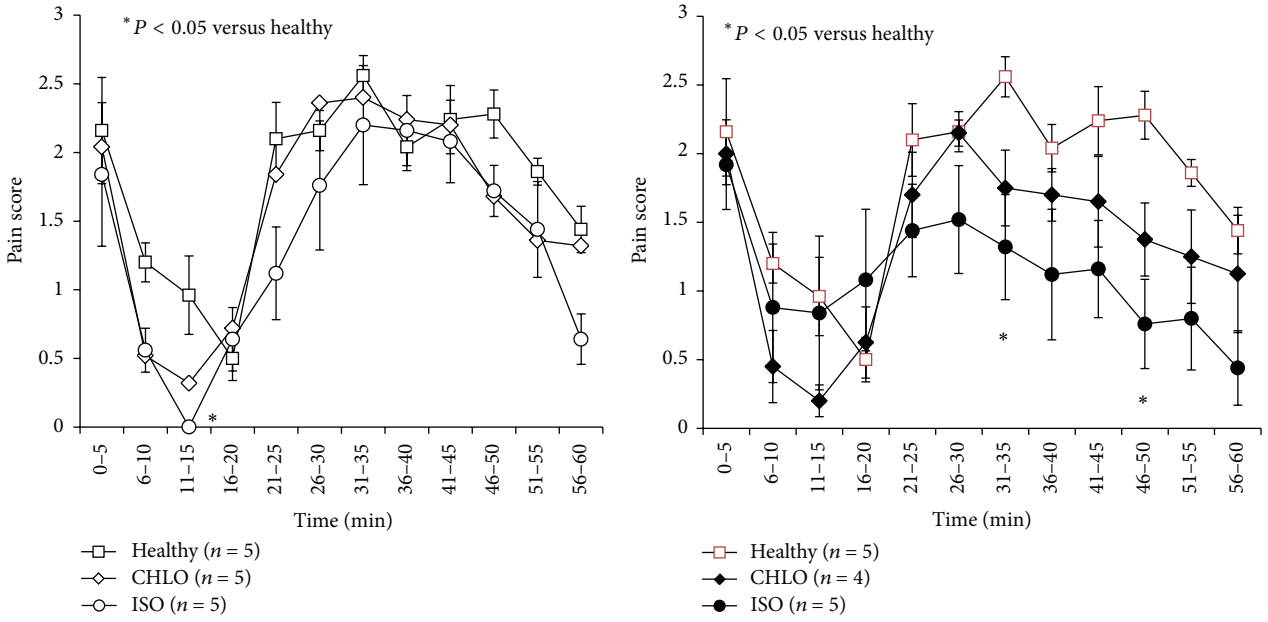
4. Discussion

In this study, we wanted to study the effect of different anesthetic agents on the extent of ischemic injury. There are different classes of anesthetics used in animal testing. Among them, we can find not only injectable anesthetics, such as



(a) Nissl staining (b) Modulation of MK801-induced neuroprotection

FIGURE 4: Neuroprotective effect of MK801 in rats anesthetized with chloral hydrate (CHLO) or isoflurane (ISO). (a) Representative brain slices after Nissl staining for each group treated with vehicle (VEH) or MK801 (MK801). (b) Infarct volumes (corrected from oedema) were determined after one-hour MCAO followed by 23 hours of reperfusion period. MK801 was injected three minutes before MCAO (IV, 0.5 mg/kg). Volumes are expressed in mm^3 (mean ± SEM); $n = 8$ animals per group. Differences in infarct volume were significant according to one-way ANOVA test ($P < 0.05$).



(a) Formalin test after 3 h (SHAM) (b) Formalin test after 3 h (I/R)

FIGURE 5: Assessment of pain after surgery including anesthesia with either chloral hydrate (CHLO) or isoflurane (ISO). Pain scores (ranging from 0 to 3) were pooled every 5 minutes. Formalin test was performed on healthy animals, sham animals, and I/R animals in each condition of anesthesia; $n = 5$ animals per group. In the SHAM group (Figure 5(a)), a significant difference was observed between isoflurane treated and healthy animals at 15 min of the formalin injection according to one-way ANOVA test ($P = 0.02$). At the other time points, no significant difference was observed between the different groups according to one-way ANOVA test. In the group I/R (Figure 5(b)), a significant difference was observed between isoflurane group and healthy animals at 35 min ($P = 0.03$) and at 50 min ($P = 0.005$) after formalin injection according to one-way ANOVA test.

barbiturates, cholinergic or morphine mimetics, but also volatile anesthetics, such as nitric oxide or halogenated gas. In the literature, pentobarbital is a widely used barbiturate, but its use is delicate because it may result in a deep sleep and respiratory distress [19, 20]. The mixture ketamine-xylazine is also commonly used in MCAO studies, but it is often reported to have an inconsistent anesthetic effect, with an extended and fluctuating induction time [21]. We therefore performed MCAO in animals asleep with either chloral hydrate or isoflurane. No significant difference was observed in terms of infarct volume and mortality in different groups. In addition, we wanted to investigate the modulation of the neuroprotection induced by MK801, an antagonist of NMDA receptor, extensively studied in stroke animal models [14–16]. Whatever the anesthetic agent used here was, the infarct volume was reduced when animals were treated with MK801. As isoflurane exerts neuroprotection through additional targets other than NMDA receptor, including two-pore-domain potassium channels, and NO-mediated cerebral artery vasodilation, we expected a potentiation of neuroprotection when coadministered with MK801 [22–27]. The surprising absence of synergistic neuroprotection in the isoflurane treated animals, compared to chloral hydrate treated animals, suggests that our single dose of isoflurane can be used without interfering with early mechanisms of stroke pathophysiology such as excitotoxicity. This hypothesis is supported by the work of Sarraf-Yazdi et al. [28], who observed the same results despite the use of lower doses of isoflurane and a higher dose of MK801 on the same MCAO model in rats. This interesting property appears with much shorter anesthesia duration, and the gas nature of isoflurane enables much easier monitoring of the anesthetic process compared to injections [29].

The second part of our study aimed at evaluating the pain felt by the animal, since it is considered as an ethical problem in experimental research [30]. The formalin test is a model of somatic pain which can be achieved in animals [31, 32]. In each experiment, we obtain a biphasic response: at the early stage (possibly related to noninflammatory processes) and the late stage (surely inflammatory) [33]. This dual response occurs through the activation of different mechanisms of pain [31], the early phase one involving the stimulation of nociceptors [18] and the late phase one resulting from peripheral inflammatory processes mediated by histamine, serotonin, or prostaglandins [34]. Besides, changes in information processing take place at the spinal level during the late phase, after the afferent stimulation previously elicited in the early phase. In 1993, Field and colleagues described chloral hydrate ability to promote a sufficient anesthesia and analgesia depth for surgical procedures [35], whereas the analgesic effect of isoflurane was poorly investigated. Here, the variability found from one animal to another in the pain felt after ischemic stroke onset, appearing less intense in ischemic than in healthy animals, suggests an impact of stroke-induced damage on brain cortical and subcortical structures involved in pain feeling. Brain infarcts may modify the perception of pain since ischemic animals were less algesic than healthy animals. Although the formalin test was carried out in the nonhemiplegic limb, some adaptive or countervailing mechanisms may modify pain signal, as put forward by the *Canadian Council on Animal Care* [36]. However, the differences between both anesthetics used in this pain study were of low magnitude. Isoflurane seemed to have a slight analgesic effect, especially at the end of the first pain phase for both sham-operated and ischemic animal groups and also at the end of the second phase in the ischemic animal groups. In the literature, isoflurane affects synaptic transmission of pain and may contribute to analgesia, up to a loss of consciousness by diminishing the strength of arousing stimuli. This effect might be involved in pain prevention during anesthesia [37]. The pain felt by sham-operated animals was thus investigated in order to check whether isoflurane could keep its analgesic effect in control condition. Pain intensity was found similar in sham-operated animals to that felt by healthy animals, except for 15 minutes after the beginning of the test, where animals under isoflurane were less sensitive than the ones of the other groups. As this time point corresponds to the very end of the first phase (noninflammatory phase) and thus to the beginning of the next (inflammatory) phase, our results suggest that isoflurane may either stimulate nociceptors or act on the peripheral inflammatory processes, as reported by Liu and colleagues at the spinal level [38].

To conclude, isoflurane seems to be a good candidate for chloral hydrate replacement and use in the rat MCAO model, with additional advantages: (i) it is easy to administer and to titrate, (ii) it has a rapid onset and recovery, (iii) it produces adequate and reproducible anesthesia depth, and (iv) it causes minimal cardiac depression. Furthermore, this anesthetic agent displays a slight analgesic effect, fulfilling an important ethical criterion for animal experimentation as referred to in the guidelines of ethics committees.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical Study

The Effects of Spinal, Inhalation, and Total Intravenous Anesthetic Techniques on Ischemia-Reperfusion Injury in Arthroscopic Knee Surgery

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Purpose. To compare the effects of different anesthesia techniques on tourniquet-related ischemia-reperfusion by measuring the levels of malondialdehyde (MDA), ischemia-modified albumin (IMA) and neuromuscular side effects. *Methods*. Sixty ASAI-II patients undergoing arthroscopic knee surgery were randomised to three groups. In Group S, intrathecal anesthesia was administered using levobupivacaine. Anesthesia was induced and maintained with sevoflurane in Group I and TIVA with propofol in Group T. Blood samples were obtained before the induction of anesthesia (t_1), 30 min after tourniquet inflation (t_2), immediately before (t_3), and 5 min (t_4), 15 min (t_5), 30 min (t_6), 1 h (t_7), 2 h (t_8), and 6 h (t_9) after tourniquet release. *Results*. MDA and IMA levels increased significantly compared with baseline values in Group S at t_2–t_9 and t_2–t_7. MDA levels in Group T and Group I were significantly lower than those in Group S at t_2–t_8 and t_2–t_9. IMA levels in Group T were significantly lower than those in Group S at t_2–t_7. Postoperatively, a temporary 1/5 loss of strength in dorsiflexion of the ankle was observed in 3 patients in Group S and 1 in Group I. *Conclusions*. TIVA with propofol can make a positive contribution in tourniquet-related ischemia-reperfusion.

1. Introduction

A proximal tourniquet is often used in lower limb surgery to provide a bloodless operative field. However, temporary occlusion of arterial blood flow during arthroscopic knee surgery and the subsequent reestablishment of perfusion after deflation of the tourniquet may result in ischemia-reperfusion injury [1–3]. When oxygen is reintroduced into ischemic tissue after tourniquet deflation, massive oxygen radicals are abruptly released into the systemic circulation. Free oxygen radicals can initiate the peroxidation of plasma lipoproteins and polyunsaturated fatty acids in cell membrane macromolecules [1, 4]. Cell injury caused by oxidative stress involves lipids, protein, and DNA, is caused by oxidative stress, and leads to the production of toxic metabolites such as malondialdehyde (MDA) and ischemia-modified albumin (IMA) [1]. MDA is a low molecular weight aldehyde and an intermediate product of lipid peroxidation. Its levels rise as an indicator of lipid peroxidation, and it has often been used as a marker of free radical formation [1–3, 5, 6]. There are many studies [7–11] concerning the rise in IMA levels in acute ischemic conditions, such as myocardial, pulmonary, cerebral, mesenteric, and skeletal muscle ischemia or infarct.

Experimental studies [12–15] have shown that neuromuscular injuries such as skeletal muscle necrosis and axonal degeneration can develop with the use of a tourniquet, especially at a pressure of 200–350 mmHg for 2 h. Tourniquet paralysis of the limb after tourniquet use is a well-recognised complication in the orthopaedic literature. This may be attributed to mechanical damage, soft-tissue oedema, contractures due to cast immobilisation, axonal compression syndrome, and vascular etiology [15, 16]. Clinical studies [16]

have demonstrated different degrees of axonal degeneration in the nerves distal to the tourniquet region. The specific injuries reported have included weakness and electromyographic changes in the upper (radial, median, and ulnar) [15] and lower (sciatic, femoral, peroneal, and tibial) limb nerves [14, 16–19]. This means that the decision whether to use a tourniquet has to be made in the light of a profit and loss equation. The measures that might be taken to reduce potential damage in the event of tourniquet use are currently the subject of research.

Earlier studies [1, 3, 4, 6, 7] demonstrated that antioxidants' free radical scavenging activity can restrict lipid peroxidation-related tissue injury. Propofol is one such antioxidant. It is chemically similar to vitamin E, an endogenous antioxidant, and butylated hydroxytoluene, one of the free radical scavengers [1, 20]. It alleviates the effects of stress-inducing hormones, such as adrenaline, noradrenaline, and cortisol [4]. Volatile anesthetics are thought to have a positive effect on free O_2 radical production in the mitochondrial electron transport chain with a mechanism similar to that of ischemic preconditioning [21]. Some studies [22, 23] suggest that inhalation anesthesia with sevoflurane is associated with less oxidative stress and fewer postoperative complications such as tourniquet-related nerve palsies than spinal anesthesia. However, other publications [24, 25] reported that spinal anesthesia, which is widely used in orthopaedic surgery, suppresses the metabolic response to surgery better than general anesthesia.

Previous studies [1, 2, 5, 11] have shown that both MDA and IMA concentrations rise during lower limb surgery involving a tourniquet; this elevation serves as a marker of ischemia-reperfusion. We hypothesised that an anesthesia technique that reduces MDA and IMA levels, which reflect IRI, might also reduce the side effects of ischemia-reperfusion injury. We thought that decreased ischemia-reperfusion injury might be achieved by a positive contribution of TIVA as a result of using propofol. In this randomised, prospective study, our aim was to evaluate the effect of different anesthetic techniques—TIVA with propofol, inhalation anesthesia with sevoflurane, and spinal anesthesia with levobupivacaine—on tourniquet-related ischemia-reperfusion by determining MDA and IMA levels and side effects in the context of arthroscopic ACL reconstruction surgery.

2. Materials and Methods

After obtaining the ethics committee approval and written informed consent from the patients, we studied 60 ASA physical status 1-2 patients, aged between 18 and 55, who were undergoing elective arthroscopic unilateral ACL reconstruction surgery requiring a pneumatic tourniquet. The patients had no metabolic, renal, hepatic, endocrinological, or immunological disturbances and were not using any pharmacological antioxidant agents (including multivitamins, pomegranates, or excessive consumption of tea or coffee) or cigarettes. Patients were allocated randomly by sealed envelope method to one of three groups: spinal anesthesia (Group S, $n = 20$), inhalation anesthesia (Group I, $n = 20$), or total intravenous anesthesia (TIVA) (Group T, $n = 20$).

The patients were not premedicated before surgery. Heart rate (HR), noninvasive arterial blood pressure (BP), peripheral oxygen saturation (SpO_2), and end-tidal partial pressure of carbon dioxide (ET CO_2) were monitored in the operating room. Venous access was achieved for anesthesia induction and saline crystalloid solution replacement. A 20 G arterial catheter was inserted on the radial line for blood sampling, and a baseline (t_1) blood sample was taken immediately. In the TIVA group (Group T), anesthesia was induced with propofol 2.5 mg/kg and remifentanil 1–1.5 μg/kg. Anesthesia was maintained with an i.v. infusion of propofol at a rate of 10 mg/kg/h, which was reduced to 8, 6, and 4 mg/kg/h at 10 min intervals and a remifentanil 0.5–1 μg/kg/h i.v. infusion. In the inhalation group (Group I), inhalation anesthesia was induced with sevoflurane mask induction and maintained with sevoflurane at 1.5-fold of the minimum alveolar concentration and 50% nitrous oxide in oxygen. In Groups I and T, i-gel LMA was used without a muscle relaxant, and the lungs were ventilated with 60% air in oxygen (6 mL/kg tidal volume, 10–12 breaths/min, and $ETCO_2$ 34–38 mmHg). In the spinal group (Group S), spinal anesthesia was performed with patients in the lateral decubitus position. Lumbar puncture was performed at the L2-3 interspace, using a 27-gauge Quincke bevel spinal needle (Spinocan, B. Braun, Germany); 0.5% plane levobupivacaine 10–12.5 mg was injected over the course of 1 min. During the procedure, a high thigh 9.5 cm circumference by 85 cm length tourniquet was used. The tourniquet was continuously inflated to 300 mmHg on the operative leg and maintained to arrest blood flow. An arthroscopic fluid pump was maintained at the 90 mmHg level throughout the operation. Arterial blood samples were obtained preoperatively from all patients (t_1), 30 min after tourniquet inflation (t_2), immediately before (t_3), and 5 min (t_4), 15 min (t_5), 30 min (t_6), 1 h (t_7), 2 h (t_8), and 6 hours (t_9) after tourniquet release. HR, systolic BP and, SpO_2 were recorded simultaneously. Mean arterial pressure (MAP) was maintained >70 mmHg, and peripheral oxygen saturation (SpO_2) was above 95% throughout surgery in all patients. In the spinal group, all patients received 2 L/min of oxygen through a nasal cannula, and $ETCO_2$ (infrared spectroscopy) was sampled from one port of the cannula. Target hemodynamic values were defined as an increase or decrease in HR and MAP of more than 25% from baseline. If increases in blood pressure or heart rate exceeded 20%, inhaler gas sevoflurane was planned to be increased at a maximum of 2.5%. In the TIVA group, the propofol infusion rate was planned to be increased gradually. In the spinal anesthesia group, if no results were achieved despite the procedures described above, we sought to bring hemodynamics under control with an additional 1 μ gr/kg fentanil. In the event of bradycardia we planned to give 0.01 mg/kg atropine, and in the event of hypotension we planned to first give 500 mL ringer lactate/30 min, and then ephedrine if no result was achieved. All operations were performed by the same surgeon. No intra-articular procedures were performed, and no unusual circumstances or complications were encountered during the operative period. All patients were monitored postoperatively in terms of the development of neurological deficits and motor loss.

Table 1: Patients characteristic data and tourniquet duration.

	Group I ($n = 20$)	Group S ($n = 20$)	Group T ($n = 20$)	P value
Age (year)	44.4 ± 12.4	36.4 ± 13.8	38.1 ± 12.2	0.131
Height (cm)	176 ± 11	170 ± 8	174 ± 10	0.368
Weight (kg)	75 ± 9	74 ± 10	77 ± 7	0.401
Sex (M/F)	9/11	8/12	10/10	0.233
ASA (1/2)	12/8	11/9	13/7	0.291
Tourniquet duration (min)	64.1 ± 18.1	68 ± 30.1	68.1 ± 22.7	0.434

Patients characteristic and surgical data are mean ± SD or number of patients: Group I: inhalation, Group S: spinal, and Group T: total intravenous anesthesia group.

The individual examining the blood samples was blinded to the group assignments. For the analysis of MDA and IMA, after plasma had been obtained from the patients, the samples were kept at −80°C until use. Lipid peroxidation in samples was determined as MDA concentrations using the method described by Erturk et al. [1] and Yagi [26]. Briefly, a 2.4 M concentration of N/12 H_2SO_4 and 0.3 mL of 10% phosphotungstic acid were added to 0.3 mL of plasma. After incubating at room temperature for 5 min, the mixture was centrifuged at 1600 g for 10 min. Discarded supernatant and sediment were suspended in 4 mL of distilled water. Subsequently, 1 mL of 0.67% thiobarbituric acid was added, and the mixture was heated in boiling water for 60 min. The colour formed was extracted into n-butanol. The mixture was again centrifuged at 1600 g for 10 min. The absorbance of the organic layer was read at 532 nm. Tetramethoxypropane was used as a standard, and MDA levels were calculated as mmol L^{-1}. After placing the blood samples in plain tubes containing separation gels, they were allowed to clot for 30 min and centrifuged before separating the serum. The samples were then immediately frozen and stored at −80°C for IMA assays. The results of a reduced cobalt to albumin binding capacity (IMA level) assay were analysed using the rapid and colourimetric method described by Erturk et al. [1] and Bar-Or et al. [27]. Two hundred microliters of patient serum was placed in glass tubes, and 50 mL of 0.1% cobalt chloride ($CoCl_2 \cdot 6H_2O$; Sigma, St. Louis, MO, USA) in H_2O was added. After gentle shaking, the solution was incubated for 10 min, to ensure sufficient cobalt albumin binding. Fifty microliters of DTT (1.5 $mgml^{-1}$ of H_2O, Sigma) was added as a colourising agent, and the reaction was quenched 2 min later by adding 1.0 mL of 0.9% NaCl. A colourimetric control was prepared for preoperative and postoperative serum samples. For the colourimetric control samples, 50 mL of distilled water was substituted for 50 mL of 1.5 mg mL^{-1} DTT. Specimen absorbencies were analysed at 470 nm using a spectrophotometer (Shimadzu UV1601, Auburn, Australia). The colour of the DTT-containing specimens was then compared with that of the colourimetric control tubes. The results were reported as absorbance units (ABSUs).

2.1. Statistical Analysis. The sample size was estimated using MDA and IMA levels as the primary endpoint. On the basis of our previous study [1] and assuming an SD of 0.7 mmol L^{-1}, 17 patients would be required in each group for an 80% probability of detecting a difference of 0.7 mmol L^{-1} in MDA values at a 5% level of significance.

The Kolmogorov-Smirnov test was used to determine the normality and homogeneity of the data distribution. Parametric data (age, weight, height, tourniquet time, and surgery time) were compared using one-way analysis of variation (ANOVA). Discrete data (sex and ASA) and side effects were compared using the chi-squared test. A repeated measures ANOVA was used for multiple comparison of all 9 repeated measures. Post hoc comparisons were performed using the Tukey test. All of these repeated measurements were compared within groups using the paired t-test. Side effects were analysed using the chi-squared test. The results are presented as the means ± SD or number. A P value of less than 0.05 was regarded as statistically significant.

3. Results

As shown in Table 1, there were no significant differences between the groups in terms of age, sex, weight, height, or tourniquet duration. The tourniquet was applied for approximately 66.7 min. Baseline MDA, IMA, and haemodynamic values were not significantly different among the groups. There was no significant difference within or between the groups with respect to HR, MAP, SpO_2, or $ETCO_2$ values (Figures 1 and 2).

Plasma concentrations of MDA increased significantly compared with the baseline values in Group S throughout the period from 30 min after tourniquet inflation (t_2) to 6 h after release (t_9) (t_2–t_9). The plasma concentrations of MDA in Group T were significantly lower than those in Group S, again from 30 min after tourniquet inflation (t_2) to 2 h after release (t_8) (t_2–t_8) (P t_2: 0.020, P t_3: 0.000, P t_4: 0.000, P t_5: 0.000, P t_6: 0.007, P t_7: 0.028, and P t_8: 0.010, $P < 0.05$ for all) (Table 2). Plasma MDA concentrations in Group I were significantly lower than those in Group S from 30 min after tourniquet inflation (t_2) to 6 h after release (t_9) (t_2–t_9) (P t_2: 0.019, P t_3: 0.001, P t_4: 0.02, P t_5: 0.001, P t_6: 0.025, P t_7: 0.001, P t_8: 0.023, and P t_9: 0.022, $P < 0.05$ for all) (Table 2). There were no differences between Group T and Group I.

Plasma IMA concentrations increased in Group S compared with the baseline values from 30 min after tourniquet inflation (t_2) to 1 h after release (t_7) (t_2–t_7). Plasma IMA concentrations in Group T were significantly lower than those in Group S, again from 30 min after inflation (t_2) to 1 h

Table 2: Changes in plasma MDA levels.

	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_9
Group S	1.8 ± 0.13	3.1 ± 0.14	3.27 ± 0.19	2.9 ± 0.15	3.5 ± 0.17	3.25 ± 0.16	3.25 ± 0.16	3.52 ± 0.17	3.46 ± 0.13
Group I	1.74 ± 0.07	1.92 ± 0.11	1.68 ± 0.09	1.73 ± 0.07	1.91 ± 0.14	2.37 ± 0.14	1.9 ± 0.13	1.83 ± 0.08	2.24 ± 0.17
Group T	1.79 ± 0.06	2.23 ± 0.20	1.59 ± 0.05	1.55 ± 0.06	1.65 ± 0.05	1.99 ± 0.07	2.15 ± 0.09	2.21 ± 0.14	2.43 ± 0.13
P	0.250	$0.009^{a,b}$	$<0.0005^{a,b}$	$<0.0005^{a,b}$	$<0.0005^{a,b}$	$0.008^{a,b}$	$0.004^{a,b}$	$0.001^{a,b}$	0.018^{a}

Plasma levels of malonyldialdehyde (MDA) (mmol/L). From all patients, preoperatively (t_1), 30 min after tourniquet inflation (t_2), immediately before (t_3), and 5 min (t_4), 15 min (t_5), 30 min (t_6), 1 h (t_7), 2 h (t_8), and 6 hours (t_9) after tourniquet release. ($P < 0.05$; for a: Group T versus S, for b: Group I versus S). Group I: inhalation, Group S: spinal, and Group T: total intravenous anesthesia group.

Table 3: Changes in plasma IMA levels.

	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_9
Group S	0.24 ± 0.04	0.30 ± 0.04	0.28 ± 0.04	0.29 ± 0.05	0.29 ± 0.05	0.28 ± 0.06	0.28 ± 0.04	0.28 ± 0.05	0.26 ± 0.07
Group I	0.22 ± 0.07	0.26 ± 0.05	0.26 ± 0.05	0.26 ± 0.05	0.25 ± 0.05	0.25 ± 0.04	0.26 ± 0.06	0.26 ± 0.05	0.27 ± 0.04
Group T	0.20 ± 0.07	0.22 ± 0.07	0.20 ± 0.07	0.22 ± 0.07	0.22 ± 0.06	0.22 ± 0.06	0.22 ± 0.05	0.25 ± 0.04	0.24 ± 0.05
P	0.220	0.001^{a}	$<0.0005^{a}$	0.001^{a}	0.001^{a}	0.010^{a}	0.001^{a}	0.126	0.251

Plasma levels of ischemia-modified albumin (IMA) (ABSU). From all patients, preoperatively (t_1), 30 min after tourniquet inflation (t_2), immediately before (t_3), and 5 min (t_4), 15 min (t_5), 30 min (t_6), 1 h (t_7), 2 h (t_8), and 6 hours (t_9) after tourniquet release. $P < 0.05$; for a: Group T versus S. Group I: inhalation, Group S: spinal, and Group T: total intravenous anesthesia group.

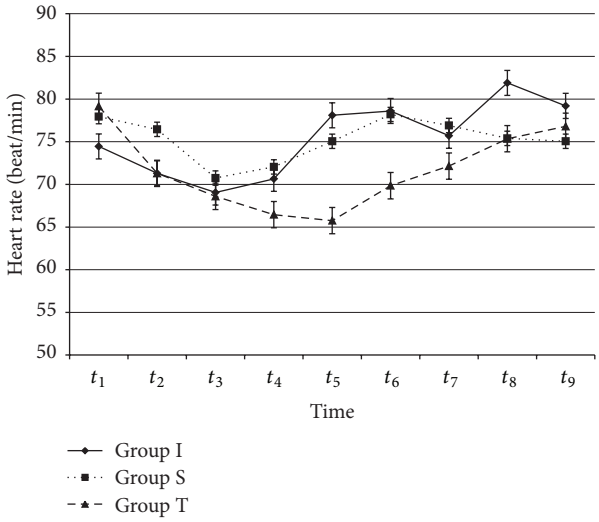

Figure 1: Heart rates values (beat/min). From all patients, preoperatively (t_1), 30 min after tourniquet inflation (t_2), immediately before (t_3), and 5 min (t_4), 15 min (t_5), 30 min (t_6), 1 h (t_7), 2 h (t_8), and 6 hours (t_9) after tourniquet release. $P > 0.05$, for all values.

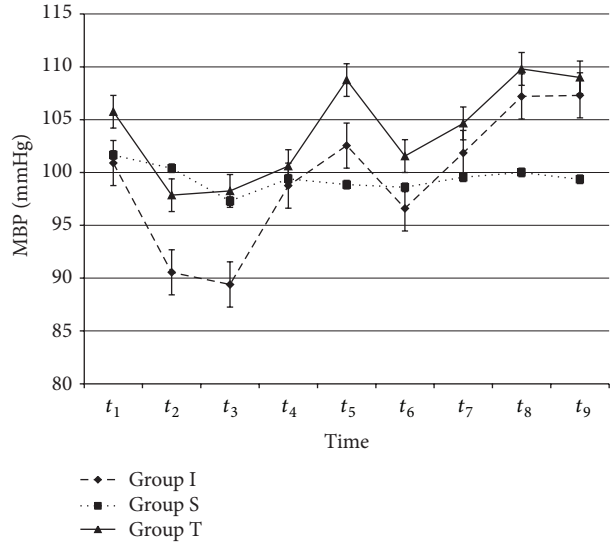

Figure 2: Mean arterial pressure values (mmHg). From all patients, preoperatively (t_1), 30 min after tourniquet inflation (t_2), immediately before (t_3), and 5 min (t_4), 15 min (t_5), 30 min (t_6), 1 h (t_7), 2 h (t_8), and 6 hours (t_9) after tourniquet release. $P > 0.05$, for all values.

after release (t_7) (t_2–t_7) (P t_2: 0.001, P t_3: 0.000, P t_4 0.001, P t_5: 0.000, P t_6: 0.007, and P t_7: 0.001, $P < 0.05$ for all) (Table 3). There were no differences between Groups T and I or between Groups I and S.

All patients were evaluated neurologically 3 h after complete recovery from anesthesia. A 1/5 loss of strength in dorsiflexion of the ankle was observed in 3 patients in Group S and 1 in Group I. No loss of strength was observed in any patient in Group T. The difference between the groups was analysed using the chi-squared test, and no difference was observed (chi-squared; 3.75, P df2, P: 0.153). The loss of strength in dorsiflexion resolved within 1 day after surgery in all patients, so no advanced test (EMG) was performed.

4. Discussion

We measured ischemia-reperfusion-associated plasma concentration changes caused by tourniquet use in MDA and IMA, which were regarded as ischemia-reperfusion markers in previous studies. From this prospective, randomised clinical study demonstrated that TIVA with propofol as an anesthetic technique may prevent the increase in the plasma concentrations of IMA and MDA as tourniquet-related ischemia-reperfusion markers in arthroscopic knee surgery.

Propofol is chemically similar to vitamin E, which is an endogen antioxidant, and butylated hydroxytoluene, one

of the free radical scavengers [11]. Propofol accumulates in biomembranes, and its radical scavenger activity as a result of the release of a hydrogen atom from its hydroxyl group occurs quite rapidly. It protects erythrocytes against oxidative stress [1, 3, 4, 28]. According to one piece of experimental research [1], propofol significantly inhibits neutrophil infiltration and MDA production in the lungs, which are target organs, after ischemia-reperfusion injury. Earlier studies [1, 3–5] confirmed that propofol alleviated tourniquet-induced ischemia-reperfusion injury in humans compared with other anesthetic agents. Studies [1, 4, 28] have used propofol as a small sedation and anesthetic dose and have demonstrated that these protocols attenuate the production of reactive oxygen species as measured by chemiluminescence in tourniquet-induced ischemia-reperfusion injury. Turan et al. [3] showed a decrease in MDA in the spinal and TIVA groups, in which propofol was used in infusion form. The positive result achieved in the spinal group in that study may be due to the use of propofol as a low-dose infusion rather than as a spinal anesthesia. We applied spinal anesthesia without using propofol or any other drug and identified this group as having the highest MDA and IMA levels of our 3 groups. Because Turan used propofol in induction in the general group and halothane in maintenance, both agents may be considered to have an effect on the outcomes. We used sevoflurane with nitrous oxide as an inhaler agent during the induction and maintenance stages in the inhalation group. In our spinal anesthesia group, which effectively represents the control group, we used no systemic anesthetic drugs, and the highest MDA and IMA levels were observed in this group.

Clinical studies [21, 29] suggest that the combined pre- and postischemic administration of potent inhalation agents has cardioprotective effects against ischemia-reperfusion injury. However, there are few studies in the literature on the physiological effect of volatile anesthetics on the skeletal muscle subjected to tourniquet-induced ischemia-reperfusion injury in humans. The effect of various anesthetic management methods on interstitial glycolysis metabolites (lactate, pyruvate, and glucose) in human skeletal muscle subjected to a tourniquet-related ischemia-reperfusion was evaluated for lower limb surgery [23]. Sevoflurane was found to involve less oxidative stress and fewer postoperative complications, such as tourniquet-related nerve palsies, than spinal anesthesia [22, 23, 30]. Inhalation anesthetic agents and propofol have been compared to examine the effect of MDA levels in different types of surgery. In one study [2], halothane and propofol were compared, and MDA levels were lower in the propofol group, although this effect did not achieve statistical significance. Another similar study [5] compared isoflurane and propofol, and the results again suggested that propofol was superior. Other studies have shown that, with the exception of isoflurane, general anesthetics provide protection against IRI at the coronary and cellular levels. All of these studies compared MDA levels and demonstrated that these were affected by general anesthesia, but they did not compare IMA levels. We investigated both MDA and IMA and observed that sevoflurane inhaled anesthesia caused a fall in MDA and IMA, although the decrease in IMA was not statistically significant. In conclusion, both sevoflurane and, to a greater extent, propofol exhibited a positive effect and lowered the rises in MDA and IMA levels. Arnaoutoglou et al. compared TIVA and inhalation anesthesia and found low MDA in the propofol group, describing this as more effective compared to the sevoflurane group [31]. Budić et al. compared TIVA and inhalation groups with a peripheral nerve block group as the control group [32]. Together with MDA, a lipid peroxidation product, they also investigated catalase activity (CAT), a first line defense mechanism antioxidant enzyme. They observed a positive effect on MDA in the TIVA and PSB groups. However, in contrast to our study, they determined high MDA values in the inhaler anesthesia group. Budić et al. used thiopental and alfentanil in induction in the inhalation group. We used only sevoflurane and nitrogen protoxide, included in the induction stage, and no other iv drug was employed. Thiopental and alfentanil employed may have given rise to these differing results.

Several experimental studies have recently been performed on the effect of remifentanil on ischemia-reperfusion injury [33–36]. These studies have reported positive effects on hepatic and intestinal ischemic reperfusion [33, 34, 36]. Remifentanil reduced hepatic apoptosis and protected against mitochondrial swelling and loss of membrane. It also reduced the TNF alpha and intracellular adhesion molecule-1 induced by ischemia-reperfusion [34]. It has also been shown to be capable of lowering superoxide dismutase and MDA levels [33, 34]. Its effects on the myocardium are unclear; however, although pre- and postconditioning targeting do establish an effect, no positive effect was established in the reperfusion and ischemia-reperfusion periods [35]. Clinical studies are needed to determine the probable effect of remifentanil on ischemia-reperfusion injury.

Literatures comparing spinal and general anesthesia show that the use of spinal anesthesia reduces the frequency of complications [25]. Ischemia-reperfusion injury and neuromuscular complications that may be regarded as-associated can primarily be prevented by techniques that are effective with regard to oxidative stress or anaerobic glycolysis metabolites. Tourniquet-associated neuromuscular deficits have been reported in the literature, with tourniquet pressure and duration being emphasised as the main causes. Our mean tourniquet pressures and durations were similar in the three groups, and there was no statistically significant difference among groups. We observed temporary neurological deficits and motor loss as side effects in 3 patients in Group S and 1 in Group I but none in Group T. Our findings may be important for selecting an anesthetic technique, particularly when a tourniquet is to be used for a longer period of time. However, further clinical studies are now needed to clarify these findings.

Our study had some limitations. First, although we investigated MDA and IMA, both of which are regarded as markers in terms of ischemia-reperfusion in the literature, had we also been able to examine antioxidant enzymes, such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), which serve as the first defence mechanism, or lactate, creatine kinase, myoglobin, and troponin as necrosis markers, these might have reinforced the results and helped to elucidate the potential mechanisms

involved. In addition, the difference in IMA levels between the spinal and inhalation groups was not statistically significant. Further studies including more patients and operations with longer tourniquet applications may produce more significant results. Additionally, we did not monitor pain and analgesic requirements.

5. Conclusion

TIVA with propofol can make a positive contribution in preventing ischemia-reperfusion-associated increases in MDA and IMA in tourniquet-related ischemia-reperfusion in arthroscopic knee surgery. Studies with a longer tourniquet period and a larger number of patients are now needed to better evaluate the effect on clinical practice of this positive contribution.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Role of Ethyl Pyruvate in Systemic Inflammatory Response and Lung Injury in an Experimental Model of Ruptured Abdominal Aortic Aneurysm

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Objectıve. The purpose of this study is to evaluate the effect of ethyl pyruvate (EP) on systemic inflammatory response and lung injury in an experimental rat model of ruptured abdominal aortic anurysm (RAAA). *Methods*. Anaesthetized 30 Sprague-Dawley male rats were randomized to sham (Sh n : 6) (Sh + EP n : 6) or shock and clamp (S/C) groups (S/C n : 9) (S/C + EP n : 9). In the S/C and S/C + EP groups, hemorrhagic shock, lower torso ischemia, and reperfusion were created, S/C group was given 1 mL saline and S/C + EP group was given 40 mg/kg EP. At the end of reperfusion process some biochemical and histological parameters were studied in serum and lung tissues. *Results*. An increase was observed in all parameters except interleukin-6 (IL-6) in the S/C group in comparison to the sham groups. In the S/C + EP group, serum myeloperoxydase (MPO), malondialdehyde (MDA), and tumor necrosis factor alpha (TNF-α) as well as lung MPO and MDA values decreased significantly ($P < 0.016$). In the lung tissues, histological injury scores and lung tissue wet/dry ratio were significantly decreased in the S/C + EP group as compared to the S/C group ($P < 0.016$). *Conclusions*. Ethyl pyruvate may reduce systemic inflammatory response and lung injury which resulted from shock and ischemia/reperfusion in an experimental model of RAAA.

1. Introduction

Ruptured abdominal aortic aneurysm (RAAA) accounts for 1-2% of deaths in the population older than 65 years of age. However endovascular therapy (EVAR), which is increasingly being performed, has decreased the mortality rate to 20–25% from 50–70% encountered in the open repair (OR) performed for the treatment of RAAA. Today, open repair is the most frequently (80%) performed method because of anatomical inconvenience, such as short neck and poor iliac arteries, or inadequate team and equipment in the vascular surgery centers [1].

Whilst ischemia and reperfusion occur only in the lower torso with the clamping and declamping during surgical treatment of intact aneurysm, preclamping hemorrhagic shock and related impaired tissue perfusion accompany the ischemia and reperfusion of the lower torso during surgical treatment of ruptured aneurysm. This complex situation leads to systemic inflammatory response syndrome (SIRS) and multiorgan failure (MOF) by causing local or distant organ damage such as lungs, liver, and heart and enhances the mortality rate in the surgical treatment of RAAA. Lindsay et al. created an experimental model and demonstrated that supramesenteric aortic clamping and hemorrhagic shock, each of which is unable to cause lung damage individually, caused distant organ damage when they are together, as in the surgical treatment of RAAA, and they used this model in numerous experimental studies [2]. Ischemia-reperfusion injury causes distant organ damage by generating systemic inflammatory response [3].

Ethyl pyruvate (EP) is a simple ester derivative of pyruvic acid. In many animal models, in which various critical

diseases including endotoxemia, sepsis, hemorrhagic shock, burn injury, pancreatitis, ileus, and myocardial, mesenteric and hepatic ischemia/reperfusion injuries were modeled; EP was demonstrated to reduce organ damage and have favorable effect on survival. EP is an effective anti-inflammatory agent and shows its efficacy by decreasing the secretion of proinflammatory proteins such as TNF-α and IL-6. EP has also been demonstrated to be an effective free oxygen radical scavenger [4–7]. Efficacy of ethyl pyruvate has not been demonstrated previously in an experimental model in which shock, ischemia, and reperfusion are found together.

The study aimed at investigating the effect of ethyl pyruvate on inflammatory response and lung injury by creating a rat model of ruptured abdominal aortic aneurysm.

2. Materials and Methods

2.1. Animal Care. The present study was performed in 30 Sprague-Dawley male rats with a mean weight of 430 ± 45 gram after the approval of Karadeniz Technical University, Animal Experiments Local Ethics Committee. The rats were kept at a room temperature of 23°C in 12/12 hours dark/light cycle and fed with standard chow and water until the study day. All rats were famished for 12 hours prior to the experiment and they were given only water

2.2. Solutions. In order to administer in the treatment groups, ethyl pyruvate (Sigma-Aldrich, Cat no. E4, 780-8) was prepared in Ringer's solution as per 1 mL would include 20 mg ethyl pyruvate. Ringer's Lactate solution was used in the control groups approximately in the same amount with EP. Ringer's solution includes 129.3 mEq/L Na^+, 5.0 mEq/L K^+, 112 mEq/L Cl^-, and 4 mEq/L Ca^{2+} and its osmolality is 275.5 mOsm/L (Polypharma, Turkey).

2.3. Experimental Design. Intraperitoneal ketamine (50 mg/kg) and xylazine (10 mg/kg) were used for the anesthesia of the rats. The anesthesia was maintained with intermittent ketamine administration allowing spontaneous respiration. Rats were randomized in two groups as sham (Sh) and shock/clamp (S/C); each group was redivided into two groups (Sh ($n = 6$), Sh + EP ($n = 6$), S/C ($n = 9$), and S/C + EP ($n = 9$)), in the way that the operator would be blind for the treatment. Sh and Sh + EP groups underwent no surgical procedure except for aortic exploration, whereas S/C and S/C + EP groups underwent shock for 60 minutes, ischemia for 60 minutes, and reperfusion for 120. Table 1 summarizes the experimental groups and the course of experiment.

Cutdown was performed and the right internal jugular vein was cannulated for the venous access, and the right carotid artery was cannulated using number 22 cannula (Novacath, Medipro, Co., Istanbul, Turkey) to monitor mean arterial pressure (MAP). Heart rate, MAP, rectal temperature, and respiratory rate were monitored (Nikon Kohden BSM-4113). In the course of experiment, 3 mL/kg/h of saline (0.9% NaCl) infusion (Perfusor Compact S, Brown) was done to meet the insensible losses. Rectal temperature was kept around 36.5°C using heat lamb. Midline laparotomy was performed in all groups and abdominal aorta was isolated at the level of proximal side of superior mesenteric artery and iliac bifurcation. Laparotomy was closed using 5/0 Prolene suture in the sham groups and the rats were kept under anesthesia for 4 hours. In the S/C groups, blood sample was drawn through the carotid artery cannula into the heparinized plastic injector (500 U Heparin) (Nevparin, 5000 U/mL, Mustafa Nevzat, Turkey) after the monitoring and stabilization periods, and shock was created keeping the MAP at 50 mmHg for 60 minutes and aneurysm rupture was simulated; the blood was stored at room temperature. The amount of blood obtained from the rats was calculated not to exceed 30% of the total blood volume. Equal amount of Ringer's solution was given to the S/C and Sh groups at the end of 60-minute hemorrhagic shock and equivalent period, respectively, whereas Sh + EP and S/C + EP groups received intraperitoneal (ip) 40 mg/kg EP. Abdominal aorta was explored through the midline laparotomy. All the groups underwent systemic heparinization by intravenous heparin given at a dose of 250 U/kg. In the S/C and S/C + EP groups, lower torso ischemia was created by clamping abdominal aorta separately at the level of superior mesenteric artery and iliac bifurcation using microvascular clamps. Half of the blood sample taken at that time was reinfused via venous route, and surgical x-clamp and resuscitation were simulated. Following 60-minute ischemic period, all of the remaining blood was reinfused just before the clamp was removed. After the clamps were removed, the abdomen was closed and the rats were kept in reperfusion for 120 minutes. The MAP was kept at 100 mmHg during reperfusion period by administering additional saline solution when needed. The amount of fluid administered in the course of experiment was recorded.

At the end of experimental period, all rats were sacrificed by drawing blood through the carotid artery. Hilar regions of both lungs were clamped and removed; a part of the left lung tissue was stored at −80°C as frozen for biochemical analyses, whereas the other part was fixed with formaldehyde for histopathological examinations. Right lungs were reserved for the calculation of the wet/dry weight ratio.

2.4. Laboratory Analysis. The serum levels of malondialdehyde (MDA), myeloperoxydase (MPO), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), ischemia modified albumin (IMA), and blood gases were measured in the blood samples.

MDA and MPO were measured in the lung tissue and histopathological examination was performed.

2.4.1. MDA Measurement. The red color that resulted from the reaction between MDA, a lipid peroxidation product, and thiobarbituric acid (TBA) was measured spectrophotometrically at 532 nm light. Plasma level was calculated as nanomol/mL (nanomoles per milliliter) [8].

2.4.2. MPO Measurement. Serum MPO levels were assessed using enzyme-linked immunosorbent assay (ELISA) kit (Hycult biotech, Catalog number HK105, The Netherlands).

Table 1: Schematic diagram of the interventions in each study group.

Groups	n	Shock 60 min	Treatment	Heparin 250 U/kg	(1/2) Blood resuscitation	Clamp 60 min	(1/2) Blood resuscitation	Reperfusion 120 min	Blood and tissue samples
Sh	6	–	RL	+	–	–	–	–	+
Sh + EP	6	–	40 mg/kg EP	+	–	–	–	–	+
S/C	9	+	RL	+	+	+	+	+	+
S/C + EP	9	+	40 mg/kg EP	+	+	+	+	+	+

Sh: sham; EP: ethyl pyruvate; S/C: shock/clamp; RL: Ringer's lactate solution.

Absorbance of the samples was measured by VERSA brand (designed by Molecular Devices in California, USA) microplate reader at 450 nm. Results were given as nanogram/mL [9].

2.4.3. Serum TNF-α Measurement. Serum TNF-α level was measured using enzyme-linked immunosorbent assay (ELISA) kit (Bender MedSystems, Catalog number BMS622, Vienna, Austria). Absorbance of the samples was measured by VERSA brand (designed by Molecular Devices in California, USA) microplate reader at 450 nm. Results were given as picogram/mL [10].

2.4.4. Serum IL-6 Measurement. Serum IL-6 levels were measured by a method similar to that of TNF-α. Results were given as picogram/mL.

2.4.5. Serum IMA Measurement. Reduced Cobalt-to-albumin binding capacity was evaluated by rapid and calorimetric assessment method developed by Bar-Or et al. Once blood samples were taken, serum and plasma specimens were prepared by centrifuging at 1.800 ×g for 15 minutes. The specimens were put into Eppendorf tubes and stored at −80°C until analysis. Reduced cobalt-to-albumin- binding capacity (IMA level) was analyzed using rapid colorimetric method. The results were given as absorbance units (ABSU) [11].

2.4.6. Blood Gas Measurement. At the end of experimental period, 0.5 mL of blood was drawn from the carotid artery to remove the probable serum residues and was disposed. Subsequently, approximately 1.5 mL of arterial blood sample taken into heparinized injector was transferred into the cartridge and blood gasses were measured (IRMA TRU point Blood Analysis System).

2.4.7. MDA Measurement in the Lung Tissue. A piece of lung tissue was used to measure MDA levels. The sample was minced and homogenized by an Ultra-TurraxT25 homogenizer (Janke and Kunkel IKA) in an ice-cold 1.15% KCl solution containing 0.05% Triton X-100. Tissue MDA levels were determined using the method described by Uchiyama and Mihara. Tetramethoxypropane was used as a standard. The MDA levels were calculated in nanomoles per gram of wet tissue [12].

2.4.8. MPO Measurement in the Lung Tissue. Tissue MPO levels were assessed using enzyme-linked immunosorbent assay (ELISA) kit (Hycult biotech, Catalog No. HK105, The Netherlands). Absorbance of the samples was measured by VERSA brand (designed by Molecular Devices in California, USA) microplate reader at 450 nm. Results were given as nanogram/mL per gram of tissue.

2.4.9. Evaluation of the Lung Edema (Wet/Dry Ratio). As the experiment was finalized, sternotomy was performed and the right lung was removed by clamping at the hilus, separated from the surrounding tissues and weighed using microbalances. It was reweighed after being stored at 70°C for 48 hours. Wet/dry weight ratio was calculated, and an increase was interpreted in favor of lung edema.

2.4.10. Histopathological Examination of the Lung Tissue. Following the finalization of the experiment, tissue samples taken from approximately the same lung sections in all rats were separately kept in numbered storages containing 10% of neutral formaldehyde solution for histopathological examination. The bloody solution was changed after 30 minutes, and the tissues were fixed with 10% neutral formaldehyde solution for 48 hours. Tissues were dehydrated by passing through the graded series of alcohol and embedded into paraffin after being made pellucid. The paraffin blocks were cut in 5 micrometer (μm) thicknesses by a microtome (Leica RM2255, Japan) and placed onto numbered slides. The sections on the slides were placed in a woven basket, kept in the incubator at 50°C for 30 minutes, and then deparaffinized and dehydrated with alcohol series. These sections were stained with Hematoxylin and Eosin (H&E); the preparations were dehydrated with alcohol series and xylene; entellan was dripped on the preparations and covered with lamella. Histopathological examination was done by an experienced histologist blind for the study groups. For the injury scoring of the lung tissues, 5 different areas in the lung preparations of each group were semiquantitatively evaluated with high magnification (400x) according to the criteria defined below [13]. Microscopic scoring criteria of lung injury were graded between 0 and 4. Grade 0: normal lung morphology, Grade 1: mild intra-alveolar edema and mild inflammatory cell infiltration, Grade 2: moderate intra-alveolar edema and moderate inflammatory cell infiltration, Grade 3: severe alveolar edema, severe inflammatory cell

Table 2: Biochemical parameters and histopathological scores in all groups (mean ± SD).

Parameters	Sh ($n = 6$)	Sh + EP ($n = 6$)	S/C ($n = 9$)	S/C + EP ($n = 9$)
MPO (ng/mL)	81.82 ± 34.21	96.91 ± 49.32	383.85 ± 38.19[a,b]	198.33 ± 69.16[a,b,c]
MDA (nmol/mL)	1.42 ± 0.43	1.45 ± 0.34	2.95 ± 1.45[a,b]	1.96 ± 0.61[a,b,c]
TNFα (pg/mL)	151.63 ± 30.64	147.52 ± 16.14	262.71 ± 18.24[a,b]	188.76 ± 56.55[a,b,c]
IL-6 (pg/mL)	164.62 ± 16.18	157.98 ± 19.93	196.53 ± 52.22	183.98 ± 56.75
IMA (ABSU)	0.42 ± 0.3	0.32 ± 0.21	0.84 ± 0.43[a,b]	0.74 ± 0.22[a,b]
Lung MDA (nmol/g)	454.29 ± 35.2	461.63 ± 51.26	522.5 ± 81.12[a,b]	499.14 ± 43.2[a,b,c]
Lung MPO (ng/mL)	5636.77 ± 404.7	5894.49 ± 971.17	6541.1 ± 613.4[a,b]	6019.12 ± 394.4[a,c]
W/D weight ratio	2.28 ± 0.18	2.21 ± 0.08	5.55 ± 0.45[a,b]	3.77 ± 0.74[a,b,c]
Histopathological score	0.5 ± 0.5	0.5 ± 0.4	2.66 ± 0.7[a,b]	1.44 ± 0.72[a,b,c]

Note: Sh: sham; EP: ethyl pyruvate; S/C: shock/clamp; MDA: malondialdehyde; MPO: myeloperoxidase; TNFα: tumor necrosis factor alpha; IL-6: interleukin-6; IMA: ischemia modified albumin; W/D ratio: wet/dry ratio.
[a]$P < 0.016$ versus Sh.
[b]$P < 0.016$ versus Sh + EP.
[c]$P < 0.016$ versus S/C.

infiltration, and focal hemorrhage, and Grade 4: disseminated inflammatory cell infiltration and destruction in alveolar structure.

2.5. Statistical Analysis. Statistical analysis was performed using SPSS 15.0. Kruskal-Wallis variance analysis (the Mann-Whitney U test with Bonferroni correction as post hoc) was used to compare the groups. The results were expressed as mean ± standard deviation (SD). Statistical significance was considered to be $P < 0.016$.

3. Results

The results of serum MPO, MDA, TNF-α, IL-6, IMA, and others are given as mean ± standard deviation in Table 2.

3.1. Serum MPO. Whilst MPO level was 81.82 ± 34.21 and 96.91 ± 49.32 ng/mL in the Sh and Sh + EP groups, respectively, it increased by 4 folds, reached to 383.85 ± 38.19 ng/mL in the S/C group, and decreased to 198.33 ± 69.16 ng/mL in the S/C + EP group. These results indicated no statistical difference between groups Sh and Sh + EP, whereas MPO was significantly increased in the S/C group versus the sham group ($P = 0.002$) and statistically significantly decreased in the S/C + EP group versus the S/C group. This result suggests that ethyl pyruvate suppresses neutrophil activation, which was enhanced by shock, ischemia, and reperfusion.

3.2. Serum MDA. Lipid peroxidation is one of the reactions caused by free oxygen radicals, and MDA is one of the end products of lipid peroxidation. MDA levels were increased by two folds in the S/C group versus the sham groups and reached to 2.95 ± 1.45 nmol/mL ($P = 0.000$) but decreased to 1.96 ± 0.61 nmol/mL in the S/C group that received EP ($P = 0.004$).

3.3. TNF-α. TNF-α, which is an important proinflammatory cytokine, was significantly increased at the end of clamping and reperfusion periods as compared to the sham groups. Comparing to the S/C group, TNF-α concentration was significantly suppressed in the S/C + EP group and decreased to 188.76 ± 56.55 pg/mL from 262.71 ± 18.24 pg/mL ($P = 0.004$).

3.4. Interleukin-6. IL-6 level was minimally increased in the S/C group versus the sham groups, but no statistically significant difference was found between the groups (whilst it was 196.53 ± 52.22 pg/mL in the S/C group, it was 183.98 ± 56.75 pg/mL in the S/C + EP group, $P = 0.48$).

3.5. IMA. This marker, which depends on the measurement of the amount of circulating albumin modified due to ischemic stress, was increased by two folds from 0.42 ± 0.3 ABSU in the sham group to 0.84±0.43 ABSU in the S/C group ($P = 0.003$) but was not significantly decreased in the S/C + EP group ($P = 0.22$).

3.6. Lung MDA and MPO. Lung tissue MDA level was significantly increased to 522.5 ± 81.12 nmol/g in the S/C group from 454.29 ± 35.2 nmol/g in the sham groups ($P = 0.000$) and decreased to 499.14 ± 43.2 nmol/g in the S/C + EP group ($P = 0.001$).

Likewise, lung MPO level increased to 6541.1 ± 613.4 ng/mL in the S/C group from 5636.77 ± 404.7 ng/mL in the sham group and decreased to 6019.12 ± 394.4 ng/mL in the S/C + EP group ($P = 0.014$).

3.7. Lung Wet/Dry Weight Ratio. There was no significant difference between the sham groups ($P = 0.748$) in terms of lung wet/dry weight ratio, but it was increased in the S/C group. The wet/dry weight ratio of the lung tissues decreased to 3.77 ± 0.74 in the group that received ethyl pyruvate from 5.55 ± 0.45 in the SIR group and lung edema was significantly reduced ($P = 0.001$).

3.8. Blood Gase. Blood gas analysis of the groups revealed increase in the acidosis and base gap in the S/C and S/C + EP groups versus the sham groups. pH decreased to 7.20 ± 0.09

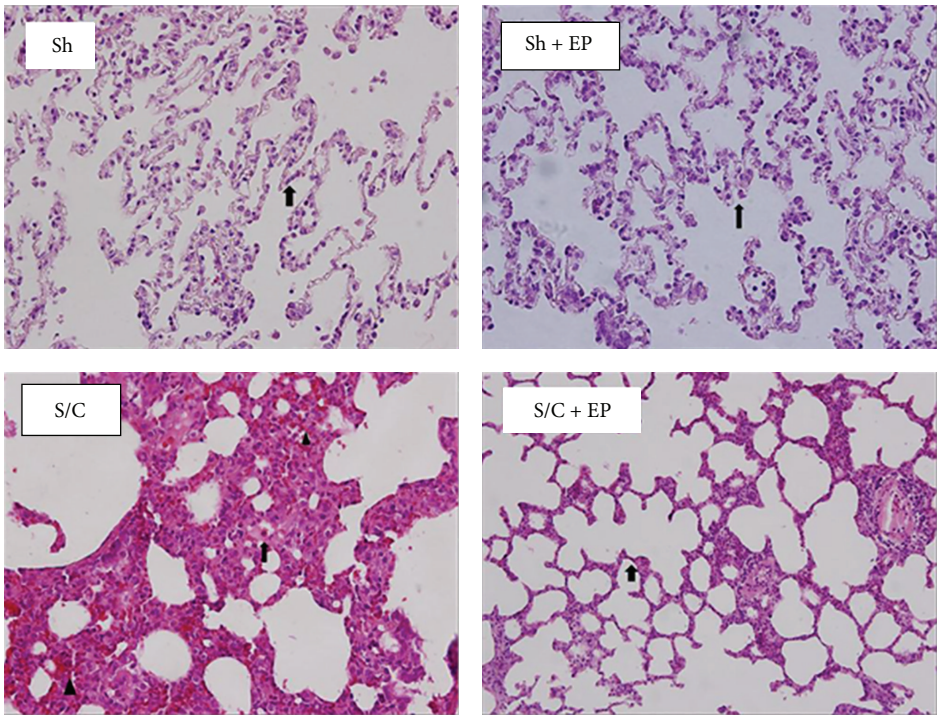

Figure 1: (Sh, Sh + EP) Normal histopathological examination of the lung tissues in Sh and Sh + EP groups. (S/C) Diffuse intra-alveolar edema (↑), intra-alveolar hemorrhage (▲), and leukocyte infiltration in the S/C group. (S/C + EP) Moderate intra-alveolar edema and alveolar epithelial thickening (↑) were observed in the S/C group that received EP. (Hematoxylin and Eosin, [H&E] ×200).

in the S/C group from 7.38 ± 0.04 in the sham groups (P = 0.014); although it decreased to 7.33 ± 0.07 in the S/C + EP group, it was not statistically significant (P = 0.4). Whilst base gap (BG) was 0.43 ± 1.38 in the Sh group, it was −9.38 ± 5.04 in the S/C group (P = 0.01) and decreased to −5.64 ± 2.8 in the S/C + EP group, but this improvement was not significant (P = 0.48). No difference was found between the groups in terms of PO_2, PCO_2, and HCO_3 values. Blood gas values are summarized in Table 3.

3.9. Lung Injury. Histopathological examination of the lung tissues revealed normal lung tissue in the sham groups but diffused intra-alveolar edema, intra-alveolar hemorrhage, and leukocyte infiltration in the S/C group. Moderate intra-alveolar edema and alveolar epithelial thickening were observed in the S/C group that received EP (Figure 1). It were observed that histopathological injury score of the lung tissue was increased by 5 folds and reached to 2.66 ± 0.7 in the S/C group from 0.5 ± 0.8 in the sham group but decreased to 1.44 ± 0.72 in the S/C group given EP (P = 0.012).

3.10. Hemodynamic Alterations and Fluid Resuscitation. Whilst the mean arterial pressure (MAP) was stable in the sham groups over the course of experiment, the amount of saline given to achieve the baseline level of MAP was low in these groups.

After the baseline level was achieved, the MAP was kept around 50 mmHg in the S/C and S/C + EP groups in accordance with the experiment protocol. The amount of blood taken from the rats was 6.7 ± 0.5 mL for each group. MAP was higher in the S/C groups than the sham groups during 60-minute aortic clamping period after the shock period. Whilst MAP was 85.9±4.2 mmHg in the sham groups, it was 132.7 ± 5.9 mmHg in the S/C groups (P = 0.0012).

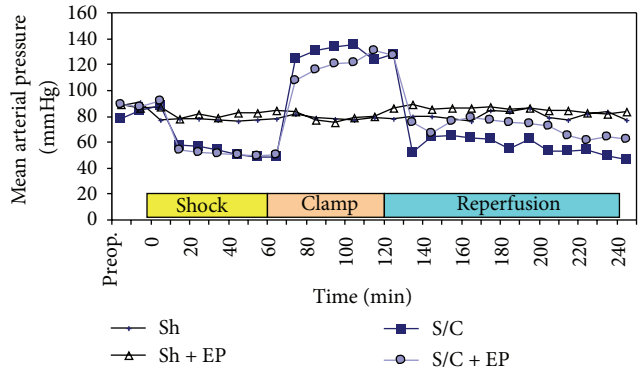

Figure 2: Mean arterial blood pressure during the experiment. Values are mean ± SEM.

During reperfusion phase, MAP decreased rapidly and became lower than that in the sham groups; decrease in MAP in the S/C group was more remarkable through the end of 120-minute reperfusion period (Figure 2). Whilst MAP was 51.34 ± 6.3 mmHg in the S/C group on the 120th minute of reperfusion period, it was 64.91 ± 5.8 mmHg in the S/C + EP group (P = 0.011).

Table 3: Blood gas values.

	pH	PO_2	PCO_2	HCO_3	BE
Sh	7.38 ± 0.04	115.75 ± 12.34	41.8 ± 5.7	25.45 ± 1.73	0.43 ± 1.38
Sh + EP	7.44 ± 0.08	107.43 ± 9.54	34.3 ± 11.6	23.51 ± 5.14	0.13 ± 2.49
S/C	7.20 ± 0.09[a,b]	79.88 ± 18.39	36.1 ± 7.05	15.4 ± 4.7	−9.38 ± 5.04[a,b]
S/C + EP	7.33 ± 0.07[a,b]	87.11 ± 14.29	36.9 ± 7.47	19.24 ± 2.88	−5.64 ± 2.8[a,b]

PaO_2: arterial oxygen pressure; $PaCO_2$: arterial carbon dioxide pressure; HCO_3: bicarbonate; BE: base excess.
[a] $P < 0.016$ versus Sh.
[b] $P < 0.016$ versus Sh + EP.
[c] $P < 0.016$ versus S/C.

Total amount of extra fluid given in addition to the maintenance fluid was 5.2 ± 0.7 mL in the S/C group, whereas it was 3.4 ± 0.6 mL in the S/C + EP group ($P = 0.0018$).

4. Discussion

In the present study, we investigated whether ethyl pyruvate reduces systemic inflammatory response and lung injury that result from shock, ischemia, and reperfusion in the surgical treatment of the rat model of ruptured abdominal aortic aneurysm. Systemic inflammatory response syndrome (SIRS) and related multiorgan failure are the most important causes of high mortality following surgical treatment of ruptured abdominal aortic aneurysm. Whilst the prevalence of multiorgan failure is 3.8% after surgical treatment of intact aneurysms, it is 64% after RAAA [14].

Unlike intact aneurysm, total body ischemia, which varies depending on the duration and deepness of hemorrhagic shock, occurs in ruptured aneurysms and lower torso ischemia and reperfusion, which result from aortic clamping, accompany this situation in the course of surgery. Although many systems including complement, coagulation, fibrinolytic, and kallixrein cascades are activated during inflammatory response triggered together by hemorrhagic shock, ischemia, and reperfusion, leukocytes play the most important role. Activation and interaction of leukocytes and endothelial surface initiate the cytokine release, endothelial microvascular permeability is increased, and transendothelial neutrophil migration occurs. Endothelial damage leads to endothelial cell swelling, capillary leak, edema, and organ dysfunction. This situation known as systemic inflammatory response is followed by multiple organ failure including the lungs and the kidneys. In many of the ischemia and reperfusion studies, some biochemical analyses are performed including cytokine levels such as TNF-α and IL-6 that indicate systemic or local organ damage, neutrophil activation marker MPO, MDA that indicates tissue hypoxia and free radical-induced lipid peroxidation, and ischemia-specific marker IMA; and damaged distant organ is examined both histopathologically and functionally.

Pyruvic acid, which is a carboxylic acid with three carbons, is the end product of glucose metabolism as a member of aliphatic amino acids. The reaction between pyruvate and coenzyme A results in Acetyl CoA, which is a rate-limiting step in the oxidative metabolism of glucose by thiocarboxylic acid. Although pyruvate is a metabolic fuel, it also functions as an endogenous oxygen radical scavenger.

Ethyl pyruvate (EP) is a simple aliphatic ester derivative of pyruvic acid. In various animal experiments, it was demonstrated to be an effective anti-inflammatory agent by inhibiting proinflammatory signal pathways such as NF-κB and p38 mitogen-activated proteinkinase. Moreover, it reduces (downregulates) the secretion of proinflammatory proteins such as TNF, IL-6, and high mobility group box 1 (HMGB1), and was demonstrated to be an effective reactive oxygen species (ROS) scavenger [15, 16]. Since ethyl pyruvate is stabilized by forming enolate with calcium, it turns into a more stable compound when dissolved in Ringer's lactate solution. Therefore, many of the studies use ethyl pyruvate dissolved in Ringer's lactate. Cruz e al. performed ischemia-reperfusion study using various solutions of pyruvate and found that ethyl pyruvate to be more effective than other pyruvate derivatives [15]. In the present study, we as well used this solution and additionally formed a sham group with Ringer's lactate similar to the literature.

Lindsay et al. conducted an experimental study and created shock, ischemia, and reperfusion, and this method was called experimental model of ruptured abdominal aortic aneurysm [2]. In this study, shock, ischemia, and reperfusion procedures were performed in the experimental groups in different periods; aortic clamp was applied to different places as supramesenteric and inframesenteric, and pulmonary permeability index and MPO activity in the lung tissue were measured in all groups and were compared with each other. As a result, it was determined that 1-hour shock, 2-hour supramesenteric clamping, and 2-hour reperfusion periods provide the highest increment in pulmonary permeability index and lead to neutrophil accumulation. Later on, this model has been used in many studies. In the present study, we as well investigated systemic inflammatory response, lung injury, and the effect of ethyl pyruvate on this injury, in a model of ruptured abdominal aortic aneurysm, in which shock, ischemia, and reperfusion could be stimulated all together. We measured serum MDA, MPO, TNF-α, IL-6, IMA, and blood gas values, as well as MDA, and MPO values in the lungs. In addition, we histopathologically examined the lung tissue and assessed the lung edema.

Lipid peroxidation is one of the reactions caused by free oxygen radicals, and MDA is one of the end products of lipid peroxidation. Measurement of MDA level in the plasma and tissues gives information about free oxygen radicals. In

a substantial proportion of experimental IR studies, information about lipid peroxidation in the serum and tissues was obtained by measuring serum MDA values using various methods. Jan et al. measured MDA values as a parameter to investigate whether ischemic preconditioning performed prior to shock reduces lung injury [17]. In the present study, it was observed that MDA values were remarkably increased both in the serum and in the lung tissues of the S/C group versus the sham group and that radical production was suppressed in the S/C + EP group. Ethyl pyruvate remarkably suppressed lipid peroxidation and accordingly free radical production. Taylor et al. created a rat model of myocardial ischemia-reperfusion and demonstrated that ethyl pyruvate reduces lipid peroxidation in the ischemic myocardial tissue [18]. Cruz et al. found that ethyl pyruvate significantly reduces hepatic MDA level in the visceral ischemia-reperfusion model [15]. Results of the present study are consistent with these two studies.

MPO, which is a peroxidase enzyme and mostly found in the neutrophils, is a lysosomal protein stored in the azurophilic granules of the neutrophils. Measurement of serum and tissue MPO values gives information about neutrophil activation. In the present study, increased MPO activity both in the systemic circulation and in the lungs due to S/C application was suppressed by EP. Wang et al. and Luan et al. demonstrated that MPO activity in the ileum was suppressed by EP in the extrahepatic cholestasis and in acute pancreatitis, respectively [19, 20]. The results of the present study are consistent with the results of above-mentioned authors. There is no evidence that ethyl pyruvate suppresses the neutrophil activation both in the systemic circulation and in the distant organ. With this regard, the present study is the first.

Tumor necrotizing factor-α is a very important proinflammatory cytokine that weighs 17 kDa, secreted from activated macrophages, monocytes, t-lymphocytes, killer cells, and fibroblasts, and binds to the cell membrane via specific receptors. In the present study, serum TNF-α levels were significantly increased in the S/C group and suppressed by EP administration. Cai et al. used ethyl pyruvate as the resuscitation fluid in hemorrhagic shock and demonstrated that TNF-α level was decreased and survival was increased in the rats [21]. This suggests that systemic inflammatory response is substantially suppressed by EP. Kung et al. created lung injury in rats by lipoteichoic acid and reported that ethyl pyruvate suppresses TNF-α and prevents lung injury via anti-inflammatory effect and this effect is dose-dependent [22]. In an experimental study in which Shahani performed the same RAAA model, it was published that TNF-α level was 10 times increased in the myocardial tissues of the rats versus the control groups and cardiac functions were improved by immune neutralization of TNF-α [23]. Harkin demonstrated that both C5 complement inhibition and, in another study, nitric oxide synthase inhibition (iNOS) provide effective protection in the serum and lung tissue by decreasing TNF-α [24]. In a review, Swartbol et al. reported that TNF-α was increased during surgery in almost all aneurism series and reached to the peak level in 6 hours, 15 times increased in the ruptured aneurysms versus the intact aneurysms, remained at peak levels for the postoperative 2 days, and increment persisted in those developed MODS [25].

İnterleukin-6 is responsible for the specific conditions in the inflammatory response of the host. It is produced as a response to the secretion of TNF-α, IL-1, or both cytokines. It is known that infusion of IL-6 alone does not generate any response. IL-6 initiates and stimulates oxidation in the neutrophils (respiratory burst), enhances ICAM-1 release from the endothelial cells, and enhances endothelial permeability. IL-6 is produced by hypoperfused skeletal muscle in the patients with peripheral artery disease and, in addition, it is systematically released in the reperfusion phase of aortic aneurysm surgery. In the present study, there was no difference between SIR and sham group in terms of IL-6 levels. There was no significant difference also in the SIR + EP group. Since IL-6 is generally secreted in the reperfusion phase being triggered by other cytokines, duration of reperfusion might have not been enough for this mediator to elevate. Kung et al. created a lung injury by LPS and determined decrease in IL-6 levels after 6 hours in the group received ethyl pyruvate [22]. It is known that IL-6 is increased in aneurysm ruptures and remains elevated when multiorgan failure is developed. Swartbol demonstrated that IL-6 reached to the peak levels within 6 hours to 7 days after surgical and endovascular treatment of intact AAA and RAAAs in various case series [25]. In the present study, absence of elevation in the SIR group as compared to the sham group could be explained by the fact that IL-6 has not been reached to the adequate serum levels yet in 2-hour reperfusion period. Probably, IL-6 levels could be measured more reliably in the postoperative surveys that would be performed in large animal models.

Various studies demonstrated that ischemia-modified albumin (IMA) is increased in the event of elevated free radical levels such as hypoxia or ischemia-reperfusion that tissues are exposed to sepsis, acute infection, advanced cirrhosis, end-stage renal disease, and advanced cancer [26]. IMA is ischemia-sensitive but not tissue-specific. Change in IMA in acute coronary syndromes has been investigated the most. Zhong et al. found a strong and independent correlation between IMA values and coronary artery disease, and Turedi et al. demonstrated that it is a prognostic marker after cardiopulmonary resuscitation in cardiac arrest patients [27, 28]. There are also various clinical studies demonstrating diagnostic value of IMA in the early stage of cerebrovascular events such as subarachnoid and intracranial hemorrhage or ischemic stroke, arterial occlusion, deep vein thrombosis, and mesenteric infarction. Gunduz et al. put forward the diagnostic value of IMA in acute mesenteric obstruction and in pulmonary embolus [29]. In the present study, IMA was significantly elevated in the S/C group, but the decrease in S/C + EP group was not found statistically significant. IMA has not been measured previously in the experimental model as we performed. Significantly, elevated IMA level in the SIR group confirms ischemia. Owing to overlapping other parameters, it can be used as an important marker that indicates ischemia in the experimental studies. The present study shows that IMA could be used in RAAA model. With regard to the lung's wet/dry weight ratio,

severe pulmonary edema was observed in the S/C group which was substantially improved with ethyl pyruvate. In the ischemia-reperfusion studies, "Pulmonary Permeability Index" gives the most valuable information about the lung tissue. It is a quantitative method obtained by proportioning the measurement of radioactive iodine 125-labeled albumin in the serum and in the bronchoalveolar fluid [2]. In the present study, we could not perform this method used by Lindsay in the experimental model of RAAA but obtained information about pulmonary edema using the lung's wet/dry weight ratio used in many studies, in which the pulmonary edema had been demonstrated quantitatively. In the present study, lung's wet/dry weight ratio was 2 times increased in the SIR group versus the sham groups but significantly decreased in the SIR + EP group versus the SIR group. That is to say, EP significantly reduced the pulmonary edema. This condition, which is associated with endothelial permeability, can be explained by the suppression of lipid peroxidation; this is also confirmed by decreased lung MDA levels.

Blood gas analysis exhibits gas change at alveolar level and acid-base balance of the organism. Serum pH level was significantly decreased in the S/C group versus the sham group and acidosis occurred, base gap was developed, and a little improvement occurred in the group that received EP although being not significant. It is obvious that great changes in blood gas parameters could not be tolerated by the rats kept in spontaneous respiration in the room air and would result in death. One of the most important limitations of this experiment is leaving the rats in spontaneous respiration in the room air without intubating.

Histopathological examination revealed diffuse edema, leukocyte infiltration, and interalveolar and intra-alveolar hemorrhage in the lung tissue of the SIR group. Injury score of this group increased to 2.66 from 0.5 in the sham group. In the SIR + EP group, a decrease was observed in the intra-alveolar edema and leukocyte infiltration and injury score decreased to 1.44 from 2.66 in the SIR group. There was no difference in the sham + EP group as compared to the sham group; that is, EP alone has not cause any damage. There are many studies demonstrating that ethyl pyruvate acts as anti-inflammatory, antiedema, and antioxidant agent and treats organ injury in many experimental studies including hemorrhagic shock, ischemia- reperfusion, burn, sepsis, and acute necrotizing pancreatitis [30, 31]. However, studies that demonstrate its efficacy on distant organ injury such as lungs are quite limited. Karabeyoglu et al. demonstrated that ethyl pyruvate reduces lung injury seen after burn in the rat model [32].

The present study is a unique experimental model, which provides the simulation of both hemorrhagic shock and ischemia-reperfusion in the same experimental model. In this respect, we think that it will provide an important contribution to the literature.

Over the course of experiment, the sham groups maintained stable blood pressure showing minimal need for fluid replacement; however, in the S/C group, a remarkable hypotension and need for fluid replacement were observed which particularly increased as the aortic clamp was removed and reperfusion was started. This resulted from the vasodilation caused by vasoactive mediators and certain metabolites that joined the systemic circulation from the ischemic tissues during reperfusion phase, as well as from the increased vascular permeability. In the group that received ethyl pyruvate, inflammatory cell activation was suppressed and microvascular permeability decreased, more stable blood pressure levels were obtained, and the need for replacement was reduced. Pyruvate is a compound that structurally resembles lactate and is shown to help survival in hemorrhagic conditions due to its antioxidant and anti-inflammatory effects [31]. Tawadrous et al. demonstrated that impaired intestinal permeability and hepatic lipid peroxidation due to hemorrhage were significantly decreased in those which received ethyl pyruvate as compared to those which received traditional fluid replacement [33].

In conclusion, ethyl pyruvate prevents the lung injury created by experimental rat model of RAAA and shows this efficacy reducing the systemic and local inflammatory response by means of suppressing neutrophil infiltration and production of free radicals.

Conflict of Interests

None of the authors have any conflict of interests related to this paper.

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Research Article

Adalimumab Ameliorates Abdominal Aorta Cross Clamping Which Induced Liver Injury in Rats

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The aim of this study was to investigate the possible protective effects of adalimumab (ADA) on cell damage in rat liver tissue during ischemia/reperfusion (I/R) injury of infrarenal abdominal aorta. Thirty male Wistar-albino rats were divided into three groups: control, I/R, and I/R+ADA, each group containing 10 animals. Laparotomy without I/R injury was performed in the control group animals. Laparotomy in the I/R group was followed by two hours of infrarenal abdominal aortic cross ligation and then two hours of reperfusion. ADA (50 mg/kg) was administered intraperitoneally as a single dose, to the I/R+ADA group, five days before I/R. The tumor necrosis factor-alpha (TNF-α) (pg/mg protein) and nitric oxide (NO) (μmol/g protein) levels in the I/R group (430.8 ± 70.1, 8.0 ± 1.1, resp.) were significantly higher than those in the I/R+ADA group (338.0 ± 71.6, $P = 0.006$; 6.3 ± 1.2, $P = 0.008$) and the control group (345.5 ± 53.3, $P = 0.008$; 6.5 ± 1.5, $P = 0.010$, resp.). I/R causes severe histopathological injury to the liver tissue, but ADA leads to much less histopathological changes. ADA treatment significantly decreased the severity of liver I/R injury. ADA pretreatment may have protective effects on experimental liver injury.

1. Introduction

Hepatic ischemia/reperfusion (I/R) injury affects the prognosis of patients in a vast clinical range, including transplantation, liver resection surgery, trauma and hemorrhagic shock, and aortic injury during abdominal surgery [1]. While aortic occlusion is carried out, the blood supply is occluded to organs such as the liver. The obstruction of the aorta and consequent reperfusion leads to distant organ injury via multiple mechanisms including neutrophilic infiltration, the production of reactive oxygen species (ROS), the release of cytokines such as the tumor necrosis factor-alpha (TNF-α) and elevation of nitric oxide (NO) levels [2–4]. Also, decreased arginase and carbamoyl phosphate synthetase-1 (CPS-1) enzyme activities are associated with tissue injury by elevated NO levels [5–8]. Reperfusion injury occurs after permitting blood reflow into an ischemic tissue, and the surge of oxygen to low oxygenated tissues causes an increased production of ROS [9]. The apoptosis pathway is activated as a result of mitochondrial damage due to increased ROS. Apoptosis plays a major role in liver injury induced by I/R [10].

TNF-α is a pleiotropic cytokine that has biological effects ranging from cell death to inducing tissue regeneration [11–13]. TNF-α is released at the beginning of reperfusion, and its level increases during the early phases of I/R [14]. The inhibition of TNF-α release, or its neutralization with anti-TNF-α antibodies, decreases the number of neutrophils infiltrating the liver, reducing liver I/R injury [15]. Adalimumab (ADA), which is the first fully human monoclonal antibody targeted against TNF-α, was first administered to study patients in 1997. It has been reported that it can be safely used for

certain diseases [16]. Previous studies have reported that infliximab, which is another inhibitor of TNF-α, can also decrease/prevent the damage of TNF-α in I/R models [17, 18].

The twofold aim of this study is to determine whether the inhibition of TNF-α ameliorates I/R-induced liver tissue injury by suppressing cell damage and whether the inhibition of TNF-α alters the NO balance by its effect on arginase and CPS-1 activity in liver I/R injury.

2. Materials and Methods

2.1. Animals. Thirty Wistar-albino male rats, weighing 250–300 g (12–15 weeks old), were used in the present study. The rats were indiscriminately divided into three groups: control group ($n = 10$), I/R group ($n = 10$), and I/R+ADA group ($n = 10$). This research was performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH, 1985) and approved by the local ethical committee at the Medical School of the Recep Tayyip Erdogan University (Approval numbers: 2012/11).

2.2. Experimental Design. The rats in the control and I/R groups received saline solution. The control group underwent a midline laparotomy and dissection of the infrarenal abdominal aortic cross (IAA) without obstruction. The I/R group underwent laparotomy and clamping of the IAA for 120 minutes, followed by 120 minutes of reperfusion. ADA (Humira; Abbott, Abbott Park, Ill) (40 mg/0.8 mL) was diluted in saline and given as one bolus applied in an intraperitoneal single dose injection of 50 mg/kg to the I/R+ADA group [19]. After five days of ADA application, the I/R+ADA group underwent 120 minutes of ischemia and 120 minutes of reperfusion.

2.3. Aortic I/R. The I/R model was designed in a way similar to previous studies [2, 20]. The rats were anesthetized with ketamine hydrochloride (50 mg/kg intramuscularly) (Ketalar; Eczacibasi, Istanbul, Turkey), and anesthesia was maintained with supplementary intramuscular injections of ketamine hydrochloride. The rats were located in a supine position under a heating lamp. The skin was prepared aseptically, and a midline laparotomy was implemented. Warm normal saline (10 mL) was dribbled into the peritoneal cavity to help maintain the fluid balance. The abdominal aorta was exposed by politely deflecting the loops of the intestine to the left with splashy gauze materials. An atraumatic microvascular clamp was located across the IAA. The abdomen was switched off, and the wound was covered with plastic wrap to minimize the loss of heat and fluid. After 120 minutes, the microvascular clamp on the IAA was removed and the lower limb reperfusion was maintained for 120 minutes. Aortic occlusion and reperfusion were corroborated by the loss and resurrection of the pulsation on the distal aorta; therefore, a no-reflow phenomenon was excluded. At the end of the reperfusion, a median sternotomy was enforced, and blood samples were drawn from the right ventricles of all rats for biochemical analyses. All rats were euthanized under anesthesia and their livers were cautiously removed. The specimens were stored for further biochemical and histological analyses.

2.4. Biochemical Parameters. Blood samples (10 mL) were taken from all rats and collected into routine tubes to evaluate the biochemical tests. The blood was separated by centrifugation @ 3,000 rpm for 10 min, after standing at room temperature for 15 min. The biochemical parameters, including activities of serum enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and concentrations of urea and creatinine were determined in the serum by using commercial kits (ARCHITECT C16000, Abbott Laboratories, USA).

2.4.1. Tissue Homogenates. After weighing the liver tissues, they were homogenized in ten volumes of ice-cold Phosphate Buffer Saline (PBS) (50 mM, pH 7.4) and centrifuged at 10,000 xg for 20 minutes. The homogenization procedure (wiseTise homogeniser, Korea) was carried out for 2 min at 10,000 rpm. All procedures were performed at 4°C. Aliquots of the supernatant were put into tubes and frozen at −80°C. Homogenate, supernatant, and extracted samples were prepared and the following determinations were made on the samples using commercial chemicals (Sigma, St. Louis, MO, USA). All the parameters were checked within one month.

2.4.2. Measurement of Protein. The tissue homogenate protein assay is a turbidimetric procedure in which benzethonium chloride is used as the protein denaturing agent. Proteins in the form of a fine suspension were quantitated turbidimetrically at 404 ηm (ARCHITECT c16000, Abbott Laboratories, USA).

2.4.3. Tissue TNF-α. The concentration of TNF-α was measured using an enzyme-linked immunosorbent assay (ELISA) method with a commercially available rat TNF-α ELISA kit (eBioscience, Vienna, Austria). The absorbance was measured at a λ of 450 ηm, using the ELISA reader. The intra-assay and interassay coefficients of variation were <5% and <10%, respectively. The limit of detection (LOD) for the TNF-α assay was 11 pg/mL. When dividing the obtained values by the protein levels, the final results were obtained as pg/mg protein.

2.4.4. Tissue NO. The concentrations of NO were measured using the colorimetric assay method, with a commercially available ELISA kit (Cayman Chemical Company, USA). The absorbance was measured at a λ of 540 ηm using the reader. The intra-assay and interassay coefficients of variation were 2.7% and 3.4%, respectively, and the LOD for the NO assay was 2.5 μM. When dividing the obtained values by the protein levels, the final results were obtained as μmol/g protein.

2.5. Immunohistological Evaluation. For immunohistochemical staining 3-4 μm thick sections of the liver tissues were cut and allowed to stand in xylene for 20 minutes before the application of an alcohol series (50–100%) then allowed to stand for 10 minutes in an H_2O_2 solution. After being

washed with PBS, these sections were heated in a citrate buffer solution at 800 W for 4-5 minutes and allowed to stand in secondary blocker substance for 20 minutes. Each slide was allowed to stand for 75 minutes in different dilutions of the primary antibody (Anti-CPS1 at 1 μg/mL and Anti-Arginase at 1/250-/500), before being stained by the Anti-Arginase (cod: ab124687, Abcam Plc., Cambridge, UK) and Anti-CPS-1 (cod: ab45956, Abcam Plc., Cambridge, UK). A diaminobenzidine solution was used as an achromogen, Mayer's hematoxylin as a counterstain for 3–5 minutes, and PBS as a negative control. The preparations were photographed after being covered with the appropriate covering materials. As a result of the immunohistochemical staining, the preparations were divided into 4 categories according to the tissue percentage of immunopositive reaction areas: mild (+), moderate (++), severe (+++), and very severe (++++). The blocked tissues were cut into 4-5 μm thick sections before being stained with hematoxylin and eosin (H&E), and then the areas found to be appropriate for histopathological evaluation were photographed. These tissues were blindly evaluated by two histologists. The results of the statistical comparisons of all of the information obtained during the evaluation of the data, within the groups and between the groups, were evaluated.

2.6. Statistical Analyses. The results were reported as the means ± standard deviation. Data analyses were performed using the statistical software SPSS for Windows (version 13.1; SPSS, USA). The Kruskal-Wallis test was used to compare the groups. A Bonferroni adjusted Mann-Whitney *U* test was used to compare the two groups. The results are given as the mean ± SD. *P* values of < 0.05 were regarded as statistically significant.

3. Result

3.1. Biochemical Parameters. The AST level of the I/R group (65.3 ± 11.5 IU/L) was strongly higher than the control group (23.3 ± 7.5 IU/L, $P < 0.001$) and the I/R+ADA group (46.7 ± 8.5 IU/L, $P = 0.003$). The AST level of the I/R+ADA group was higher than the control group ($P < 0.001$). The ALT level of the I/R group (59.2 ± 17.5 IU/L) was strongly higher than the control group (41.1 ± 11.7 IU/L, $P = 0.010$). The urea level of the I/R group (22.2 ± 9.3 mg/dL) was significantly lower than the I/R+ADA group (42.0 ± 13.8 mg/dL, $P = 0.002$) and the control group (39.8 ± 4.6 mg/dL, $P < 0.001$). The TNF-α level of the I/R group (430.8 ± 70.1 pg/mg protein) was significantly higher than the I/R+ADA group (338.0 ± 71.6 pg/mg protein, $P = 0.006$) and the control group (345.5± 53.3 pg/mg protein, $P = 0.008$). The NO level of the I/R group (8.0 ± 1.1 μmol/g protein) was strongly higher than the I/R+ADA group (6.3 ± 1.2μmol/g protein, $P = 0.008$) and the control group (6.5 ± 1.5 μmol/g protein, $P = 0.010$). All results are shown in Table 1.

3.2. Histological Parameters. There were no textural or cellular deformities found in the histopathological examinations of the control livers stained using the H&E method. The morphological structures were observed to have a normal histological appearance (Figure 1(a)).

Table 1: All the biochemical results of three groups.

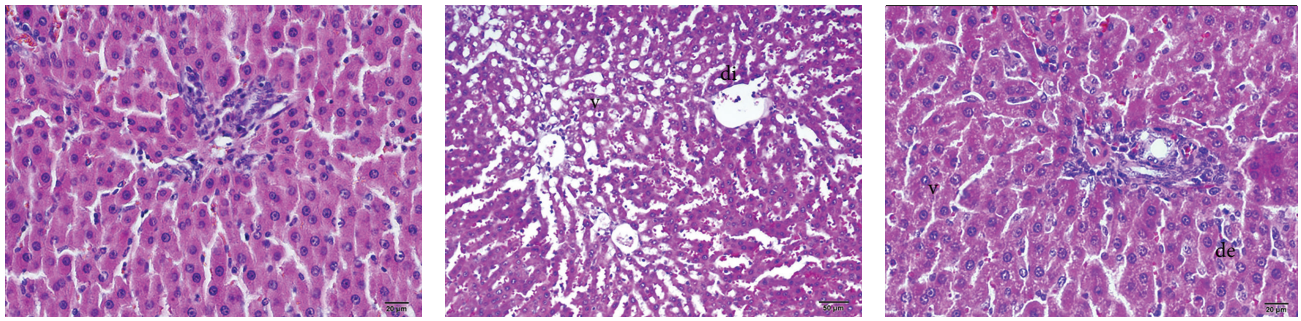
	Control (mean ± sd)	I/R (mean ± sd)	I/R + ADA (mean ± sd)
ALT (IU/L)	41.1 ± 11.7	59.2 ± 17.5^{F}	51.4 ± 8.8
AST (IU/L)	23.3 ± 7.5	$65.3 \pm 11.5^{*,¶}$	$46.7 \pm 8.5^{*}$
Creatinine (mg/dL)	0.4 ± 0.05	0.5 ± 0.05	0.5 ± 0.07
Urea (mg/dL)	39.8 ± 4.6	$22.2 \pm 9.3^{*,W}$	42.0 ± 13.8
TNF-α (pg/mg protein)	345.5 ± 53.3	$430.8 \pm 70.1^{X,\alpha}$	338.0 ± 71.6
NO (μmol/g protein)	6.5 ± 1.5	$8.0 \pm 1.1^{F,\dagger}$	6.3 ± 1.2

ADA: adalimumab; I/R: ischemia/reperfusion; TNF-α: tumor necrosis factor-alpha; NO: nitric oxide.
For ALT: $^{F}P = 0.010$ versus control group.
For AST: $^{*}P < 0.001$ versus control group; $^{¶}P = 0.003$ versus I/R + ADA group.
For Urea: $^{*}P < 0.001$ versus control group; $^{W}P = 0.002$ versus I/R + ADA group.
For TNF-α: $^{X}P = 0.008$ versus control group; $^{\alpha}P = 0.006$ versus I/R + ADA group.
For NO: $^{F}P = 0.010$ versus control group; $^{\dagger}P = 0.008$ versus I/R + ADA group.

The histopathological examination of the I/R group livers stained using the H&E method revealed hepatocyte necrosis with severe cellular deformities (Figure 1(b)). A vasoconstriction was detected in the early reperfusion or acute phase with an increase in the amount of leukocytes, platelet aggregation in the sinusoids, endothelial cell swelling, cell surface recesses and protrusions, and vacuolization related to intracellular edema and neutrophilic infiltration. In the sinusoids, there were partial dilatations and Kupffer cells protruding into the lumen observed to be flat, round, and bulging. Necrotic losses related to degeneration were observed in the hepatocytes near the central vein and the area surrounding the portal vein. Acute liver capillaries in the connective tissue surrounding areas of severe congestion were seen; an increase in the number of neutrophils was found. In the acute phase there was an increase in the number of neutrophils in the connective tissue surrounding the areas of severe congestion in the liver capillaries (Figure 1(b)).

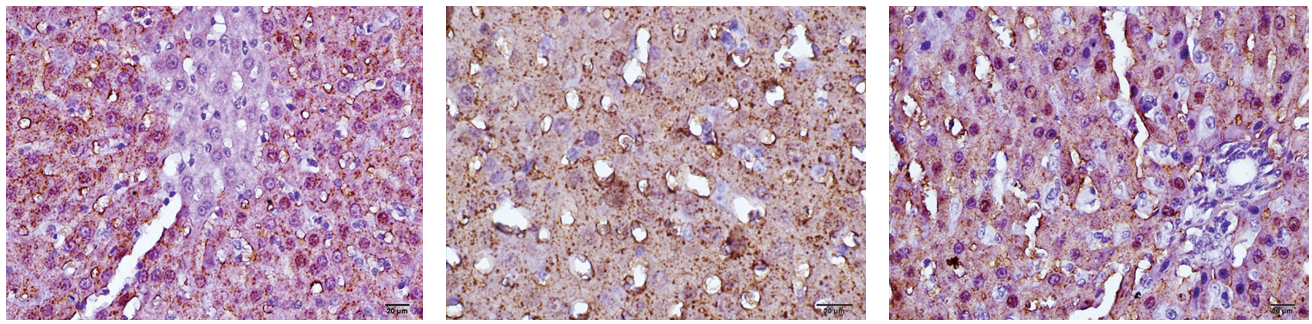
In the I/R+ADA group, the histopathological examination after the H&E staining method revealed lower tissue and cellular deformities than the I/R group (Figure 1(c)). Although the sinusoidal dilatation was decreased, there were long-course structures similar to those in the control group. The sinusoidal wall-settled Kupffer cells were observed to be more flat and stained deep basophilically (Figure 1(c)).

3.2.1. Immunohistochemical Parameters. In the control group, while the surroundings of the central vein and portal area were less immunoreactive, the endothelial cells found in the dilatation areas were stained heavily positive. Although the hepatocyte nuclei of the I/R group were negatively immunoreactive, the immunoreactivity of the hepatocyte nuclei of the I/R+ADA group was observed to be very intense.



(a) (b) (c)

Figure 1: Histopathologic examination of liver tissue by light microscopy; (a) control group, (b) I/R applied group, di: dilatation and v: vacuolization, (c) I/R+ADA applied group, de: degenerative cell, H&E stain.



(a) (b) (c)

Figure 2: Histopathologic examination of liver tissue by light microscopy; immunohistochemical staining of liver tissues with immunoperoxidase method revealed strong and diffuse reactivity; (a) control group, (b) I/R applied group, (c) I/R+ADA applied group, immunoperoxidase stained Antiliver arginase antibody.

Staining of the liver tissues using the immunoperoxidase method revealed the antiliver arginase immunopositivity to be 15% (++), 55% (+++), and 30% (++++) in the control group; 65% (++), 30% (+++), and 5% (++++) in the I/R group; and 15% (++), 40% (+++), and 45% (++++) in the I/R+ADA group.

In the adult rat hepatic parenchyma of the control group, there was a heterogeneous distribution of CPS-1 in all hepatocytes, except for a narrow area around the terminal hepatic venules. There was CPS-1 reactivity observed in the hepatocyte cytoplasm and mitochondrial matrix stained using immunohistochemical methods.

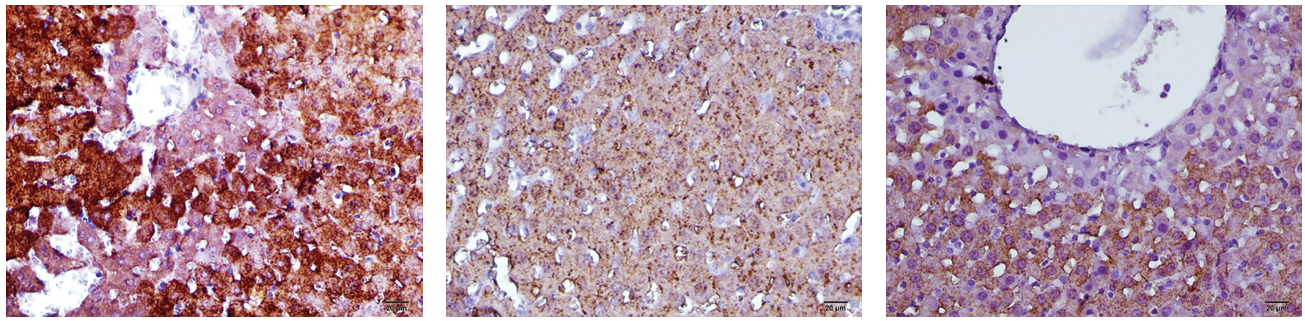
Staining of the liver tissues using the immunoperoxidase method revealed the anti-CPS-1 immune positivity to be 5% (++), 30% (+++), and 65% (++++) in the control group; 50% (++), 45% (+++), and 5% (++++) in the I/R group; and 5% (++), 65% (+++), and 30% (++++) in the I/R+ADA group (Figure 3).

The arginase results are shown in Figure 2, and the CPS-1 results are shown in Figure 3. All histological results are shown in Table 2.

4. Discussion

In our study, the tissue TNF-α and NO levels of the I/R group were found to be significantly high and the serum urea level to be significantly low. The tissue TNF-α and NO levels of the I/R+ADA group were significantly lower than the I/R group; however, the TNF-α, NO, and urea levels were similar to the control group. In the tissues stained with H&E, it was observed that cell damage in the I/R group was significantly high, while it was low in the I/R+ADA group. Immunohistochemical staining of the arginase and CPS-1 activities were significantly low in the I/R group. Serum AST and ALT levels showed a significant increase during I/R. The findings of the present study have shown that two hours of aortic occlusion, followed by 2 hours of reperfusion, induce severe liver parenchymal damage.

NO plays an important and controversial role in I/R injury. Previous studies reported that overabundant NO levels induce apoptosis [21, 22]. Some other studies reported that increased NO concentration protects cells from apoptosis by vasodilatation [23, 24]. NO is synthesized from arginine and O_2 by NO synthase (NOS) [25]. NO may mediate protection from I/R injury by the activation of the intracellular pathway, guanylate cyclase, which in turn activates protein kinases G and C, which leads to the opening of the mitochondrial ATP-dependent K+ channels [26]. A previous study has shown that the upregulation of vascular arginase inhibits NO-mediated vasodilation during I/R. In particular, the authors have demonstrated that the protein expression of arginase was augmented by I/R [27]. The inhibition of arginase activity



(a) (b) (c)

Figure 3: Histopathologic examination of liver tissue by light microscopy; immunohistochemical staining of liver tissues with immunoperoxidase method revealed strong and diffuse reactivity; (a) control group (b) I/R applied group, (c) I/R+ADA applied group, immunoperoxidase stained Anti-CPS1 antibody.

Table 2: Histopathologic examination of liver tissue.

	Sinusoid dilatation (mean ± sd)	Hepatocyte degeneration (mean ± sd)	Neutrophil infiltration (mean ± sd)	Antiliver arginase reactivity (mean ± sd)	Anti-CPS-1 reactivity (mean ± sd)
Control	0 ± 0	0 ± 0	0 ± 0	3.0 ± 0.8	3.8 ± 0.4
I/R	$3.0 \pm 0.4^{*,¶}$	$3.1 \pm 0.3^{*,A}$	$2.8 \pm 0.8^{*,W}$	$2.1 \pm 0.8^{¥,B}$	$2.0 \pm 0.6^{*,¶}$
I/R + ADA	$2.2 \pm 0.8^{*}$	$2.2 \pm 0.7^{*}$	$1.2 \pm 0.4^{*}$	3.7 ± 0.6	3.0 ± 0.8^{X}

CPS 1: carbamoyl phosphate synthetase 1; I/R: ischemia/reperfusion; ADA: adalimumab.
For sinusoid dilatation: $^{*}P < 0.001$ versus control group; $^{¶}P = 0.015$ versus I/R + ADA group.
For hepatocyte degeneration: $^{*}P < 0.001$ versus control group; $^{A}P = 0.005$ versus I/R + ADA group.
For neutrophil infiltration: $^{*}P < 0.001$ versus control group; $^{W}P < 0.001$ versus I/R + ADA.
For Antiliver arginase reactivity: $^{¥}P = 0.011$ versus control group; $^{B}P = 0.002$ versus I/R + ADA.
For Anti-CPS1 reactivity: $^{*}P < 0.001$, $^{X}P = 0.015$ versus control group; $^{¶}P = 0.015$ versus I/R + ADA group.

in the I/R vessels induces the stimulated NO production, and thus, restores NO-mediated vasodilatation. An arginase inhibitor increases NO production and dilatation in normal vessels and also restores the NO-mediated dilatation after I/R [28].

The release of NO is related to oxidative stress, and previous I/R studies have shown the NO level to be high in the I/R group and low in the treatment group [21, 29]. NO-induced cell death is generally considered to be associated with DNA damage or mitochondrial damage. In the previous study, it was demonstrated that the endoplasmic reticulum stress pathway was involved in NO-mediated apoptosis [30]. Previous studies reported that infliximab therapy decreased serum NO levels [31]. Similarly, current study showed that ADA treatment diminished tissue NO levels during I/R injury. In our study, the NO and TNF-α levels of the I/R group were higher than in the control and I/R+ADA groups. The examination of the histological preparations (H&E) revealed many findings of cell degeneration in the I/R group. ADA treatment may ameliorate damage of I/R injury.

CPS-1 and arginase are key enzymes in the urea cycle [32]. Arginase exists in two isoforms, liver-type arginase I and nonhepatic-type arginase II. The former is a cytosolic enzyme found primarily in the liver [33]. CPS-1 is a liver-specific, intramitochondrial, rate-limiting enzyme in the urea cycle, which plays a staminal role in protein and nitrogen metabolism. Previous studies have shown that a quantitative change in this enzyme's expression and function can affect NO production by limiting substrate availability [34]. It was reported that inhibiting the degradation of arginine by arginase and CPS-1 increases NO synthesis [28, 35], so that the tissues of the I/R group were preserved. Therefore, decreased activity of arginase is a potential factor that excessive NO levels [36]. In our study, low levels of urea and increased NO in the I/R group indicate the inhibition of arginase and CPS-1.

In the current study, arginase and CPS-1 activities were low in the I/R group. Due to the activation of the NO synthesis pathway from the arginine in this group, we have found the NO level to be high in the control group and I/R+ADA group. The NO level, which was also higher than in the control group, was higher than that required for the basal body level, suggesting that NO triggers cell damage, rather than tissue protection, via the vasodilatation effect. The results of the I/R+ADA group were similar to the control group. ADA treatment protects the tissues from I/R damage by providing maintenance of the body's equilibrium state of the oxidative stress mechanism.

NO bioavailability may be critically regulated by arginase by competing with NOS for their extensive substrate L-arginine [37]. Arginase, CPS-1, and NOS are immensely important for maintaining the delicate balance in the

organism [38]. ADA treatment during I/R may maintain the balance between arginase and NOS, prevent excessive NO release by decreasing both arginase and CPS-1 activities, and prevent NO related vasodilatation by increasing both arginase and CPS-1 activities. Therefore, this treatment reduces NO formation through the suppression of NOS expression in liver I/R. The I/R+ADA group may be preserved from cell cytokines and ROS-mediated apoptosis caused by NO. Previous study reported that NO levels may be increase during I/R; however, NO-mediated vasodilatation functions are inhibited by increased H_2O_2 due to ROS in I/R [6]. We speculate that arginase and CPS-1 may be downregulated by increased NO level during I/R and their tissue levels may be decreased. Therefore, increased NO levels may lead to cell damage by oxidative stress rather than vasodilatation in I/R. ADA may maintain this balance.

Various factors are involved in I/R injury, including ROS production, calcium overload, neutrophilic infiltration, and cytokine release. The destructive effects of I/R result from the generation of ROS, subsequent to reoxygenation, that causes direct tissue damage and initiates a cascade of destructive cellular responses, leading to inflammation, cell death, and organ damage [39, 40]. Among these mediators, TNF-α, which plays a key role in the inflammatory reaction, is thought to play a major role in I/R injury. High TNF-α increases ROS, causing increased apoptosis [41]. It has been demonstrated by some studies that a prophylactic anti-TNF-α treatment, such as infliximab, may be an effective therapeutic strategy for preventing I/R-induced injury [18, 42, 43]. ADA is a potent antibody against TNF-α, which can neutralize all forms (extracellular, transmembrane, and receptor-bound) of TNF-α. In this study, the issue TNF-α level of the I/R group was significantly higher than the control and I/R+ADA groups. It was observed that the TNF-α level of the I/R+ADA group was similar to the control group.

The increase of AST and ALT observed in I/R group can be explained by the hepatocyte damage which is caused by the ROS and cytokines during the I/R phase. The lower increase of AST and ALT levels was observed in animals of I/R+ADA group when compared to I/R group. Our results have shown that ADA may have protective effects against liver I/R injury, because of its anti-inflammatory and antioxidant properties, which reduce TNF-α release. The studies of ADA in rats were limited, and it has been reported that the maximum serum level of ADA was reached after an average of five days, after subcutaneous administration in humans [44]. In the current study, we administered the ADA intraperitoneally five days before I/R, and we have shown it to be protective in I/R.

5. Conclusion

During I/R, arginase and CPS-1 enzymes are excessively inhibited; therefore, excessive NO synthesis and the accompanied cytokine increases have been shown to cause apoptosis. Treatment with ADA as an inhibitor of TNF-α during I/R decreases cytokines, prevents the increase and decrease of NO by maintaining the balance between arginase, CPS-1, and NOS, and consequently protects the cells from death.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

The Effect of Intravenous Anesthetics on Ischemia-Reperfusion Injury

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The effects of intravenous anesthetics on ischemia-reperfusion injury (IRI) have been investigated in both animals and clinical studies. The protective effects and the dosages of the intravenous anesthetics on IRI were discussed in this paper. The prevention of the tissue injury after the IRI was demonstrated with intravenous anesthetics in some studies. In the future, the studies should be focused on the dosage of the anesthetics related to diminishing the tissue injuries. Further studies might be required in order to investigate the effects of the anesthetics on molecular levels.

1. Introduction

Ischemia-reperfusion injury (IRI) can be resulted from many factors such as the release of free oxygen radicals and consecutive lipid peroxidation, cell death by apoptosis or necrosis, inflammatory cytokines, and damage to the microvasculature [1, 2]. Reactive oxygen species (ROS) that appear with reperfusion injury damage cellular structures through the process of the lipid peroxidation of cellular membranes and yield toxic metabolites such as malondialdehyde (MDA), that is, used as a sensitive marker of ischemia-reperfusion injury. There is a balance between ROS and the scavenging capacity of antioxidant enzymes. Therefore, the total antioxidant capacity (TAC) is a functional outcome of both the oxidation capacity and the consumption rate of antioxidants during oxidative stress [1, 2]. There are three time frames in which protection against ischemia-reperfusion injury can be induced: before ischemia occurs, during ischemia, and after the ischemia at the onset of reperfusion.

Klune and Tsung [3] reported in a review article that the mechanism of organ damage after IRI has been studied extensively and consists of complex interactions of multiple inflammatory pathways. The major contributors to IRI include production of reactive oxygen species, release of proinflammatory cytokines and chemokines, and activation of immune cells to promote inflammation and tissue damage. Recent research has focused on the mechanisms by which these immune responses are initially activated through signaling molecules and their cellular receptors. Thorough understanding of the pathophysiology of liver IRI may yield novel therapeutic strategies to reduce IRI and lead to improved clinical outcomes [3]. Both experimental and clinical studies focusing on the reduction of IRI report that tissue injury may be prevented through the use of anesthetic agents or anesthesia methods. A variety of investigations using experimental animals have shown that intravenous anesthetic agents have a protective effect against ischemia and reperfusion injuries [4].

2. Opioids (Morphine, Remifentanil, and Fentanyl)

Evidence has now accumulated that intravenous anesthetics and some narcotics may be cardioprotective [4]. The cardioprotective effects of opioid receptor agonists have been consistently demonstrated in different models of ischemia-reperfusion injury in vivo as well as in vitro [5].

Morphine, remifentanil, and fentanyl as opioids have been effectively used for anesthesia/analgesia regimens in some surgical procedures [6–8]. Neuraxial morphine given during a postischemic period had been reported to have

the potential to exacerbate ischemic spinal cord injury [9]. However, it remains unknown whether synthetic opioids administered systemically exacerbate ischemic injury. Therefore, Shirasawa and colleagues sought to compare the damage of the spinal cord after transient spinal cord ischemia in rabbits anesthetized with three different regimens: isoflurane, fentanyl with isoflurane, and remifentanil with isoflurane. Their results suggested that neither i.v. fentanyl nor i.v. remifentanil added to 0.5 MAC isoflurane exacerbated ischemic spinal cord injury in rabbits when compared to 1 MAC isoflurane [10]. In an experimental study, the authors determined whether morphine, administered via either the i.v. or intrathecal routes, can ameliorate hepatic IRI in rats with either normal or cirrhotic livers. They also tested whether morphine-mediated hepatoprotection involves μ-opioid receptor activation and the PI3K/Akt and Jak2/STAT3 pathways as these pathways have previously been shown to be involved in morphine-mediated cardioprotection. In the study morphine as an opioid was administered either i.v. or intrathecally 10 min before initiating 1 h of ischemia followed by 6 h of reperfusion in normal rat liver. The authors reported that morphine preconditioning protects against IRI in both normal and cirrhotic rat livers. The mechanisms of morphine-induced hepato-protection are most likely multifactorial. These multifactorials involve opioid receptors, phosphatidylinositol-3-kinase, and Akt [11].

Remifentanil is a new, potent, ultra-short-acting selective opioid receptor agonist in the clinical use of anesthesia and analgesia [7, 12]. In an animal study, it was evaluated whether remifentanil was cardioprotective when administered in postconditioning fashion and compared its effect with that of ischemic postconditioning. The relative role of opioid receptor subtypes in both regimes was also investigated by the use of subtype-specific opioid receptor antagonists. Remifentanil postconditioning was evaluated using a 5 min infusion of the drug at 1, 5, 10, or 20 μg/kg/min of body weight. The results of the study indicated that remifentanil postconditioning protected the heart from ischemia-reperfusion injury to a similar extent as of ischemic postconditioning. This protection involves κ and δ but not μ opioid receptor activation [13].

An experimental mice study had demonstrated that a single bolus of 1 μg/kg of remifentanil given before tissue ischemia was protected against IRI in the small intestine, allowing us to bypass the inherent adverse effects of conventional μ-opiods such as persistent inhibition of gastrointestinal motility and respiratory drive. A marked amelioration of mucosal injury in remifentanil-treated mice was accompanied by a reduction of oxidative stress locally and inflammation systemically, as evidenced by decreased concentrations of gut tissue MDA and plasma IL-6 [14].

Another study determined the effects of remifentanil in focal brain ischemia and ischemia-reperfusion injury. Mechanisms linked to mitogen-activated protein kinases, including extracellular signaling-regulated kinase (ERK) 1/2, p38 kinases, and c-Jun N-terminal kinase (JNK), and various cytokines were also examined. Remifentanil (5 μg/kg/min) was given alone or combined with naltrindole (δ-opioid receptor antagonist; 1 mg/kg). Remifentanil infusion was started 10 minutes before middle cerebral artery occlusion (MCAO) and continued throughout. It was concluded from the study that remifentanil may be neuroprotective against focal ischemia-reperfusion injury, possibly through the activation of δ-opioid receptors and attenuation of ERK 1/2 activity and TNF-α production, in the rat brain [15].

Fentanyl, a synthetic derivative of morphine, is widely used for patients undergoing cardiovascular surgeries. Clinical and experimental evidences suggest that most of the cardiovascular effects of fentanyl are mediated by opioid receptors (ORs) acting. In an experimental study, the cardioprotective effects of IV-administered fentanyl using a model of myocardial ischemia-reperfusion injury associated with pharmacologically induced central sympathetic over activity were investigated. The results of the study concluded that fentanyl's effects for limiting myocardial ischemic injury are mediated via peripheral ORs, while opioid's antiarrhythmic actions are mediated via central OR agonism [16].

3. α-2a Adrenergic Agonists (Dexmedetomidine)

Dexmedetomidine, a potent and highly selective α-2 adrenoreceptor agonist, is widely used for sedation in intensive care units (ICU). Dexmedetomidine also offers good perioperative hemodynamic stability and an intraoperative anesthetic sparing effect [17]. The results of an experimental study clearly demonstrated that oxidative stress parameters were significantly altered in experimental hepatic IR injury in the rats. Dexmedetomidine was found to be a protective agent against the oxidative alterations in hepatic IR injury on the liver and remote organs, when given before induction of ischemia. Moreover, dexmedetomidine protected against the harmful effects of IR in terms of the histopathological changes in the liver. Therefore, dexmedetomidine may be used as an adjuvant anesthetic agent before surgery for patients with potential hepatic IR injury [18].

Yagmurdur and colleagues examined the effect of dexmedetomidine on ischemia-reperfusion injury due to tourniquet application during upper-extremity surgery by determining blood malondialdehyde and hypoxanthine levels. Alterations in aspartate aminotransferase, alanine aminotransferase, creatine phosphokinase, lactate dehydrogenase, uric acid, and creatinine levels were also assessed. In the dexmedetomidine group, a continuous infusion of dexmedetomidine (1 μg/kg for 10 minutes, followed by 0.5 μg/kg h^{-1}) was used until the end of surgery, whereas the control group received an equivalent volume of saline. Their results suggest that dexmedetomidine may offer advantages by inhibiting lipid peroxidation in the case of anticipated ischemia-reperfusion injury, which would occur in upper-extremity surgery requiring tourniquet application [19].

A clinical study evaluated the effects of dexmedetomidine on tourniquet-induced ischemia-reperfusion injury during general anesthesia by measuring MDA and TAC levels when dexmedetomidine was added to the general anesthesia. The main findings of the study demonstrated that serum MDA levels were decreased when compared to basal values at 5 and 20 minutes ATR and that TAC was lower than basal values

at 1 minute before and at 5 minutes ATR and reached the basal level at 20 minutes ATR. However, these findings were similar to the results obtained from the group that was not given dexmedetomidine [20].

Dexmedetomidine has been used for purposes of anesthesia and sedation, and experimental studies have demonstrated its neuroprotective effects. However, it has also been shown that the constriction of cerebral vessels in response to high doses of dexmedetomidine decreases cerebral blood flow. A study tested the hypothesis that dexmedetomidine-induced cerebral hypoperfusion exacerbates ischemic cerebral injury. The effects of dexmedetomidine on cerebral blood flow and mean arterial blood pressure were studied first in this study. Six rats received intravenous infusions of dexmedetomidine in doses ranging from 0.01 to 10 μg/kg min^{-1}. Hypertension following the administration of high-dose dexmedetomidine is associated with cerebral hypoperfusion and the exacerbation of ischemic brain injury, possibly through alpha-2-induced cerebral vasoconstriction [21].

4. Propofol

Propofol is a rapidly acting intravenous hypnotic agent, that is, frequently used in clinical anesthesia administrations [7, 22, 23]. Propofol is an intravenous anesthetic with neuroprotective effects against cerebral ischemia-reperfusion injury [24]. Few studies regarding the neuroprotective and neurobehavioral effects of propofol have been conducted, and the underlying mechanisms are still unclear. Because IRI may result in neuronal apoptosis, the apoptosis regulatory genes B-cell leukemia-2 (Bcl-2) and Bcl-2-associated X protein (Bax) may be involved in the neuroprotective process. In a study, cerebral ischemia was induced by clamping the bilateral common carotid arteries for 10 min. Propofol (1.0 mg/kg/min) was administered intravenously for 1 h before the induction of ischemia. The results of this study showed that neurobehavioral scores were higher in propofol-treated rats compared to ischemia-reperfusion injury-induced rats with no propofol treatment. Moreover, the hippocampal expression of Bcl-2 was significantly higher, while the expression of Bax was significantly lower in propofol-treated rats compared to IRI-induced rats at 24 h after ischemia. Hence, this study suggests that the neuroprotective effects of propofol against neuronal apoptosis may be a consequence of the regulation of Bcl-2 and Bax [25].

Remote pulmonary injuries after hepatic reperfusion are frequently caused by reactive oxygen species (ROS) induced damage. The choice of anesthetics may affect the balance between oxidants and antioxidants, and propofol, a commonly used anesthetic, has an antioxidant effect. In a study a model was developed to study the effect of propofol on pulmonary function with hepatic ischemia-reperfusion injury manipulation. The aim of the study was to determine remote pulmonary dysfunction after hepatic reperfusion and determine if propofol affects this dysfunction by altering ROS production from the liver or lungs. Remote pulmonary dysfunction and reperfusion injury in the liver were demonstrated in this rat model, as well as massive ROS production and lipid peroxidation. As a conclusion of this study, propofol infusion attenuated remote pulmonary injury by lessening oxidative injury from the reperfused liver [26].

In an another clinical study, it was demonstrated that total intravenous anesthesia with propofol and regional anesthesia techniques provided better antioxidant defense and reduced endothelial dysfunction than general inhalational anesthesia with sevoflurane during tourniquet application in pediatric extremity surgery [27].

The antioxidant properties of propofol have been shown to improve ischemia-reperfusion injury. In an animal study, the authors investigated whether anesthesia with propofol can ameliorate remote lung injury induced by intestinal ischemia-reperfusion (IIR) in rats. Using propofol to induce and maintain anesthesia efficiently prevented IIR-induced lung injury. Systemic antioxidant protection, improvement of intestinal injury, inhibition of the inflammatory response, and preservation of the alveolar-capillary permeability seem to be crucial, mediating mechanisms for this simple and clinically relevant intervention [28].

5. Ketamine

Ketamine is an anesthetic with anti-inflammatory properties, which has shown protective effects on IRI in various organs [29, 30]. An experimental study investigated the effects of ketamine on intestinal IRI. Male Wistar rats underwent either sham surgery or 30 min of intestinal ischemia followed by 60 min of reperfusion. Ketamine pretreatment was administered by intraperitoneal injections at doses of 100, 50, 12.5, or 6.25 mg/kg. The intestinal morphology, mucosal damage, leukocyte infiltration, serum P-selectin, serum intracellular adhesion molecule-1 (ICAM-1), serum antithrombin-III (ATIII), and myenteric ganglion cell structure were evaluated. Intestinal IRI led to severe mucosal damage, leukocyte (especially neutrophil) infiltration, P-selectin and ICAM-1 elevations, ATIII depletion, and myenteric ganglion cell morphological alterations. According to the results of this study, the ketamine dose dependently diminished these alterations (except for ICAM-1 serum levels), reaching statistical significance at 100, 50, and 12.5 mg/kg. Ketamine protects the intestine against ischemia-reperfusion injury [29].

In an another experimental study, the authors sought to determine whether a higher dose of ketamine-xylazine (KX) protected isolated guinea pig hearts against myocardial ischemia-reperfusion injury. Male Hartley guinea pigs (Crl : HA; 275 to 300 g; $n = 14$) were anesthetized with either of the 2 doses of KX (K: 85 mg/kg, X: 15 mg/kg; or K: 200 mg/kg, X: 60 mg/kg). They found that higher doses of KX used to anesthetize guinea pigs led to reduction in myocardial infarct size and improved hemodynamic function after experimental ischemia-reperfusion. These results suggest that supplementation of KX to ensure an adequate anesthetic plane may introduce unwanted variability in ischemia-reperfusion studies [30].

The effect of ketamine on IRI was compared to other intravenous anesthetics. The aim of the study was to investigate the effects of ketamine and pentobarbital anesthesia on

the motility alterations and tissue injury caused by ischemia-reperfusion injury in rats. In the study rats were anesthetized with either pentobarbital sodium (50 mg/kg) or ketamine (100 mg/kg). The results of the study showed that ketamine anesthesia is associated with diminished intestinal injury and abolishes the intestinal transit delay induced by ischemia-reperfusion injury [31].

Propofol and ketamine were compared in an animal study. This study examined the cardioprotective effects of propofol and ketamine with and without N-acetylcysteine (NAC). Creatine kinase (CK), myocardial band of creatine kinase (CK-MB), and troponin-I (Tn-I) levels CK, CK-MB, and Tn-I levels did not differ significantly between the ketamine groups (1–3) and the propofol groups (4–6) $P > .05$). Malondialdehyde levels in the propofol groups (4–6) were significantly lower than in the ketamine groups (1–3; $P < .05$). In this rat model of global cardiac ischemia, propofol with NAC attenuates myocardial injury more than ketamine (with or without NAC) [32].

6. Barbiturates (Thiopental and Pentobarbital), Etomidate, and Midazolam

Pentobarbital and thiopental are barbituric acid derived, sedative acting anesthetic agents. Dogan and colleagues reported that thiopental and propofol, especially thiopental, are more effective to protect renal ischemia-reperfusion injury in an experimental study [33]. Another study was demonstrated to compare the influence of three anesthetics (60 mg/kg thiopental, 1200 mg/kg urethane, and 60 mg/kg pentobarbital intraperitoneally) on ventricular arrhythmias and to combine it with measured hemodynamic parameters to find the most suitable agent for such experiments. In the model of ischemia- and reperfusion-induced arrhythmias in Sprague-Dawley rats, after left anterior descending coronary artery occlusion (7 minutes) and reperfusion (15 minutes), the following parameters had been measured or calculated: mortality index; ventricular fibrillation and tachycardia incidence and duration; systolic, diastolic, and mean arterial blood pressure; heart rate; myocardial index of oxygen consumption; and plasma creatine kinase concentration. According the results of this study, pentobarbital is the most suitable anesthetic offering stable hemodynamic values during arrhythmia studies. These hemodynamic values were similar to physiological values in awake rats; the long arrhythmia duration during reperfusion and approximately 50% mortality index are crucial parameters for evaluating antiarrhythmic drugs [34].

Etomidate, commonly used for cardiac patients, is an intravenous anesthetic agent. Etomidate, thiopental, propofol, and ketamine were compared in a study. The aim of the study was to compare the use of different anesthetic drugs in a skeletal IRI model. Rats in each group were anesthetized either with thiopental, ketamine, propofol, or etomidate. Malondialdehyde, superoxide dismutase, catalase, and glutathione peroxidase were measured in skeletal muscle via a spectrophotometer. In this study rats anesthetized with ketamine (60 mg/kg), propofol (100 mg/kg), or etomidate (20 mg/kg) did not show increased malondialdehyde levels in comparison to control levels. While the drugs did not cause a distinction in the levels of superoxide dismutase, catalase, glutathion peroxidase, iron, and copper, zinc was in a lower level in IRI group compared to sham control. It was concluded that ketamine, propofol, and etomidate, with anesthetic doses, denoted efficacious effects on IRI; hence the drugs might be preferred in certain operations with the risk of IRI [35].

Interest in the doses of multiple anesthetics in ischemia-reperfusion injury has studied. Etomidate, thiopental, propofol, and intralipid were compared in a study. The purpose of this study was to investigate and compare the efficiency of ketamine, thiopental, propofol, etomidate, and intralipid in reducing the injury induced by free radicals in a rat model of renal IRI. Rats in the IRI groups were given ketamine (20 mg/kg), thiopental (20 mg/kg) propofol (25 mg/kg), etomidate (10 mg/kg), and 10% of intralipid (250 mg/kg) intraperitoneally 15 min prior to the ischemia for 60 min, followed by reperfusion for 60 min. Biochemical malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), blood urea nitrogen (BUN), creatine (Cr), and aspartate aminotransferase (AST) were determined, and histopathological analysis was performed with these samples. MDA levels were lower in the ketamine, thiopental, and propofol groups compared to the control group ($P < 0.05$). In the thiopental and propofol groups, the levels of histopathological scores were significantly lower than control and etomidate groups in ischemia-reperfusion injury. These results demonstrated that IRI was significantly reduced in the presence of propofol and thiopental. The protective effects of these drugs may belong to their antioxidant properties. These results may indicate that propofol and thiopental anesthesia protects against functional, biochemical, and morphological damage better than control in renal ischemia-reperfusion injury [36].

Erturk and colleagues compared the effects of propofol and N-acetyl cysteine (NAC) on tourniquet-induced ischemia-reperfusion injury by determining malonyldialdehyde, ischemia-modified albumin, lactate, blood gas, and hemodynamic levels in arthroscopic knee surgery. Sixty ASA I or II patients were randomized into three groups. Intrathecal anesthesia was administered using 0.5% heavy bupivacaine in all patients. In group P, propofol was administered in a 0.2 $mg\,kg^{-1}$ bolus, followed by infusion at a rate of 2 $mg\,kg^{-1}\,h^{-1}$; in group NAC, NAC was administered as an infusion at a rate of 5 $mg\,kg^{-1}\,h^{-1}$, and, in group C (the control group), an equal volume of isotonic saline was administered in patients until 30 min after reperfusion. Plasma concentrations of malonyldialdehyde, ischemia-modified albumin, and lactate in groups P and NAC were significantly lower than those in group C. The author reported that comparisons of groups P and NAC revealed no significant differences. Small-dose infusions of both propofol and NAC appear to provide similar protection against ischemia-reperfusion injury in arthroscopic knee surgery [37].

Midazolam, a benzodiazepine, is used for sedation anesthesia in some surgical procedures [22, 23]. An animal study

Table 1: The dosage studies of the intravenous anesthetics on IRI.

Anesthetic	Dosage	Clinical/animal	Organ/tissue	Marker	IRI
Morphine	(i) 1–100 μg/kg (ii) 30 μg	(i) Animal (11) (ii) Animal (9)	(i) Liver (ii) Spinal cord	(i) Opioid receptors (ii) Opioid receptors	(i) Protect (ii) Exacerbate
Fentanyl	5 or 50 μg/kg,	Animal (16)	Myocard	Opioid receptors	Protect
Remifentanil	(i) 1 μg/kg (ii) 5 μg/kg/min (iii) 1, 5, 10 or 20 μg/kg/min	(i) Animal (14) (ii) Animal (15) (iii) Animal (13)	(i) Small intestine (ii) Brain (iii) Heart	(i) MDA, IL-6 (ii) ERK, TNF-α (iii) Opioid receptors	(i) Protect (ii) Protect (iii) Protect
Dexmedetomidine	(i) 1 microg/kg for 10 minutes, followed by 0.5 microg kg^{-1} h^{-1} (iii) 100 μg/kg	(i) Clinical (19) (ii) Animal (18)	(i) Upperextremity (ii) Liver	(i) MDA (ii) No	(i) Protect (ii) Protect
Propofol	(i) 25 mg/kg/h (ii) 25 mg/kg (iii) 0.2 mg kg^{-1} bolus, followed by infusion at a rate of 2 mg kg^{-1} h^{-1}	(i) Animal (26) (ii) Animal (36) (iii) Clinical (37)	(i) Pulmonary (ii) Renal (iii) Knee surgery	(i) No (ii) MDA (iii) MDA, IMA	(i) Protect (ii) Protect (iii) Protect
Ketamine	(i) 85–200 mg/kg (ii) 100 mg/kg	(i) Animal (30) (ii) Animal (29)	(i) Heart (ii) Intestinal	(i) No (ii) No	(i) Protect (ii) Protect
Thiopental	60 mg/kg	Animal (34)	Coronary	No	Protect
Pentobarbital	60 mg/kg	Animal (34)	Coronary	No	Protect
Etomidate	(i) 20 mg/kg (ii) 10 mg/kg	(i) Animal (35) (ii) Animal (36)	(i) Skeletal muscle (ii) Renal	(i) MDA (ii) MDA	(i) Protect (ii) Protect
Midazolam	3 mg/kg	Animal (38)	Mitochondrial	No	Protect
Lidocaine	1.5 mg kg^{-1} bolus and 2.0 mg kg^{-1} h^{-1} infusion	Clinical (40)	Myocardial	CK-MB, TnI	Protect

compared the neuroprotective effects of propofol, thiopental, etomidate, and midazolam as anesthetic drugs in fetal rat brain in the ischemia-reperfusion (IR) model. In the study pregnant rats of day 19 were randomly allocated into eight groups. Fetal brain ischemia was induced by clamping the utero-ovarian artery bilaterally for 30 min and reperfusion was achieved by removing the clamps for 60 min. One milliliter intralipid solution, 40 mg/kg propofol, 3 mg/kg thiopental, 0.1 mg/kg etomidate, and 3 mg/kg midazolam were administered intraperitoneally in the vehicle group, propofol group, thiopental group, etomidate group, and midazolam group, respectively, 20 min before IR procedure. The results of the study reported that the anesthetic drugs including propofol, thiopental, etomidate, and midazolam decrease the lipid peroxidation back to control values, while only propofol and midazolam have a protective effect on mitochondrial damage [38].

7. Local Anesthetics (Lidocaine)

Lidocaine is a widely used local anesthetic and antiarrhythmic agent that has been shown to have cardioprotective effects against myocardial ischemia and reperfusion injury by blocking cardiac sodium channels, reducing intracellular calcium loading, reducing ROS production, and modulating mitochondrial bioenergetics [39]. In a randomized, double-blinded trial, 99 patients received i.v. lidocaine 2% (i.e., a 1.5 mg kg^{-1} bolus at induction of anesthesia followed by a 2.0 mg kg^{-1} h^{-1} infusion intraoperatively) or an equal volume of saline. Serum creatine kinase-myocardial band (CK-MB) and troponin I (TnI) concentrations were measured before surgery. The result of this study found that the continuous i.v. infusion of lidocaine during surgery reduced myocardial injury in patients undergoing off-pump coronary artery bypass graft surgery [40].

Interest in the dosage of anesthetics in skeletal ischemia-reperfusion injury has recently increased. Thus, the aim of the study was to compare the effects of subanesthetic doses of ketamine, propofol, and etomidate on a skeletal IRI model. The results of the study showed that subanesthetic doses of ketamine, propofol, and etomidate displayed beneficial effects in IRI [41].The dosage studies of the intravenous anesthetics on IRI were shown in Table 1.

In a conclusion, the prevention of the tissue injury after the IRI was demonstrated with most of the intravenous anesthetics (e.g., propofol, midazolam, and thiopental) in both animal and clinical studies. In the future, the studies

should be focused on the dosage of the anesthetics related to diminishing the tissue injuries. Further studies might be required in order to investigate the effects of the anesthetics on molecular levels.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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Research Article

The Effects of Remote Ischemic Preconditioning and N-Acetylcysteine with Remote Ischemic Preconditioning in Rat Hepatic Ischemia Reperfusion Injury Model

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Background. Remote ischemic preconditioning (RIP) and pharmacological preconditioning are the effective methods that can be used to prevent ischemia reperfusion (IR) injury. The aim of this study was to evaluate the effects of RIP and N-Acetylcysteine (NAC) with RIP in the rat hepatic IR injury model. *Materials and Methods*. 28 rats were divided into 4 groups. Group I (sham): only laparotomy was performed. Group II (IR): following 30 minutes of hepatic pedicle occlusion, 4 hours of reperfusion was performed. Group III (RIP + IR): following 3 cycles of RIP, hepatic IR was performed. Group IV (RIP + NAC + IR): following RIP and intraperitoneal administration of NAC (150 mg/kg), hepatic IR was performed. All the rats were sacrificed after blood samples were taken for the measurements of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels and liver was processed for conventional histopathology. *Results*. The hepatic histopathological injury scores of RIP + IR and RIP + NAC + IR groups were significantly lower than IR group ($P = 0.006$, $P = 0.003$, resp.). There were no significant differences in AST and ALT values between the IR, RIP + IR, and RIP + NAC + IR groups. *Conclusions*. In the present study, it was demonstrated histopathologically that RIP and RIP + NAC decreased hepatic IR injury significantly.

1. Introduction

Liver ischemia/reperfusion injury (IRI) may occur during surgery, like hepatectomy or transplantation, or systemic hypoxia, like respiratory or circulatory failure. Reperfusion can cause more damage than ischemia itself [1]. During ischemia toxic oxygen radicals are produced in the tissues. These oxygen and superoxide radicals can cause endothelial injury, an increase in microvascular permeability and tissue edema in the reperfusion period [2, 3]. Besides activated adhesion molecules and cytokines can start systemic inflammatory response, which are known as IRI [3]. Although tissue ischemia is the main starter of the pathophysiological changes, reperfusion causes inflammation [4].

Liver IRI can cause hepatic failure especially in the presence of coexisting hepatic disease [5] or even multiple organ failure [6–8].

Ischemic preconditioning (IP) is one of the most common techniques that are used to reduce hepatic IRI [9]. After a short period of ischemia/reperfusion, prolonged ischemia reperfusion (IR) causes less injury in the tissue, which is called direct IP [10]. Similarly, brief ischemia/reperfusion stimulus to an organ can protect a remote organ against IRI and this is called remote ischemic preconditioning (RIP) [11]. Pharmacological method is another way to protect the tissue from IRI and many different drugs were studied for this purpose [9, 12]. N-Acetylcysteine (NAC) is one of the most commonly used drugs in several IRI studies [13–17].

The aim of this study is to compare the effectiveness of RIP and NAC addition to RIP in the rat hepatic IRI model.

2. Material and Methods

After approval of the Experimental Animal Research Committee of our institution, the study was conducted at the experimental animals' laboratory of our institute. Twenty-eight adult male Wistar albino rats weighing 250–300 g were used in this study.

Rats were randomized into four groups: Group I (sham, $n = 7$): following laparotomy, at the 65th minute of the anesthesia hepatic pedicle dissection was performed and waited till the 270th minute of the anesthesia without any procedure. Group II (IR, $n = 7$): At the 65th minute of the anesthesia, total hepatic ischemia for 30 minutes and four hours of reperfusion were performed. Group III (RIP + IR, $n = 7$): following laparotomy, three cycles of RIP applied to left hind limb were performed 5 minutes before hepatic IR. Group IV (RIP + NAC + IR, $n = 7$): 150 mg/kg NAC (*Asist amp* 300 mg/3 mL *amp, Hüsnü Arsan İlaç Sanayi, Turkey*) was administered intraperitoneally at the 60th minute of the anesthesia in addition to the procedures of group III.

2.1. Experimental Protocol. Rats were anesthetized with intraperitoneal 50 mg/kg ketamine (*Ketalar flk., Pfizer Pharma GMBH, Germany*) and 10 mg/kg xylazine hydrochloride (*Alfazyne* % 2, *Alfasan International, Holland*). Anesthesia was maintained with intraperitoneal 25 mg/kg ketamine when needed. Durations of anesthesia were equal in all groups.

Laparotomy was performed with a midline incision. After the liver and the hepatic pedicle were visualized a microvascular clamp was used for performing ischemia. Successful occlusion of the pedicle was confirmed by the change of the color of the liver. Rats were heated with a heating lamp during the operation to maintain a rectal body temperature of 37–37.5°C. Hydration was maintained with subcutaneous infusion of 3 mL/kg/h saline solution.

The effectiveness of the RIP method that we used has been shown previously [18, 19]. For this purpose an elastic bandage (1 cm width and 30 cm length) to the proximal left hind limb was wrapped circularly 3 times and squeezed. Three cycles of 10-minute ischemia and 10-minute reperfusion were performed.

At the end of the study, sternotomy was performed under anesthesia to all rats and blood samples were taken from the right atrium. Then hepatectomy was performed for histopathology and rats were sacrificed by exsanguination. Liver samples were fixed for 24–48 hours in 10% buffered formaldehyde and examined by a light microscope.

Congestion, necrosis, cytoplasmic vacuolization, cytoplasmic hypereosinophilia, nuclear pyknosis, and inflammatory cell number were examined to assess the degree of the liver injury. Hepatic histopathological injury score (HHIS) [20] was classified according to the severity of the injury (grade 0: minimal or no injury; grade 1: mild injury; grade 2: moderate injury; grade 3: severe injury).

Blood samples were centrifuged for 10 minutes at 3000 rpm and plasma samples were stored at −20°C until the measurement. *Cobas Integra 800, Roche, USA,* analyzer was used for the measurements of Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels.

For statistical analysis, SPSS 15.0 (Statistical Package for the Social Sciences version 15, Chicago, IL, USA) was used. Kruskal-Wallis variance analysis was performed to analyze the data. All data were expressed as mean ± standard deviation (mean ± SD) using Mann-Whitney U test for pair wise comparisons of groups. The level of statistical significance was accepted as $P < 0.05$.

3. Results

A total of 28 rats were included in the study. None of the rats died during the study period.

3.1. Hepatic Histopathological Injury Scores. The HHIS of the sham operated group was significantly lower than the IR, RIP + IR, and RIP + NAC + IR groups (resp., $P = 0.001$, $P = 0.001$, $P = 0.002$). The scores were significantly higher in the IR group than in the RIP + IR, and RIP + NAC + IR groups (resp., $P = 0.006$, $P = 0.003$). The difference between the scores of the RIP + IR and RIP + NAC + IR groups was not significant ($P = 0.334$) (Table 1).

In the sham group, normal morphological features were observed. Overall injury grade was 0 (Figures 1(a), 1(b), 1(c), and 1(d)).

In the IR group, disintegration and hemorrhage in the hepatic chords, sinusoidal dilatation, and mononuclear cell infiltration was observed. In some regions focal necrosis were also detected (Figures 2(a), 2(b), 2(c), and 2(d)).

In the RIP + IR group, integration of the hepatic chords was better and less sinusoidal dilatation, mononuclear cell infiltration, and degeneration of hepatic cells were observed compared to the IR group (Figures 3(a), 3(b), and 3(c)).

In the RIP + NAC + IR group histopathological findings were similar to the RIP + IR group. Although no significant difference was observed, HHIS was lower than the RIP + IR group (Figures 4(a), 4(b), and 4(c)).

3.2. Biochemical Parameters. The values of AST and ALT for the IR (resp., $P = 0.018$, $P = 0.018$), RIP + IR (resp., $P = 0.0003$, $P = 0.003$), and RIP + NAC + IR (resp., $P = 0.002$, $P = 0.002$) groups were significantly higher than the sham group (Table 1). No statistically significant differences were determined for the values of AST and ALT in comparisons between the IR group and RIP + IR group (resp., $P = 0.886$, $P = 0.086$) and between the IR group and RIP + NAC + IR group (resp., $P = 0.406$, $P = 0.064$). Also there were no significant differences between the AST and ALT values of the RIP + IR and RIP + NAC + IR groups (resp., $P = 0.775$, $P = 0.475$).

4. Discussion

In the present study, it was demonstrated histopathologically that RIP and RIP + NAC decreased hepatic IR injury significantly. Although HHIS was better in the RIP + NAC

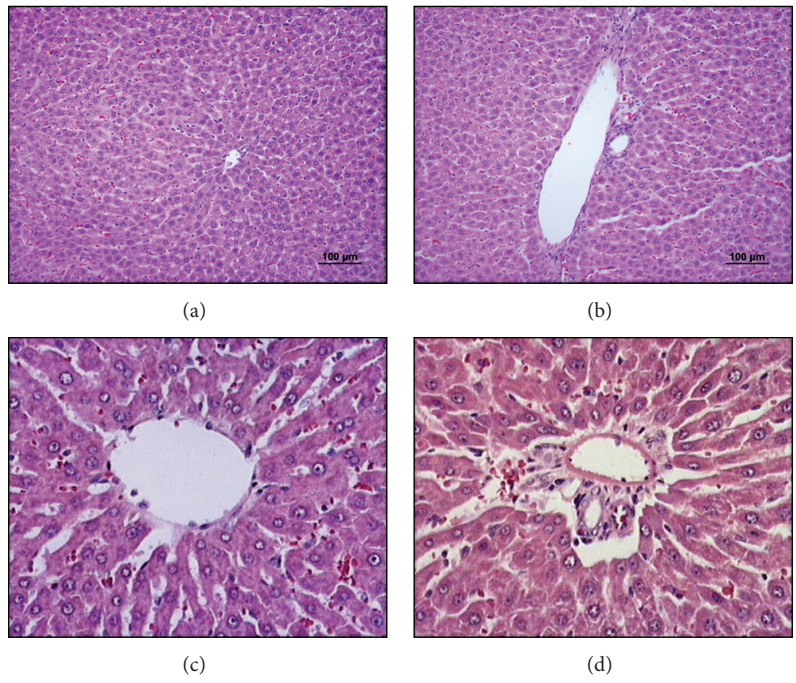


Figure 1: The liver sections of sham group (×20 and ×40). ((a) and (c)) normal sight of vena centralis and ((b) and (d)) normal sight of portal region.

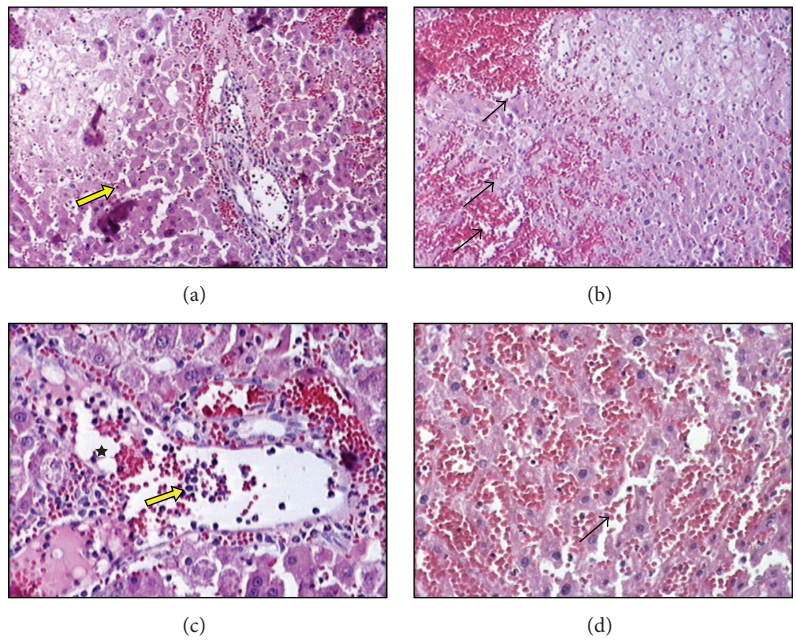
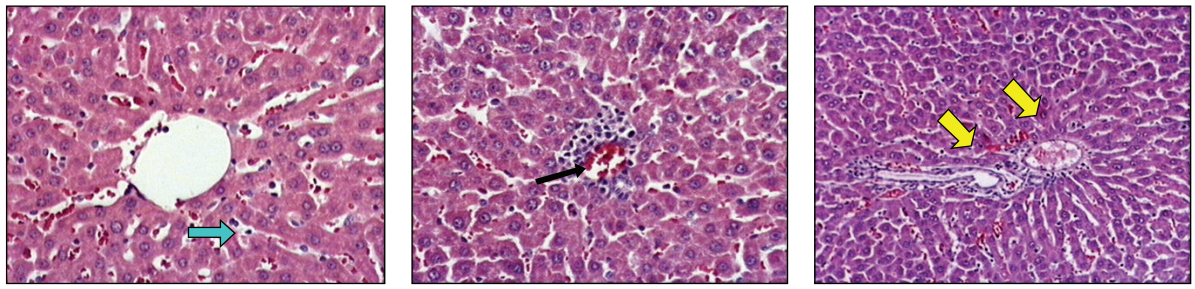


Figure 2: The liver sections of ischemia reperfusion group (×20 and ×40). ((a) and (c)) The integrity of hepatocyte cell cords is destroyed, sinusoidal dilatation, mononuclear cell infiltration (star), and focal necrosis of the hepatocytes (yellow arrows) ((b) and (d)). Erythrocyte extravasation (black arrows).

Table 1: Aspartate aminotransferase, alanine aminotransferase, and hepatic histopathological injury score values for the study groups.

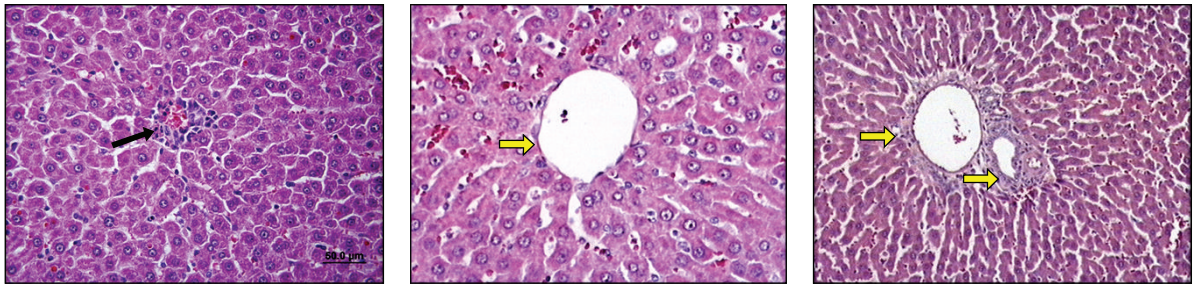
	AST (U/L)	ALT (U/L)	HHIS
Sham group	138.4 ± 30.0	50.0 ± 9.7	0.00 ± 0.00
IR group	1034.7 ± 521.0*	834.2 ± 427.6*	2.28 ± 0.48*
RIP + IR group	1081.8 ± 227.5*	1203.6 ± 297.8*	1.28 ± 0.48*#
RIP + NAC + IR group	1703.8 ± 1145.3*	1237.4 ± 540.5*	1.00 ± 0.57*#

*$P < 0.05$, compared to sham group.
#$P < 0.05$, compared to IR group.
AST: aspartate aminotransferase, ALT: alanine aminotransferase, HHIS: hepatic histopathological injury score, IR: ischemia-reperfusion, RIP: remote ischemic preconditioning, NAC: N-Acetylcysteine.



(a) (b) (c)

Figure 3: The liver sections of remote ischemic preconditioning + ischemia reperfusion group (×20 and ×40). (a) Blue arrow is showing the integrity of hepatocyte cell cords is more regular and less sinusoidal dilatation compared to ischemia reperfusion group. (b) Black arrow shows less focal necrosis. (c) Yellow arrows point the portal region which is close to normal.



(a) (b) (c)

Figure 4: The liver sections of remote ischemic preconditioning + N-Acetylcysteine + ischemia reperfusion group (×20 and ×40). (a) Focal necrosis of the hepatocytes has been seen rarely (black arrow). ((b) and (c)) Less sinusoidal dilatation compared to ischemia reperfusion group and yellow arrows point the portal region which is close to normal.

group, there was no significant difference between RIP + IR and RIP + NAC + IR groups. According to the biochemical parameters, both methods could not prevent IR injury.

Ischemia/reperfusion injury induces cholestasis and reduces bile secretion temporarily. The changes of the bile flow result with an increase in AST/ALT levels, liver myeloperoxidase (MPO) activity, and plasma bilirubin levels and return to normal in 1–3 days [21]. The best indicators of hepatic IRI are enzyme activities like plasma AST, ALT, and histopathologic changes [6, 22]. Therefore we choose AST, ALT, and HHIS for detecting IRI.

Total [23] or partial [7] hepatic ischemia models can be used for hepatic IR studies. Partial ischemia causes less mesenteric congestion but besides its technical difficulty this model does not reflect the clinical practice. The total ischemia time that hepatic IRI can occur but does not impair the hemodynamic stability was determined as at least 25 minutes [19, 23]. In this study, 30 minutes of total hepatic ischemia was used because of the similarity to the clinical practice (Pringle manoeuvre).

In the present study hepatic IRI has been shown after 30 minutes of total hepatic ischemia with both biochemical and histopathological methods. Histopathological and biochemical changes in the IR group compared to the sham operated group indicated that hepatic IR model was applied correctly.

Remote IP was first described by Przyklenk et al. [24] who showed that brief occlusion of coroner arteries protects the heart against prolonged ischemia. The protective effect of

RIP was also shown in lung [7], kidney [25], muscle [26], and bowel [27] IRI. RIP was suggested to be an effective and easy method for protecting the liver against IRI without generating a direct trauma to the liver [23, 28]. Lai et al. [28] showed that 4 cycles of IP by clamping the femoral artery before partial ischemia of the liver were effective to reduce the hepatic IRI. Küntscher et al. [18] reported that using a tourniquet would be as effective as direct clamping of the femoral artery for generating RIP. Abu-Amara et al. [29] showed that six cycles of RIP for four minutes before hepatic IR significantly reduced the hepatic IRI. Saita et al. [26] found that the most effective IP method is 10 minutes ischemia and 10 minutes reperfusion for 3 cycles for skeleton muscle IRI. Kanoria et al. [23] demonstrated that 3 cycles of RIP significantly reduce plasma levels of transferases and increase hepatic blood flow and peripheral oxygen saturation in a total hepatic ischemia model. Similarly, Şahin et al. [19] reported that 3 cycles of 10 minutes hind limb RIP protect liver from IRI biochemically and histopathologically. In the present study, 3 cycles of hind limb RIP, whose effectiveness was shown, were used but the protective effect of RIP could only be detected histopathologically.

N-Acetylcysteine participates in the glutathione syntheses in the lung and liver as a cysteine source and increases glutathione syntheses and it also bounds free oxygen radicals and protects the cell by preventing cell injury [9]. The hypothesis of this study was combination of RIP and NAC would provide a better tissue protection. This method was chosen because this technique could be easily performed before liver resection or transplantation.

The administration of NAC alone can provide tissue protection [13–17]. In the study of Smyrniotis et al. [16] it was shown that NAC (0.3 mg/kg IV) can reduce hepatic IRI biochemically and histopathologically. Galhardo et al. [14] found that 150 mg/kg IV NAC administration to rats before hepatic ischemia, reduced necrosis, apoptosis, and microvesicular steatosis compared to IR group. But controversial to these findings Ghosh et al. [30] and Baumann et al. [31] could not show the beneficial effects of 150 mg/kg IV NAC before hepatic ischemia.

In the present study we found that NAC (150 mg/kg IP) combination with RIP before hepatic total ischemia significantly decreased the HHIS compared to IR group. The HHIS of RIP + NAC group was lower than RIP but there was no statistically significant difference between these groups.

We could not confirm the beneficial histological effects of RIP and RIP + NAC with biochemical parameters that show the hepatic functions. Kanoria et al. [32] studied the effects of RIP, 3 cycles of 10 minutes, after 25 minutes of total hepatic ischemia and two hours of reperfusion histologically and biochemically. Unlike our results, they reported a significant reduction in plasma transferase levels and they were compatible with the histological findings. Likewise, Wang et al. [33] detected a significant reduction in ALT levels with RIP after hepatic IR at the first and third hours of reperfusion in mice. But the researchers could not find a significant effect at the second hour of reperfusion. We could not explain the biochemical results that did not confirm our histological findings but the difference of the study animals could be the reason of our varied results. Besides in this study only the early periods of reperfusion are investigated. The possible long-term effects of the drugs could be masked. Therefore newer studies that investigate long-term effects in different animal types are needed.

In conclusion, this study demonstrated that RIP and NAC addition to RIP decreased significantly hepatic IRI histopathologically.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

The Effect of Dexmedetomidine on Oxidative Stress during Pneumoperitoneum

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Purpose. This study was intended to investigate the effect of dexmedetomidine on oxidative stress response in pneumoperitoneum established in rats. *Methods*. Animals were randomized into three groups, group S: with no pneumoperitoneum, group P: with pneumoperitoneum established, and group D: given 100 mcg intraperitoneal dexmedetomidine 30 min before establishment of pneumoperitoneum. Plasma total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI) activity were measured 30 min after conclusion of pneumoperitoneum. *Results*. The mean TOS level was significantly higher in group P than in the other two groups, and the TOS level was significantly higher in group D than in group S ($P < 0.05$). Plasma TAS level was found to be lower in group P than in the other two groups, and the TAS level was lower in group D than in group S ($P < 0.05$). Consequently, the OSI was significantly higher in group P than in groups D and S ($P < 0.05$). *Conclusions*. Ischemia-reperfusion phenomenon that occurs during pneumoperitoneum causes oxidative stress and consumption of plasma antioxidants. Dexmedetomidine decreases oxidative stress caused by pneumoperitoneum and strengthens the antioxidant defense system.

1. Introduction

Laparoscopic surgery is performed widely because it causes less tissue trauma associated with shorter healing time compared with open surgery. Nevertheless, concerns regarding systemic complications and pathophysiology are still being investigated [1].

Clinical and experimental studies have established that the increase in intra-abdominal pressure that develops depending on the degree of pneumoperitoneum during laparoscopic surgery can cause hypoperfusion of intra-abdominal organs [2–6]. Increases in ischemia and the oxidative stress response were observed with pneumoperitoneum-dependent impairment of splenic perfusion [7–11]. After desufflation, reperfusion injury occurred with the fall in intra-abdominal pressure [3, 12].

Reactive oxygen products (ROS) like superoxide and hydroxyl radicals released during reperfusion are considered to contribute to reperfusion injury, because excessive ROS and their toxic products cause DNA damage, lipid peroxidation, and mitochondrial membrane and cell damage [13, 14]. Therefore ROS is considered a significant mediator of tissue injury. ROS is strictly controlled by complex antioxidants containing enzymes like superoxide dismutase, catalase, and glutathione peroxide [15, 16]. Free radicals formed as a result of pneumoperitoneum cause plasma antioxidants to decrease [17]. Thus one of the main results of ischemia-reperfusion due to pneumoperitoneum is the disturbance of the balance between the oxidative and antioxidative systems. The imbalance is defined as oxidative stress [18]. The severity of oxidative stress is determined by the measurement of total oxidant status (TOS) and consumed antioxidant status [17].

Various agents have been used in animal studies to protect organs from ischemia-reperfusion induced oxidative stress. Although these agents have been recommended for

the protection of organs during various pathological conditions and surgical procedures in humans, their development and approval for clinical use are a lengthy process. One agent approved for clinical use that has been shown to protect the kidney against ischemia-reperfusion injury [19] is dexmedetomidine, a selective and potent α2-adrenoceptor agonist. It is frequently used for anesthesia in daily practice and for sedation, anxiolysis, and analgesia in the intensive care unit.

In this experimental study we aimed to investigate the effect of dexmedetomidine on oxidative stress in the ischemia-reperfusion injury due to pneumoperitoneum. We planned to use plasma total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI) parameters for determining the effect.

2. Materials and Methods

This study was approved by the ethics committee of our university and was performed in compliance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.1. Animal Preparation. Twenty-four adult female Sprague-Dawley rats weighing 250–300 g were used in this study. The animals were kept in a windowless, light-controlled environment at 20 ± 2°C and were allowed free access to food and water. They were fasted for one night before the experiment. The animals were anesthetized with 50 mg/kg ketamine (Ketalar; Parke Davis, Berlin, Germany) and 20 mg/kg xylazine (Rompun; Bayer, Leverkusen, Germany), and were placed in a supine position on an operating table. The tail vein was cannulated with a 24 G intravenous catheter. After the tracheal region was cleaned, the trachea was isolated with a midline incision and cannulated with a 16 G intravenous catheter. Mechanical ventilation was initiated in volume-controlled mode with a respiratory frequency of 40/min, tidal volume of 10 mL/kg, inspirium/expirium ratio of 1 : 1, and fractional inspiratory oxygen concentration (FiO_2) of 1.0. Spontaneous respiration was suppressed with intravenous pancuronium (1 mg/kg).

2.2. Experimental Protocol. Following an initial stabilization period, the animals were randomized into three groups (n = 8 in each): group S (sham group), no pneumoperitoneum was established; group P (pneumoperitoneum group), 60 min pneumoperitoneum was established under 12 mm Hg pressure; and group D (ischemia-reperfusion/dexmedetomidine treatment group), intraperitoneal dexmedetomidine (100 μg) was administered 30 min before abdominal insufflation to establish 60 min pneumoperitoneum under 12 mm Hg pressure.

2.3. Establishment of Pneumoperitoneum. Pneumoperitoneum was established by inserting an 18 G intravenous catheter into the abdominal right lower quadrant of the peritoneal cavity and insufflating the abdomen with CO_2 to a pneumoperitoneal pressure of 12 mm Hg. The intra-abdominal pressure was maintained for 60 min with an electronic laparoflator (Karl-Starz GmbH, Tutlingen, Germany).

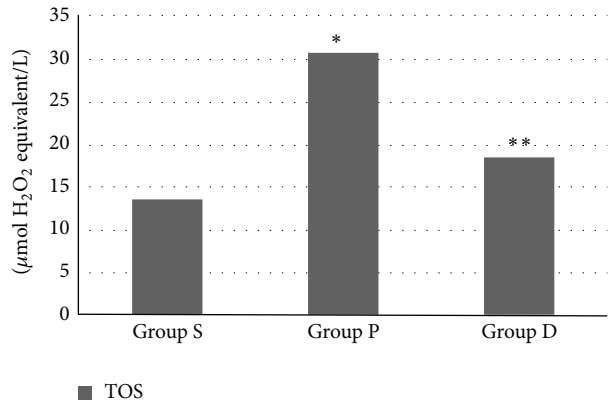

Figure 1: Total oxidant status (TOS) levels (group S: 13.56 ± 1.72, group P: 30.67 ± 3.06, and group D: 18.43 ± 1.93). $^*P < 0.0001$ group P when compared with D and S groups and $^{**}P < 0.01$ group D when compared with group S.

2.4. Blood Samples. Blood samples were obtained in tubes containing 3.8% sodium citrate as an anticoagulant. Plasma and serum were separated by centrifugation at 3000 rpm for 10 min. The serum and plasma samples were kept at −80°C until biochemical analysis.

2.5. Measurements. Blood plasma total antioxidant status (TAS) and total oxidant status (TOS) were measured 30 min after desufflation.

Plasma TOS was determined using a method as previously described by Erel [20] and is expressed as μmol H_2O_2 equivalent/L. Serum TAS was determined using an automated measurement method developed by Erel [21] and is expressed as mmol Trolox equivalent/L. The OSI was calculated from the TOS and TAS values: OSI = [(TOS, μmol H_2O_2 equivalent/L)/(TAS, μmol Trolox equivalent/L)] × 100 [22].

2.6. Statistical Analysis. Statistical analysis was performed using two-way and three-way ANOVA. All values are expressed as means ± SD. Significance was set at $P < 0.05$.

3. Results

All rats survived until the end of the experiment. Body weight was similar among the groups (202.62 ± 28.86, 211.00 ± 14.45, and 212.87 ± 15.71 g in groups S, D, and P, resp.).

The mean TOS level was significantly higher in group P than in the other two groups ($P < 0.0001$), and the TOS level was significantly higher in group D than in group S ($P < 0.01$) (Figure 1). Plasma TAS level was found to be lower in pneumoperitoneum group than in groups S and D ($P < 0.0001$ and < 0.015, resp.), and TAS level was lower in D group than in group S ($P < 0.0001$) (Figure 2). Consequently,

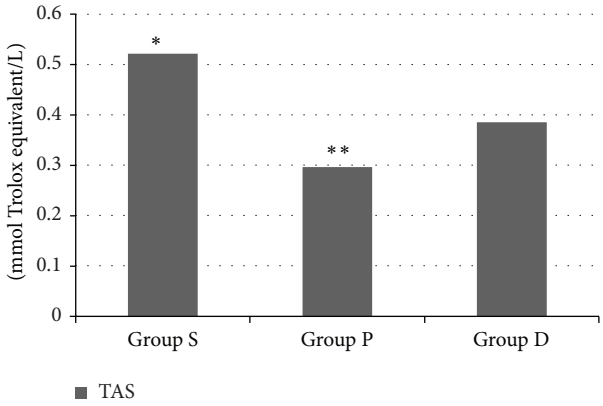

Figure 2: Total antioxidant status (TAS) levels (group S: 0.52 ± 0.49, group P: 0.29 ± 0.06, and group D: 0.38 ± 0.05). $^{*}P < 0.001$ group S when compared with D and C groups and $^{**}P < 0.015$ group P when compared with group D.

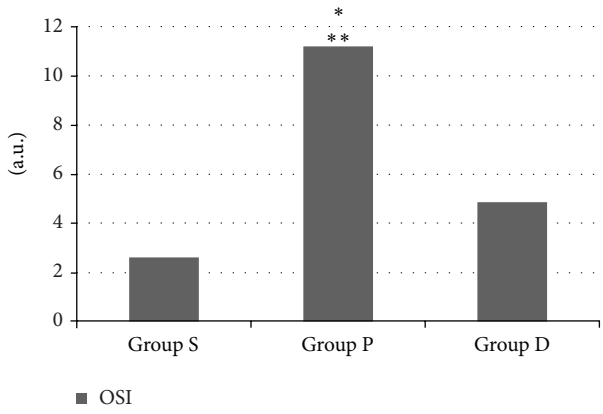

Figure 3: Oxidative stress index (OSI) levels (group S: 2.61 ± 0.48, group P: 11.2 ± 4.90, and group D: 4.86 ± 0.89). $^{*}P < 0.0001$ group P when compared with groups S and $^{**}P < 0.001$ group P when compared with group D.

the OSI was significantly higher in group P than in groups D and S ($P < 0.001$ and < 0.0001, resp.) (Figure 3).

4. Discussion

In our study we determined that plasma TOS level and OSI score decreased, and antioxidant defense system strengthened with dexmedetomidine administration before pneumoperitoneum in rats. We observed that dexmedetomidine prevented oxidative damage caused by pneumoperitoneum.

The free oxygen radicals are known to be released during this reperfusion period and have been proposed as important mediators of tissue injury [23]. Free oxygen radicals induced lipid peroxidation associated with a decrease in plasma antioxidant capacity [7]. Reactive oxygen radicals play a vital role in the injury caused by ischemia-reperfusion. Many studies for animals [2, 7, 11] and human beings [24, 25] have investigated ischemia-reperfusion injury caused by pneumoperitoneum. In these studies oxidative stress and lipid peroxidation markers were evaluated with measurements of different markers like thiobarbituric acid reactive substances (TBARS), endogen antioxidant level, and histological findings to obtain protein carbonyl and protein sulfhydryl content [26]. These measurements showed that pneumoperitoneum may cause an increase in the oxidative stress response and ROS may cause damage to lipids and proteins during oxidative stress.

In this study we observed a significant increase in total plasma level after pneumoperitoneum. Hydrogen peroxide and other peroxide derivatives are produced biologically in organisms, but in some pathological cases it is produced at high levels [20]. In particular high oxygen concentration in the circulation during the reperfusion causes the formation of free radicals. Baysal et al. determined that free radicals formed during laparoscopy caused oxidative stress in children, increased plasma oxidant status and OSI level after pneumoperitoneum, and decreased total antioxidant status [17]. Similarly we observed an increase in plasma oxidative stress index and a decrease in total antioxidant status level after pneumoperitoneum deflation.

To reverse the oxidative stress caused by pneumoperitoneum, various pharmacological agents and protective methods have been tested [12, 23, 27, 28]. Ates et al. discovered that erythropoietin administration before pneumoperitoneum caused a significant decrease in LDH, TNF-α, and MDA levels [27]. Imamoğlu et al. showed that melatonin administration before pneumoperitoneum insufflation and deinsufflation decreased kidney MDA level [23]. Similarly zinc, pentoxifylline, and NAC administration to renal ischemia-reperfusion injury caused by laparoscopy decreases kidney tissue MDA level [28].

Dexmedetomidine, an α2-adrenergic agonist, has been shown experimentally to prevent ischemia-reperfusion injury by producing vasodilation and is frequently used in anesthesia and intensive care practice [19, 29]. Apart from sedative, analgesic characteristics of dexmedetomidine it has been shown that it has the ability to relieve the lung injury caused by renal ischemia-reperfusion [30]. Dexmedetomidine provides local protection in ischemic kidney and following that generates anti-inflammatory effect by decreasing systemic accumulation of cytokines. This effect is determined with evidence at preclinical and clinical level [19, 30].

Positive effect of dexmedetomidine has been shown on ischemia-reperfusion experimental models where oxidative injury plays a significant role. But systemic effect on oxidative stress is not shown yet.

In the light of findings of our study we determined that oxidative stress response decreases and antioxidant defense system strengthens with the administration of dexmedetomidine before pneumoperitoneum. We determined that plasma total oxidant status and OSI level were lower and total antioxidant status level was higher in groups to which dexmedetomidine was administered before pneumoperitoneum. We observed that dexmedetomidine can play a role in preventing the oxidative injury caused by pneumoperitoneum.

Laparoscopic procedure can be used widely for patients from different age groups in early future. Administration of laparoscopic procedure can increase the severity of ischemia-reperfusion injury in old patients with malignity and cardiovascular problems. Therefore dexmedetomidine as anesthetic agent may be preferred as protective agent in preventing adverse effects of oxidative injury.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical Study

The Comparison of the Effects of Sevoflurane Inhalation Anesthesia and Intravenous Propofol Anesthesia on Oxidative Stress in One Lung Ventilation

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Background. The aim of this study is to compare the effects of sevoflurane and propofol on one lung ventilation (OLV) induced ischemia-reperfusion injury (IRI) by determining the blood gas, ischemia-modified albumin (IMA), and malonyldialdehyde (MDA). *Material and Methods*. Forty-four patients undergoing thoracic surgery with OLV were randomized in two groups (sevoflurane Group S, propofol Group P). Anesthesia was inducted with thiopental and was maintained with 1–2.5% of sevoflurane within the 40/60% of O_2/N_2O mixture in Group S. In Group P anesthesia was inducted with propofol and was maintained with infusion of propofol and remifentanil. Hemodynamic records and blood samples were obtained before anesthesia induction (t_1), 1 min before two lung ventilation (t_2), 30 min after two lung ventilation (t_3), and postoperative sixth hours (t_4). *Results*. Heart rate at t_2 and t_3 in Group P was significantly lower than that in Group S. While there were no significant differences in terms of pH and pCO_2, pO_2 at t_2 and t_3 in Group S was significantly lower than that in Group P. IMA levels at t_4 in Group S were significantly lower than those in Group P. *Conclusion*. Sevoflurane may offer protection against IRI after OLV in thoracic surgery.

1. Introduction

One-lung ventilation (OLV) is usually performed to provide wide surgical area in thoracic surgery. During the OLV hypoxic pulmonary vasoconstriction occurs in nonventilated lung (NVL). While the blood flow of other lobe increases, perfusion and oxygenation of NVL decrease. As a result of this, tissue ischemia occurs in nonventilated site. After resuming two-lung ventilation (2LV), the reperfusion of the blood and reentry of oxygen to ischemic tissue cause sudden and significant increase in reactive oxygen species (ROS) production [1]. Increased ROS induce lipid peroxidation of polyunsaturated fatty acid in biological membranes and plasma lipoproteins [2]. These events, reentry of the oxygen to ischemic tissue and peroxidative reaction of some biological structure, are called ischemia-reperfusion injury (IRI). IRI may cause some cardiac complications [3].

The total antioxidant status (TAS) of human body counteracts oxidative stress. Resuming the 2LV from OLV or after treatment of pneumothorax [4, 5] hydrostatic pressure rises may cause increase in alveolocapillary membrane permeability leading to pulmonary oedema. Bowler et al. reported that TAS was decreased by pulmonary edema fluids in acute lung injuries [6]. It was stated that after 2LV severe oxidative injuries may be important in patients without adequate TAS [1].

There are a lot of studies carried out to prevent IRI [7–14]. Some antioxidant agents can restrain lipid peroxidation and reperfusion injury. Propofol, chemically similar to phenol based free radical scavengers, was used for this purpose [7–11]. On the other hand some studies emphasized that halogenated inhalation agent, sevoflurane, can lead to reduction in IRI [12–14].

After reperfusion of ischemic tissue malonyldialdehyde (MDA), toxic intermediate product of lipid peroxidation and

ischemia-modified albumin (IMA) levels increase in blood. Thus both MDA and IMA were used as a marker of IRI studies [2, 9].

The aim of this randomized, prospective, double-blind study is to compare the effects of propofol and sevoflurane on IRI in patients undergoing thoracic surgery in which OLV/2LV was used. MDA, IMA, blood gas levels, and hemodynamics were measured for this purpose.

2. Material and Methods

After obtaining the ethics committee approval and patient informed consent the study was carried out in 44 patients, aged between 18 and 65, ASA physical status I or II, undergoing OLV/2LV for thoracic surgery. Sealed envelope method was used for randomization and the patients were divided into two groups (sevoflurane: Group S, $n = 22$ and propofol: Group P, $n = 22$). Patients with ASA score of III or more and severe metabolic, renal, or hepatic diseases, using cigarettes or antioxidant agents, were excluded from the study.

All patients were sedated with 3 mg of midazolam intramuscularly 30 min before the operation. In the operating room, electrocardiography, peripheral arterial oxygen saturation, and invasive arterial blood pressure were monitored. First blood samples for blood gas, MDA, and IMA were obtained and vital parameters were recorded at this time (t_1). Thiopental (6 mg/kg) in Group S and propofol (1.5–2.5 mg/kg) in Group P were used for induction of anesthesia. After the administration of fentanil 2 μ/kg and rocuronium 0.6 mg/kg all patients were intubated with double lumen tubes. In Group S anesthesia was maintained with 1–2.5% of sevoflurane within the 40/60% of O_2/N_2O mixture. In Group P anesthesia was maintained with total intravenous anesthesia using infusion of 125–250 μ/kg/min of propofol and 0.1–0.25 μ/kg/min of remifentanil. Ventilation was mechanically controlled and OLV was put into practice for surgical intervention using tidal volume: 6–8 mL/kg, with respiratory rate: 12–20 and fraction of inspired O_2: 1 adjusted to CO_2: 35–45 mmHg. After the required procedure was carried out 2LV was resumed from OLV. The blood samples were obtained 1 min before 2LV (t_2) and 30 min after 2LV (t_3). Hemodynamics was also recorded at these intervals. At the end of the operation patients were extubated and transferred to Surgical Intensive Care Unit. Last blood samples and hemodynamic record were obtained at postoperative sixth hour (t_4).

The Kolmogorov-Smirnov test was used to determine normality and homogeneity of data distribution. Parametric data (age, blood pressure, OLV time) were compared using one-way analysis of variation (ANOVA). Nonparametric data were compared using the Kruskal-Wallis test. MDA, IMA, and blood gas analysis were compared using Student's t-test between two groups.

3. Results

There were no significant differences between the groups with respect to age, sex, and OLV time (Table 1).

Table 1: Patients characteristic data.

	Group S	Group P
Age (years)	52.31 ± 13.22	52.45 ± 11.80
Sex (M/F)	6/16	8/14
OLV time (min)	111.59 ± 44.891	135.68 ± 45.021

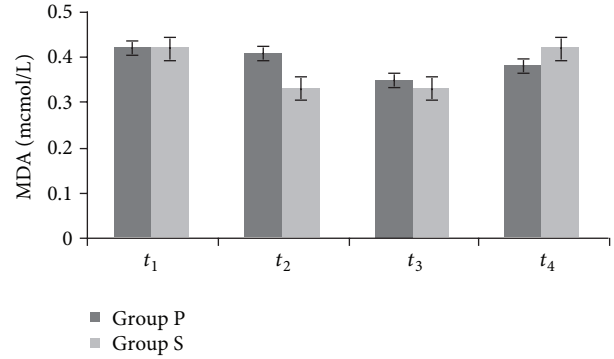

Figure 1: Plasma concentration of malonyldialdehyde ($P > 0.05$).

Although there were no significant differences between the mean arterial pressures, heart rate at t_2 and t_3 in Group P was significantly lower than the parameters in Group S (t_2; 65.05 ± 11.32, 73.95 ± 13.00, t_3; 62.91 ± 12.21, 72.05 ± 15.57, resp.) ($P < 0.05$) (Table 2).

In blood gas analyses, there were no significant differences in terms of pH and pCO_2. In Group S, pO_2 at t_2 and t_3 was significantly lower than Group P (t_2: 151.45 ± 71.85, 240.17 ± 117.43, t_3: 186.55 ± 67.62, 259.51 ± 102.98, resp.) ($P < 0.01$) (Table 2).

There were no significant differences between the groups in terms of MDA (Figure 1). IMA levels at t_4 in Group S were significantly lower than Group P (0.76 ± 0.09, 0.83 ± 0.09, resp., $P < 0.05$) (Figure 2).

4. Discussion

This study showed that sevoflurane seemed to provide more protection than propofol by lower increasing in IMA and MDA. These findings have also been clinically important even if there were no complications in patients, because our patients were of ASA I or II score. If this study was performed with ASA III or more scored patients, cardiac or pulmonary complications due to lipid membrane peroxidation could occurr.

Cheng et al. [1] studied the effect of OLV on oxidative stress by measuring ROS and TAS in patients undergoing thoracic surgery with OLV. Their study showed that while ROS increases TAS decreases. In addition authors stated that extravascular lung fluid and intrathoracic blood volume were increased after 2LV. However, mostly patients do not counteract severe complication despite increasing ROS. They explained this condition by the fact that patients with normal TAS can tolerate these negative effects of oxidative stress. However, some critically ill [15], older aged [16], traumatic, or cancer patients have decreased TAS in their plasma. In these

Table 2: Heart rate (HR), mean arterial pressure (MAP), and blood gases.

	t_1	t_2	t_3	t_4
Group S				
HR	81.77 ± 12.78	73.95 ± 13.00	72.05 ± 15.57	79.27 ± 13.93
MAP	90.41 ± 15.00	76.05 ± 9.44	80.64 ± 14.31	84.91 ± 17.84
pH	7.37 ± 0.06	257.31 ± 0.04	7.31 ± 0.03	7.36 ± 0.04
pO_2	154.95 ± 92.03	151.45 ± 71.85*	186.55 ± 67.62*	147.43 ± 71.41
pCO_2	44.35 ± 6.75	45.01 ± 8.09	45.15 ± 7.6	39.88 ± 5.99
Group P				
HR	78.41 ± 18.42	65.05 ± 11.32#	62.91 ± 12.21#	79.45 ± 17.19
MAP	93.05 ± 10.98	81.59 ± 17.37	77.23 ± 16.09	88.32 ± 13.98
pH	7.37 ± 0.04	7.32 ± 0.06	7.33 ± 0.06	7.38 ± 0.03
pO_2	184.66 ± 83.30	240.17 ± 117.43	259.51 ± 102.98	163.22 ± 64.43
pCO_2	42.84 ± 6.09	44.38 ± 8.78	42.11 ± 8.09	39.47 ± 4.98

#$P < 0.05$ when heart rate at t_2 and t_3 in Group P was compared with that in Group S.
*$P < 0.01$ when pO_2 at t_2 and t_3 in Group S was compared with that in Group P.

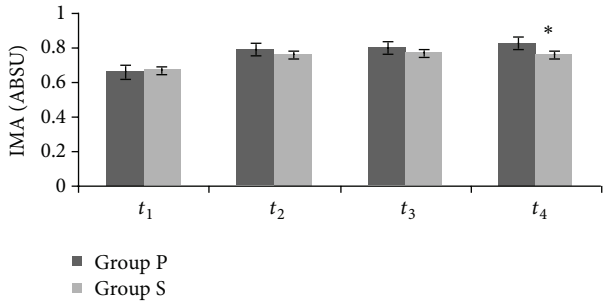

Figure 2: Plasma concentration of ischemia-modified albumin. IMA at t_4 in Group S compared with Group P (*$P < 0.05$). ABSU: absorbance unit.

patients oxidative stress may cause destruction to DNA and protein and lipid structures.

Propofol was used to decrease IRI in a lot of clinical or experimental reperfusion studies. In an experimental reperfusion model, Akyol et al. [2] found that propofol was effective in protecting lung injury caused by increased oxidative stress and neutrophil accumulation. Huang et al. [8] investigated the effect of propofol infusion anesthesia on reperfusion injury compared to isoflurane inhalational anesthesia during OLV in thoracic surgery. They studied ROS and TAS and stated that propofol infusion shortens and attenuates oxidative stress during OLV. This protective effect of propofol was attributed to its antioxidant properties. However, they also stated that in critically ill patients, the use of total intravenous anesthesia with propofol infusion may be limited because of their unstable hemodynamics. Limited usage of propofol for this reason brings up different agent to prevent IRI.

The effects of inhalational anesthetics on ischemic myocardium have been investigated for many years. The protective effects of halogenated inhalational anesthetics were shown by different studies. Zaugg et al. [17] stated that inhalational anesthetics (sevoflurane and isoflurane) provide protection to IRI in cardiomyocytes by selectively priming K_{ATP} channels through multiple triggering protein kinase C-coupled signaling pathways. In another study, Novalija et al. [18] showed that anesthetic preconditioning with sevoflurane improved adenosine triphosphate synthesis and reduced ROS formation in mitochondria after ischemia by a redox dependent mechanism. One of the good IRI models is cardiac surgery in which reperfusion may cause deterioration of rhythm and contraction of myocardium. Garcia et al. [19] stated at the end of their study that pharmacological preconditioning by sevoflurane provided protective role in cardiac events in coronary bypass patients.

Although there are a lot of studies in different ischemia-reperfusion models, there is no study in which the effect of sevoflurane on IRI was compared with propofol in OLV. Annecke et al. [12] compared the effects of sevoflurane on IRI with propofol after thoracic aortic occlusion in pig. After removing the clamp severe shock occurred in both study groups. While norepinephrine requirements in the sevoflurane group were significantly reduced during reperfusion, serum lactate dehydrogenase, aspartate transaminase, and alanine aminotransferase were also lower with sevoflurane. They state that the use of sevoflurane compared with propofol attenuated the hemodynamic sequelae of reperfusion injury in their model.

Another explanation of protective effect of sevoflurane against IRI may be the effect of it on hypoxic pulmonary vasoconstriction (HPV). During OLV while the perfusion of nonventilated lung is decreased, other lung's is increase. Nonventilated lung remains not only atelectatic, but also hypoperfused and ischemic. While inhalational anesthetics, sevoflurane, can inhibit HPV, intravenous anesthetics, propofol, are unaffected on HPV. Thus, nonventilated lung does not remain severely hypoperfused, and reperfusion injury was limited in patients with sevoflurane anesthesia. Lower pO_2 levels in Group S at t_2 and t_3 show continuing of the perfusion of nonventilated/atelectatic lung in our patients.

IMA reaches a peak level of sixth hour of reperfusion and begins to decrease at twelfth hour. In this study, lower IMA

level in Group S than Group P at postoperative sixth hour showed that sevoflurane provided protection against IRI. However, we did not show the protection with MDA level. There were no different MDA levels between the groups at all measurement times. Cheng et al. [1] stated that resection of lung cancer can decrease MDA levels. Some patients in both groups were operated on for lung cancer. Probably, we cannot support our findings with MDA.

Although these findings encourage us to use sevoflurane to provide protection against IRI, there were limitations in our study. The last blood sample was obtained at postoperative sixth hour. If we investigate postoperative twelfth hour or later, we can show the earlier return to normal IMA level in sevoflurane group. Another limitation of our study is the lacking of another control group. If another control group was formed with an agent with no protection to IRI, the study could be more powerful.

In conclusion we consider that sevoflurane may offer protection against reperfusion injury after one-lung ventilation in thoracic surgery.

Conflicts of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Ischemia-Reperfusion Injury and Volatile Anesthetics

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Ischemia-reperfusion injury (IRI) is induced as a result of reentry of the blood and oxygen to ischemic tissue. Antioxidant and some other drugs have protective effect on IRI. In many surgeries and clinical conditions IRI is counteract inevitable. Some anesthetic agents may have a protective role in this procedure. It is known that inhalational anesthetics possess protective effects against IRI. In this review the mechanism of preventive effects of volatile anesthetics and different ischemia-reperfusion models are discussed.

1. Introduction

After the ischemic period reentry of the blood to tissue causes massive release of oxygen free radicals. These free radicals trigger enzymatic reactions, such as peroxidation of polyunsaturated fatty acids or plasma lipoproteins, which leads to oxidative destruction of cell membranes and the productions of toxic reactive metabolites and cell injury involving DNA, proteins, and lipids [1]. All of these events are called ischemia-reperfusion injury (IRI).

2. Pathophysiology and Clinical Presentation

IRI occurred mostly during anesthesia and intensive care practice. In cardiac surgery or tourniquet application for extremity surgery, thromboembolic events and revascularization, severe hypotension, and restoration of hypovolemic shock, in organ transplantation, can cause IRI. During the ischemia anaerobic glycolysis is activated and then establishment of reperfusion accompanied by pro- and anti-inflammatory cytokine release, polymorphonuclear neutrophil activation, and platelet adhesion to the vascular endothelium occur with production of reactive oxygen species and release of vasoactive factors [2–4]. On the other hand, plasma concentration of some enzymes such as catalase, glutathione peroxidase, superoxide dismutase, lactated dehydrogenase, and some metabolites such as malonyldialdehyde (MDA), ischemia-modified albumin (IMA), lactate, and reactive oxygen species (ROS) increases during postreperfusion period. As a result of these pathophysiological phenomena, local and systemic inflammatory responses are formed by different mechanisms [5–7].

The total antioxidant status (TAS) of human body counteracts oxidative stress and reperfusion injury. It was found that while ROS increased, TAS decreased as a result of oxidative stress [8]. However, most patients do not counteract severe complication despite increasing ROS. It was explained that patients with normal TAS can tolerate these negative effects of oxidative stress. However, advanced age, severe ill, traumatic, or cancer patients have lower TAS in their plasma [9, 10]. In these patients oxidative stress may cause destruction of DNA and some structures with protein and lipid.

Severe systemic inflammatory reactions as a result of massive inflammatory mediator release and reperfusion injury may activate endothelial cells in remote organs which are not exposed to initial ischemic injury [11]. The distant effect of ischemia reperfusion causes microvascular injury with leukocyte invasion on endothelium [12]. These events may lead to multiorgan failure and increased postoperative morbidity and mortality. It was reported that IRI may cause cardiopulmonary complication such as tachyarrhythmia and hypoxia [13].

A lot of studies are conducted to prevent IRI. Some of these are related to anesthesia method such as regional anesthesia, inhalation general anesthesia, or total intravenous anesthesia.

3. The Mechanisms of Protective Effects of Volatile Anesthetics

The effects of volatile anesthetics on IRI were investigated for several years [14–17]. It is known that volatile anesthetics, especially halogenated, have a protective role against IRI. These protective effects have been attributed to pre- and postconditioning effects with apoptosis. The mechanisms of these effects have been investigated, and new pathways are asserted continuously. Kowalski et al. [18] stated that polymorphonuclear neutrophils (PMN) lead to reperfusion injury in many organs and tissues via adhesioning to vascular endothelial cells. They investigated the effects of halothane, isoflurane, and sevoflurane on postischemic adhesion of human PMN in the intact coronary system of isolated perfused guinea pig hearts. As a result of this study they found that volatile anesthetics had inhibitory effect on ischemia induced adhesion of PMN and concluded that it may be beneficial for the heart during general anesthesia. Similarly, it was stated that volatile anesthetics were able to modulate the interaction of PMN with the endothelial cell, and this may play a crucial role in the initiation of IRI in other studies [17, 19].

However, protective effects of volatile anesthetics against IRI are wondered and some studies were carried out to explain the mechanism. Novalija et al. [20] performed anesthetic preconditioning with sevoflurane and gained positive outcomes with isolated guinea pig hearts. They explained the positive effect of sevoflurane with improved adenosine triphosphate synthesis and reduced ROS formation in mitochondria after ischemia by a redox dependent mechanism. Kersten et al. [21] stated that volatile anesthetics improved recovery of contractile function of postischemic, reperfused myocardium, and activated KATP channels. For the same purpose Zaugg et al. [22] studied to test whether volatile anesthetics mediate this effect by activation of the mitochondrial adenosine triphosphate-sensitive potassium (mitoKATP) or sarcolemmal KATP channel in rat ventricular myocytes and to evaluate the signaling pathways involved. At the end of their study they found that volatile anesthetics mediate their protection in cardiomyocytes by selectively priming mitoKATP channels through multiple triggering protein kinase C-coupled signaling pathways. And also in another study Marinovic et al. [23] investigated "an innative" protective mechanism of volatile anesthetics against IRI. They made an effort to reveal whether KATP channels are triggers initiating the preconditioning signaling and/or effectors responsible for the cardioprotective memory and activation during ischemia reperfusion. Adult rat cardiomyocytes were exposed to oxidative stress. To induce preconditioning, the myocytes were pretreated with isoflurane. The involvement of sarcolemmal and mitochondrial KATP channels was investigated using specific inhibitors. At the end of the study they concluded that both sarcolemmal and mitochondrial KATP channels play essential and distinct roles in protection afforded by isoflurane. They also stated that sarcolemmal KATP channel seemed to act as an effector of preconditioning, whereas mitochondrial KATP channel played a dual role as a trigger and an effector.

Lucchinetti et al. [24] explored in their study the effects of sevoflurane, propofol, and intralipid on metabolic flux rates of fatty acid oxidation (FOX) and glucose oxidation (GOX) in hearts exposed to ischemia and reperfusion. They studied on isolated, paced working rat hearts that were exposed to 20 min of ischemia and 30 min of reperfusion. Study groups were treated with sevoflurane or propofol. They observed that sevoflurane improved the recovery of left ventricular work and myocardial efficiency. This functional improvement was accompanied by reduced increases in postischemic diastolic and systolic intracellular Ca^{+2} concentrations. Sevoflurane increased GOX and decreased FOX in hearts exposed to ischemia and reperfusion. GLUT4 expression was markedly increased in lipid rafts of sevoflurane treated hearts. Increased GOX closely correlated with reduced Ca^{+2} overload. As a result of their study they concluded that enhanced glucose uptake via GLUT4 fuels recovery from Ca^{+2} overload after ischemia and reperfusion in sevoflurane treated hearts.

The protective effects of volatile anesthetics have been longstanding subject in many studies. It was shown that one of the mechanisms of IRI was an intracellular calcium overload. Volatile anesthetics might also protect the myocardium from IRI by altering myocardial calcium fluxes. They also preserve myocardial energetics and protect from ROS derived injury. Louvier and Lançon stated that enflurane and halothane seemed to be more efficient than isoflurane. They explained these cardiovascular effects by a specific effect on myocardial cells. They also stated that halothane and enflurane mainly decreased intracellular calcium availability by a direct effect on sarcoplasmic reticulum, while isoflurane only decreased the transsarcolemnal calcium entry [25].

4. Organ Specific IRI Models and Volatile Anesthetics

Oxidative stress and reperfusion injury may develop in different ischemia-reperfusion models. In these models enzymatic reactions and cellular destructions can affect not only related system but also remote organ and system. Multiorgan involvement may occurr as a result of IRI. The main organs in which IRI occurs are heart, lung, brain, liver, kidney, and intestine. Preconditioning and postconditioning with volatile anesthetics confer protection against reperfusion injury in these organs. There are a lot of studies that investigated protective effects of volatile anesthetics on these organs.

4.1. Heart Surgery and IRI. One of the most studied reperfusion model is open heart surgery. It was known that cardiac surgery using cardiopulmonary bypass is associated with release of inflammatory mediators and severe systemic inflammatory reactions. Cardiopulmonary bypass was performed and heart was exposed to ischemia in this surgery. After declamping the aorta ischemic tissue was reperfused and reoxygenated. Reperfusion and reoxygenation of the myocardium may lead to dysrhythmia or hypotension called "myocardial stunning." That is why the effects of volatile anesthetics on reperfusion injury were mostly studied on open heart surgery. On the other hand some antioxidative agents

were used for prevention of IRI in both clinical and experimental studies. Propofol is chemically similar to phenol based on free radical scavengers and endogenous antioxidant vitamin E [26, 27]. Therefore, it was used in many clinical and experimental reperfusion injury studies. In consequence of these studies, it was usually concluded that propofol shortens and attenuates oxidative stress and IRI [28–32]. As the protective effects of propofol is well known, some studies compared propofol and volatile anesthetics such as halothane, isoflurane, and sevoflurane carried out to exhibit the effect of volatile anesthetics on oxidative stress and IRI. Conzen et al. [33] carried out a study on 20 patients scheduled to undergo elective offpump coronary artery bypass surgery. They maintained anesthesia with either sevoflurane or propofol. For assessing myocardial injury, troponin I and myocardial fraction of creatine kinase were determined during the postoperative 24 hours. They found that troponin I concentration increased significantly in propofol infusion group. They concluded that cardiac output improved with sevoflurane but not with propofol, suggesting better maintenance of myocardial function. In another study Julier et al. [34] investigated the effects of sevoflurane preconditioning on myocardial and renal function by measuring postoperative release of brain natriuretic peptide. They found that sevoflurane preconditioning significantly decreased postoperative release of brain natriuretic peptide and concluded that sevoflurane preconditioning preserves myocardial and renal function. Garcia et al. [35] stated at the end of their study that pharmacological preconditioning by sevoflurane provided protective role in cardiac events in coronary bypass patients.

4.2. Thoracic Surgery/One Lung Ventilation and IRI. One lung ventilation (OLV) is frequently used for thoracic and some other surgeries. During OLV, vessels in nonventilated lung (NVL) are constructed and blood flow mainly goes towards other lung lob. In such condition called hypoxic pulmonary vasoconstriction (HPV), while the blood flow of other lobe increases, perfusion and oxygenation of NVL decrease. As a result of this, tissue ischemia occurs in nonventilated site. After resuming 2-lung ventilation, reentry of the blood to ischemic tissue causes sudden and significant increase in ROS production leading to IRI.

We carried out a study to compare the preventive effects of sevoflurane and propofol from IRI in thoracic surgery with OLV by measuring blood gases, IMA, and MDA. We observed lower arterial oxygen pressure in sevoflurane group than in propofol group during OLV as a demonstration of HPV. IMA level at postoperative sixth hour was lower in sevoflurane group than in propofol group. We conclude that sevoflurane was superior in preventing in IRI compared to propofol [36]. Casanova et al. [14] emphasized importance of ischemia-reperfusion induced lung injury in thoracic surgery due to association with ventilation damage to one lung. They studied and evaluated the cytoprotective effects of sevoflurane compared with propofol in a pulmonary autotransplant model in pigs. They found increased oxidative stress markers and proinflammatory mediators in the propofol group. In a consequence of their study, they concluded that sevoflurane decreased the inflammatory response and oxidative stress and provided a protection in a live ischemia reperfusion lung model. In another study Liu et al. [37] investigated the effects of administration of isoflurane and sevoflurane before the intervention on ischemia-reperfusion induced lung injury in an isolated buffer-perfused rat lung model by measuring the coefficient of filtration of the lung, lactate dehydrogenase activity, tumor necrosis factor alpha, nitric oxide metabolites in the perfusate, and the wet-to-dry lung weight ratio. They found that administration of 1 MAC isoflurane or sevoflurane before ischemia significantly attenuates ischemia-reperfusion induced filtration and the wet-to-dry lung weight ratio. This 1 MAC inhibits increase of lactate dehydrogenase activity and tumor necrosis factor alpha in the perfusate and abrogates the decrease in nitric oxide metabolites in the perfusate. They concluded that isoflurane and sevoflurane administered before ischemia could attenuate ischemia-reperfusion induced injury in isolated rat lungs.

4.3. Tourniquet Induced Extremity IRI. Another frequently studied model is tourniquet induced ischemia-reperfusion model. Application of tourniquet is liberally used for providing bloodless surgical field and control of intraoperative bleeding in extremity surgery. Therefore, muscle ischemia occurs in distal area of tourniquet. IRI occurs after deflation of tourniquet and reoxygenation of ischemic tissue. It was stated that muscle ischemia is accompanied by hypoxic cellular challenge and anaerobic glycolysis, reperfusion by neutrophil activation, formation of reactive oxygen species, and release of vasoactive factors [2]. Carles et al. [38] investigated the effects of sevoflurane compared with propofol in tourniquet induced ischemia reperfusion by measuring with microdialysis probes interstitial metabolite levels of anaerobic glycolysis. They found that lactate, pyruvate, and glucose remained at a significantly higher level in the sevoflurane group during reperfusion. Their results indicated that there is a better availability of interstitial glycolysis metabolites in the skeletal muscle during ischemia and reperfusion after sevoflurane exposure. They concluded that sevoflurane had a potential preconditioning effect on tourniquet-induced skeletal muscle IRI. It may be considered that there is a more efficient anaerobic glycolysis after sevoflurane exposure because of higher availability of energetic substratum, that is, pyruvate, allowing higher production of lactate and therefore higher mitochondrial ATP [39, 40]. Higher interstitial glycolysis substratum levels resulting from sevoflurane exposure may participate in the preservation of ATP synthesis in the skeletal muscle.

4.4. Major Vascular Surgery and IRI. In some surgical procedures, such as aneurysm repair of big vessels, traumatic vessel injuries, and procedures related to artery being clamped to provide bloodless surgical area, volatile anesthetics were used to show their preventive effects from IRI in these procedures. Aortic ischemia and reperfusion may induce pulmonary sequestration of neutrophil granulocytes. Kalb et al. [41] investigated the effects of pre- or postconditioning with sevoflurane showing pulmonary neutrophil accumulation

after IRI of the aorta. Anesthetized and mechanically ventilated rats underwent laparotomy and developed ischemia by clamping of the infrarenal aorta. Pre- and postconditioning with sevoflurane were applied. Following reperfusion, the lungs were removed for microscopic determination of neutrophil accumulation. They found that preconditioning, but not postconditioning, with sevoflurane reduced pulmonary neutrophil accumulation after IRI. They concluded that since neutrophil accumulation played a major role in the pathophysiology of acute lung injury, their data suggested a protective effect of sevoflurane preconditioning on remote pulmonary IRI.

Annecke et al. [15] compared the effects of sevoflurane with propofol on IRI after thoracic aortic occlusion in pigs. The animals received sevoflurane or propofol anesthesia before, during, and after lower body ischemia. Fluid and catecholamine requirements were assessed. Serum samples and intestinal tissue specimens were obtained. All animals displayed a severe reperfusion injury following 90 min occlusion of the thoracic aorta. However, animals receiving sevoflurane showed less signs of IRI as assessed by systemic hemodynamic instability than animals receiving propofol for the same intervention. Norepinephrine requirement in the sevoflurane group was significantly reduced during reperfusion. Animals tested with sevoflurane had a less pronounced increase of serum enzyme activities indicative of tissue injury. Serum activities of lactate dehydrogenase, aspartate transaminase, and alanine aminotransferase were lower with sevoflurane. In a consequence of the study they concluded that use of sevoflurane compared with propofol attenuated the hemodynamic sequelae of IRI. Koşucu et al. [42] investigated the effects of sevoflurane anesthesia combined with epidural anesthesia on IRI in patients undergoing surgical revascularization due to aortoiliac occlusive disease, by measuring plasma MDA and IMA levels. They found that serum levels of MDA and IMA were lower in study group compared to control group. In consequence of their study, they concluded that the sevoflurane anesthesia combined with epidural anesthesia might decrease the IRI in aortoiliac occlusive disease.

4.5. Cerebral Events and IRI. Cerebral IRI is encountered from various neurological, vascular, and cardiovascular procedures. This typically causes a disorder of water homeostasis and has been associated with oxidative stress, inflammatory response, lipid peroxidation, and apoptosis [43–46]. Preconditioning with volatile anesthetics can limit the cerebral IRI. Bedirli et al. [47] carried out a study to examine the effects of sevoflurane or isoflurane preconditioning on cerebral ischemia-reperfusion induced inflammation, oxidative stress, and lipid peroxidation and test the hypothesis that the underlining mechanism of the protective effect of preconditioning involves changes in the apoptotic gene expression profiles in an experimental model of middle cerebral artery occlusion in rats. In consequence of their study they found that sevoflurane and isoflurane preconditioning ameliorates inflammation, cerebral lipid peroxidation, and histologic injury. They also concluded that downregulation of proapoptotic molecules and upregulation of antiapoptotic molecules may be associated with this effect.

For the same purpose Wang et al. [48] investigated the postconditioning neuroprotective effect of sevoflurane in rats with middle cerebral artery occlusion. They found that postconditioning with sevoflurane not only reduced infarct volume but also improved learning and memory. They concluded that this neuroprotective effect may be partly due to the activation of PI3K/Akt pathway and inhibiting neuronal apoptosis. In another study, Ishiyama et al. [49] compared the effects of sevoflurane with propofol on cerebral pial arteriolar and venular diameters during global brain ischemia and reperfusion. Twenty rabbits were anesthetized with sevoflurane or propofol and then global brain ischemia was induced by clamping the brachiocephalic, left common carotid, and left subclavian arteries. They observed pial microcirculation microscopically through closed cranial windows and measured using a digital video analyzer. They found that pial arterioles and venules did not dilate immediately after reperfusion and subsequently constricted throughout the reperfusion period in propofol group. In contrast, pial arterioles and venules dilated temporarily and returned to baseline in sevoflurane group. Adverse effects in sevoflurane group (pulmonary edema and acute brain swelling) were higher than propofol group. In addition, blood pressure, heart rate, and plasma glucose were stable in sevoflurane group.

4.6. Liver and IRI. Temporary interruption of blood flow of liver causes hepatic ischemia. IRI may cause removal of interruption and subsequent reperfusion in some surgical procedures. Since volatile anesthetics are capable of providing relevant organ protection from IRI, several studies are conducted in this field [50, 51]. Bedirli et al. [52] investigated the effects of isoflurane and sevoflurane in a warm liver ischemia-reperfusion model on cytokines, hepatic tissue blood flow, energy content, and liver structure. They found that sevoflurane given before, during, and after hepatic ischemia protected the liver against IRI, whereas the effects of isoflurane on hepatic IRI were not notable.

4.7. Kidney and IRI. Renal IRI is an inflammatory process involving multiple cellular and systemic responses, including complement activation, activation of proinflammatory cytokines and chemokines, and infiltration by leukocytes such as neutrophils, macrophages, and T cells [53]. Lee et al. [54] reported that volatile anesthetics, including isoflurane, protected from renal IRI injury by attenuating the inflammatory response as well as necrosis. Daqing et al. [55] used preconditioning with noble gas, xenon, to show its protective effect in renal IRI. They observed that xenon was a natural inducer hypoxia-inducible factor 1a. Providing their data confirmation in the clinical setting, they suggested that xenon preconditioning before renal ischemia can prevent acute renal failure arising from IRI. Guye et al. [56] examined a possible protective effect of desflurane preconditioning on the kidney in renal ischemia-reperfusion model in rabbits. They investigated tubular cell damage histologically. They found lower histological damage in desflurane group and concluded that desflurane preconditioning reduced renal IRI.

4.8. Opposing Views. There are lots of studies in the literature investigating the effects of volatile anesthetics in other ischemia-reperfusion models. However, recently, many of these studies have been performed with sevoflurane and isoflurane. Halothane and enflurane are no longer investigated for this purpose due to decreased usage of these agents. However, some studies suggested that there is no protection [29, 57, 58], moreover harmful [59] effects of volatile anesthetics.

4.9. In the Future. IRI may occur in different clinical conditions without surgical intervention. After successful cardiopulmonary resuscitation, reperfusion is established in tissues and different organs that remained ischemic and hypoxic. As a result of reperfusion, IRI is inevitably encountered. On the other hand, severe hypotension connected with hypovolemic, hemorrhagic, or septic shocks also causes tissue hypoxemia. After treatment of these clinical conditions IRI may also occur.

Recently, tissue and organ transplantation is rapidly improved. As the transplanted organs are exposed to ischemia and reperfusion, IRI will be encountered more frequently. Therefore novel treatment modality will be offered to clinical practice. Perhaps usage of volatile anesthetics may be a part of this in the future.

5. Conclusion

Although there are a lot of studies in the literature suggesting potential protective effects of volatile anesthetics, further studies are required to show the effects of volatile anesthetics on IRI.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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Research Article

Efficacy and Safety of Hepatectomy Performed with Intermittent Portal Triad Clamping with Low Central Venous Pressure

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Background. This retrospective study was designed to investigate the efficacy and safety of intermittent portal triad clamping (PTC) with low central venous pressure (CVP) in liver resections. *Methods*. Between January 2007 and August 2013, 115 patients underwent liver resection with intermittent PTC. The patients' data were retrospectively analyzed. *Results*. There were 58 males and 57 females with a mean age of 55 years (±13.7). Cirrhosis was found in 23 patients. Resections were performed for malignant disease in 62.6% ($n = 72$) and for benign disease in 37.4% ($n = 43$). Major hepatectomy was performed in 26 patients (22.4%). Mean liver ischemia period was 27.1 min (±13.9). The mortality rate was 1.7% and the morbidity rate was 22.6%. Cumulative clamping time ($t = 3.61$, $P < 0.001$) and operation time ($t = 2.38$, $P < 0.019$) were significantly correlated with AST alterations (D-AST). Cumulative clamping time ($t = 5.16$, $P < 0.001$) was significantly correlated with D-ALT. Operation time ($t = 5.81$, $P < 0.001$) was significantly correlated with D-LDH. *Conclusions*. Intermittent PTC under low CVP was performed with low morbidity and mortality. Intermittent PTC can be safely applied up to 60 minutes in both normal and impaired livers.

1. Introduction

Operative blood loss is one of the main factors associated with perioperative prognosis of patients undergoing liver resection [1–6]. Bleeding during liver resection generally occurs in the dissection phase, during the parenchymal transection or during the revascularization phase of the procedure. Especially resection of lesions in close proximity or infiltrating major vascular structures (i.e., the cava hepatic junction) or an extended hepatectomy can be unpredictably complicated by life-threatening hemorrhage. Various strategies to reduce intraoperative bleeding during hepatectomy have been described in the literature [7]. Afferent or complete devascularization before parenchymal transection and precise hemostasis during parenchymal transection with the assistance devices (including ultrasonic dissection, heat coagulation, or bipolar vessel sealing), hepatectomy performed under low central venous pressure (CVP)s and temporary occlusion of blood inflow with or without outflow control are important strategies for prevention from blood loss.

Temporary occlusion of blood inflow of the liver, also called Pringle maneuver (PTC, portal triad clamping), is simply applied without complex anesthetic management [7]. However, total vascular exclusion of the liver including PTC and occlusion of the inferior vena cava below and above the liver may need complex surgical technique and anesthetic management [7, 8]. Both types of vascular occlusion techniques are effective in limiting bleeding, but they also

produce liver ischemia. To minimize ischemia-reperfusion injury to the liver remnant, intermittent PTC is commonly used [7]. It is not yet fully known how much vascular occlusion influences the amount of parenchymal bleeding, the rate of hepatocyte damage, subsequent recovery, and the surgical outcome. The efficacy and safety of intermittent PTC during liver resection under low CVP were evaluated in the present study. Outcome variables included the degree of ischemia-reperfusion injury, intraoperative blood loss, alteration of liver functions, and the incidence and severity of postoperative complications.

2. Patients and Methods

One hundred and thirty-six patients who were candidates for elective liver resections between January 2007 and August 2013 were considered eligible for this study. Exclusion criteria were an age of 16 years or younger, surgery on the liver without parenchymal resection (including inoperable cases, $n = 9$), and liver resection performed without intermittent PTC ($n = 12$). Overall, 115 patients had undergone 116 liver resections. The patients' data were prospectively recorded and retrospectively analyzed. The study was approved by the institutional committee on human subjects.

2.1. Patient Selection. All patients considered for resection underwent preoperative assessment. Laboratory tests included liver function tests, coagulation tests, and measurement of serum creatinine and electrolytes. Each patient was preoperatively evaluated by an abdominal triphasic computed tomographic scan to plan the liver surgery. For malignant diseases, patients were considered operable if all diagnosed tumors could be treated by radical resection with macroscopically negative surgical margins and a sufficient future liver remnant. Functional liver status was evaluated by using the Child-Pugh-Turcotte (CPT) score. Patients with CPT score greater than 7 and model for end-stage liver disease score greater than 16 were considered for the liver transplantation program instead of the liver resection procedure. Sufficient future liver remnant is assessed with the help of manual volumetry from CT images. Additionally, 2 of 3 major hepatic veins were preserved during major liver resection in patients with chronic viral hepatitis or presence of cirrhosis.

2.2. Hepatectomy and Perioperative Care. Central vein catheterization was performed routinely and central venous pressure (CVP) was maintained at less than 5 cm H_2O during liver resection. Conventional liver resection was performed through a J incision. Extraparenchymal control of ipsilateral inflow and outflow was attempted before resection. Resection was performed under intermittent PTC in general ($n = 115/127$). Intermittent PTC in cycles of 15/5 min of clamp/unclamp times was used in all patients. Liver transection was performed with the combination of clamp crushing method and vessel sealing system (Ligasure, Covidien AG, CA, USA) [9]. We used Bismuth's terminology for hepatectomy (segmental and sectorial division of liver parenchyma) in this study [10]. Major hepatectomy was defined as the resection of three or more segments. All patients received antibiotic prophylaxis. Hemorrhage during liver resection was replaced with fresh frozen plasma ($n = 93/116$). Transfusion with erythrocyte suspension was required in 37 of 116 resections. Extubation of the patient in the operating room was achieved in 113 of 115 patients. Patients with uneventful operative course were transferred to the surgical ward after extubation ($n = 113/115$). Prophylactic daily subcutaneous injection of low-molecular-weight heparin sodium was started on postoperative day (POD) 0.

2.3. Postoperative Followup and Data Collection. Patients received standardized pulmonary care a day after extubation [9]. Patients were seen daily by the surgical team until hospital discharge. Parameters on postoperative liver function (i.e., INR, levels of bilirubin, liver transaminases (aspartate aminotransferase, AST; alanine aminotransferase, ALT), γ-glutamyltransferase (GGT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH)) were measured preoperatively and daily during the first postoperative week. Duration of warm ischemia and operative time were recorded. Intraoperative blood losses were calculated by adding the blood volume into the suction canister to the blood loss as calculated by weighing the sponges. Patients were observed until the day of discharge. Postoperative complications including pulmonary complications, wound infection, biliary leak, deep venous thrombosis, and liver failure were recorded. All postoperative complications determined in the study group were classified according to *Dindo's* description [11]. Routine abdominal ultrasound was carried out in any patient with a suspected infected collection. All fluid collections were drained percutaneously with bacteriologic cultures. The length of hospital stay was recorded.

2.4. Statistical Analysis. Data were expressed as mean (± standard deviations) or median (range). Data were collected to a computer using SPSS software (version 11.0; SPSS Inc., Chicago, IL). To assess the hepatic injury response, the delta (D)-AST (maximum level minus preoperative level) and D-ALT were calculated. To assess the ischemic insult, the D-LDH was calculated. To identify factors affecting D-AST, D-ALT and D-LDH various clinical variables were evaluated by multiple linear regression analysis. Comparative analysis of categorical variables was performed using Mann-Whitney U test and Kruskal-Wallis test. Comparative analysis of numeric variables was performed using Spearman's correlation coefficient. After making the logarithmic transformation, logistic regression employing a Wald statistic backward stepwise selection was performed. Differences at $P < 0.05$ were considered statistically significant.

3. Results

There were 58 males and 57 females who had undergone elective liver resection with intermittent PTC. Majority of patents ($n = 98$, 85.2%) were classified as ASA 1 or 2 (Table 1).

HBV or HCV related chronic viral hepatitis was determined in 27 patients (23.5%). Pathologically confirmed cirrhosis was found in 23 patients in the study group. Indications for resection were malignant disease in 62.6% of the patients ($n = 72$) and benign disease in 37.4% ($n = 43$). Giant hemangiomas and hydatid cyst or alveolar hydatid disease were leading benign causes for liver resection, whereas hepatocellular carcinoma (HCC) and liver metastasis from colorectal carcinoma were leading malign causes for liver resection (Table 1). Double primary liver malignancy (HCC and intrahepatic cholangiocarcinoma) was determined in one patient after pathological examination of the resection material. Major hepatectomy was performed in 26 patients (22.4%) (Table 2). Reresection was performed in one patient for liver metastasis from colorectal carcinoma (two times and interval time between two resection were 14 months). Mean liver ischemia period during liver resection was 27.1 minutes (±13.9 minutes). Cumulative ischemia period of the liver reached up to 60 minutes in 7 of 115 patients. With the use of intermittent PTC, 23.1% of major liver resections ($n = 6/26$) and 81.1% of less extensive liver resections ($n = 73/90$) were performed without any blood transfusion.

The mortality rate was 1.7% ($n = 2$) after hepatectomy. The causes of death were pulmonary embolism after segment 7 resection ($n = 1$) and pneumonia after left hepatectomy ($n = 1$). The morbidity rate was 22.6% ($n = 26$) after hepatectomy (46.2% for major hepatectomy ($n = 12/26$), 18.2% for sectorectomy ($n = 2/11$), 22% for segmentectomy ($n = 11/50$), and 3.4% for nonanatomical subsegmentary liver resection ($n = 1/29$)). The causes and the severity of postoperative complications were summarized in Table 3. Only one patient with Child A cirrhosis was faced with postoperative liver failure after left hepatectomy for hepatocellular carcinoma. Liver failure recovered after 6 cycles of plasmapheresis and medical support.

3.1. Biochemical Evaluation of Hepatocyte Injury and Ischemic Insult. The changes in perioperative serum AST and ALT are shown in Figures 1 and 2, respectively. The postoperative serum AST and ALT levels rose rapidly to a peak on day 1 and then decreased gradually in the first postoperative week. The curves of the perioperative serums AST and ALT showed no marked difference. Cumulative clamping time ($t = 3.61$, $P < 0.001$) and operation time ($t = 2.38$, $P = 0.019$) were significantly correlated with D-AST (Table 4). Cumulative clamping time ($t = 5.16$, $P < 0.001$) was significantly correlated with D-ALT (Table 4). The changes in perioperative serum LDH are shown in Figure 3. The postoperative serum LDH levels rose rapidly to a peak on day 0 (day of operation) and then decreased gradually in the first postoperative week. Operation time ($t = 5.81$, $P < 0.001$) was significantly correlated with D-LDH (Table 4).

3.2. Postoperative Alteration on Cholestatic Enzymes. The changes in perioperative serums ALP and GGT are shown in Figures 4 and 5, respectively. The postoperative serum ALP and GGT levels decreased gradually after operation and increased gradually after the 2nd postoperative days.

Table 1: Patient characteristics.

Variable	Number (%)
Age (mean, ±SD)	55 (±13.7)
Gender	
Male	58 (50.4)
Female	57 (49.6)
BMI (kg/m^2) (mean, ±SD)	26.5 (±5.2)
Comorbidities	
Presence of anemia	3 (2.6)
Diabetes	16 (13.9)
Systemic arterial hypertension	18 (15.7)
Coronary artery disease	6 (5.2)
Chronic pulmonary disease	2 (1.7)
ASA score	
1-2	98 (85.2)
3-4	17 (14.8)
Chronic HBV infection	23 (20)
Chronic HCV infection	4 (3.5)
Presence of cirrhosis	23 (20)
Benign liver tumors	43 (37.4)
Giant hemangioma	27 (23.5)
Hydatid cyst of the liver	2 (1.7)
Alveolar hydatid disease	6 (5.2)
Hepatolithiasis	4 (3.5)
Granulomatous lesions	4 (3.5)
Malignant liver tumors	72 (62.6)
HCC	32 (27.8)
Metastasis from colorectal carcinoma	22 (20)
Klatskin tumor	8 (7)
Gallbladder cancer	6 (5.2)
ICC	2 (1.7)
Metastasis from breast carcinoma	2 (1.7)
Hemangiosarcoma of the liver	1 (0.9)

BMI: body mass index, ASA: American Society of Anesthesia; HBV: hepatitis B virus; HCV: hepatitis C virus; HCC; hepatocellular carcinoma; ICC: intrahepatic cholangiocarcinoma; SD: standard deviation.

3.3. Postoperative Hepatic Functional Reserve. The changes in perioperative serum total bilirubin and INR are shown in Figures 6 and 7, respectively. The perioperative serum total bilirubin rose gradually after operation and then decreased in 2 or 3 days. The perioperative INR rose very gradually after operation. The recovery of INR levels was observed as nearly flat curving graphic at the end of first postoperative week.

4. Discussion

It is now accepted that liver parenchyma is more tolerant to prolonged continuous normothermic ischemia than to the consequences of massive bleeding and blood transfusions [7, 12–14]. The first priority is therefore to reduce intraoperative blood loss. In this series, 68% of liver resections were performed without any blood transfusion, and this could be achieved with the use of intermittent PTC.

Table 2: Intraoperative and postoperative parameters of the study group.

Variable	Number (%)
Major hepatectomy	26 (22.4)
Right hepatectomy	5 (4.3)
Extended right hepatectomy	5 (4.3)
Left hepatectomy	11 (9.5)
Extended left hepatectomy	5 (4.3)
Sectorectomy	11 (9.5)
Posterior sectorectomy	1 (0.9)
Medial sectorectomy	1 (0.9)
Lateral sectorectomy	9 (7.8)
Segmentectomy	50 (43.1)
Nonanatomical subsegmentary liver resection	29 (25)
Reresection	1 (0.9)
Portal triad clamping period (minutes) (mean, ±SD)	27.1 (±13.9)
Additional surgery	2 (1.7)
Operative time (minutes) (mean, ±SD)	207.7 (±93.3)
Intraoperative bleeding amount (milliliters) (median, min–max)	382 (20–2000)
Intraoperative or postoperative blood transfusion	37 (32)
Intraoperative or postoperative fresh frozen plasma transfusion	93 (80.2)
Morbidity	26 (22.6)
Reoperation	2 (1.7)
Mortality	2 (1.7)
Requirement of ICU care	4 (3.5)
Length of postoperative hospital stay (days) (mean, ±SD)	11.5 (±7.1)

ICU: intensive care unit; SD: standard deviation.

Table 3: Distribution of postoperative complications according to Dindo's gravity index.

Type of complication	Severity of complication	*N* (%)
Atelectasis	Dindo I	4 (3.5)
	Dindo IIIa	1 (0.9)
Pleural effusion	Dindo I	2 (1.7)
	Dindo II	4 (3.5)
	Dindo IIIa	3 (2.6)
Pneumonia	Dindo II	4 (3.5)
	Dindo IVb	1 (0.9)
Pulmonary embolism	Dindo V	1 (0.9)
Wound infection	Dindo I	7 (6)
	Dindo IIIb	1 (0.9)
Biliary leak	Dindo II	4 (3.5)
	Dindo IIIb 2	2 (1.7)
	Dindo IIIa 2	2 (1.7)
Liver failure	Dindo IVa	1 (0.9)

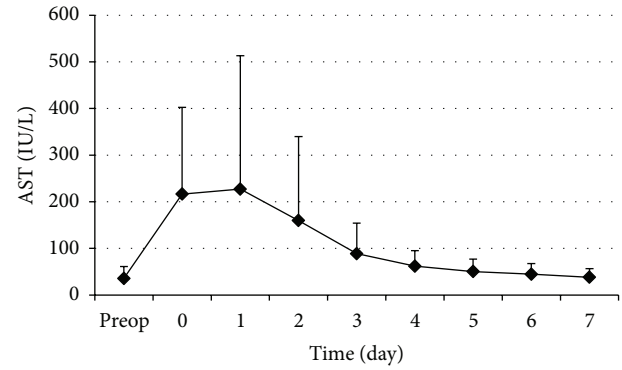

Figure 1: Perioperative changes of serum AST. Data are expressed as mean ± SD.

PTC (Pringle maneuver) is the oldest method of hepatic vascular control [15]. The PTC is performed by encircling the hepatoduodenal ligament with a tape and then applying a tourniquet (we also preferred tourniquet) to or a vascular clamp until the pulse in the hepatic artery disappears distally. An aberrant left hepatic artery originating from the left gastric artery should also be occluded if present [7]. After pedicle clamping, a moderate decrease in venous return due to pooling of blood in the mesenteric basin results in a 10% decrease in the cardiac index. Simultaneously, a sympathetic reflex produced by clamping causes a 40% increase in systemic vascular resistance and a 40% increase in mean arterial pressure. Unclamping of the hepatic pedicle leads to a transient decrease in blood pressure because of deactivation of the above-mentioned reflex [16–18]. PTC is generally well tolerated because caval flow is not interrupted and specific anesthetic management is not required [7]. A number of clinical studies have established 60 minutes as the safe duration of continuous PTC under normothermic conditions for both normal and pathologic (mainly cirrhotic) livers [7, 12, 19, 20]. However, it has been reported that continuous PTC has some potential drawbacks. These include portal vein emboli, spontaneous rupture of the spleen [21], induction of hepatic ischemia-reperfusion (IR) injury [22], and splanchnic congestion [13].

The process of warm IR injury involves activation of immune pathways and is dominated by hepatocellular injury. There are 2 distinct phases that occur in warm IR injury. The initial phase is defined as the period less than 2 hours after reperfusion and the late phase of injury, which occurs at 6 to 48 hours after reperfusion [13, 23]. The early phase is marked by activation of immune cells and production of oxidant stress; the later injury is mediated by neutrophil accumulation and hepatocellular injury. In addition to oxidant-mediated damage, the production of cytokines and chemokines also plays a key role in the pathogenesis of IR injury locally and systemically [24]. To minimize adverse effects of continuous PTC, intermittent PTC method has been evolved [7]. It has been shown that the intermittent PTC reduces splanchnic congestion and decreases hepatic IR injury [25]. The initial

Table 4: Factors affecting delta-aspartate aminotransferase, delta-alanine aminotransferase, and delta-lactate dehydrogenase.

	Parameter estimate	Standard error	95% confidence interval	t	P
Factors affecting D-AST					
Cumulative clamping time	0.010	0.003	0.005–0.015	3.609	<0.001
Operative time	0.0010	0.0004	0.0002–0.002	2.386	0.019
Factors affecting D-ALT					
Cumulative clamping time	0.016	0.003	0.010–0.022	5.165	<0.001
Factors affecting D-LDH					
Operative time	0.001	0.0002	0.0007–0.0016	5.813	<0.001

D-AST: delta-aspartate aminotransferase; D-ALT: delta-alanine aminotransferase; D-LDH: delta-lactate dehydrogenase.

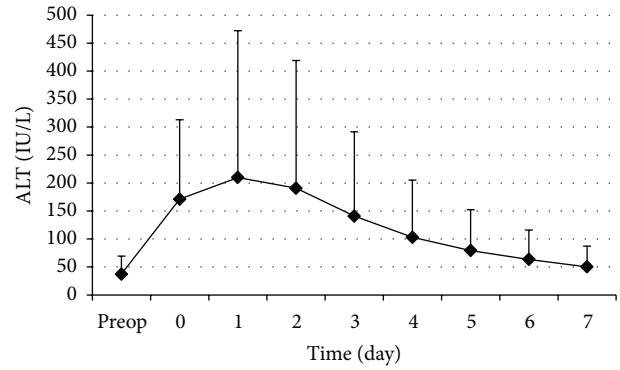

Figure 2: Perioperative changes of serum ALT. Data are expressed as mean ± SD.

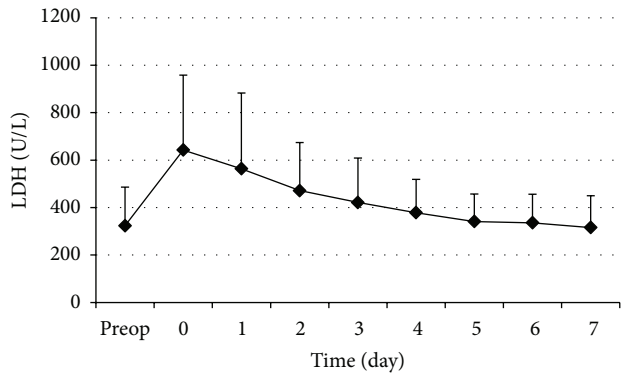

Figure 3: Perioperative changes of serum LDH. Data are expressed as mean ± SD.

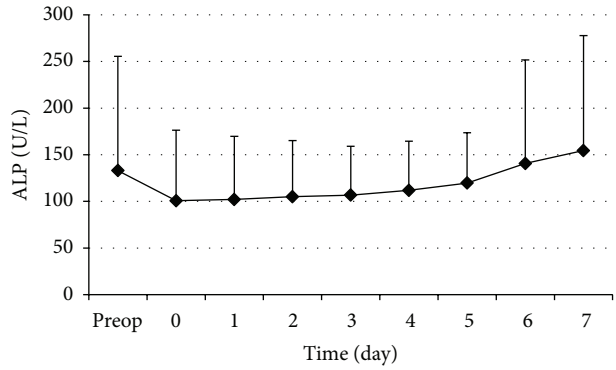

Figure 4: Perioperative changes of serum ALP. Data are expressed as mean ± SD.

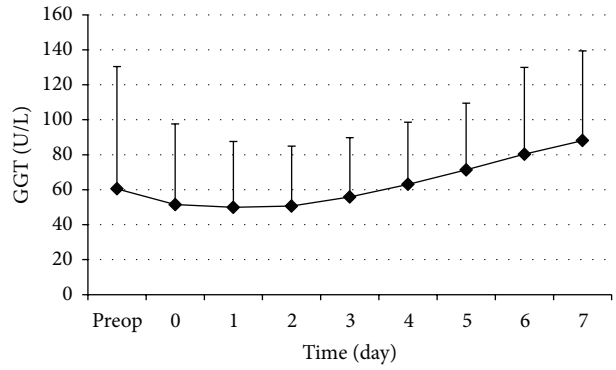

Figure 5: Perioperative changes of serum GGT. Data are expressed as mean ± SD.

cycle of clamping/unclamping during intermittent PTC could have a preconditioning hepatoprotective effect [7]. Although the exact mechanisms are not completely understood, the protective effects of ischemic preconditioning include inhibition of apoptosis via the decrease of Kupffer cell activation (activated Kupffer cells release TNF-a, which binds to the TNF-R1 receptor of hepatocytes and initiates the apoptotic process), activation of polymorphonuclear leukocytes, preservation of cellular adenosine triphosphate content, and release of substances such as adenosine and nitric oxide by the ischemic tissue which protect the liver against the subsequent prolonged ischemia [26–28]. Another technical advantage of intermittent PTC is that intermittent release of the portal clamp allows gradual hemostasis over smaller transection areas [29, 30]. However, repeated clamp removal during intermittent PTC may result in fluctuations of systemic blood pressure, multiple episodes of hepatic IR injury, and repeated bleeding from the transection surfaces. However, prospective clinical studies proved the hepatoprotective effect of intermittent PTC [30–32]. Also intermittent PTC permits a significant increase (almost doubling) of the ischemia times that can be achieved with continuous PTC. It can be safely applied up to 120 minutes in both normal and impaired livers [7, 31, 33, 34]. The proven effectiveness of intermittent PTC in reducing bleeding together with its hepatoprotective profile

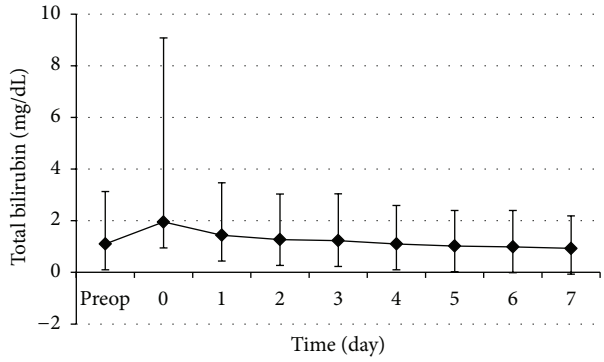

Figure 6: Perioperative changes of serum total bilirubin. Data are expressed as mean ± SD.

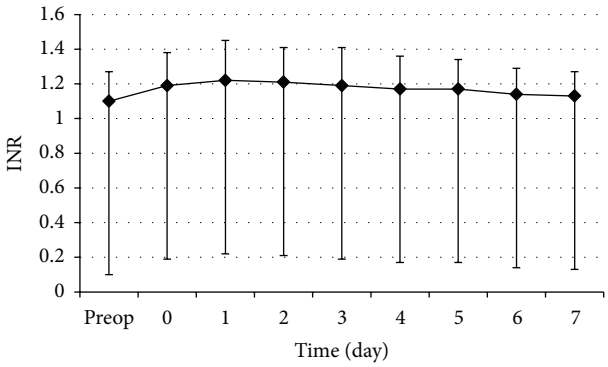

Figure 7: Perioperative changes of serum INR. Data are expressed as mean ± SEM.

encourages wide application of the method. Application of intermittent PTC under low CVP reduces bleeding during parenchymal transection and during unclamping [33, 35–37]. A low perioperative CVP has been suggested to limit blood loss during liver resection [38, 39]. By lowering the pressure inside the inferior caval vein, the hepatic venous pressure and, thus, the hepatic sinusoidal pressure would drop, possibly resulting in less bleeding during resection [38].

The biochemical investigation of liver IR injury has been a well-evolved issue [40–43]. Alteration on liver transaminase levels reflects hepatocellular injury, whereas accumulation of lactate reflects the severity of tissue ischemia. Postoperative transaminase levels may be related to the volume of liver resection. Clavien and colleagues reported that, for matched patients with the same ischemia time, patients with extended liver resections had lower postoperative peak AST levels than patients with smaller resection volumes. Smaller remnant liver masses might have been associated with lower postreperfusion serum AST and ALT levels than larger residual liver volumes [44]. In a recent study from Japan, it was claimed that the cirrhotic liver releases smaller amounts of aminotransferase than normal liver after IR [45]. The alteration of transaminase levels after liver resection with intermittent PTC in this study seems comparable with the previous studies [46–48]. In the current study, age, gender, BMI, and ASA score of patients, the presence of chronic viral hepatitis or cirrhosis, etiology (benign/malign) for liver resection, the extension of liver resection, intraoperative bleeding amount, and transfusion requirement were found to be unrelated with the postoperative liver injury. Our strict exclusion criteria for patients with chronic liver disease might be a factor for better tolerance of cirrhotic patients to normothermic intermittent inflow occlusion. As well demonstrated in the literature, cumulative clamping time is a major determinant of liver injury due to PTC. In addition to cumulative clamping time, the adverse effects of long operation time on the liver functions were also demonstrated in our study.

Despite the general opinion on the level of alkaline phosphatase and gamma-glutamyltransferase levels not being indicators of liver function and not useful tools to predict liver function after hepatectomy, the postoperative alteration curves of both parameters showed similar characteristics in the first postoperative week. However, postoperative ALP and GGT alteration curves in patients presented with jaundice (Klatskin tumor of gallbladder carcinoma) were discordant from other patients. In jaundiced patients requiring surgery for tumor resection, biliary drainage is suggested before hepatic resection for the elimination of negative effects of cholestasis in liver [49]. The studies from Japan insisted that radical surgery be performed after complete recovery from jaundice; therefore, we performed liver surgery after decrement of total bilirubin level under 2.0 mg/dL. According to this policy, the alteration of bilirubin levels after liver resection might be demonstrated in a common curve without the division of patients with obstructive jaundice or not. When compared to results of Scatton et al., the alteration curve of bilirubin levels in the current study was shown to have similar characteristics.

The absence of randomization and the heterogeneity of the group were main limitations of the study. The effects of comprehensive comorbidities on the development of IR injury may not be shown clearly in such a small study population. Preoperative hospital stay of patients with obstructive jaundice was longer than other patients in the study, at least 3 week. Therefore, to impede any bias on this issue, we analyzed postoperative hospital stay of patients rather than analysis of overall hospital stay. However, all operations were performed by the same surgical team with uniform surgical technique or surgical trauma.

In conclusion, according to our experience, intermittent PTC is an easily applied, flexible method with the inherent risk of bleeding during the reperfusion periods. The morbidity and mortality rates, bleeding amounts, and hospital stay in the current study were found comparable with the previous studies. Intermittent PTC with low CVP permits execution of complex, time consuming resections even in patients with an abnormal liver. Intermittent PTC can be safely applied up to 60 minutes in both normal and impaired livers.

Authors' Contributions

Serdar Topaloglu and Adnan Calik are liver surgeons of K.T.U. Liver Study Group. Coskun Aydın and Sema Kocyigit

are residents of the Department of Surgery, and they are responsible for the care of patients who underwent liver surgery. Kıymet Yesilcicek Calik, Coskun Aydın, Sema Kocyigit, Asim Orem, and Huseyin Yaman are also responsible for collection of data according to patients. Dilek Kutanis and Davut Dohman are anesthesiologists of K.T.U. Liver Study Group. Erdem Karabulut is responsible for the statistical analyses of data. Mithat Kerim Arslan is Chairman of the Department of Surgery.

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