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RESEARCH ARTICLE

Cardioprotective Effect of Intravenous Lipid Emulsion in Bupivacaine-Induced Experimental Cardiac Toxicity

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ABSTRACT

The intravenous lipid emulsion (ILE) therapy is known to alleviate clinical symptoms in cases of bupivacaine-induced cardiac toxicity. However, there is insufficient information regarding histopathological damage. This study aimed to investigate whether the use of ILE therapy in rats with experimentally induced bupivacaine-related cardiac toxicity can ameliorate histopathological damage. 28 Wistar albino rats were divided into four groups: control (A), lipid (B), bupivacaine (C), and bupivacaine + lipid (D). After providing monitoring in all groups, group B received 1.5 mL ILE + 0.25 µg/kg/min ILE infusion, group C received 3 µg/kg/min bupivacaine infusion, and group D received 3 µg/kg/min bupivacaine infusion followed by 1.5 mL ILE + 0.25 µg/kg/min ILE infusion after observing cardiac toxicity. Heart rate and respiratory rate were recorded. Blood samples were collected post-procedure to measure LDH, CK-MB, and troponin levels. Cardiac tissue samples were obtained for histopathological examination. There was no significant difference in baseline heart rate and respiratory rate among the groups ($P>0.05$). However, in the second measurements, heart rate and respiratory rate were higher in group D compared to group C ($P<0.05$). LDH and CK-MB levels were higher in group C compared to the other groups ($P<0.05$). Irisin and asprosin scores were higher in group D compared to the other groups ($P<0.05$). ILE was found to have a cardioprotective effect in the treatment of bupivacaine-induced cardiac toxicity, as it improved both clinical and laboratory parameters. However, histologically, cardiac damage persisted.

Keywords: Bupivacaine, Cardiac toxicity, Histopathology, Lipid emulsion

INTRODUCTION

Unlike general anesthesia, local anesthetics (LA) exert their main mechanism of action by providing temporary anesthesia through the blockade of sodium channels in the targeted operative area without affecting the patient's consciousness. LAs are commonly preferred in emergency departments, procedural rooms, and surgeries performed under local anesthesia in the daily practice of anesthesiology. LAs have a wide range of side effects. While transient and minor complications are frequently observed, local anesthetic systemic toxicity (LAST) can also occur due to accidental intravascular injection or rapid absorption from the application site. LAST is known as the most fatal complication of LAs [1,2].

LAST usually begins with nonspecific symptoms such as restlessness, agitation, nausea, and vomiting. Also, it can progress to central nervous system depression, respiratory arrest, and cardiovascular collapse if appropriate and timely intervention is not performed. In addition to essential life support, intravenous lipid emulsion (ILE) treatment has been accepted as one of the treatment options for LAST and has found its place in the LAST treatment algorithm in recent years [3].

The effectiveness of ILE in treating LAST has been reported in the literature through numerous case presentations and animal studies [4-8]. These reports have demonstrated that ILE provides hemodynamic stabilization and resolves clinical symptoms. However, the histologic effects of ILE treatment on cardiac tissue in LAST cases are not clear.

This study aimed to investigate whether ILE treatment improves both the clinical findings and histopathological damage on cardiac tissue in rats with bupivacaine-induced experimental cardiac toxicity.

Material and Methods

Ethical Approval

Ethical approval for this study was obtained from the Animal Studies Ethics Committee of Adıyaman University (ADIYAMAN-HADYEK: 25.11.2021 - 2021/050).

Animals

The study was conducted in the Animal Laboratory of Adıyaman University in 2022. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health. The study was conducted in the Laboratory Animal Facility (Adıyaman, Türkiye) in 2022. Twenty-eight adult male Wistar-Albino rats weighing between 300-350 g were housed and fed with a standard diet and water ad libitum. Room temperature (22-25°C) and humidity (50-55%) were monitored daily. Lighting was provided with 12 h light-dark cycles (06:00-18:00) using cool white fluorescent lamps.

Experimental Groups

To calculate the sample size, we use data from a study that correlated myocardial bupivacaine concentration between control and lipid group (respectively, 30.0/5.0 and 22.4/4.8) [9]. Thus, in order to reproduce these findings with a maximum allowable error estimation of 5%, a statistical power of 80%, and an effect size of 1.55, a sample size of 7 rats per group would be sufficient.

In the study, all animals were anesthetized using a combination of ketamine/xylazine (50 mg/kg 20 mg/kg; i.m.). Electrocardiogram (ECG) recordings in lead II were measured in anesthetized rats using the MP36 system and AcqKnowledge software (BIOPAC Systems Inc.). Respiratory rates were countered. The animals were divided into four groups. Seven subjects were used in each group.

Group A (Sham Group): No agents were administered to the rats in this group.

Group B (Lipid Group): Rats in this group underwent cardiac monitoring under anesthesia, followed by intravenous access via the tail vein. They received a 1.5 mL/kg ILE (Intralipid 20%, Fresenius Kabi AB, Uppsala, Sweden) plus a continuous infusion of 0.25 µg/kg/min of ILE.

Group C (Bupivacaine Group): After cardiac monitoring under anesthesia, rats in this group also received intravenous access via the tail vein. A continuous infusion of 3 µg/kg/min of bupivacaine (Marcaine 0.5%. AstraZeneca Ltd, İstanbul, Türkiye) was initiated to induce experimental LAST and achieve cardiac toxicity symptoms (arrhythmia, bradycardia). The bupivacaine infusion was stopped at the onset of cardiac toxicity.

Group D (Bupivacaine + ILE Group): In addition to Group C, rats in this group received intravenous ILE infusion (1.5 mL/kg intravenous bolus + 0.25 µg/kg/min infusion for 15 min) under O_2 support after discontinuation of bupivacaine infusion. The procedure was terminated upon achieving hemodynamic stability.

At the end of the defined procedures in all groups, cardiac tissues were collected for histological examination.

Biochemical Analysis

Blood samples were collected from the extracted cardiac tissue in EDTA tubes, and creatine kinase-myocardial band (CK-MB), lactate dehydrogenase (LDH), and troponin levels were determined using the Biosite Triage Meter Plus (San Diego, USA) device.

Immunohistochemical Analyses

According to the immunohistochemical staining method using the avidin-biotin-peroxidase (ABC) complex, minor modifications were applied [10,11]. Liver tissues were blocked using this method, and sections with a 4-6 µm thickness were obtained and deparaffinized. Primary antibodies Asprosin (Rabbit polyclonal IgG antibody, Fine Test, FNab09797, China) and irisin (Rabbit polyclonal IgG antibody, Phoenix Pharmaceuticals, H-067-17, USA) were used at a dilution of 1/200 with the Thermo Scientific™ TP-015-HA commercial kit. After applying 3,3-diaminobenzidine (DAB) chromogen, staining was performed using Mayer's Hematoxylin, and the samples were examined and photographed under a light microscope using the Leica DM500 microscope.

Histoscores were created based on the prevalence (0.1: <25%, 0.4: 26-50%, 0.6: 51-75%, 0.9: 76-100%) and intensity (0: none, +0.5: very weak, +1: weak, +2: moderate, +3: intense) of immunoreactivity [12].

Statistical Analysis

Descriptive statistics, including mean, standard deviation, median, minimum, maximum, frequency, and ratio, were used for the data. The distribution of variables was tested using the Kolmogorov-Smirnov test. Non-parametric tests such as Kruskal-Wallis and Mann-Whitney U tests were used for the analysis of quantitative independent variables. SPSS 28.0 software was used for the analyses.

Results

The study initially involved 28 rats and concluded with the same number. After the monitoring phase at the beginning of the study, the minimum and maximum values for heart rate were determined as 318 and 384, respectively, while the minimum and maximum values for respiratory rate were 71 and 88, respectively. Second measurements were taken in Groups C and D, where toxicity was induced. In

Table 1. *Descriptive characteristics of groups*					
Parameters		**Group A**	**Group B**	**Group C**	**Group D**
Heart rate	First measurement min-max (mean)	318-351 (340.6)	343-350 (347.6)	312-384 (339.1)	326-350 (338.4)
	Second measurement min-max (mean)			157-193 (171.9)	302-330 (317)
Respiratory rate	First measurement min-max (mean)	73-85 (79.57)	74-88 (79.86)	71-84 (75.57)	75-84 (79.29)
	Second measurement min-max (mean)			48-70 (61.71)	64-81 (72.43)
Irisin score	min-max (mean)	0.6-0.9 (0.77)	0.6-0.9 (0.79)	0.9-1.2 (1.11)	1.2-1.8 (1.46)
Asprosin score	min-max (mean)	0.8-0.9 (0.87)	0.8-0.9 (0.86)	0.8-1.2 (1.06)	1.8-2.7 (2.29)

Table 2. *Comparison statistical results of groups*								
Vital Signs, Laboratory and Histopathologic Parameters			**Group A**	**Group B**	**Group C**	**Group D**	**P Value**	
Heart rate	First measurement	Mean.±sd	340.6±12.6	347.6±3.2	339.1±25.1	338.4±7.6	0.220	K
		Median	348.0	350.0	332.0	340.0		
	Second measurement	Mean.±sd			171.9±13.0	317.0±10.5	0.002	K
		Median			172.0	319.0		
Respiratory rate	First measurement	Mean.±sd	79.57±3.99	79.86±5.05	75.57±4.50	79.29±3.20	0.217	K
		Median	80.0	80.0	74.0	80.0		
	Second measurement	Mean.±sd			61.71±10.24	72.43±5.68	0.045	K
		Median			69.0	72.0		
LDH		Mean.±sd	1141±435	579±281	2141±411	1315±428	0.000	K
		Median	903	547	2243	1344		
CK-MB		Mean.±sd	522±165	827±278	1623±351	875±155	0.000	K
		Median	498	798	1758	820		
Troponin		Mean.±sd	1.96±2.34	0.93±0.32	4.52±2.46	3.96±2.23	0.001	K
		Median	1.20	0.83	3.4	4.2		
Irisin Score		Mean.±sd	0.77±0.13	0.79±0.09	1.11±0.12	1.46±0.32	0.000	K
		Median	0.80	0.80	1.20	1.20		
Asprosin Score		Mean.±sd	0.87±0.05	0.86±0.05	1.06±0.18	2.29±0.41	0.000	K
		Median	0.90	0.90	1.20	2.50		

[K] *Kruskal-wallis(Mann-whitney u test)*

these second measurements, the minimum and maximum values for heart rate were found to be 157 and 330, respectively, while the minimum and maximum values for respiratory rate were 48 and 81, respectively *(Table 1)*.

There were no significant differences observed in the initial values of heart rate and respiratory rate among the groups (P>0.05). However, in the second measurement, heart rate and respiratory rate were significantly higher in Group D compared to Group C (P<0.05) *(Table 2)*.

Regarding LDH and CK-MB levels, Group C showed significantly higher levels than other groups (P<0.05). Regarding troponin levels, Group C and Group D were significantly higher compared to other groups (P<0.05), but no significant difference was found between Group C and Group D (P>0.05).

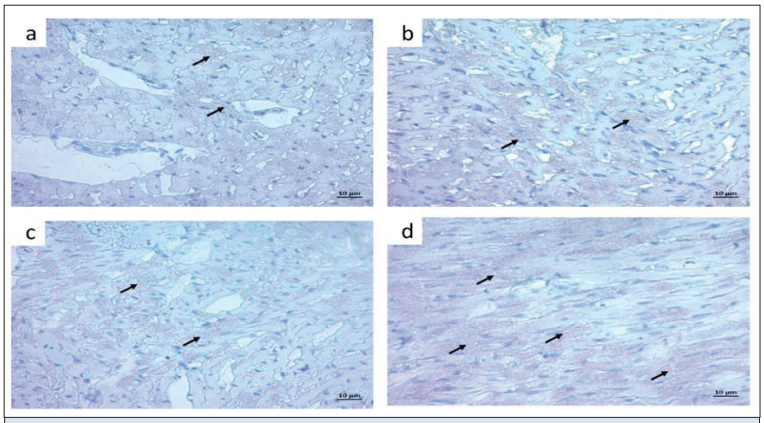

Fig 1. Irisin immunoreactivity of study groups. Scala bar: 10 µm

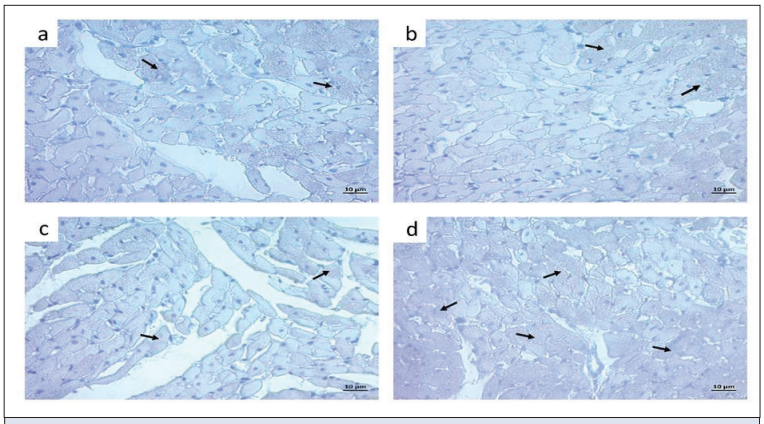

Fig 2. Asprosin immunoreactivity of studygroups. Scala bar: 10 µm

In Group D, the irisin score was significantly higher compared to all other groups (P<0.05), while no significant difference was observed between Group A and Group B (P>0.05). In Group D, asprosin score was significantly higher compared to all other groups (P<0.05), while no significant difference was observed between Group A and Group B (P>0.05) *(Table 2)*.

In the histological examination of cardiac tissues, it was observed that both asprosin and irisin immunoreactivity were higher in Group D compared to the other groups *(Fig. 1, Fig. 2)*.

Discussion

LAST typically initiates with non-specific symptoms such as restlessness, agitation, nausea, and vomiting, these cortical symptoms can be masked in cases where sedation is administered [13]. In our study, the use of sedation in rats subjected to experimental bupivacaine toxicity did not result in non-specific symptoms; instead, it presented with arrhythmia and respiratory depression.

Lazar et al.[14] found that in the group receiving Lipid + ropivacaine, there was less decrease in heart rate. Wu et al.[15] reported in their study where they investigated ILE treatment in rats with local anesthesia-induced central nervous system toxicity that ILE treatment reduced the detection of respiratory arrest and apnea, and resulted in less decline in heart rate. In our study, it was observed that ILE corrected the decrease in respiratory rate and heart rate associated with LAST.

LDH, CK-MB, and troponin are commonly used as clinical markers of cardiac damage. There are some experimental animal studies in the literature using these markers to assess cardiac damage. In these studies, elevated CK-MB, LDH and Troponin levels were associated with myocardial damage [16,17]. In our study, cardiac markers were found to be higher in the bupivacaine group compared to all other groups. When comparing the bupivacaine + lipid group to the bupivacaine group, although troponin levels were higher in the bupivacaine group, no statistically significant difference was found as observed with other cardiac markers. Unlike other markers, troponin has a later peak and a longer duration of elevation [24]. In the bupivacaine+lipid group, the lower levels of all cardiac markers compared to bupivacaine further support the association between the cardioprotective effect of ILE.

Lazar et al.[14] reported no differences in the histological evaluation of tissues obtained from the groups. In our

study, however, the irisin and asprosin scores were higher in the bupivacaine + lipid group compared to all other groups. This indicates that the highest cardiac tissue damage occurred in the bupivacaine + lipid group. In Lazar et al.[14]'s study, tissue analysis was performed after administering the determined drug doses in both the bupivacaine and bupivacaine + lipid groups without observing any toxicity. In our study, histological analyses were conducted after observing cardiac toxicity in both the bupivacaine and bupivacaine+lipid groups.

Irisin is a myokine derived from the cleavage of fibronectin type III domain-containing 5 (FNDC5). Irisin plays a role in mitochondrial energy regulation, fatty acid oxidation, and glucose metabolism. Changes in irisin levels have been shown to be associated with cardiovascular diseases and myocardial damage [18,19]. In our study, it was observed that the irisin score in the bupivacaine + ILE group was higher than that in the bupivacaine group when examining the heart tissues.

Asprosin is a newly identified centrally acting orexigenic adipokine that is secreted from white adipose tissue and regulates glucose metabolism. Increased levels of asprosin have been reported to be associated with coronary artery diseases and myocardial damage [20]. In our study, it was observed that the asprosin score in the bupivacaine + ILE group was higher than that in the bupivacaine group when examining the heart tissues.

Many studies in the literature examine the protective effects of ILE without the development of toxicity or initiate ILE treatment after the induction of cardiac arrest. In our study, ILE treatment was used in accordance with clinical practice for the treatment of cardiac toxicity related to LAST. However, the low number of experimental animals and basic-level monitoring are among the main limitations of this study. Further studies conducted with larger sample sizes and more advanced cardiac and respiratory monitoring will provide more extensive information.

In conclusion, ILE has a cardioprotective effect in the treatment of cardiac toxicity caused by bupivacaine and improves both clinical and laboratory parameters. However, it should be noted that cardiac damage still exists histologically. Therefore, continued monitored follow-up is recommended after ILE treatment in the context of LAST.

Availability of Data and Materials

The datasets during and/or analyzed during the current study available from the corresponding author (N. Yılmaz) on reasonable request.

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Conflict of Interest

The authors declare that they no conflict of interest.

Author Contributors

NY and MD planned the study, designed the experiments and helped manuscript writing; AT and FT helped with data analyses and bioinformatics and wrote the manuscript; NY, MD, AT and FT collected samples, conducted laboratory process and histopathologic examination; NY and FT analysed the statistics data. All authors read and approved the final manuscript.

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