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The Cardiotoxicity of Bupivacaine and Levobupivacaine in Combination with Carnitine in Rats

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Abstract

Background: Cardiotoxic effects of local anesthetics are much more common in the presence of carnitine deficiency.

Objective: We examined the cardiotoxic effects of levobupivacaine and bupivacaine in combination with carnitine, in rats.

Methods: 21 female rats were divided into three groups [carnitine, carnitine + bupivacaine, carnitine + levobupivacaine]. All groups received 100 $mg.kg^{-1}$ I.V. carnitine slowly in five minutes, and 2 $mg.kg.min^{-1}$ bupivacaine or levobupivacaine were given to second and third groups respectively. The study was continued 16 minutes in control group and until cardiac arrest of rats in second and third group. Average heart rate, median arterial blood pressure, PR and QRS complex intervals on ECG of D1, D2, D3 derivations of rats were evaluated. Onset of prolongation of these intervals and then every two minutes PR and QRS complex intervals were recorded afterwards. Time until asystole developed and total doses of local anesthetic agents given during this time were recorded.

Results: We observed idioventricular rhythm and nodal rhythm in both local anesthetic groups and in one case second degree AV block in the bupivacaine group. PR and QRS complex intervals on ECG were significantly different between groups. Total doses of local anesthetics and cardiac arrest times were significantly different, in favor of levobupivaine.

Conclusion: Using the same dose, levobupivacaine was less cardiotoxic when compared to bupivacaine with the existence of carnitine. The current study does not provide any evidence for prophylactic use of carnitine to avoid local anesthetic related cardiotoxicity.

Keywords: Cardiotoxicty; Carnitine; Local anesthetics; Bupivacaine; Levobupivacaine

Introduction

Local anesthetics block the Na^+ channels from opening in excitable cell membranes and decrease fast Na^+ flow into cell in a dose-dependent manner [1-3]. Since, in addition to blocking ion channels in nerve cell membranes, local anesthetics also block channels in other excitable tissues, they have potential cardiovascular toxicities. Local anesthetics inhibit sinoatrial [SA] and atrioventricular [AV] conduction and they cause prolongation in PR and QRS complex intervals on ECG and AV block at varying degrees [4]. Toxicity risk is higher in local anesthetics with long-acting effect and lipid solubility such as bupivacaine [5]. When administered fast in high dose or injected into a vein as a mistake, the signs of acute cardiac toxicity occur, first the atrioventricular conduction slows down. Therefore, bupivacaine demonstrates its effect with disruptions in conduction before myocardial depression.

Levobupivacaine is a long-acting local anesthetic in amide structure that is an S(-) isomer of racemic bupivacaine [6-8] and its pharmacodynamic characteristics are similar to bupivacaine. However, it is less cardiotoxic than bupivacaine [9-11].

Carnitine's most significant function is to transport long-chain free fatty acids to mitochondrial matrix for β-oxidation [6]. Roberts et al. showed in rats that, carnitine deficiency in otherwise healthy skeletal muscle results in loss of muscle fibers [12]. There are some studies on rats showing that, L-carnitine reduces susceptibility to bupivacaine-induced cardiotoxicity [13]. And also there are some case reports suggest that, L-carnitine deficiency increases susceptibility to bupivacaine induced ventricular arhymias, even with non-toxic doses bupivacaine. In studies reported some intraoperative cardiac arrests with nontoxic doses of local anesthetics in patients who were known to have L-carnitine deficiency [14,15].

In this study, we aimed to investigate cardiotoxic effects of infusion of I.V. 2 $mg.kg.min^{-1}$ bupivacaine and levobupivacaine when carnitine was administered 100 $mg.kg^{-1}$ prophylactically before administration of local anesthetics to rats.

Materials and Methods

Approval and study permit were obtained from the Ethics Committee of the Animal Laboratory of the Department of Physiology, School of Medicine, Akdeniz University. 21 female Wistar albino rats of 4 months with weights ranging from 210 to 300g [grams] were randomly divided into 3 groups.

Group 1: Carnitine group (7)

Group 2: Canitine + Bupivacaine group (7)

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Group 3: Carnitine + Levobupivacaine group (7)

The study was carried out with 5 rats in Group 1, 7 rats in Group 2, and 7 rats in Group 3.

Study planned 7 rats in all groups. But during catheterization before administration of drug 2 of them died. For this reason the control group [carnitine group] continued with 5 rats.

Rats in each group were administered intraperitoneal anesthetics with 1 $g.kg^{-1}$ ethyl carbamate [Urethane, U-2500, Sigma Chemical, USA]. Rats were placed on the operation table in supine position. Dissection was conducted on the neck area and 22 G I.V. cannula [22.G I.V. Cannula, Bicakcilar, Turkey] was placed in right vena jugularis interna. Then, dissection was conducted on the groin area and 22 G I.V. cannula (22.G I.V. Cannula, Bicakcilar, Turkey) was placed in left femoral artery, and a pressure transducer (Transducer, Model TP-400 T, Nihon Kohden, Japan) was placed in order to observe systemic artery pressure changes, thus creating a connection between arterial cannula and monitor (Polygraph System RM-600 Nihon Kohden, 1999, Japan). I.V. local anesthetics and carnitine were administered through internal jugular vein. Invasive hemodynamic monitoring was conducted from femoral artery. Four needle electrodes belonging to polygraph were placed inside extremity muscles of rats for ECG measurement. For each of the rats, systolic blood pressure, diastolic blood pressure, mean arterial pressures (mmHg), and heart rates were recorded in two-minutes intervals. Records of D1, D2, and D3 derivations of ECG (PR interval, QRS interval) were printed out. Entry data of each rat were recorded, and then rats in Group 1 were only given I.V. 100 $mg.kg^{-1}$ carnitine (Carnitine 1 gr/5mL injectable solution, Sigma-tau) at a slow pace. Rats in Group 2 were administered 100 $mg.kg^{-1}$ I.V. carnitine within five minutes at a slow pace. Then, the initial values were recorded and two minutes later, bupivacaine HCL (Marcain, 0.5%, Astra Zeneca) was administered with an infusion pump (Compact Infusion Pump 975, Harvard Apparatus, USA) at a 2 $mg.kg^{-1}.min^{-1}$ infusion pace. Rats in Group 3 were given 100 $mg.kg^{-1}$ carnitine in five minutes at a slow pace. Then, the initial values were recorded and levobupivacaine (Chirocaine, 0.5%, Abbott) was administered at 2 $mg.kg^{-1}.min^{-1}$ infusion pace two minutes later. Infusions were continued until rats developed cardiac arrest the time between start of the local anesthetic administration and asystole was recorded. In order to compensate blood losses during cannulation, 10±2 $mL.kg^{-1}$ colloid fluid was infused and drug infusions were initiated in five minutes.

The study continued for 16 minutes in carnitine group after recording the initial values and until the rats were arrested in bupivacaine and levobupivacaine group. Heart rate (beats/min) and mean arterial blood pressure (mmHg), PR intervals measured in D1, D2, and D3 derivations and QRS complex widths (msn), rat weights (gram-g), local anesthetics dosage until asystole developed (milligram-mg) and duration (minute-min) were included in statistical evaluation.

In statistical analyses of the study, Repeated Measures Analysis of Variance test and Mann-Whitney-U test were used, and $p<0.05$ was established to be statistically significant.

Statistical evaluations in all 3 groups were carried out with data obtained until the 10th minute, since the animals in Group 2 lived up to 10 min maximum. Animals in Group 3 lived up to 14 min maximum.

Results

In our study, mean values of the body weights of Wistar albino rats were found to be within physiological range [210-300 g]. A statistically significant difference was not observed between the groups ($p>0.05$] (Table 1).

One animal in Group 2 was arrested sooner then the 10th min following secondary AV block and thus, it was not included in the statistical evaluation. And one animal in Group 3 was not included in the statistical evaluation due to arrest following idioventricular rhythm sooner then 10th min.

Animals that were included in the study in Groups 2 and 3 were established to have nodal rhythm and idioventricular rhythm in general. One animal in Group 2 developed second degree AV block.

PR values in D1, D2 and D3 derivations

Mean values of the PR intervals of ECG belonging to D1, D2 and D3 derivations between groups were compared. In each group, a significant difference was not established in terms of time-based PR values ($p>0.05$). PR intervals were observed to prolong based on initial values. Also, statistically significant difference was not established in terms of PR values belonging to D1, D2 and D3 derivations, between groups ($p>0.05$) (Figure 1).

A statistically significant difference was established between the groups in terms PR interval values in D1, D2, and D3 derivation from the 6th minute at 8th and 10th minutes ($p<0.05$) (Figures 1 and 2).

QRS values

QRS complex mean width values belonging to ECG D1, D2, and D3 derivations were compared between the groups. A statistically significant difference was observed in terms of QRS values based on time within each group ($p<0.05$). Also, statistically significant

Group	N	Minimum	Maximum	Mean	SD
Carnitine weight	5	220	280	260,0000	25,49510
Carnitine Bupivacaine weight	7	210	300	242,8571	28,11541
Carnitine Levobuivacaine weight	7	240	280	251,4286	14,63850

Data are ± Standard Deviation.
SD: Standard Deviation
Kruskal-Wallis test was used for comparison of the groups

Table 1: Mean body weights of the groups.

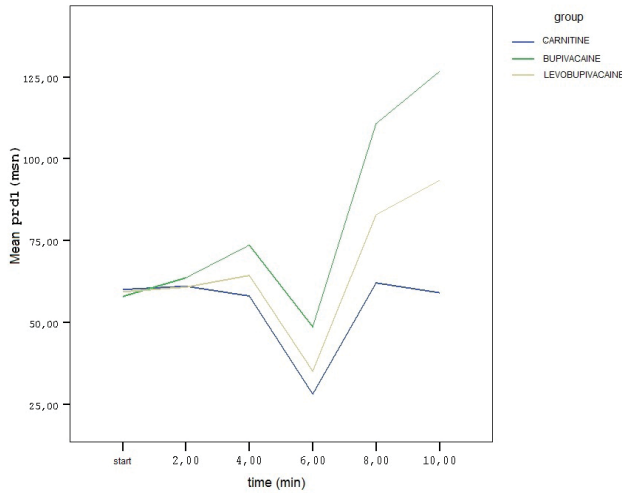

Figure 1: Change in PR interval belonging to D1 derivation of ECG of the groups based on time.

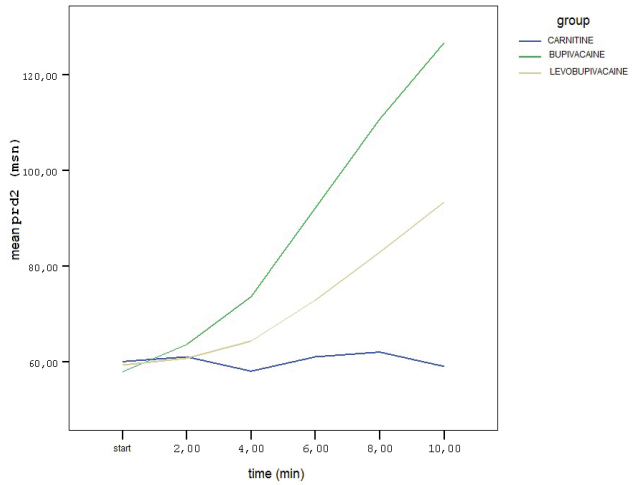

Figure 2: Change in PR interval belonging to D2 derivation of ECG of the groups based on time.

differences were established in terms of QRS values belonging to D1, D2, and D3 derivations, between groups ($p<0.05$) (Figure 3). In the bupivacaine group, the measured QRS width value was observed to be significantly high compared with other groups.

A statistically significant difference was observed in terms of PRD2 [PR interval length in D2 derivation] from the 6th min at 8th and 10th min between the groups ($p<0.05$) (Figure 4).

A statistically significant difference was observed in terms of PRD3 [PR interval length in D3 derivation] from the 6th min at 8th and 10th min between the groups ($p<0.05$) (Figure 5).

Mean heart rate values

In each group, a statistically difference was established in the time-based mean heart rate ($p<0.05$). A statistically significant difference was not established between Group 2 and Group 3 ($p> 0.05$). A statistically significant difference was found between Groups 1 and 3 and also Groups 1 and 2 ($p <0.05$) (Figure 6).

A statistically significant difference was not established between Group 2 and Group ($p>0.05$). A statistically significant difference was found between Groups 1 and 3 and also Groups 1 and 2 ($p<0.05$) (Figure 6).

Mean arterial pressure values

In each group, statistically significant decreases were established in time-based mean arterial pressure values ($p<0.05$). A statistically significant difference was not found between the groups in mean arterial pressure ($p>0.05$) (Figure 7).

In Group 2, cardiac asystole developed with 4.32 mg local anesthetics on average. In Group 3, asystole developed with 5.95 mg local anesthetics on average. A statistically significant difference was established between Groups 2 and 3 in terms of the dosage of local anesthetics given until asystole developed in rats ($p<0.05$) (Figure 8).

While, in Group 2, cardiac asystole developed after 8.85 min on average, cardiac asystole developed after 11.71 min in Group 3 on average. A statistically significant difference was established between Groups 2 and 3 in terms of the time until cardiac asystole developed ($p<0.05$) (Figure 8).

Discussion

Local anesthetics reduce fast Na^+ flow into cell in a dose dependent manner by impeding the opening of Na^+ channels in excitable cell membranes [heart, brain, peripheral nerves]. Affinity of Na^+ channels of local anesthetics are typically high when the channel is open or inactive [1,2]. Local anesthetics also prolong cardiac action potential time by blocking voltage-dependent K^+ channels, and this contributes to cardiotoxicity by increasing the inactive Na^+ channel block [16,17]. In addition, local anesthetics cause intracellular Ca^{+2} reduction through L-type Ca^{+2} channels activation and Ca^{+2} release inhibition from sarcoplasmic reticulum and thus cardiac depression [18]. Systemic effects of local anesthetics appear by the absorption of the drug from the area where it is injected in a dose-dependent manner or by its systemic administration [1]. This means that the acute cardiotoxicity findings appear when the drug is administered fast in high doses or intravascularly. Systemic toxic effects of local anesthetics occur on cardio vascular system [CVS] and central nervous system [CNS] [1]. CVS is more resistant to toxic effects compared to CNS. This means

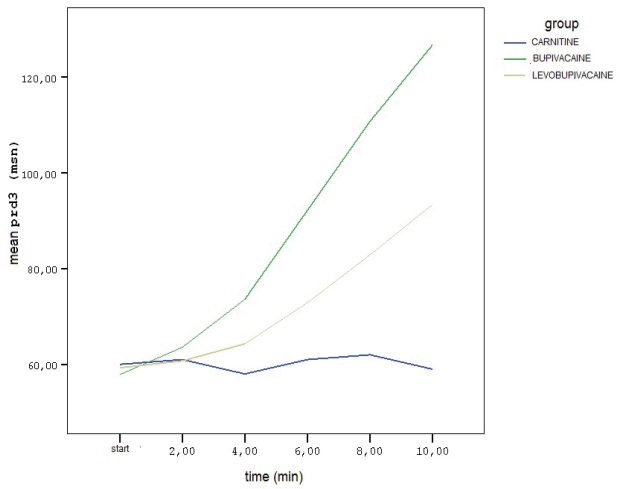

Figure 3: Change in PR values belonging to D3 derivation of ECG of the groups based on time.

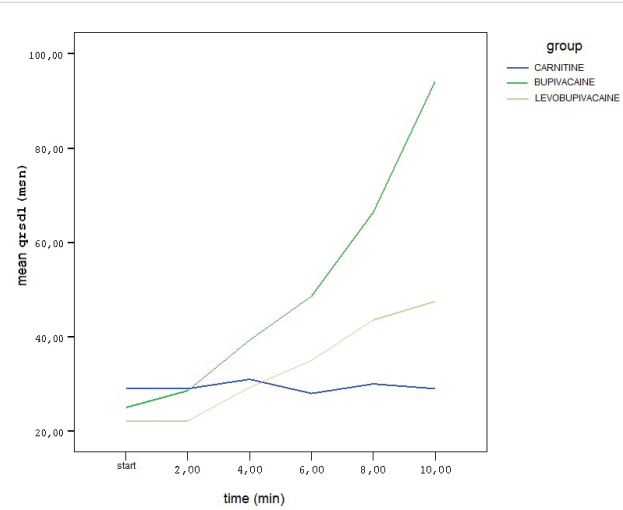

Figure 4: Change in QRS values belonging to D1 derivation of ECG of the groups based on time.

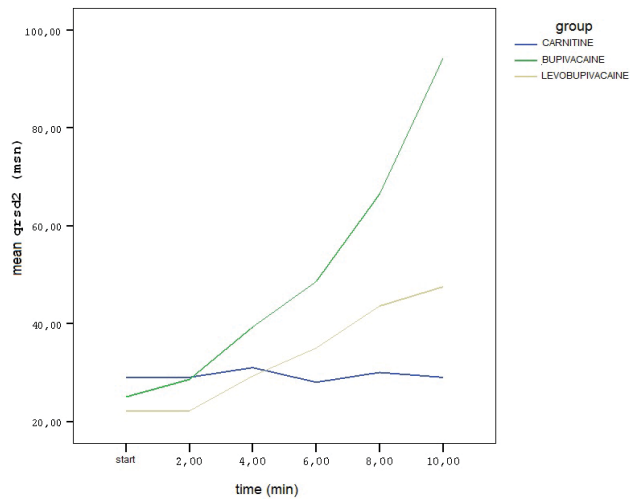

Figure 5: Change in QRS values belonging to D2 derivation of ECG of the groups based on time

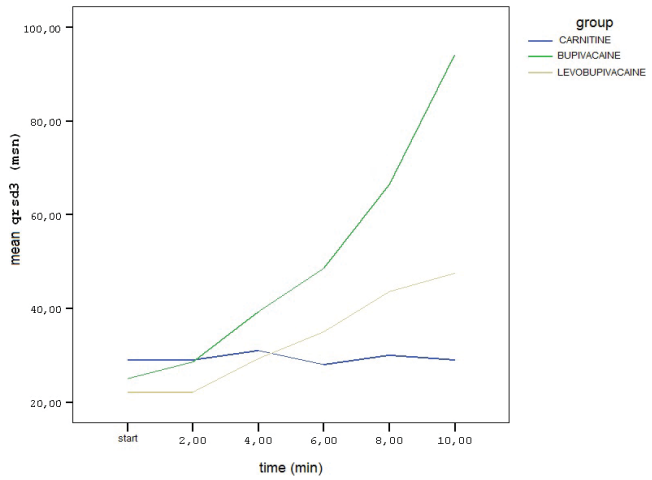

Figure 6: Change in QRS values belonging to D3 derivation of ECG of the groups based on time.

that such CNS findings as convulsions appear rather more primarily. Toxicity risk is higher in long-acting and high-lipid solubility local anesthetics. The highest cardiotoxic local anesthetic agent is bupivacaine. It may cause ventricular tachycardia [VT], ventricular fibrillation [VF], asystole, and electromechanic dissociation in plasma levels above 4 µg/mL [5].

Local anesthetics blocks conduction in sinoatrial [SA] and atrioventricular [AV] node and prolong bradycardia, PR interval and QRS complex interval and cause AV block in varying degrees, QT prolongation, and ventricular arrhythmia in varying degrees [4,19,20]. Also, they cause hypotension by myocardial depression and vasodilation through expanding vein smooth muscle. This is to say that local anesthetics cause a disruption first in conduction function [21]. In our study, it was established that bupivacaine and levobupivacaine given i.v, first caused prolongation in PR interval and QRS complex width in a dose dependent manner, and then hypotension.

It was demonstrated in studies that levobupivacaine was less cardiotoxic compared to bupivacaine [7,10,20-26]. In their study conducted on papillary muscle of guinea pigs, Vanhoutte et al. showed that bupivacaine slowed down cardiac conduction more compared to levobupivacaine and caused a deeper Na blockage [23]. In studies conducted on isolated rabbit hearts, levobupivacaine was shown to cause less arrhythmia and QRS prolongation compared to bupivacaine [8,10,22]. In similar studies, levobupivacaine was reported to cause less delay in AV conduction [24] and less expansion in QRS [20]. In our study, when checked the mean QRS expansion values caused by bupivacaine and levobupivacaine, it was established that levobupivacaine caused less expansion in QRS compared to bupivacaine ($p<0.05$). In addition, while in our study the animals in bupivacaine group died in 8.8 min on average, animals in levobupivacaine group died in 11.7 min on average ($p<0.05$). Thus, in compliance with literature information, bupivacaine was found to be more cardiotoxic compared to levobupivacaine.

Convulsion developed in patients that underwent peripheral nerve blockage and were falsely administered I.V. 142-150 mg levobupivacaine, however, no cardiac pathology was reported [27-30]. In a study conducted on volunteer subjects, levobupivacaine was administered

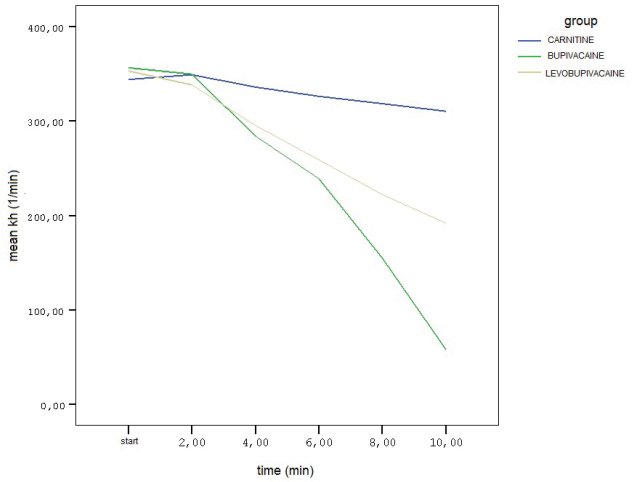

Figure 7: Change in mean heart rate values of the groups based on time.

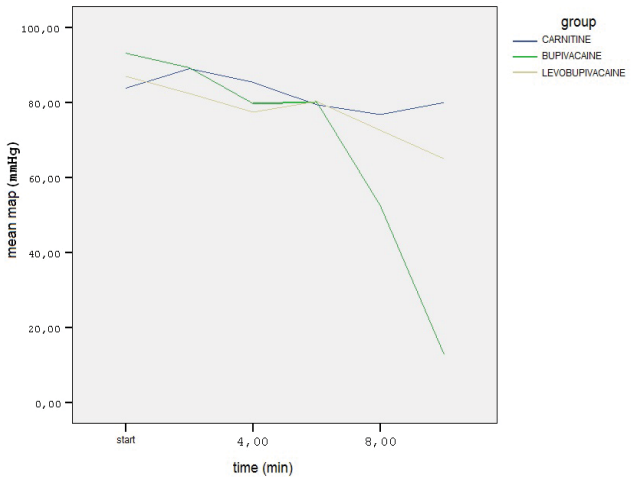

Figure 8: Change in mean arterial pressure of the groups based on time.

until the formation of SSS finding at a 10 mg/min dose infusion or total 150 mg and a severe cardiac pathology was not observed, but only increases in blood pressure and heart rate were reported [31,32]. In a patient that was falsely given I.V. 125 mg levobupivacaine, hypotension, bradycardia, ST-T changes, and supraventricular rhythm were reported to develop [33]. It was reported that bupivacaine administered at a dose of I.V. 2 $mg.kg^{-1}$ in anesthetized rats caused severe bradycardia and progressive hypotension, that resuscitation did not produce response, and all 12 animals died [34]. In the same study, moderate bradycardia developed in rats administered levobupivacaine, and 10 out of 12 animals were revived through resuscitation. In our study, a statistically significant change was not observed in terms of change of time-based heart rate due to bupivacaine and levobupivacaine administered I.V. 2 $mg.kg.dk^{-1}$ ($p>0.05$).

In our study, we did not apply resuscitation when cardiotoxic findings appeared, and thus, we do not have any opinion as to how many animals in groups could benefit from resuscitation. It was reported that the I.V. use of bupivacaine increased plasma catecholamine level and thus autonomous nervous system activation transforms short-term VT into persisting VT or VF [35]. It was noted that I.V. bupivacaine in anesthetized rats caused increases in mean arterial pressure and bradycardia in a dose-dependent manner [36]. In our study, increases in systolic blood pressures and thus mean arterial pressures and bradycardia were established about 4-6 min after initiating bupivacaine infusion [2 $mg.kg.min^{-1}$] in three animals in the bupivacaine group. This situation was not observed in levobupivacaine group. We believed that the reason for this, was sympathetic nervous system activation, but we did not come across with a serious finding such as VT or VF.

In animal studies, lethal dose of levobupivacaine was reported to be 1.3-1.6 times higher compared to bupivacaine [10,27,37-39]. It was established in our study that the animals in bupivacaine group died following 4.32 mg local anesthetic and the ones in levobupivacaine group died following 5.95 mg local anesthetic administration. It was found that the lethal dose of levobupivacaine was 1.37 times higher than bupivacaine. A significant difference was established between groups in terms of the administered local anesthetic amount ($p<0.05$).

The most important function of carnitine is to transport long-chain free fatty acids to mitochondria matrix for β-oxidation [6]. Major energy sources in heart is provided by long-chain fatty acid β-oxidation. In the case of carnitine deficiency, fatty acid synthesis increases and triglyceride is accumulated in tissue [heart]. Under anoxic conditions, glycolysis is preferred in heart and fatty acid use is limited. Therefore, the accumulated acyl carnitines inhibit Na^{+}-K^{+} ATP_{az} and Na^{+}-Ca^{+2} channels and cause arrhythmia. Carnitine increases mitochondrial energy production that slows down during ischemic process, transfer of fatty acids to mitochondria, and their oxidation there. As a result, more ATP is produced, and lactic acid production, acidosis, and cellular damage is reduced [40]. In some patients, cardiac toxicity were reported with local anesthetics even before toxic doses were reached when patient has carnitine deficiency [14,15].

In our study, we administered I.V. 100 $mg.kg^{-1}$ carnitine for prophylaxis of cardiac toxicity due to I.V. 2 $mg.kg.min^{-1}$ local anesthetics. In literature in some studies carried out with carnitine for LA toxicity [41] Weinberg et al. showed in their study conducted with bupivacaine that bupivacaine hinders carnitine-dependent mitochondrial lipid transport in cardiac mitochondria of rats [41]. In this study; bupivacaine was demonstrated to carnitine inhibited acyl-carnitine translocase. This enzyme is necessary to pass long-chain acyl CoAs through inner membrane of mitochondria. Through this enzyme inhibition, acyl-carnitine change is inhibited in mitochondria, and as a result of this inhibition, arrhythmia occurs due to accumulation of cytoplasmic acylcarnitines. Therefore, cases with secondary carnitine deficiency are more prone to local anesthetic cardiotoxicity and carnitine is believed to be beneficial for cardiotoxicity occurring in such patients [41].

In a study, Wong et al. applied 100 mg/kg subcutaneous bupivacaine with 2 mg/kg/min of I.V. infusion, until asistoly observed. They mentioned that carnitine deficiency can play a role for bupivacaine induced cardiotoxicity and L carnitine plays a prophylactic role for bupivacaine induced cardiotoxicity [14], but in a further study they failed to show preemptive administration of L-carnitine to the general population in the preoperative setting in order to avoid local anesthetic toxicity [13].

The acute cardiac toxicity results from accidental intravenous administration of local anesthetics. In the study, we aimed to investigate the effectiveness of carnitine in preventing acute cardiac toxicity.

Conclusion

In our study, we investigated the effect of 100 $mg.kg^{-1}$ carnitine administered before infusion prophylactically in bupivacaine and levobupivacaine cardiotoxicity given to rats with I.V. 2 $mg.kg^{-1}.min^{-1}$ infusion. Consequently, we observed in this study that levobupivacaine is less cardiotoxic compared to bupivacaine. However, we failed to show preemptive carnitine administration is beneficial for cardiotoxicity developed by levobupivacaine and bupivacaine infusion. We believe that controlled and further studies are necessary on this subject, including concentrations of the local anesthetics.

Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

Author's Contributions

This work was carried in collaboration between all authors. Bilge Karslı and Metin Cıngı, designed the study and wrote the protocol. Metin Cıngı and Bilge Karslı managed the experimental process. Bilge Karslı, Nurten Kayacan and Yeşim Çetintaş wrote the first draft of the manuscript. Metin Cıngı, Bilge Karslı, Nurten Kayacan, Yeşim Çetintaş and Zekiye Bigat managed the literature searches, analyses of the study, statistical datas and wrote the manuscript. Bilge Karslı, Nurten Kayacan and Yeşim Çetintaş have prepared the article for publication. Yeşim Çetintaş has edited the manuscript. All authors read and approved the final manuscript.

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