Journal of Inflammation Research

Dovepress
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ORIGINAL RESEARCH

Investigation of the Protective Effects of Dexmedetomidine, Midazolam, Propofol, and Intralipid on Oxidative Stress and Inflammation in Rats with Lidocaine-Induced Toxicity

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Aim: The aim of this study was to compare the effects of dexmedetomidine, midazolam, propofol, and intralipid on lidocaine-induced cardiotoxicity and neurotoxicity.

Methods: Forty-eight male Sprague-Dawley rats were randomly divided into six groups (n = 8 per group): control (C), lidocaine (L), lidocaine + dexmedetomidine (LD), lidocaine + midazolam (LM), lidocaine + propofol (LP), and lidocaine + intralipid (LI). Dexmedetomidine (100 µg/kg), midazolam (4 mg/kg), propofol (40 mg/kg), and intralipid (10 mg/kg) were administered intraperitoneally as pretreatment. Lidocaine (90 mg/kg) was administered intraperitoneally to induce oxidative stress in all groups except the control. After 60 minutes of electrocardiography (ECG) recording, the rats were sacrificed, and heart and brain tissue samples were collected. Comparative measurements of total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), and inflammatory parameters were conducted.

Results: In heart tissue samples, TAS was significantly higher in LI and LD groups ($p < 0.05$). Additionally, oxidative stress was significantly higher in the LM group ($p < 0.05$). Despite an increase in oxidative stress in brain tissue samples across all groups, it was found that all groups exhibited antioxidant protective effects ($p < 0.05$). Inflammatory parameters in heart and brain tissues significantly decreased in all groups, especially in the LI group ($p < 0.05$).

Conclusion: It was observed that pretreatment with midazolam increased oxidative stress induced by lidocaine, while dexmedetomidine and intralipid exhibited greater antioxidant effects. Dexmedetomidine and intralipid used as pretreatment were shown to be more effective in protecting against oxidative stress and inflammation.

Keywords: oxidative stress, inflammation, dexmedetomidine, midazolam, propofol, intralipid

Introduction

Local anesthetic systemic toxicity (LAST) arises depending on the preferred type of medication, duration and concentration of administration, patient-related factors, and evolving anesthesia application techniques with regional anesthesia methods.[1–3] In the event of LAST development, it is recommended to treat it with a multidisciplinary approach following

Received: 28 August 2024
Accepted: 15 December 2024
Published: 10 January 2025

the guidelines recommendations. The importance of using intravenous lipid solutions is particularly emphasized in guidelines when cardiovascular and central nervous system symptoms are present.[4] The widely accepted mechanism of lipid emulsion therapy in the treatment of LAST is the indirect mechanism of "lipid shuttle". According to the lipid shuttle theory, the lipid emulsion absorbs the highly lipid-soluble local anesthetic lidocaine from the heart and brain. Then, the lipid emulsion containing lidocaine is transported to the liver, muscles, and adipose tissue for detoxification and storage. In addition, the lipid emulsion itself exhibits several beneficial effects, including positive inotropic effect, fatty acid supply, reduction of mitochondrial dysfunction, phosphorylation of glycogen synthase kinase-3β, and inhibition of nitric oxide release. Lipid emulsion was shown to reduce bupivacaine-induced apoptosis in rat cardiomyoblasts via inhibition of ROS production.[5]

Reactive oxygen species (ROS) are oxygen-based molecules that play critical roles in both normal and pathological processes due to their structural instability.[6] ROS exert harmful effects on membrane lipids, proteins, nucleic acids, and deoxyribonucleic acid (DNA).[7–9] There are endogenous and exogenous antioxidant defense mechanisms that either prevent ROS formation or mitigate the harmful effects of oxygen radicals.[10]

Dexmedetomidine is a selective α-2 adrenoceptor agonist with sedative, anxiolytic, hypnotic, analgesic, and sympatholytic properties. It is known that dexmedetomidine enhances antioxidant enzymatic mechanisms and suppresses proinflammatory cytokines.[11] Midazolam is a hydrophilic drug with rapid onset of action, exhibiting anesthetic, anxiolytic, and hypnotic effects. It exerts its effects through affinity to γ-aminobutyric acid (GABA) and benzodiazepine receptors. Midazolam is known to have antioxidative and cytoprotective effects.[12] Propofol is a sedative intravenous anesthetic used for induction and maintenance of anesthesia. It is known to reduce neuroinflammation by inhibiting oxidative stress and preventing apoptosis and necrosis through its antioxidative effect.[13]

Although there are many studies in the literature investigating the effects of lipid emulsion, dexmedetomidine, midazolam, and propofol pretreatments in the development of oxidative stress,[14–20] there is no study comparing the protective effects of lipid emulsion, dexmedetomidine, propofol, and midazolam pretreatments in lidocaine toxicity. We believe that the drugs we will use in our study can prevent toxicity and organ damage that may occur after the use of local anesthetics with their antioxidant and anti-inflammatory activities, but the bioavailability and metabolism of the drugs to be used may vary significantly between individuals. This may be caused by factors such as genetic polymorphisms, age, gender, and comorbidities and may affect the results of the study. In addition, the concentration of lipid emulsions, the types of oils they contain and the application time have a significant impact on the effectiveness of the treatment. Animal models are a valuable tool to study the effects of lidocaine toxicity, but these models have limited compatibility with human biology, which can affect study results. In this experimental model, we aimed to evaluate and compare the effectiveness of the drugs used in pretreatment on oxidative stress and inflammatory response.

Materials and Methods

Ethical approval for this experimental study was obtained from the Local Ethics Committee for Animal Experiments of the University of Health Sciences, with decision number 03 dated 18.05.2022, and the procedures on experimental animals were conducted at our university's Laboratory Animal Production and Research Laboratory and Medical Biochemistry Department Research Laboratory. Institutional review board number of experiment is 80, date is 10.02.2023. In our study, power analysis was used to determine the number of animals. G Power 3.1 Dusseldorf program was used for power analysis, and as a result of the power analysis; a total of 48 rats were planned to be included in the study, with 8 animals in each group. A total of 48 male Sprague-Dawley rats, aged 10–12 weeks and weighing 200–250 grams, were used. Each rat was observed in the laboratory environment for one week before the experiment. Throughout the study, the rats were provided with ad libitum tap water and standard rodent feed. The animals were kept in cages in a room with controlled temperature (23–25°C) and a 12/12-hour light/dark cycle, with humidity maintained at 50–60%. The principles of the *Guide for the Care and Use of Laboratory Animals* formed the basis of the guidelines we applied regarding the care and welfare of the laboratory animals we used in our study.[21]

Distribution of Study Groups

Group K: Control group (n = 8)

Group L: Lidocaine group (n = 8)
Group LD: Lidocaine + Dexmedetomidine group (n = 8)
Group LM: Lidocaine + Midazolam group (n = 8)
Group LP: Lidocaine + Propofol group (n = 8)
Group LI: Lidocaine + Intralipid group (n = 8)

Study Design

Anesthesia of the experimental animals was achieved by intraperitoneal administration of 1.25 g/kg urethane, and ECG recording was performed using an electrophysiological data acquisition unit (PowerLab 16/30, AD Instruments, Castle Hill, Australia). ECG recording was conducted based on lead II derivation. LabChart v8.0 software was used for ECG recording and data analysis. To prevent hypothermia in anesthetized animals, a homeothermic blanket was used to maintain body temperature at 37°C. After the pinch reflex test was negative in animals under urethane anesthesia, the drugs were administered intraperitoneally.

Group K

Control group (n = 8): After urethane anesthesia, 1 mL of 0.95% saline solution (Incpharma Pharmaceuticals Inc., Istanbul, Turkey) was administered intraperitoneally, and ECG recording was performed for 60 minutes.

Group L

Lidocaine group (n = 8): After urethane anesthesia, 90 mg/kg lidocaine (Aritmal 2%, Osel Pharmaceuticals Inc., Istanbul, Turkey) was administered intraperitoneally, and ECG recording was performed for 60 minutes.

Group LD

Lidocaine + Dexmedetomidine group (n = 8): After urethane anesthesia, 100 μg/kg dexmedetomidine (Precedex 200 μg/2 mL, Hospira Inc., USA) was administered intraperitoneally. Thirty minutes later, 90 mg/kg lidocaine (Aritmal 2%, Osel Pharmaceuticals Inc., Istanbul, Turkey) was administered intraperitoneally, and ECG recording was performed for 60 minutes.

Group LM

Lidocaine + Midazolam group (n = 8): After urethane anesthesia, 4 mg/kg midazolam (Dormicum 15 mg/3 mL, Deva Holding Inc., Istanbul, Turkey) was administered intraperitoneally. Thirty minutes later, 90 mg/kg lidocaine (Aritmal 2%, Osel Pharmaceuticals Inc., Istanbul, Turkey) was administered intraperitoneally, and ECG recording was performed for 60 minutes.

Group LP

Lidocaine + Propofol group (n = 8): After urethane anesthesia, 40 mg/kg propofol (1%, Fresenius Kabi Pharmaceuticals Ltd., Turkey) was administered intraperitoneally. Thirty minutes later, 90 mg/kg lidocaine (Aritmal 2%, Osel Pharmaceuticals Inc., Istanbul, Turkey) was administered intraperitoneally, and ECG recording was performed for 60 minutes.

Group LI

Lidocaine + Intralipid group (n = 8): After urethane anesthesia, 10 mg/kg intralipid (Clinoleic 20% lipid solution, Lessines, Belgium) was administered intraperitoneally. Thirty minutes later, 90 mg/kg lidocaine (Aritmal 2%, Osel Pharmaceuticals Inc., Istanbul, Turkey) was administered intraperitoneally, and ECG recording was performed for 60 minutes.

After sacrifice, heart and brain tissue samples were obtained from all groups. Tissue samples were collected immediately from the animals that died before 60 minutes after lidocaine injection. Protein analysis was performed on tissue samples for normalization of other biomarkers. Comparative measurements were made for TOS, TAS, OSI, and inflammatory parameters.

Protein Determination of Tissue Samples

Protein determination was analyzed using a commercial kit based on the Bradford method (Coomassie Plus, Protein Assay, Thermo fisher Scientific, Massachusetts, USA).

Measurement of TAS, TOS, and OS

Tissue TAS (mmol ascorbate eq/L [mg/protein]; Rel Assay, Mega Tıp, Gaziantep, Turkey, RL0024) and TOS (μmol H 2 O 2 eq/L [mg/protein]; Rel Assay, Turkey, RL0017) were analyzed using commercial enzyme-linked immunosorbent assay (ELISA) kits. The results were obtained by spectrophotometric method and expressed in units as specified in the protocol of each kit for serum and tissue samples. OSI value was calculated using a mathematical equation. OSI=TOS/TAS

Measurement of Inflammatory Parameters

Tissue interleukin-1beta(IL-1β) (pg/mL [mg protein]; Elabscience, Shanghai, China- E-EL-R0012), interleukin-6 (IL-6) (pg/mL [mg protein]; Elabscience, Shanghai, China- E-EL-R0015), and tumor necrosis factor alpha (TNF-α) (pg/mL [mg protein]; Elabscience, Shanghai, China- E-EL-R0019) were analyzed using commercial ELISA kits. The results were obtained by spectrophotometric method and expressed in units as specified in the protocol of each kit for serum and tissue samples.

Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences (IBM® SPSS Statistics for Windows, Version 25.0, Armonk, NY, USA) and RStudio program. The distribution of variables was examined using the Shapiro–Wilk test. Non-normally distributed continuous variables were analyzed using the Kruskal–Wallis *H*-test, and between group differences were analyzed using the Dunn's test. Bonferroni correction was used as a correction in within-group analyses. The confidence level was set at 95%.

Results

Group K had statistically higher TAS values in both heart and brain tissues compared to Group L ($p < 0.001$). Group L exhibited statistically higher TOS and OSI values in heart and brain tissues compared to Group K ($p < 0.001$ for both) (Table 1 and Table 2) (Figure 1).

In heart and brain tissues, IL-1β, IL-6, and TNF-α levels were found to be statistically higher in Group L compared to Group K (Tables 1 and 2) (Figure 1). Similarly, in Group LD and Group LI, TAS values in the heart were statistically higher compared to Group L ($p < 0.001$), while TOS and OSI values were statistically lower ($p < 0.01$). Additionally, no

Table 1 Comparison of Biochemical Variables Among the Study Groups in Heart Tissue

Heart				
Variables	**Groups**	**Median (Min - Max)**	**p**	**Subgroup Comparisons**
TAS	L	0.103(0.07–0.16)	<0.01	2 > 1,3 5 > 1,2,3 6 > 1,2,3
	LD	0.169(0.15–0.19)		
	LM	0.1(0.08–0.27)		
	LP	0.131(0.11–0.23)		
	LI	0.186(0.16–0.21)		
	Control	0.214(0.18–0.23)		

(*Continued*)

Table 1 (Continued).

Heart				
Variables	**Groups**	**Median (Min - Max)**	**p**	**Subgroup Comparisons**
TOS	L	0.536(0.47–0.63)	<0.01	1 > 5,6 2 > 6 3 > 6
	LD	0.453(0.34–0.58)		
	LM	0.519(0.37–0.74)		
	LP	0.442(0.31–0.92)		
	LI	0.368(0.29–0.54)		
	Control	0.321(0.27–0.4)		
OSI	L	5.216(3.45–7.41)	<0.01	1 > 2,5,6 3 > 2,5,6 4 > 6 5 > 6
	LD	2.624(1.8–3.78)		
	LM	5.107(2.03–6.95)		
	LP	2.506(1.95–8.71)		
	LI	2.051(1.62–2.92)		
	Control	1.556(1.25–2.17)		
IL1β	L	424.86(383.84–486.46)	<0.01	1 > 2,3,4,5,6 2 > 6 3 > 5.6 4 > 5.6
	LD	323.23(239.33–391.56)		
	LM	336.15(304.04–439.74)		
	LP	344.21(251.78–393.74)		
	LI	256.92(152.12–284.95)		
	Control	587.56(551.08–607.03)		
IL6	L	422.78(313.89–475.26)	<0.01	1 > 2,3,4,5,6 2 > 6 3 > 5.6 4 > 5.6
	LD	492.09(429.8–561.78)		
	LM	452.48(419.01–485.53)		
	LP	329.33(312.46–417.91)		
	LI	702.04(640.98–767.25)		
	Control	508.3(486.4–601.92)		
TNFα	L	620.04(593.22–687.47)	<0.01	1 > 2,3,4,5,6 3 > 2,5,6 4 > 2,5,6 5 > 6
	LD	601.84(547.69–637.73)		
	LM	429.88(401.29–548.31)		
	LP	424.86(383.84–486.46)		
	LI	323.23(239.33–391.56)		
	Control	336.15(304.04–439.74)		

Notes: 1:L, 2:LD, 3:LM, 4:LP, 5:LI, 6:Control.

Abbreviations: L, Lidocaine; LD, Lidocaine + Dexmedetomidine; LM, Lidocaine + Midazolam; LI, Lidocaine + Intralipid; TAS, Total antioxidant status; TOS, Total oxidant status; OSI, Oxidative stress index; IL1β, interleukin-1β; IL6, interleukin-6; TNFα, Tumor necrosis factor-α.

https://doi.org/10.2147/JIR.S493566

Table 2 Comparison of Biochemical Variables Among the Study Groups in Brain Tissue

Brain				
Variables	**Groups**	**Median (Min-Max)**	**p**	**Subgroup Comparisons**
TAS	L	0.017(0.01–0.02)	<0.01	2 > 1,3 5 >1,2,3 6 > 3,2,1
	LD	0.027(0.02–0.03)		
	LM	0.022(0.02–0.02)		
	LP	0.024(0.02–0.03)		
	LI	0.035(0.02–0.04)		
	Control	0.042(0.03–0.05)		
TOS	L	0.485(0.4–0.58)	<0.01	1 > 2, 5, 6 3 > 6
	LD	0.348(0.22–0.48)		
	LM	0.419(0.36–0.52)		
	LP	0.436(0.06–0.54)		
	LI	0.303(0.23–0.44)		
	Control	0.218(0.11–0.34)		
OSI	L	31.097(22.44–39.29)	<0.01	1 > 2,3,4,5,6 2 > 6 3 > 5,6 5 > 6
	LD	12.353(7.82–17.53)		
	LM	18.255(15.72–31.47)		
	LP	16.928(2.62–22.22)		
	LI	8.925(7.04–19.64)		
	Control	5.589(2.49–8.85)		
IL1β	L	424.1(400.84–435.67)	<0.01	1 > 2,3,4,5,6 2 > 6 3 > 5,6 4 > 5 5 > 6
	LD	305.97(300.45–343.46)		
	LM	324.33(312.7–373.55)		
	LP	315.3(311.13–346.96)		
	LI	302.41(256.72–318.64)		
	Control	257.21(214.12–292.52)		
IL6	L	607.79(583.65–625.24)	<0.01	1 > 2,3,4,5,6 2 > 4,5,6 3 > 2,4,5,6 4 > 5,6
	LD	506.88(481.76–528.9)		
	LM	577.65(532.65–601.42)		
	LP	530.86(514.01–562.91)		
	LI	419.03(410.93–444.98)		
	Control	407.93(383–427.6)		

(*Continued*)

Table 2 (Continued).

Brain				
Variables	**Groups**	**Median (Min-Max)**	**p**	**Subgroup Comparisons**
TNFα	L	723.88(643.52–867.86)	<0.01	1 > 2,4,5,6 3 > 2,5,6 4 > 2,5,6
	LD	508.3(328.22–714.04)		
	LM	655.65(451.75–725.84)		
	LP	608.13(571.43–631.19)		
	LI	465.58(399.03–521.15)		

Notes: 1:L, 2:LD, 3:LM, 4:LP, 5:LI, 6:Control.

Abbreviations: L, Lidocaine; LD, Lidocaine + Dexmedetomidine; LM, Lidocaine + Midazolam; LI, Lidocaine + Intralipid; TAS, Total antioxidant status; TOS, Total oxidant status; OSI, Oxidative stress index; IL1β, interleukin-1β; IL6, interleukin-6; TNFα, Tumor necrosis factor-α.

statistically significant differences were observed in TAS, TOS, and OSI between Group L and other drug groups in the heart (Table 1) (Figure 1).

Regarding brain TOS, statistically significant differences were observed between Group L and Group LD ($p < 0.05$), Group LM ($p < 0.05$), and Group LI ($p < 0.05$). Similarly, significant differences were observed in brain OSI between Group L and all other groups ($p < 0.05$) (Table 2) (Figure 1).

In terms of heart TAS, Group LI had statistically higher values compared to Group LD, Group LM, and Group LP ($p < 0.05$). Regarding heart TOS, significant differences were observed only between Group LM and Group LI ($p < 0.05$). Regarding heart OSI, significant differences were observed between Group LD and Group LM ($p < 0.05$), and between Group LM and Group LI ($p < 0.05$) (Table 1) (Figure 1). Regarding brain OSI, significant differences were observed only between Group LD and Group LM ($p < 0.05$), and between Group LM and Group LI ($p < 0.05$) (Table 2) (Figure 1).

In both heart and brain tissues, IL-1β, IL-6, and TNF-α levels were statistically lower in Group LD, Group LM, Group LP, and Group LI compared to Group L (Tables 1 and 2) (Figure 2). In the heart, all inflammatory parameters were significantly lower in Group LI compared to other groups (Table 1) (Figure 2).

In the comparison of inflammatory parameters in the brain among all drug groups, statistically significant differences were observed in IL-1β levels between Group LD and Group LM ($p < 0.05$), Group LM and Group LI ($p < 0.05$), and Group LP and Group LI ($p < 0.05$). Similarly, significant differences were observed in IL-6 levels between Group LD and Group LM ($p < 0.05$), Group LD and Group LP ($p < 0.05$), Group LD and Group LI ($p < 0.05$), Group LM and Group LP ($p < 0.05$), Group LM and Group LI ($p < 0.05$), and Group LP and Group LI ($p < 0.05$). Regarding TNF-α levels in the brain, significant differences were observed between Group LD and Group LM ($p < 0.05$), Group LM and Group LI ($p < 0.05$), and Group LP and Group LI ($p < 0.05$) (Table 2) (Figure 2).

Discussion

This study examined the effects of lidocaine toxicity on tissue oxidant–antioxidant balance, marked by increased TOS and OSI, decreased TAS, and elevated IL-1β, IL-6, TNF-α. Pretreatment with dexmedetomidine, midazolam, propofol, or intralipid showed significant antioxidant effects. Dexmedetomidine and intralipid provided the most prominent protection in heart tissue, while dexmedetomidine, propofol, and intralipid were more effective than midazolam in brain tissue. However, midazolam pretreatment led to a greater oxidative burden. All study drugs reduced inflammation, with intralipid achieving the most substantial reductions in both heart and brain tissues.

Previous research demonstrated that lidocaine-induced oxidative imbalance is dose-dependent and linked to elevated free oxygen radicals.[22] Consistent with the literature, our study showed that lidocaine toxicity disrupts antioxidative protective mechanisms.[23] While lidocaine toxicity disrupts antioxidative mechanisms, it may not solely result from injection but from excessive serum levels or prolonged administration. Numerous studies in the literature have shown

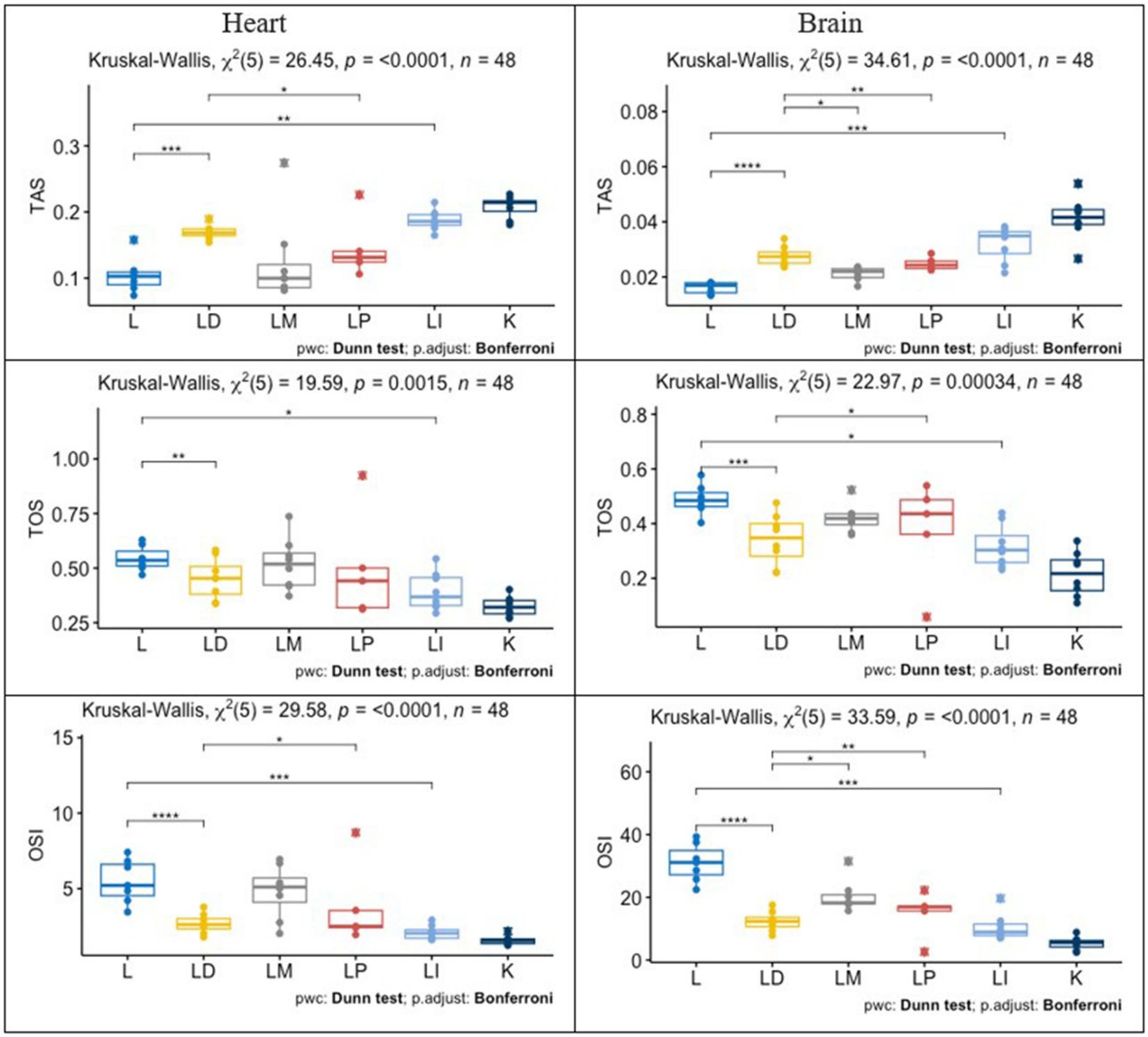

Figure 1 Comparison of individual tissue oxidation parameters.

that lidocaine stimulates antioxidant mechanisms in oxidative balance.[24–27] Several studies have highlighted the antioxidative potential of drugs like dexmedetomidine, propofol, midazolam, and intralipid.

Han C et al[28] conducted a prospective randomized controlled study comparing the antioxidative effects of the sedative agents propofol, midazolam, and dexmedetomidine under in vitro conditions. They found that both propofol and dexmedetomidine produced fewer free oxygen radicals and demonstrated greater antioxidative effects than midazolam. Similarly, a study investigating the effects of propofol, midazolam, and dexmedetomidine on diaphragm function in mechanically ventilated mice reported that midazolam exhibited lower antioxidative activity than the other sedative drugs, leading to increased lipid peroxidation and oxidative damage.[29]

Dexmedetomidine is well known for its anti-inflammatory properties.[30] In a study examining neurotoxic effects induced by local anesthetics on sciatic nerve cells, dexmedetomidine was found to reduce oxidative stress and protect neuronal cells through its antioxidative effects.[31] Notably, this study differed from ours, as local anesthetic toxicity was induced with bupivacaine instead of lidocaine. Ding X et al[15] explored the benefits of dexmedetomidine in mitigating neurotoxic effects, oxidative stress, and cytotoxic outcomes in a rat model of lidocaine toxicity. Consistent with our

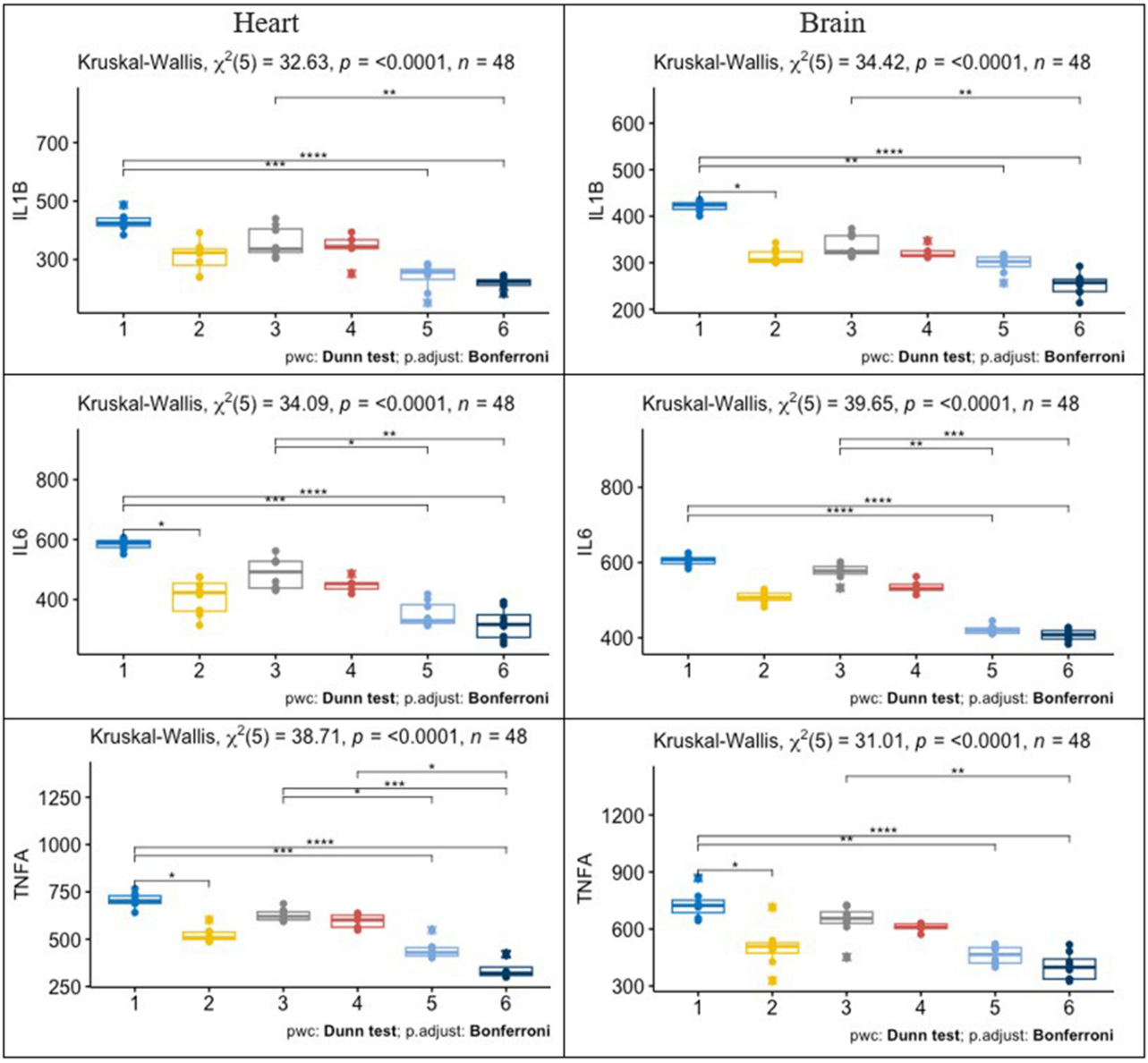

Figure 2 Comparison of individual tissue inflammatory parameters.

findings, their results showed that dexmedetomidine reduced oxidative stress, exhibited anti-inflammatory effects, and inhibited apoptosis and necrosis.

Wang C et al[19] studied the effects of midazolam and dexmedetomidine on cell proliferation in neuroblastoma and glioma cells under both in vitro and in vivo conditions. Dexmedetomidine demonstrated the ability to sustain cell viability for a longer duration by activating protective mechanisms against oxidative stress, even in cancer cells. In contrast, midazolam-induced apoptosis and reduced cell proliferation in cancer cells. While dexmedetomidine'santiox-idative effects were observed as favorable in our study, midazolam was associated with an increased oxidative burden.

In an in vitro study evaluating midazolam as an adjuvant in lidocaine toxicity, midazolam was shown to induce oxidative stress and apoptosis in rat astrocytes and human neuroblastoma cells, acting synergistically with lidocaine toxicity.[32] Tsuchiya M et al[33] compared the antioxidant activity of propofol and midazolam in rat erythrocyte membranes exposed to oxidative stress. Propofol exhibited significantly greater antioxidant activity than midazolam, although midazolam still showed some antioxidative effects. In alignment with these findings, our study demonstrated that propofol provided better antioxidative protection than midazolam.

https://doi.org/10.2147/JIR.S493566

An in vitro study on propofol's antioxidant effects on endothelial cells revealed that propofol enhanced endothelial nitric oxide synthase and protein expression under oxidative stress induced by lipopolysaccharides. When added to the study groups, propofol reduced protein expression and nitric oxide synthase production in endothelial cells, protecting them from oxidative damage.[17] Another study focusing on neonatal rat cardiomyocytes under oxidative stress demonstrated that propofol inhibited both intrinsic and extrinsic apoptotic pathways, effectively preventing free oxygen radical formation and showing antioxidative effects.[34] Although our study employed different methods to induce oxidative stress, propofol's protective effects were consistent with previous findings from in vitro experiments.

The neuroprotective effects of propofol were further evaluated in a cerebral ischemia-reperfusion injury model in mice. Propofol was shown to reduce the cerebral infarct size and suppress the release of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α, thereby demonstrating protective effects against focal cerebral ischemia.[35] In line with this, our study found that propofol attenuated the release of proinflammatory cytokines in response to lidocaine-induced toxicity.

In an in vivo study evaluating the protective effects against free radicals through lipid peroxidation induced in rat livers, propofol demonstrated a greater protective effect compared to both intralipid and midazolam.[36] However, in our study, the protective effect of propofol was found to be less than that of intralipid but greater than midazolam. This discrepancy could be attributed to the continuous infusion of propofol at anesthetic doses used in our study. The cytoprotective effects of intralipid observed in our research align with findings reported in the literature.

In an experimental in vitro study investigating the effects of intralipid and caffeic phenolic acid on rats exposed to oxidative stress induced by chlorpyrifos, an organophosphate insecticide, measurements of TOS and TAS levels were conducted following oxidative stress. The study noted that in all groups, including the intralipid group, serum TAS levels increased, while serum TOS levels decreased.[37]

In our study, which evaluated the protective effects of dexmedetomidine, midazolam, propofol, and intralipid against toxicity and organ damage caused by local anesthetics, it was particularly evident that inflammation caused by lidocaine was significantly reduced when used in combination with intralipid. Analysis of heart tissue samples revealed no significant difference in inflammation between the midazolam and propofol groups. Among all study drug groups, lidocaine combined with intralipid led to the greatest reduction in inflammatory markers, including IL-1β, IL-6, and TNF-α. Similarly, in brain tissue samples, the group treated with intralipid alongside lidocaine exhibited the strongest suppression of inflammation, mirroring the findings in heart tissue. While the overall inflammatory response was reduced in all groups, midazolam demonstrated the least effect compared to the other study groups.

Our study demonstrated that pretreatment with intralipid in lidocaine-induced toxicity has significant antioxidant effects. The widely accepted mechanism for lipid emulsion therapy in local anesthetic systemic toxicity is the *lipid shuttle hypothesis*. This theory suggests that lipid emulsions absorb lipid-soluble anesthetics, such as lidocaine (log P = 2.44), from critical organs like the heart and brain, facilitating their transport to metabolically active tissues such as the liver, muscles, and adipose tissues for detoxification and storage.[5] This indirect mechanism likely contributed significantly to the observed reduction in oxidative stress in our study.

Beyond the lipid shuttle effect, lipid emulsions also exert direct protective effects, including mitigating mitochondrial dysfunction, inhibiting nitric oxide release, and activating recovery pathways such as glycogen synthase kinase-3β phosphorylation. Previous studies have demonstrated that lipid emulsions reduce apoptosis in bupivacaine-induced toxicity by inhibiting ROS production.[5] These findings suggest a dual role of intralipid in alleviating the harmful effects of lidocaine toxicity.

Regarding propofol, our study observed increased TAS activity in the propofol pretreatment group compared to lidocaine alone. Since propofol is dissolved in 10% intralipid, it is plausible that the observed antioxidant effects are partially due to the intralipid vehicle. This observation aligns with previous findings that lipid emulsions can scavenge local anesthetics from critical tissues. However, as high-performance liquid chromatography (HPLC) was not performed to quantify lidocaine concentrations in serum or lipid emulsion, it remains uncertain whether the antioxidant effects are solely attributable to the lipid shuttle mechanism or to direct antioxidant actions of the lipid emulsion.

Future studies should aim to differentiate these mechanisms by incorporating HPLC analysis to measure lidocaine concentrations in serum and tissues. Such an analysis would clarify the relative contributions of the lipid shuttle and

direct antioxidant effects. Furthermore, comparative studies using lipid emulsions without propofol could help isolate the specific contributions of propofol versus its solvent.

Limitations

In our study, only lidocaine was used as a local anesthetic. The drugs used in pretreatment with other local anesthetics may have different effects, which represents one of the major limitations of our study. Additionally, only brain and heart tissues were examined. Another limitation is that the number of animal experiments was minimized, and a larger sample size could not be achieved due to ethical committee restrictions.

The most significant limitation is that such a study cannot yet be conducted on humans due to insufficient evidence. In our study, we observed that inflammation was reduced with the use of intralipid, which is consistent with existing literature. Further studies with larger sample sizes are needed to confirm the effectiveness and superiority of dexmedetomidine in local anesthetic toxicity compared to other anesthetics. To better understand the relationship between the antioxidant effects of intralipid and the clearance rate of lidocaine in serum, it is recommended to measure serum and tissue levels using high-performance liquid chromatography (HPLC). Additionally, exploring the synergistic effects of combining propofol and dexmedetomidine may help achieve stronger protective effects against oxidative stress and inflammation.

Conclusions

The use of intralipid in local anesthetic systemic toxicity is recommended in the guidelines.[4] The widespread adoption of ultrasound has contributed to a reduction in the incidence of side effects and complications related to local anesthetic agents. However, despite this advancement, the incidence of LAST has been reported to be 1.8 per 1000 nerve blocks.[38]

In our study, we focused on damage to brain and heart tissue. It is crucial to understand the mechanisms of action of these agents and the protective effects they may provide in other tissues in future studies.

Based on the data from our study, dexmedetomidine appears to be a promising agent for sedation in regional anesthesia due to its ability to reduce inflammation more effectively.

Acknowledgment

ChatGPT 3.5 was used to translate this study into English. Once authors have used this service, they have reviewed and edited the content as necessary and are fully responsible for the content of the publication.

Disclosure

The authors report no conflicts of interest in this work.

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