Original Article

The effects of Ropivacaine and its Combination with Dexmedetomidine and Dexamethasone on Neural Apoptosis

Behnam Hosseini[1], Ali Dabbagh[1], Faramarz Mosaffa[1], Shideh Dabir[1], Mohammadreza Moshari[1], Seyed Mohammad Seyed Alshohadaei [1], Fereshteh Baghizadeh[1*]

Abstract

Background: Neurotoxicity effects of anesthetics in different age groups is a major concern regarding neural injuries. It is reasonable to recognize the neurotoxicity risks of anesthetic drugs and their combinations**.**

Materials and Methods: A total of 16 mice (Mus musculus) weighing 22 to 30 gr were randomly divided into four drug groups (control, Ropivacaine, Ropivacaine + Dexmedetomidine, Ropivacaine + Dexamethasone). 24 hours after unilateral injection of drugs into the femoral nerve of mice, the mice were killed and their femoral nerve was removed. Hematoxylin-eosin tissue staining was used to evaluate changes in the effects of the drugs, and nerve samples were extracted to measure TLR4 and caspase 3 expressions. After Western blotting, the protein expression level was checked between different groups.

Results: Ropivacaine in combination with dexamethasone caused less damage to the rat nerve cells. The combination of ropivacaine with dexamethasone (p=0.53 and p=0.46) compared to the combination of ropivacaine with dexmedetomidine relatively had better results in terms of cytotoxicity.

Conclusion: A combination of ropivacaine with dexamethasone reduces neurotoxicity risk.

Keywords: Ropivacaine, Dexmedetomidine, Dexamethasone, Toll-like receptors, Glyceraldehyde 3-phosphate dehydrogenase, Cysteine-aspartic acid protease

1. Anesthesiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Corresponding Author: Fereshteh Baghizadeh, MD, Anesthesiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Tel: +98 21 2243 2572
Email:
fereshteh_baghizadeh@yahoo.com

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Introduction

Induction of anesthesia is always an integral part of most surgeries. The use of different types of drugs to perform this process has different effects. Meanwhile, neurological damage and subsequent disorders have always been a concern for many anesthesiologists and researchers. Therefore, in the last two decades, many studies have been conducted on the effects of anesthetics on the nervous system of children and the elderly (1). In this regard, a large number of studies have discussed the potential unwanted effects of anesthetic drugs; both on the central neural network and peripheral nerve cells (2, 3).

Ropivacaine is an amino amide that has fewer toxic effects than other environmental anesthetics (4, 5). On the other hand, it has a lower lipophilic level than other amides, which causes it to pass through myelinated motor fibers, with a longer duration of block and longer analgesic effects than lidocaine and mepivacaine (6-8).

Many studies show Dexmedetomidine alone has

less neurotoxic effects, possibly due to fewer effects on the GABA or NMDA receptor pathways (9, 10), with some reports about its organ protective effects including neuroprotection (11).

Corticosteroids are another class of drugs whose anti-apoptotic effects have been discussed; while some reports have shown their potential effects as cognitive impairment in immature rodents (12, 13).

N-Methyl-D-Aspartate (NMDA) and Gamma-Aminobutyric Acid (GABA) are the most important receptors involved in the pathway of brain development while being among the main functional mechanisms of anesthetic drugs (14, 15). Possibly, these anesthetics might affect cellular neuro-apoptosis by disrupting the pathway of these receptors (16).

Toll-like receptors (TLR4) are another category is cellular receptors that are introduced as an endotoxin detector (LPS), a cellular stressor, and a cell content-related injury such as DNA, RNA, and the cytoplasm (17).

Previous studies have discussed the role of this protein in nervous system damage and the formation of oxygen-free radicals and inflammation as the etiology and pathogenicity of TLR4. So, TLR4 level assay could be an appropriate surrogate measure in neural cell cytotoxicity (18). Sequential activation of caspases plays a central role in the execution phase of cell apoptosis (19). However, caspase-3 seems necessary for normal brain development; besides the role in apoptosis (20).

Nervous system inflammation (one of the side effects of anesthetics) is caused by the activation of microglia and the presence of astrocytes along with the involvement of neurons (21). However, this does not include all cases and there are several other ways to do this type of cell damage (22).

This study was designed to investigate the neurotoxic effects of Ropivacaine and its association with two dexamethasone and dexmedetomidine drugs after injection into the rat femoral nerve.

Methods

This study was reviewed and approved by the Research Ethics Committee of Shahid Beheshti University of Medical Sciences, with the registration code of IR. SBMU. RETECH.REC.1397.1030.

For evaluating neurotoxicity of Ropivacaine, Dexmedetomidine, and Dexamethasone, Mice (*Mus musculus*) with a similar weight (22 to 30 gr) were used. Mice were kept and used according to the companion animal ethics (23). Four random groups of mice were formed (Figure 1).

After 24 h, the mice sacrificed using gas CO2 and the femoral nerve removed. Hematoxylin-eosin staining was used to evaluate changes in the effects of the drugs on isolated nerve tissues, and nerve samples extracted to measure TLR4 and Caspas3 expression.

Radio-immuno-precipitation assay buffer (RIPA buffer) then extracts the protein. The expression levels of the proteins evaluated by electrophoresis and western blotting. At this stage, 20-40 µg of protein used for Western blotting. Image J software is used to detect and analyze images from Western blotting.

Data were analyzed by Statistical Package for Social Sciences, version 14 (SPSS Inc., Chicago, IL, USA) on a Microsoft Windows-based setting. After testing for normality of pairwise differences with the Shapiro-Wilk normality test, the effect of Ropivacaine, Dexmedetomidine- Ropivacaine, and Dexamethasone-Ropivacaine on compared between groups using independent t-tests. A value of $P<0.05$ was considered statistically significant.

Results

The expression of TLR4 and caspase 3 (as contributing factors to apoptosis induction and toxicity) were compared with GAPDH (as a housekeeping gene and baseline) in four different groups. As can be seen in figure 2, the expression index of caspase 3 biomarkers was presented based on the amount and thickness of the band produced on SDS gel following the western blot technique. Figure 3 represents the qualitative range of affinity of the target protein (caspase 3 marker) to the standard GAPDH index. According to the electrophoresis affinity index (similar band thickness), the highest expression of Caspase 3 protein compared to GAPDH is related to Ropivacaine +

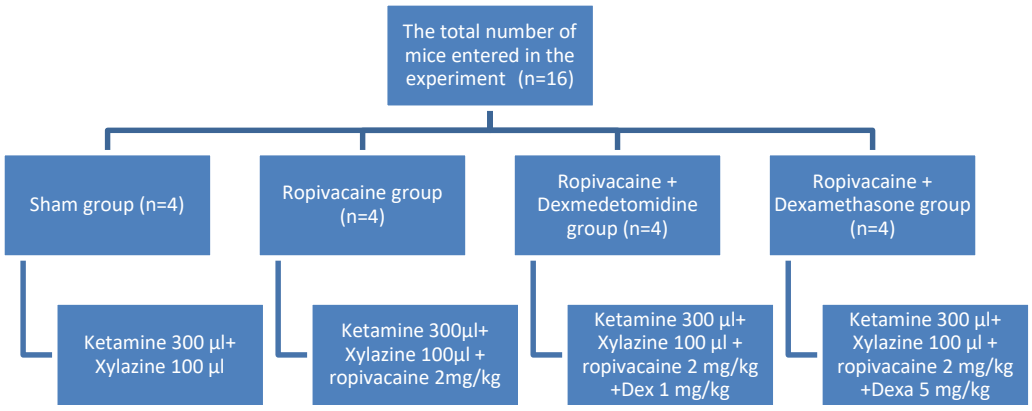

Figure 1. Flowchart related to how to sample and divide the groups included in the study.

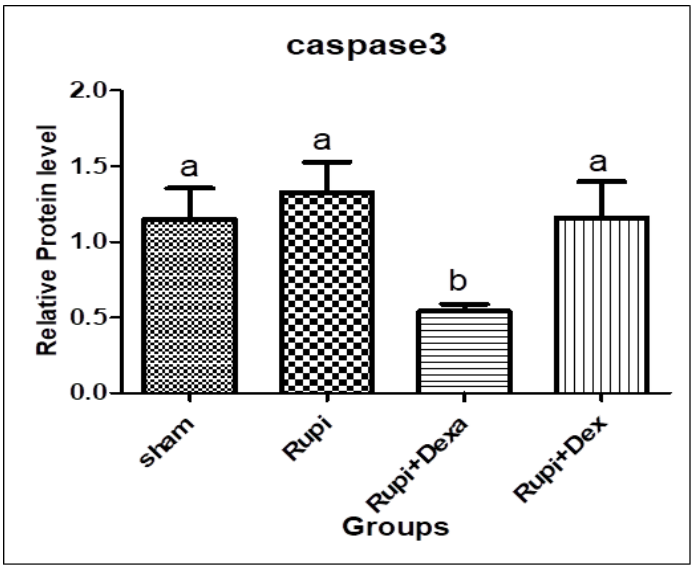

Figure 2. Diagram (mean P. Value) of caspase3 expression compared to GAPDH in rat femoral nerve (24 h after injection) in four groups (sham, Ropivacaine, Ropivacaine + dexamethasone, and Ropivacaine + dexmedetomidine).

Dexmedetomidine composition.

Figure 4 presents the quantification of TLR4 biomarker based on the amount and density of the band produced on SDS gel, followed by the Western blot technique. This table presents the results of TLR4 protein extraction 24 h after injection into the rat femoral nerve compared to the standard GAPDH index for the four groups (control, bupivacaine, bupivacaine + dexamethasone, and bupivacaine + dexmedetomidine).

Figure 5 shows the electrophoresis results of the proteins extracted on the gel. This table presents the qualitative range of affinity of the target protein (TLR4 marker) by group and extraction site compared to the standard GAPDH index.

Discussion

Numerous studies have focused on the neurotoxicity effects of all kinds of anesthetic drugs. This type of disorder often manifests itself as damage or death in the cells of the neurons, resulting in different outcomes such as Alzheimer's, behavioral changes, and even transient and lasting cognitive changes (24). However, given the many limitations of the ability to repair the damaged central nervous system, it is important to

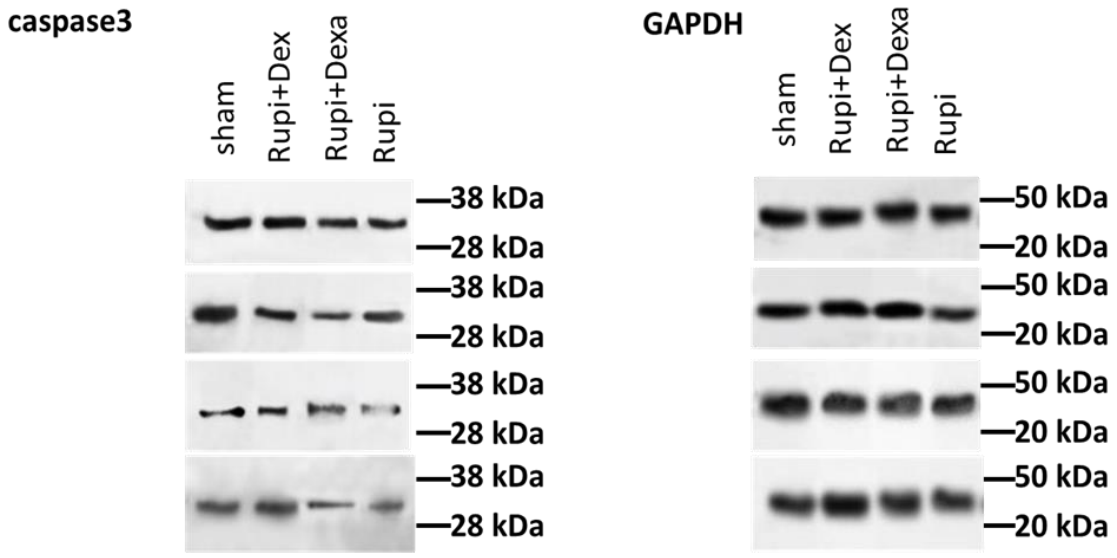

Figure 3. Qualitative comparison of caspase3 protein expression in rat femoral nerve for four groups (sham, Ropivacaine, Ropivacaine + dexamethasone, and Ropivacaine + dexmedetomidine) compared to standard GAPDH index protein.

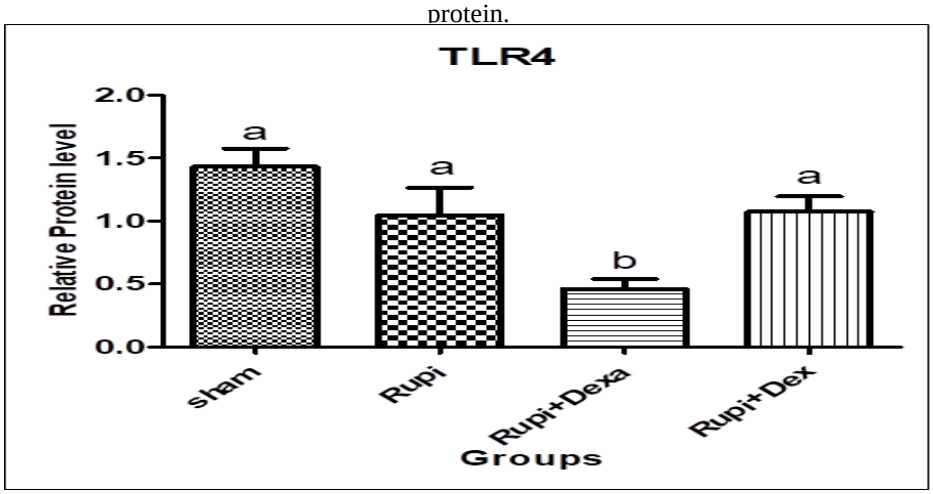

Figure 4. Diagram (mean P. Value) of TLR4 expression compared to GAPDH in rat femoral nerve (24 h after injection) in four groups (sham, Ropivacaine, Ropivacaine + dexamethasone, and Ropivacaine + dexmedetomidine).

reduce the damage caused by inhibiting apoptotic neural cell death (25). The mechanism of this type of damage at the cellular level is still unclear, but what is being speculated about is the activation of microglia and the presence of astrocytes, which in turn causes devastating effects by involving neurons (26, 27). These injuries often increase the likelihood of disorders such as Alzheimer's by causing inflammation on the surface of the nervous system. A more detailed classification of nerve injury induction routes is demonstrated in Figure 6.

Ropivacaine is a local anesthetic widely used in surgical procedures worldwide (28). Prescription of these drugs administration is associated with an increased inflammatory response, altered nerve permeability, and myotoxicity (29). This amino amide has toxic effects such as other environmental anesthetics. Therefore, it is important to consider the approach to reduce the toxic effects of taking this drug.

The primary aim of the present study was to evaluate the neurotoxicity of Ropivacaine and combination with Dexmedetomidine and Dexamethasone in mice neural apoptosis when injected perineurally. Furthermore, this study was designed to test this hypothesis that Dexmedetomidine and Dexamethasone in combination with Ropivacaine have different effects on nerve injury in rats. Based on the results, it is observed that the use of ropivacaine in

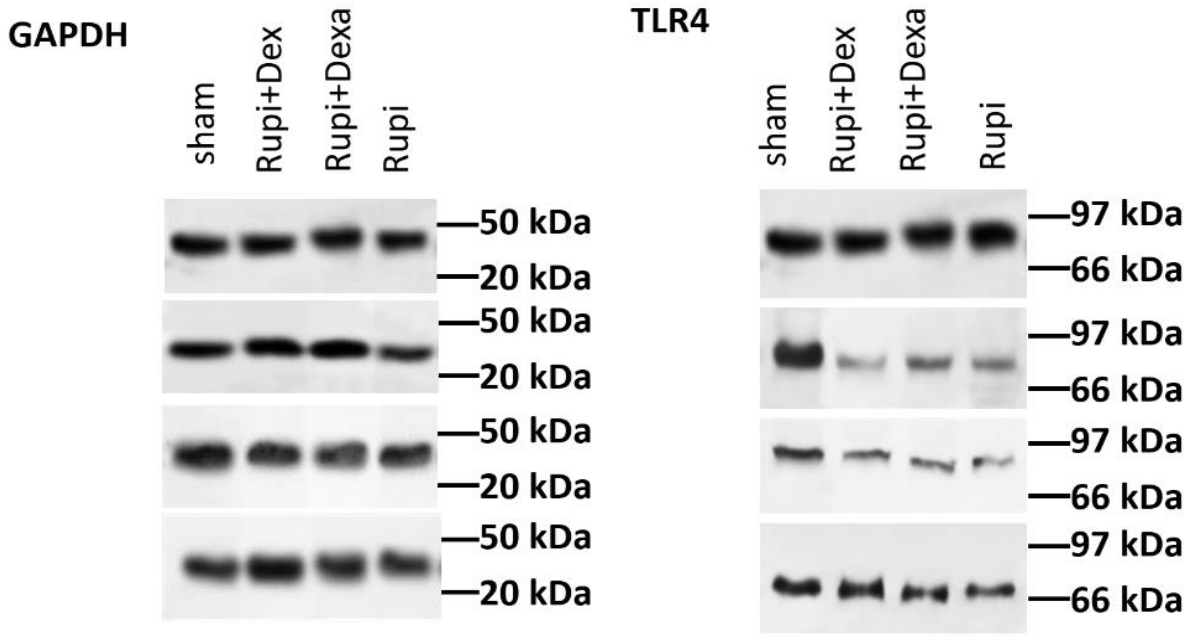

Figure 5. Qualitative comparison of TLR4 protein expression in rat femoral nerve for four groups (sham, Ropivacaine, Ropivacaine + dexamethasone, and Ropivacaine + dexmedetomidine) compared to standard GAPDH index protein.

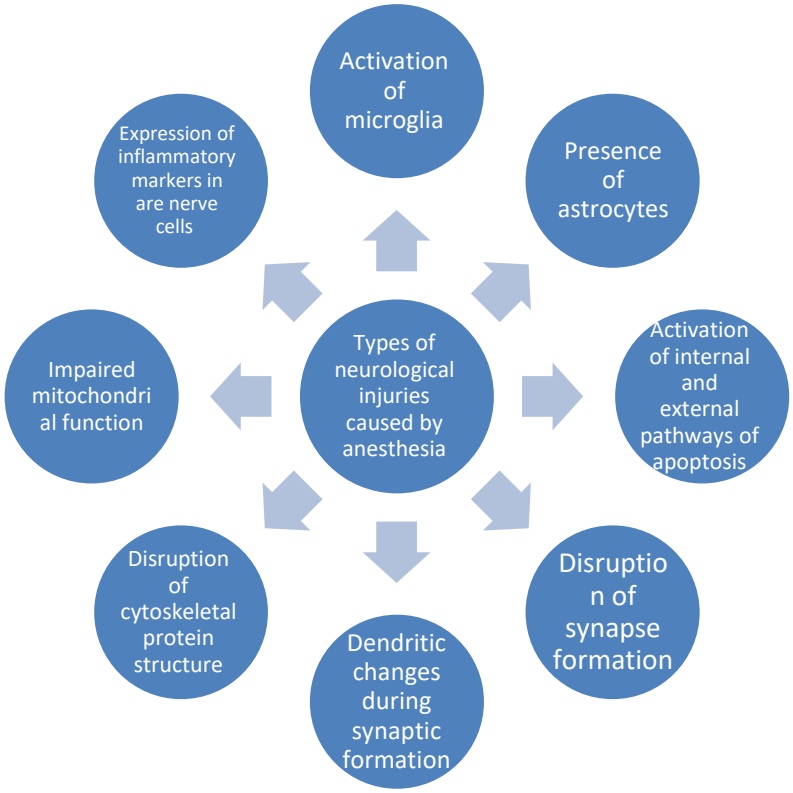

Figure 6. Routes of nerve injury induction.

combination with dexamethasone caused less damage to the rat nerve cells. Importantly, the combined use of ropivacaine with dexamethasone (p=0.53 and p=0.46) compared to the combination of ropivacaine with dexmedetomidine (p=1.158 and p=1.074) show relatively better conditions in terms of cytotoxicity

(Figs. 3 and 5). Indeed, we demonstrated that the combined use of ropivacaine with dexamethasone reduces its neurotoxicity risk. An earlier study of the effects of clonidine and dexmedetomidine on the effects of ropivacaine as a local anesthetic showed that neurotoxicity leading to apoptosis of nerve cells decreased due to increased microglia activity following a combined administration of ropivacaine and clonidine. As a result, it has been shown that the use of ropivacaine with clonidine or dexamethasone in addition to improving the quality of anesthetic effects also reduces neural toxicity (30, 31). This type of change can also be seen in the central nervous system, with the simultaneous use of both Dexmedetomidine and ropivacaine increasing the quality of anesthesia and reducing the activity of astrocytes. It should be noted that the combination of these two drugs is useful for improving the effect of anesthesia and reducing inflammation in the central nervous system (31, 32).

Conclusion

Based on the results, the combined use of ropivacaine with dexamethasone reduces its neurotoxicity risk. However, the human phase of this research and closer scrutiny of the cellular mechanisms behind the use of these drugs could paint a brighter horizon for physicians and researchers.

Acknowledgment

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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