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The Effect of Dexmedetomidine and Levobupivacaine in an Experimental Ischemia Reperfusion Model

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Abstract. *Background/Aim: Although it has been reported that different molecules are effective in preventing ischemia-reperfusion (I/R) injury, the most effective treatment is still unknown. Materials and Methods: The rats were divided into four groups of eight rats each. Group C: 1 ml intraperitoneal (IP) isotonic + laparotomy + IP 2 ml isotonic +I/R. Group D: 100 μg kg^{-1}/1 ml IP dexmedetomidine + laparotomy + IP 2 ml isotonic +I/R. Group L: 1 ml IP isotonic + laparotomy + IP levobupivacaine (2.5 mg kg^{-1}/2 ml) +I/R. Group DL: 100 μg kg^{-1}/1 ml IP dexmedetomidine + laparotomy + IP levobupivacaine (2.5 mg kg^{-1}/2 ml) +I/R. Brain, heart, lung, and liver tissue samples were collected for histopathological examination. Biochemically, levels of aspartate amino transaminase, alanine amino transaminase, serum glucose, total antioxidant status (TAS), total oxidant status, ischemia modified albumin, and malondialdehyde were measured in blood samples. Results: Group D mean blood TAS levels were found to be statistically significantly higher than those in Group C and Group L (p=0.037, p=0.048 respectively). Group DL oxidative stress index (OSI) value was found to be statistically significantly lower than that of Group C (p=0.010). Conclusion: Both dexmedetomidine and levobupivacaine demonstrated protective effects in I/R injury. When used in combination, the effects of these treatments were further enhanced, reaching statistical significance. As our literature review found no studies on the combined use of dexmedetomidine and levobupivacaine in I/R injury, it is anticipated that supporting these results with clinical studies may significantly contribute to clinical practice.*

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Clinically, ischemia/reperfusion (I/R) injury is a vascular crisis with high mortality that frequently occurs after acute mesenteric ischemia, shock, and major operations (1). It is known that I/R injury develops due to oxidative stress, lipid peroxidation, and oxygen-derived free radicals (2). I/R injury not only damages the ischemic region but also causes varying degrees of damage to other organ systems (3). Although it has been reported that different molecules are effective in preventing I/R injury, the most effective treatment is still unknown (2).

Dexmedetomidine (Dex) is a highly selective α2-adrenergic agonist that has sedative, analgesic, anxiolytic, and sympatholytic properties (4). It is frequently used in the intensive care units and during perioperative periods as an

anesthetic adjuvant (1). Dex prevents I/R injury by reducing the oxidative stress response and suppressing the release of inflammatory mediators (5). Some studies demonstrated that that Dex has a protective effect on the liver, kidney, brain, and retina upon I/R damage (5-7). However, in these studies, there were not enough data to simultaneously demonstrate its effects on the brain, lung, and liver damage. For this reason, unlike other studies, our study examined the effects of Dex in the cranial cavity, thorax, and abdomen simultaneously.

Local anesthetics (LA) are widely used in many surgical procedures for the purpose of postoperative analgesia. Bupivacaine (B) and levobupivacaine (LVB) are among the local anesthetic agents frequently used for this purpose (8). LVB is an amide local anesthetic that is the negative enantiomer of bupivacaine. Aside from its local anesthetic properties, it also has an adrenergic antagonist effect (9). The anti-inflammatory effects of LAs have been demonstrated in several studies (10, 11). Considering that Dex can prevent I/R damage through its anti-inflammatory mechanisms, it is hypothesized that LAs, which also possess this property, could reduce I/R damage as well. There is a limited number of studies on the effects of B on I/R injury whereas there is only 1 study on the effects of LVB (12-14). In studies on the effects on IR injury, B is frequently administered *via* thoracic epidural injection and although its mechanism is not fully known, it may be associated with a reduction of organ hypoperfusion through the induction of sympathetic preganglionic blockade (12, 13, 15). It has also been stated that intraperitoneal (IP) application of B in surgery is effective for postoperative analgesia (16). However, no studies were found regarding the effects of IP bupivacaine administration on I/R injury. In addition, it has been reported that LVB is as effective as B whilst having a lower risk of cardiac and central nervous system toxicity (17). The reason we studied LVB is that although there are a few studies on the effect of B on I/R injury, there is a lack of studies on LVB, which possesses a lower risk of toxicity. Studies demonstrate that LVB and Dex can be used safely together while achieving a more effective analgesia and anesthesia (18). Therefore, in our study, the effects of combined use of Dex and LVB were investigated, with the hypothesis that they may be more effective in preventing IR damage, as they are thought to provide a stronger anti-inflammatory effect when used in combination.

The primary aim of this study was to reveal the effects of Dex and LVB on oxidative stress parameters in I/R injury. The secondary aim was to investigate the effects of Dex and LVB on blood biochemical parameters indicating organ functions, ischemia biomarkers and histopathological organ damage.

Materials and Methods

This study was conducted at the University of Health Science, Hamidiye Animal Experiments Laboratory between 03-28 October 2022 with the approval of the Health Science University, Hamidiye Animal Experiments Local Ethics Committee (Approval Date/Approval No: 28/07/2022/07-02). A total of 32 Spraque Dawley male rats with an average weight of 360±50 grams, 20-24 weeks of age, were used. Throughout the study, all rats were kept in metal cages, with four rats in each cage, in a 12-h light-12-h dark environment with normal room temperature (21±2˚C) and humidity (40-60%). The rats were fed with standard rat chow and given tap water. Cage maintenance was carried out regularly with daily checks, and all rats were treated humanely during the course of the study in accordance with the 'Guide for the Care and Use of Laboratory Animals'. All surgical procedures performed on rats were performed under anesthesia. In anesthesia induction, Ketamine Hydrochloride (Ketalar® vial, 50 mg/ml, Eczacıbaşı, Istanbul, Turkey) 80 mg/kg IP and Xylazine Hydrochloride (Rompun® vial, 23.32 mg/ml, Bayer, Istanbul, Turkey) 10 mg/kg IP was used.

The rats were divided into four groups of eight rats each. Group C (Control): 1 ml IP isotonic solution + Laparotomy + IP 2 ml isotonic solution +I/R. Group D (Dexmedetomidine): 100 μg kg^{-1}/1 ml IP Dex + Laparotomy + IP 2 ml isotonic solution +I/R. Group L (Levobupivacaine): 1 ml IP isotonic solution + Laparotomy + IP LVB (2.5 mg kg^{-1}/2 ml) +I/R. Group DL (Dex+LVB): 100 μg kg^{-1}/1 ml IP Dex + Laparotomy + IP LVB (2.5 mg kg^{-1}/2 ml) +I/R.

After 8 h of fasting, the weights of all rats were measured under anesthesia and recorded. Vascular access was established from the tail vein of all rats with a 26G intravenous cannula under anesthesia. Rats were administered 10 ml/kg/h saline infusion during the procedure. Three ml IP isotonic solution were administered to the rats in Group C, 100 μg kg^{-1}/1 ml IP Dex and 2 ml isotonic solution to the rats in Group D, 1 ml IP isotonic solution and LVB (2.5 mg kg^{-1}/2 ml) to the rats in Group L, and 100 μg kg^{-1}/1 ml IP Dex and LVB (2.5 mg kg^{-1}/2 ml) to the rats in Group DL. A 3 cm long full-thickness abdominal midline incision was made 30 min after the administration of IP isotonic solution or Dex according to the groups. Immediately after laparotomy, 2 ml IP isotonic solution or LVB was administered according to the groups as stated above, and the superior mesenteric arteries (SMA) of the rats in all groups were dissected and closed with a vascular clamp. After 60 min of ischemia, 60 min of reperfusion was achieved. Approximately 4-6 ml of blood samples were collected *via* cardiac puncture for biochemical analysis. The rats were sacrificed, and the brain, lung and liver tissue samples were taken for histopathological examination.

Biochemical method (Blood). The person performing the blood sample tests was blinded to group assignments. Aspartate amino transaminase (AST), alanine amino transaminase (ALT) and serum glucose in blood samples were measured with an Abbott Architect c16000 analyzer (Abbott, Abbott Park, IL, USA). Total antioxidant status (TAS) and total oxidant status (TOS) were measured with Real Assay Total Antioxidant Status Assay Kit (Mega Medikal, Ankara, Turkey) (2). TAS values were calculated as mmol ascorbic acid equivalent/l, and TOS values were calculated as μmol H_2O_2 equivalent/l. Oxidative stress index (OSI) was measured using the formula:

OSI (AU)=(TOS μmol/l)/(TAS [mmol Trolox equiv/l] ×100) (19). Ischemia modified albumin (IMA) and malondialdehyde (MDA) concentrations were determined according to the method described by Ertürk *et al*. (20).

Table I. *Blood biochemical parameters and ischemia markers of the rats in the groups.*

Rat number (n)	Group C 8	Group D 8	Group L 8	Group DL 8	*p*-Value
Glucose					**<0.001**[1]
Mean±Sd	86.5±1.6	96.1±7.8	104.5±2.6	100.1±7.4	
Med (min-max)	86 (85-89)	97.5 (79-106)	104.5 (101-109)	99 (90-113)	
BUN					0.075[1]
Mean±Sd	32.3±4.1	37.3±4.2	38±4.9	37.3±6.7	
Med (min-max)	31.5 (2-740)	38 (31-43)	39 (31-46)	36 (31-52)	
Creatinine					**<0.001**[1]
Mean±Sd	0.4±0	0.5±0	0.5±0	0.5±0	
Med (min-max)	0.4 (0.4-0.5)	0.5 (0.4-0.5)	0.5 (0.5-0.5)	0.5 (0.5-0.6)	
ALT					0.258[1]
Mean±Sd	64.6±7.8	65.3±8.3	71.1±10	69.8±4.5	
Med (min-max)	63.5 (53-76)	62.5 (55-80)	72 (58-82)	69 (65-76)	
AST					0.029[1]
Mean±Sd	73.5±11.8	75.9±12.4	88.5±5.3	80.9±8.5	
Med (min-max	72 (60-97)	75.5 (55-92)	89.5 (80-95)	83.5 (68-93)	
IMA					**0.002**[1]
Mean±Sd	11±1.8	9.3±1.4	13±1.5	10.7±1.7	
Med (min-max)	11 (9-14.4)	8.8 (7.6-11.2)	12.6 (11.3-15)	10.6 (8.5-13.6)	
MDA					0.678[1]
Mean±Sd	1.5±0.3	1.5±0.2	1.4±0.4	1.4±0.2	
Med (min-max)	1.6 (1.1-1.8)	1.5 (1-1.8)	1.4 (0.9-2)	1.4 (1.1-1.6)	

[1]Kruskal-Wallis Test. Statistically significant p-values are shown in bold. Group C: Control; Group D: Dexmedetomidin; Group L: Levobupivacaine; Group DL: Dexmedetomidine + Levobupivacaine. Sd: Standard deviation; Med: Median; min: minimum; max: maximum; BUN: Blood urea nitrogen; ALT: alanine transaminase; AST: aspartate transaminase; IMA: ischemia modified albumin; MDA: malondialdehyde.

Histopathological examination. Tissue samples stained with hematoxylin-eosin were examined under a light microscope. Liver histopathological evaluation was performed according to the histopathological activity index (HAI) (21). Lung injury was rated from 1 (best, Grade 0) to 4 (worst, Grade 3) (22). For brain tissue damage, the presence of congestion, necrosis and gliosis was evaluated (23). The heart was evaluated semi-quantitatively as no damage (0), mild damage (1), moderate damage (2) and severe damage (3) according to myocardial damage, edema, inflammatory cell infiltration, and loss of striation (24).

Statistical analysis. Analyses were performed using MedCalc Statistical Software version 12.7.7 (MedCalc bvba, Ostend, Belgium).

Results

Mean blood glucose levels in Group L and Group DL were found to be statistically significantly higher than those in Group C (p=0.001 and p=0.016, respectively). Mean blood creatinine levels in Group L and Group DL were statistically significantly higher than those in Group C (p=0.008, p<0.001 respectively). It was also found that Group L mean blood AST levels were statistically significantly higher than those in Group C (p=0.031). Group L mean blood IMA levels were statistically significantly higher than those in Group D (p=0.001). Blood biochemical parameters and ischemia markers of the rats in the groups are shown in Table I whereas *post-hoc* pairwise comparisons of blood biochemical parameters and ischemia markers between the groups are shown in Table II.

Table II. *Post-hoc pairwise comparisons of the values of blood biochemical parameters and ischemia markers between the groups.*

Group	Glucose	Creatinine	AST	IMA
C-D	0.210	0.112	1.000	0.330
C-DL	**0.016**	**<0.001**	1.000	1.000
C-L	**0.000**	**0.008**	**0.031**	0.330
D-DL	1.000	0.652	1.000	0.151
D-L	0.178	1.000	0.126	**0.001**
DL-L	1.000	1.000	0.790	0.659

Bonferroni correction Mann–Whitney *U*-test. Group C: Control; Group D: Dexmedetomidine; Group L: Levobupivacaine; Group DL: Dexmedetomidine + Levobupivacaine; AST: aspartate transaminase; IMA: ischemia modified albumin. Statistically significant *p*-values are shown in bold.

Group D mean blood TAS levels were found to be statistically significantly higher than those in Group C and Group L (p=0.037 and p=0.048 respectively). Group DL OSI value was found to be statistically significantly lower than that in Group C (p=0.010). The average blood oxidative

Table III. *The average values of blood oxidative stress parameters of the rats in the groups.*

Rat number (n)	Group C 8	Group D 8	Group L 8	Group DL 8	*p*-Value
TAS					**0.021**[1]
Mean±Sd	0.8±0.1	1±0.1	0.8±0.1	0.9±0.1	
Med (min-max)	0.8 (0.7-0.9)	1 (0.7-1.1)	0.7 (0.7-1.1)	0.9 (0.7-1)	
TOS					0.072[1]
Mean±Sd	4.1±0.5	4.2±0.4	3.9±0.5	3.5±0.5	
Med (min-max)	4.1 (3.4-5.2)	4.1 (3.7-4.9)	3.9 (3.1-4.7)	3.5 (3-4.2)	
OSI					**0.006**[1]
Mean±Sd	523.8±73	437.6±98.3	488.5±87	405.6+54.9	
Med (min-max)	509.5 (424.7-639)	413.8 (344.9-667.1)	467.1 (412.4-674.3)	398.4 (343.4-522.9)	

[1]Kruskal–Wallis test. Statistically significant *p*-values are shown in bold. Group C: Control; Group D: Dexmedetomidin; Group L: Levobupivacaine; Group DL: Dexmedetomidine + Levobupivacaine; Sd: standard deviation; Med: median; min: minimum; max: maximum; TAS: total antioxidant status; TOS: total oxidant status; OSI: oxidative stress index.

stress parameters of the rats in the groups are shown in Table III. *Post-hoc* pairwise comparisons of blood oxidative stress parameters between groups were shown in Table IV.

Since the lung tissue sample of rat C6 was lost during the study, the Lung Histopathology Score could not be calculated. Upon analyzing the histopathology scores of rats in the groups, no statistically significant differences were observed in terms of mean Liver Histopathology Score (LHS), Pulmonary Histopathology Score (PHS), and Heart Histopathology Score (HHS) (p=0.793, p=0.720, and p=0.123 respectively). Table V presents the group-wise data for average histopathology scores of the rats.

Table IV. *Post-hoc pairwise comparisons of the values of blood oxidative stress parameters between groups.*

Group	TAS	OSI
C-D	**0.037**	0.085
C-DL	1.000	**0.010**
C-L	1.000	1.000
D-DL	0.527	1.000
D-L	**0.048**	0.733
DL-L	1.000	0.151

Bonferroni correction Mann–Whitney *U*-test. Statistically significant *p*-values are shown in bold. Group C: Control; Group D: Dexmedetomidin; Group L: Levobupivacaine; Group DL: Dexmedetomidine + Levobupivacaine; TAS: total antioxidant status; OSI: oxidative stress index.

Discussion

In this study, it was observed that Group L exhibited significantly elevated levels of blood glucose, creatinine, and AST, whereas Group DL displayed significantly higher levels of blood glucose and creatinine when compared to the control group. The study revealed that the blood TAS level was significantly lower in Group D compared to Group C and Group L, whereas the OSI level in Group DL was notably lower when compared to Group C. Blood IMA level was found to be significantly higher in Group L compared to Group D.

The results regarding the protective effects of Dex have been found to vary depending on the route of administration, time, dose and model of ischemia (2). Therefore, the results regarding the effects of Dex on I/R injury are controversial and new studies are needed. Dex is frequently used in the perioperative period. In addition to its effects on the central locus coeruleus α2 adrenergic receptors that prevent stress and reduce hyperglycemia, Dex also acts on α2 adrenaline receptors on peripheral islet tissue cells, inhibiting insulin secretion and increasing blood glucose levels. However, the ultimate effect of dexmedetomidine on perioperative glucose levels is unclear (25, 26). In our study, while the administration of Dex resulted in a rise in average blood glucose levels compared to the control group, it was determined that this increase did not yield a statistically significant difference. The results obtained from our study and other studies highlight the uncertainty regarding Dex's final effect on blood glucose level.

It has been shown that Dex generally reduces blood AST and ALT levels (2, 27). In our study, unlike these studies in the literature, it was determined that Dex application did not cause a statistically significant change in AST and ALT levels. It was considered that this difference might be attributed to the variations in the I/R model, particularly regarding the shorter duration of reperfusion.

In the literature, while it is stated that Dex has a renal protective effect on I/R injury, the exact mechanism of this effect has not yet been definitively elucidated. Studies have generally shown that it has protective effects on renal histopathology without causing any significant change in blood urea nitrogen (BUN) and creatinine levels (Cr) (28-30). In our

Table V. *Mean histopathology scores of rats in groups.*

Rat number (n)	Group C 8	Group D 8	Group L 8	Group DL 8	*p*-Value
LHS					0.785[2]
0	5 (62.5)	5 (62.5)	6 (75)	4 (50)	
1	3 (37.5)	3 (37.5)	2 (25)	4 (50)	
					0.793[1]
Mean±Sd	0.4±0.5	0.4±0.5	0.3±0.5	0.5±0.5	
Med (min-max)	0 (0-1)	0 (0-1)	0 (0-1)	0.5 (0-1)	
PHS					0.138[2]
0	-	-	2 (25)	-	
1	3 (42.9)	2 (25)	-	5 (62.5)	
2	2 (28.6)	5 (62.5)	4 (50)	2 (25)	
3	2 (28.6)	1 (12.5)	2 (25)	1 (12.5)	
					0.720[1]
Mean±Sd	1.9±0.9	1.9±0.6	1.8±1.2	1.5±0.8	
Med (min-max)	2 (1-3)	2 (1-3)	2 (0-3)	1 (1-3)	
BHS					NA
HHS					0.114[2]
0	4 (50)	8 (100)	5 (62.5)	4 (50)	
1	4 (50)	-	3 (37.5)	4 (50)	
					0.123[1]
Mean±Sd	0.5±0.5	0±0	0.4±0.5	0.5±0,.5	
Med (min-max)	0.5 (0-1)	0 (0-0)	0 (0-1)	0.5 (0-1)	

[1]Kruskal–Wallis Test; [2]Pearson Chi-Square. Group C: Control; Group D: Dexmedetomidin; Group L: Levobupivacaine; Group DL: Dexmedetomidine + Levobupivacaine; Sd: standard deviation; Med: median; min: minimum; max: maximum; LHS: liver histopathology score; PHS; pulmonary histopathology score; BHS: brain histopathology score; HHS: heart histopathology score.

study, although the reperfusion period was short, it was determined that Dex did not cause a significant change in blood BUN and Cr levels, which is consistent with the results of other studies (28-30). Some studies reported that Dex reduced blood BUN and Cr levels (31, 32). According to the results obtained from studies in the literature and our own research, it is evident that Dex does not induce changes in blood BUN and Cr levels in the early hours of reperfusion but reduces them in the later hours; weeks later their levels are restored. Therefore, blood BUN and Cr levels may vary depending on the duration of ischemia and the degree of injury.

MDA is the product of lipid peroxidation and is considered the most important marker of oxidative stress (33). The results of studies in the literature show that the effect of Dex on blood MDA levels may vary depending on the reperfusion time (31, 33). In our research, consistent with the study conducted by Cakir *et al*. (31), Dex did not yield any statistically significant alterations in blood MDA levels. The results of our study as well as of studies in the literature demonstrate that the effect of Dex on MDA levels in I/R injury varies depending on the ischemia model and duration. Therefore, it is thought that the effect of Dex on MDA levels can be elucidated by measuring MDA levels in blood samples taken at different times with short intervals after I/R.

IMA is the modified form of albumin, that is formed because of its interaction with superoxide radicals caused by ischemia and oxidative stress. It is used in the diagnosis of many ischemic diseases (30). Studies in the literature generally state that Dex has a reducing effect on IMA levels (30, 34). In our study, consistent with the results of the study conducted by Acar *et al*. (30), it was determined that Dex administration reduced IMA levels, however the effect was not statistically significant. The results of other studies and those of our study reveal that Dex has a protective effect by reducing blood IMA levels in I/R injury.

Although studies in the literature have generally reported that Dex has a protective effect on organs, its effects on biochemical parameters indicating organ functions are controversial (2, 31, 32). Our study showed that Dex administration caused an increase in blood glucose, BUN, Cr, AST, and ALT values, but the change was not statistically significant. Therefore, more comprehensive clinical and experimental studies are needed to clearly demonstrate the effects of Dex on biochemical parameters indicating organ functions in I/R injury. Studies in the literature indicate that Dex generally reduces MDA and IMA values, which are also used as biomarkers in I/R injury, although the decrease varies according to the ischemia reperfusion time (30, 34). Likewise,

in our study, it was determined that the average MDA and IMA levels decreased in Dex-treated rats, although the decrease was not statistically significant. The outcomes of other studies and those of our own study suggest that Dex has a protective benefit by decreasing IMA and MDA in I/R injury.

Under normal conditions, free oxygen radicals in the body are kept in balance by protective mechanisms. Oxidant molecule formation rates and the effectiveness of all antioxidant molecules determine the level of oxidative stress. Since oxidant and antioxidant molecules have a synergistic effect with each other, their separate measurements may be insufficient to determine the total intracellular oxidant stress. Additionally, these measurements have high costs. Hence, due to their ease and practicality, TAS, TOS, and OSI (TOS/TAS ratio) are measured for the assessment of oxidative stress. OSI is used to determine the oxidant-antioxidant balance of the body (30, 35). For this reason, TAS, TOS and OSI were evaluated in this study. The primary aim of our study was to reveal the effects of the agents used on TAS, TOS and OSI. Studies in the literature generally report that Dex has an oxidative stress-reducing effect (2, 36). Our study, consistent with the study results in the literature, showed that Dex application caused a statistically significant increase in TAS level, a slight increase in TOS level although not statistically significant, and a decrease in OSI that was also not statistically significant.

The only study in the literature examining the impact of LVB on I/R injury was conducted by Kosucu *et al*. (14), and this clinical study employed an intrathecal method. However, no studies were found regarding IP application of the LVB in I/R injury. Additionally, there is a lack of research on the combined use of Dex and LVB. Kosucu *et al*. (14) reported that intrathecal LVB did not reduce blood IMA and MDA levels when compared to preoperative values and was disadvantageous compared to total intravenous anesthesia. In our study, it was found that LVB increased average IMA levels and decreased MDA levels, although the change was not statistically significant. In addition, it was determined that Dex significantly reduced IMA levels compared to LVB. However, it did not cause a significant change in MDA levels. This result suggests that LVB is disadvantageous compared to Dex regarding IMA. Additionally, when LVB was used together with Dex, the negative effect of LVB on IMA was reduced and no statistically significant difference was seen compared to the control group. This result suggests that Dex may reduce the negative effect of LVB on IMA. In an experimental study conducted by Sarikus *et al*. (13), it was reported that epidural application of B did not result in a significant change in MDA levels in blood samples, but it did lead to a statistically significant decrease in blood AST and ALT levels. It has been reported that it reduces hepatic apoptosis histopathologically. In our study as well, in accordance with the results of the aforementioned study, LVB did not induce a significant change in blood MDA levels. However, contrary to the results of this study, our study showed a statistically significant increase in ALT levels. It was thought that this difference might arise from the fact that B and LVB are different molecules, even though they have a similar structure, and the differences in the I/R model and duration. Additionally, it was observed that the levels of AST and ALT decreased in the rats receiving a combination of LVB and Dex compared to the group that received LVB alone, whereas there was no statistically significant increase in AST levels. This result suggests that Dex may reduce the possible negative effects of LVB on liver function tests. However, in our study, LVB had no negative effect on the liver, either histopathologically or regarding the oxidative stress parameters. Therefore, it was thought that the most likely reason for the increase in liver function parameters in the group using LVB was the difference in the I/R model or the difference in the use of B and the method of LA application in other studies. Bedirli *et al*. (12) reported that epidurally administered B reduced blood MDA levels, histopathological intestinal injury score and apoptosis. Since no effect on oxidative stress parameters was found in the studies investigating I/R damage of LVB and B in the literature, no comparison could be made with the oxidative stress parameters in our study. Our primary aim was to discuss the effects of I/R injury on oxidative stress parameters in the early period. We observed that although both Dex and LVB reduced OSI in the early period of I/R injury, there was no statistically significant difference. However, OSI decreased statistically significantly in the group where Dex and LVB were used together. This result reveals that the most effective method in reducing oxidative stress in I/R injury is the combined use of Dex and LVB. Due to the scarcity of research on the impact of B and LVB on I/R injury, the ongoing debate surrounding the effects of Dex, and the absence of any studies concerning the concurrent use of Dex and LVB in our English literature review, we conclude that the findings of this study should be supported by clinical trials. Supporting our study results with clinical trials could contribute significantly to reducing patient morbidity/mortality and treatment costs through providing an organ-protective effect in cases of I/R injury which can cause serious damage.

In the clinical studies found in the literature, it is commonly noted that Dex exhibits cardioprotective effects, however Tosun *et al*. (37) reported the absence of such protective effects (38). The study conducted by Bouwman *et al*. (39) reported that B infusion reduced cardiac infarction rates assessed histopathologically. Our study, in line with the results of the study mentioned earlier, also found that LVB, while not statistically significant, reduced the mean HHS values. The results from our study showed that, although not statistically significantly, Dex and LVB reduced HHS when

used alone. This decrease was found to be greater with Dex. Additionally, it was concluded that when DEX and LVB were used together, they had no cardioprotective effects assessed histopathologically.

In our literature review, no study was found that explored the effects of LVB and B on histopathological lung damage following I/R injury. It is reported in the literature that Dex has positive effects on lung histopathology in I/R injury (40). In contrast to these studies, our study did not observe a significant change in the PHS values in the group subjected to Dex treatment. Although not statistically significant, it was found that LVB reduced mean PHS compared to the control group. In our study, it was determined that Dex alone did not reduce PHS and LVB alone reduced it slightly. Notably, when Dex and LVB were administered together, although the statistical significance was not established, a more substantial decrease in pulmonary I/R damage was observed histopathologically. These results indicated that the Dex-LVB combination may have a pulmonary protective effect.

In the literature, the effects of B on organ damage in I/R are controversial (13). A study on the effects of B on the liver following I/R injury was conducted by Sarikus *et al*. (13) but new studies are needed on this subject. Sarikus *et al*. (13) reported that epidurally administered B increased hepatic damage assessed histopathologically. In our literature review, we could not find any studies showing the histopathological effects of LVB on liver damage in I/R injury. In I/R injury Dex is generally reported to have a hepatic protective effect (2, 27). The results obtained in our study reveal that, although not statistically significantly, LVB reduced the average LHS. Unlike studies in the literature, it was determined that Dex alone did not cause a significant change in the mean LHS. The results of our study demonstrate that, even though statistical significance was not established, in I/R injury, LVB has a protective effect on the liver whereas Dex has no effect. Additionally, the results show that the combined use of Dex and LVB leads to an increase in I/R damage in liver tissue. However, these results were not supported by oxidative stress parameters.

Conclusion

Based on our results, it was determined that both Dex and LVB had protective effects on I/R injury. Particularly, when used in combination, these effects were further enhanced and reached statistical significance. Considering that the combined use of Dex and LVB further increases analgesic and anesthetic effectiveness as demonstrated in studies in the literature, we conclude that the risk of patient morbidity and mortality can also be decreased by reducing I/R damage with the combined use of Dex and LVB in major surgical interventions where the risk of I/R damage is high. Since no studies on the combined use of Dex and LVB in I/R injury were found in our literature review, it is anticipated that supporting the results of this study with clinical studies may substantially contribute to clinical practice.

Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

Authors' Contributions

H.I.T: Study design, all surgical examinations, literature review. S.K: Study design, all surgical examinations, literature review, article writing. S.O: Literature review, English editing, study coordination. A.S.C: Literature review, English editing, study coordination. Y.K.K: Surgical examination, literature review. K.B: Biochemical analysis. G.K: Histopathological examination. E.M.G: Biochemical analysis. N.B.S.D: Literature review and English editing. U.K: Study design, all surgical examination, literature review, article writing.

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