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Examination of the effect of bupivacaine on brain tissue in rats with induced experimental renal failure

Nezir Yılmaz, Mehmet Tepe, Öznur Uludağ

Department of Anesthesiology and Reanimation, Adıyaman University, Faculty of Medicine, Adıyaman, Turkey

ORCID ID of the author(s)

NY: 0000-0002-4351-6256
MT: 0000-0002-2596-1284
ÖU: 0000-0002-6017-5836

Abstract

Background/Aim: Local anesthetics are frequently used and often considered harmless, but they can precipitate local anesthetic systemic toxicity (LAST) when accidentally administered intravascularly or when a toxic dose is rapidly absorbed, which can result in mortality. In cases of renal function impairment, the altered pharmacokinetics of local anesthetics lead to a lowered toxicity threshold. In this study, the aim was to histopathologically investigate the increase in neurotoxicity in the central nervous system due to bupivacaine in experimental renal failure.

Methods: In the study, a total of 28 male Wistar albino rats, aged 8-10 weeks, were evenly divided into four groups: Group C (control group) received intraperitoneal 1 mL/kg saline; Group G (glycerol group) received intramuscular 10 mL/kg glycerol, Group GB (glycerol+bupivacaine group) received intramuscular 10 mL/kg glycerol followed by intraperitoneal 4 mg/kg bupivacaine; and Group B (bupivacaine group) received intraperitoneal 4 mg/kg bupivacaine. All rats were sacrificed after the experimental period. Tissue samples were preserved and stained with hematoxylin-eosin for histopathological analyses. TRPM2 and Reelin levels in brain tissue were measured using immunohistochemical methods.

Results: In the histopathological examination, Group G exhibited higher Reelin and TRPM2 levels compared to all other groups ($P<0.001$). In Group GB, both Reelin and TRPM2 immunoreactivity were significantly higher compared to Group B ($P<0.001$).

Conclusion: It can be concluded that renal dysfunction increases neurotoxicity in brain tissue associated with bupivacaine.

Keywords: bupivacaine, neurotoxicity, renal failure

Corresponding Author
Nezir Yılmaz
Department of Anesthesiology and Reanimation,
Adıyaman Training and Research Hospital,
Adıyaman, Turkey
E-mail: yilmaznezir@hotmail.com

Introduction

Local anesthetics (LA) are pharmacological agents that induce anesthetic effects in the administered area by blocking impulse transmission in neurons responsible for pain and temperature through a temporary Na$^+$ channel blockade [1]. LA are applied in a wide range of medical procedures, from minor cosmetic interventions to complex surgical procedures, and serve as a cornerstone of multimodal analgesia practices [2,3]. Despite their widespread use, complication rates remain low. While minor and transient side effects are commonly identified, life-threatening systemic toxicity of LA, known as local anesthetic systemic toxicity (LAST), can occur, presenting with central nervous system toxicity and cardiotoxicity manifestations [4,5].

In the context of impaired kidney function, alterations occurring in tissues as well as changes in the pharmacokinetics of local anesthetics lower the toxic dose threshold of the drug, potentially increasing the rate of complications [6]. Glycerol, often employed as a pharmacological agent, profoundly mimics acute kidney injury caused by rhabdomyolysis-induced acute renal damage in humans, manifesting exaggerated myoglobin release, tubular necrosis, and renal vasoconstrictive effects [7].

This study aims to histopathologically examine the increase in neurotoxicity in the central nervous system associated with bupivacaine in rats with experimentally induced renal failure.

Materials and methods

This is an experimental animal study and ARRIVE guidelines were used for this manuscript.

Animal procurement

In our study, a total of 28 male Wistar albino rats, aged 8-10 weeks, weighing from 240 to 260 grams, were systematically allocated into four groups, with each group consisting of seven rats. Throughout the experimental period, the rats were housed in rooms with a light-dark cycle of 12 hours each, at a room temperature ranging from $22 \pm 20°C$, and with unrestricted access to both food and water.

Ethical approval

Approval for this study was granted by the Adıyaman University Animal Experiments Ethics Committee (15.09.2022-2022/054). Animals were obtained from the Experimental Research Center unit of Adıyaman University, where experimental applications and care were conducted. At the conclusion of the study, rat tissues were processed at the Department of Histology and Embryology, Faculty of Medicine, Adıyaman University, and sera were worked on in the Medical Biology Laboratory of the Faculty of Medicine, Adıyaman University.

Formation of experimental groups

- Group C (Control Group): At the onset of the experiment, injections of 1 mL/kg saline solution were administered intraperitoneally to each subject.

- Group G (Glycerol Group): At the commencement of the experiment, intramuscular injections of 10 mL/kg glycerol solution were administered to each subject.

- Group GB (Glycerol+Bupivacaine Group): At the initiation of the experiment, each subject underwent a dual-phase administration, starting with a single intramuscular injection of 10 ml/kg glycerol, followed by an intraperitoneal injection of 4 mg/kg bupivacaine.

- Group B (Bupivacaine Group): At the outset of the experiment, each subject was administered a single intraperitoneal injection of 4 mg/kg bupivacaine.

At the conclusion of the experimental period, subsequent to intracardiac blood collection while under anesthesia, the rats were humanely euthanized under anesthesia, and tissue and serum samples were carefully preserved under suitable conditions for subsequent histological and biochemical analyses. As per previous literature knowledge, rats were dehydrated by withholding water for 12 hours before glycerol injections [8].

Collection and preservation of tissues

Immunohistochemical assessment was conducted at the Histology Laboratory of Adıyaman University Faculty of Medicine. Tissue samples obtained from euthanized animals underwent standard processing for subsequent light microscopic examination, following a one-week post-fixation period in a 10% formalin solution. Standard histological sections, involving a series of alcohol, xylene, and paraffin treatments, were prepared using the Leica TP1020 automated tissue processor (Leica TP1020, Nussloch, Germany), and 7 μm thick sections were meticulously sliced using the Thermo Shandon Finesse ME microtome (Thermo Fisher Scientific, Cheshire, UK).
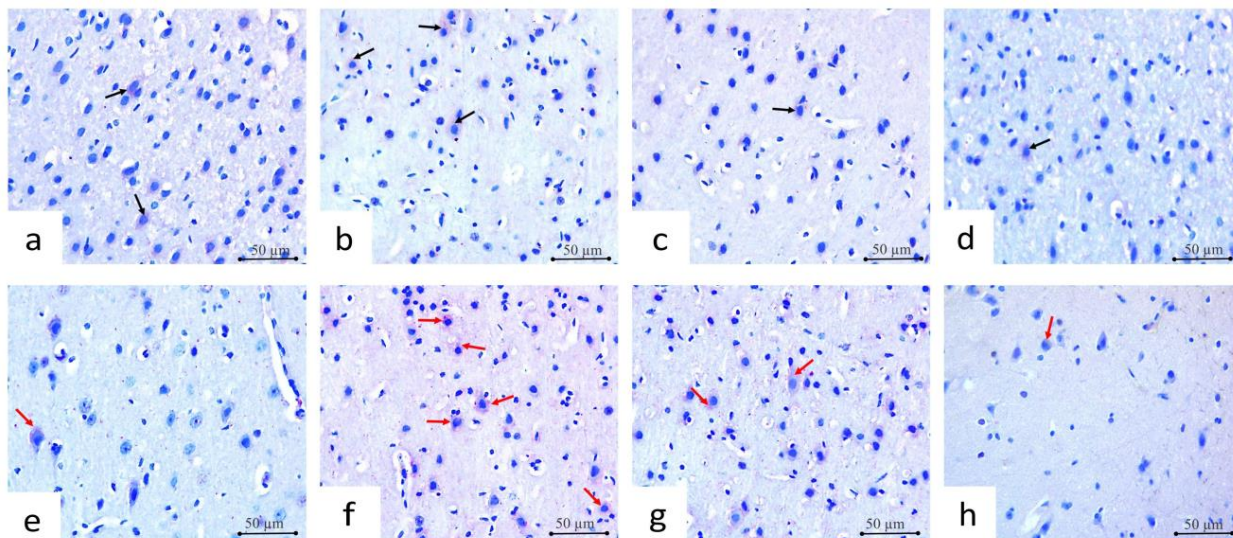
Immunohistochemical staining

Immunohistochemical staining was conducted using minor adaptations to the avidin-biotin-peroxidase (ABC) complex method [9]. Sections, 7 μm thick, were acquired from paraffin-embedded tissues. Primary antibodies targeting Reelin (Rabbit polyclonal Ig [Reelin E5] Rabbit Polyclonal sc-25346, Santa Cruz Biotechnology, Inc., California, USA) and TRPM2 (anti-TRPM2 Rabbit polyclonal IgG antibody, ab101738 Abcam, London, UK) were appropriately diluted at ratios of 1/50 and 1/200 using the Thermo Scientific™ TP-015-HA commercial kit. Subsequent to AEC chromogen application, the sections were counterstained using Mayer's hematoxylin and subsequently assessed under a light microscope. Prepared slides were scrutinized and captured using the Leica DM500 microscope. The histoscore calculation was based on immunoreactivity prevalence (0.1= <%25, 0.4= %26-50, 0.6= %51-75, 0.9= %76-100) and intensity (0= none, +0.5= very low, +1= low, +2= moderate, +3= intense), with the histoscore being computed as the product of prevalence and intensity (histoscore = prevalence x intensity).

Statistical analysis

To calculate the sample size, we used data from a study that correlated TPRM2 levels with pathologic changes in the hippocampus (respectively, 5.40/1.14 and 8.40/2.07) [10]. Thus, in order to reproduce these findings with a maximum allowable error estimation of 5%, a statistical power of 90%, and an effect size of 1.8, a sample size of seven rats per group was determined sufficient.

Descriptive statistics encompassed mean, standard deviation, median, minimum, and maximum values for the dataset. The distribution of variables was assessed using the Kolmogorov-Smirnov test. Quantitative independent data were

Figure 1: The light microscope images of brain cortex tissues obtained from the study groups.

subjected to analysis through the Kruskal-Wallis and Mann-Whitney U tests. The statistical analyses were executed utilizing SPSS 28.0 software.

Results

The immunohistochemical staining results for Reelin were examined under light microscopy, revealing Reelin immunoreactivity in the brain tissue (black arrow) (Figure 1). Reelin immunoreactivity in the brain cortex tissue was similar in the control (Figure 1a) and bupivacaine (Figure d) groups (*P*=0.173). When compared to the control group, it was found that Reelin immunoreactivity significantly increased in the glycerol group (Figure 1b) (*P*<0.001). Conversely, when compared to the glycerol group, Reelin immunoreactivity was reduced in the glycerol+bupivacaine group (Figure 1c) (*P*<0.001) (Table 1, Reelin histoscore).

Upon examining the results of immunohistochemical staining for TRPM2 under light microscopy, TRPM2 immunoreactivity was observed in the brain cortex tissue (red arrow) (Figure 1). TRPM2 immunoreactivity in the brain cortex tissue was similar in the control (Figure 1e) and bupivacaine (Figure 1h) groups (*P*=0.528). It was observed that TRPM2 immunoreactivity significantly increased in the glycerol group (Figure 1f) when compared to the control group (*P*<0.001). In contrast, TRPM2 immunoreactivity was reduced in the glycerol+bupivacaine group (Figure 1g) compared to the glycerol group (*P*<0.001) (Table 1, TRPM2 histoscore).

Table 1: Comparison of immunohistoscores among the groups

	Min-Max	Median	Mean (SD)	*P*-value
Reelin				
Group C	0.10 - 0.30	0.20	0.229 (0.076)	<0.001 [K]
Group G	0.80 - 1.20	0.90	0.914 (0.135)	
Group GB	0.40 - 0.80	0.60	0.571 (0.138)	
Group B	0.10 - 0.30	0.20	0.171 (0.076)	
TPRM2				
Group C	0.10 - 0.30	0.30	0.229 (0.095)	<0.001 [K]
Group G	0.80 - 1.20	0.90	0.971 (0.160)	
Group GB	0.60 - 0.80	0.60	0.657 (0.098)	
Group B	0.20 - 0.30	0.20	0.214 (0.038)	

[K] Kruskal-Wallis (Mann-Whitney U test)

Discussion

The onset time, potency, and duration of action of LA depends on factors such as pKa, lipid solubility, and protein binding [11]. LA primarily binds to two plasma proteins: α1-acid glycoprotein with high capacity and low affinity, and albumin with low affinity and high capacity. The capacity of albumin to bind LA becomes significant when the concentrations of LA increase. In cases of metabolic acidosis, the levels of free LA unbound to plasma proteins increase, with bupivacaine showing the most prominent effect [12].

Both acute and chronic kidney failure can impact the pharmacokinetics of LA, manifesting in four distinct stages (absorption, distribution, metabolism, and excretion). Due to relative alkalization by LA, absorption is increased. Enhanced blood flow due to hyperdynamic circulation leads to rapid increases in plasma concentrations of LA. In cases of kidney failure, the elimination of LA metabolites through the urinary system will be reduced [13]. Lili et al. [14] demonstrated that renal dysfunction caused by glycerol in rats with acute kidney injury may alter plasma protein binding and vascular permeability, leading to higher plasma drug concentrations. Considering all this pharmacological information, it is recommended to reduce LA doses by 25% in patients with end-stage kidney failure [12].

LAST is a complex and fatal complication that can extend to seizures and cardiac arrest due to the blockade of Na^+ channels in both myocardial cells and thalamocortical neurons [15,16]. LAST initially presents with nonspecific symptoms, like restlessness and agitation, followed by central nervous system (CNS) excitation-inhibition findings and cardiovascular symptoms. While LAST is presented with CNS symptoms in 77% of cases, the most common manifestation is seizures, seen in 53-68% of cases [17,18]. The literature includes numerous case presentations discussing clinical manifestations related to LAST [19-21] as well as studies investigating neurotoxicity on motor neurons due to intrathecal use [22-24]. However, studies investigating the CNS histopathology associated with local anesthetics are limited.

Spitzer et al. [25] reported complications in two patients who experienced CNS toxicity following an interscalene block.

Although initial central nervous system imaging on the day of complications did not reveal any pathology, subsequent control imaging conducted within one to five days indicated the presence of T2/FLAIR hyperintensities accompanied by apparent diffusion coefficient (ADC) restriction in the cortical grey matter and basal ganglia of the left hemisphere.

Reelin is an extracellular matrix protein that controls neuronal migration during the developmental stages of brain tissue. Mice with Reelin deficiency show abnormal neuronal morphology and behavior. Reelin levels have been associated with neuropsychiatric pathologies and brain injuries [26-28]. In our study, the higher Reelin levels observed in the group with experimentally induced kidney failure suggest that renal failure exacerbates CNS damage associated with bupivacaine.

TRPM2 (melastatin-like transient receptor potential 2 channel) is a non-selective calcium channel. When exposed to oxidative stress, it becomes activated, resulting in elevated intracellular free Ca2+ concentrations and subsequent cellular damage. Elevated levels of TRPM2 expression have been linked to brain injury [29]. Similarly, in our study, the higher TRPM2 levels observed in the group with experimentally induced kidney failure suggest that kidney dysfunction exacerbates CNS damage associated with bupivacaine.

Limitations

The limitations of this study include the lack of CNS monitoring, and the inability to demonstrate the physiological effects of kidney failure.

Conclusion

In conclusion, both TPRM2 and Reelin scores, utilized both immunohistochemically and histopathologically, have demonstrated increased neurotoxicity associated with bupivacaine in cases of renal function impairment. In this context, we believe that a reduction in dosage is necessary when using local anesthetics in renal function impairment cases. Furthermore, we anticipate that larger-scale studies involving the monitoring of the central nervous system (such as EEG), and the assessment of acidosis through blood gas analysis will provide additional insights.

Acknowledgements

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